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Knopf et al.(10) **Pub. No.: US 2010/0183695 A1**(43) **Pub. Date: Jul. 22, 2010**(54) **CERBERUS/COCO DERIVATIVES AND USES THEREOF****Related U.S. Application Data**(76) Inventors: **John Knopf**, Carlisle, MA (US);
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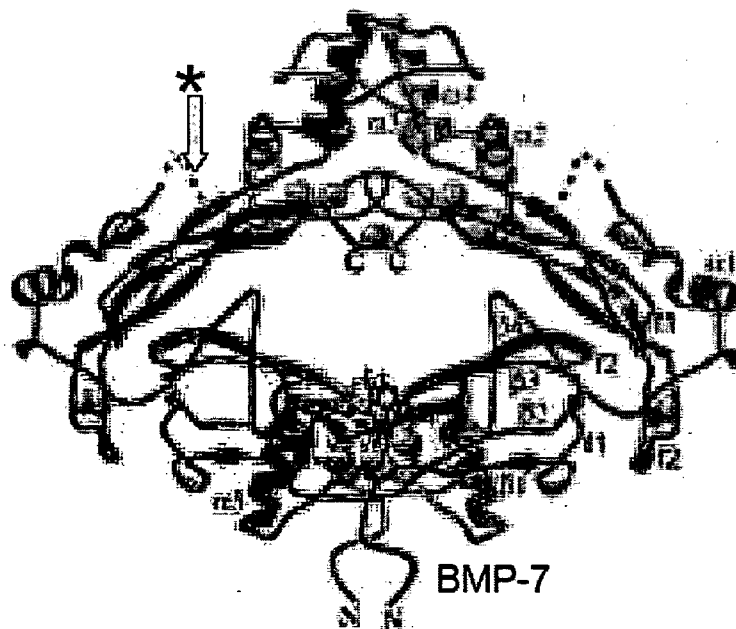
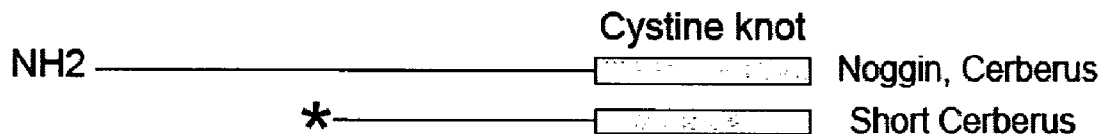
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A61P 21/00 (2006.01)(21) Appl. No.: **11/597,182**(52) **U.S. Cl. 424/422; 514/14; 514/2**(22) PCT Filed: **May 27, 2005**(57) **ABSTRACT**(86) PCT No.: **PCT/US05/18928**

§ 371 (c)(1),

(2), (4) Date: **Mar. 22, 2010**

The invention relates to Cerberus/Dan/Gremlin polypeptides or variants thereof for use in treating a variety of disorders associated with myostatin, nodal and GDF-11. Preferred polypeptides are Coco or Cerberus derivatives.



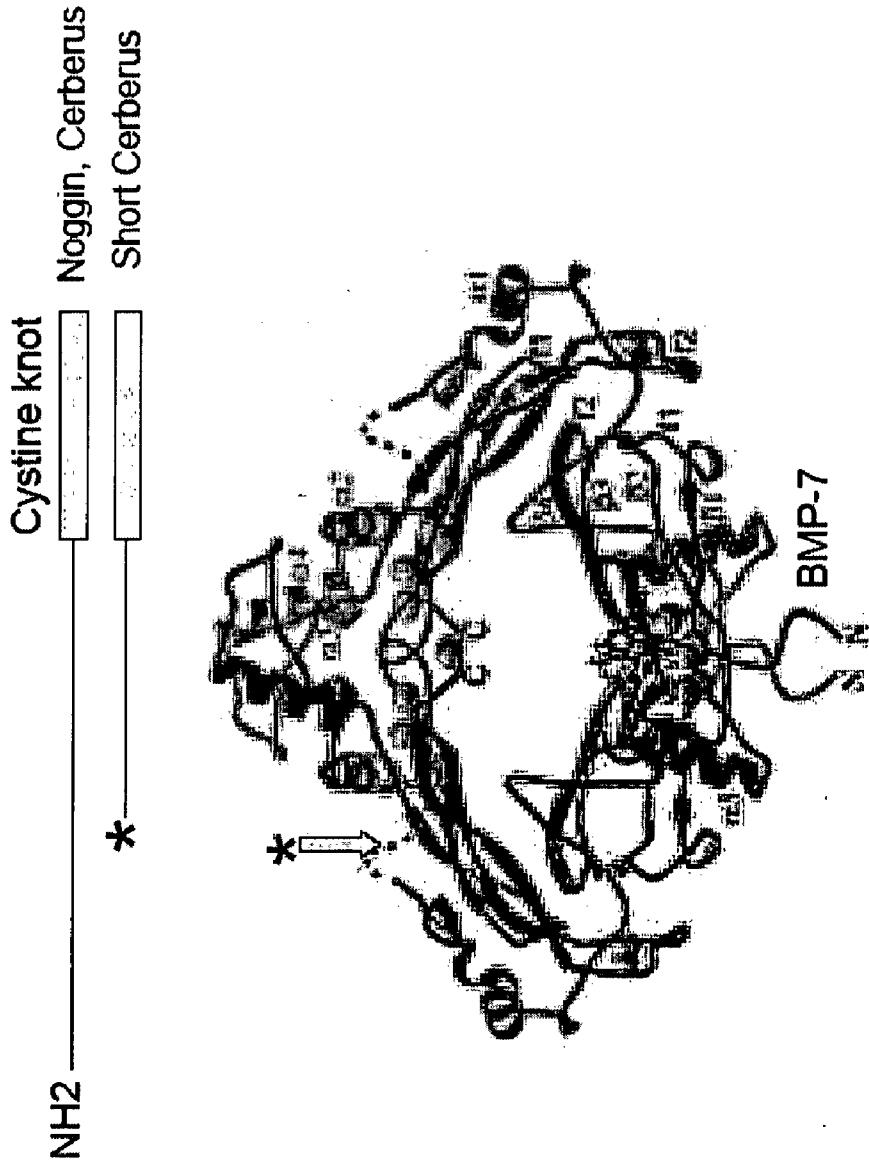


Figure 1

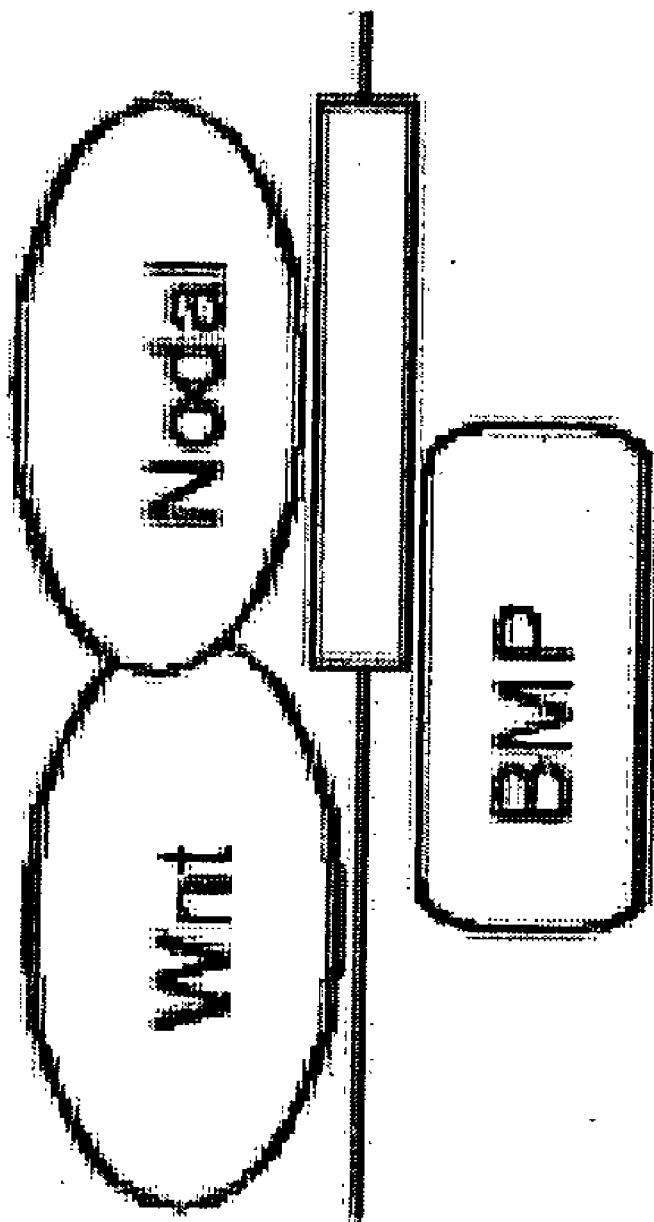


Figure 2

A

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1  CCA CGC GTC CGA GAA AAT TGG AAA CCA GCG ACT CTG GGT CAA TCT TGA CCC TCC AGC CAG ACG ACA
    M L L
67  GGG GAC CGA CAA ACC CCC AGA CCC CAA ACC CTA ATA AGG AGG ATT GTG TAA GAA GAC ATG CTG TTG
    F Q A T S L L A L L C F T V R A F P F M E E
133  TTC CAG GCC ACC AGC CTA TTG GCC CTT CTC TGT TTC ACG GTC AGG GCA TTT CCC TTT ATG GAA GAA
    E G S A S F A Q N V L H S R S F P V S H H G
199  GAA GGG TCG GCT TCG TTT GCC CAG AAT GTG CTC CAC AGC AGA TCC TTC CCA GTC TCC CAC CAT GGA
    A F M D L P L F R Q N R R K I S Q N F I L H
265  GCT TTC ATG GAC CTG CCG CTA TTC AGA CAG AAC AGG AGG AAA ATA TCC CAG AAT TTC ATC CTG CAC
    S D F R E H M D E E A L R R K L V W E S A I
331  TCA GAT CCC AGA GAG CAC ATG GAT GAG GAG GCG CTG AGG AGA AAA CTG GTG TGG GAG AGC GCC ATC
    R R D R M R S Q P D Q V L P I G Q D A L K R
397  CGC AGG GAC AAG ATG AGA TCC CAA CCA GAC CAG GTG CTG CCC ATT GGG CAA GAT GCT CTG AAA CGC
    S R C H A L P F I Q N V F R K N C F P V R L
463  TCC AGA TGC CAT GCC TTG CCT TTC ATA CAG AAT GTG TTC AGG AAG AAC TGC TTC CCA GTG CGC CTC
    F N K F C F G Q C N S F Y V F G W P A G L S
529  CCT AAC AAG TTC TGC TTT GGC CAG TGC AAC TCC TTC TAT GTG CCT GGT TGG CCT GCC GGA CTC TCC
    Q P C T S C A P S R S R R I S L P L R C R S
595  CAG CCC TGT ACG TCC TGC GCC CCC AGT CGA TCC CGG CGC ATT TCG CTG CCG TTA CGC TGT CGT TCC
    G H L A W Q E V E L V E E C E C E T R Y D R
661  GGT CAC CTT GCT TGG CAA GAG GTG GAG CTG GTG GAA GAG TGC GAG TGC GAA ACC CGC TAC GAC AGG
    N T V E P A G S G E D Y L P V S *
727  AAT ACG GTG GAG CCA GCT GGC AGC GGA GAC TAC CTG CCC GTT TCA TAG GCC CAA ACC GCT CTA
793  CAT GTG CCC AAA TGG ACC AAA CTA TGT GAC CCG GGG CCT TGG CGC TCC CAG TAA CTT CAC CCT TTG
859  ACA GGC GTC CCT TTG CCA AAG CAG GGG AAG TTC CAA CGA CTT GAC GAC TTT ATT TAA GAA TGA ACA
925  GTT CTG ACC GAC GGC TGA TTT ATA CGC TTG TCT AGA TTC CCA GAA TCC CTA GGG GGA AAG CCT GTG
991  ACG AAG CTC CCG CTT TAT TGG GAG GGT GTG AGT AAT GAT TCA ACA TGG CGG AAT TCT GCC TCT TAT
1057  TGT ACC CGG GAG ACT GGT CTC CTG CCA TAT TGT GCT GTG TAT AAG GGC TGT GCC CGT CCT GCT CAT
1123  CTT TTA TAC AAG TGA TGC TTT TAT TGG TAG TTA TTA TGC ATT GTC TGT GCC AGT CCT GTT GTC AAT
1189  TCA ACT TTC CAG ACC GCG TCT ATA AGT GTC ATG GTT ATT GAT GGC GTT GGT TTT GCC TTT CTG CAC
1255  TGC TGG TTC TGA CTC TTA AAA TAA TGT AGG TTC TTG TCC AGG TCA GTT GAT CCG CTG GCT GCT GCA
1321  TTG TTT TGG GAG TCT GAG CCA GCA GTG CAG AGA ATA TAA ACA GAC ATG GCT TCC AAT AGC AGT GAC
1387  TTT TAC CTA GAA CCA GTG GAA ATG AGG AAT GGA TAT TGG GAA GTC TTA TTC CTG ATT TTA AGC A

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B

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xCoco (1) ALKRSRCHALPFIQNVFRNCPPVRLPNKFCFGQCSFYVEGWP-----AGLSQPCTSCAPSRSRRLSLPLR
mCoco (1) -----VISRPGCTSAARVLNHLCPGRCSSFYIPSSDP-----TPVVFCSNCSVPARKRWTSVTLW
hCoco (1) -----VFSRPGCSAARLRLNHLCPGHCSLLYIEGSDP-----TPLVLCSNCSMPARKRWAPVVLW
fugu Coco (1) KEGKQSCSGVPPPTQRYTAAGCSAVTVHNKLCFGQCSSLFVPSAELPGTGMGLLHHRGPCSRCAFSKAHAVVLPDL
xCerberus (1) EIMKEACKTLPPFTQNIHENCDDRMVIONHLCPGKCSISLHVFNQ-----QDRRNTCSHCLEPSKFTLNHLPLN
hCerberus (1) EVHWETCRTPVPSQTIHETGCEKVVVQNNLCPGKCGSVHFFGAA-----QHSHTSCSHCLPAKFTTNHLPLN
cCerberus (1) EVHWETCRTPVFNQTIHEDCQNVVQNNLCPGKCSSIRFFEGEG-----ADAHSFCSHCSPKFTTNHLPLN
cCaronte (1) EMBQETCRTPFSQSVAHESCEKVIVQNNLCPGKCSSFHVPGPD-----DRLYTFCSKCLBTKFSMKHFDLN

xCoco (68) CRSGHL-----AWQEVLEVCECECTRYDRNTVEPAGSGEDYLPVS----
mCoco (54) CGAGQLASPRRVKISTVLVQKCQCRPKL-----
hCoco (54) CLTGSSASRRRVKISTMLIEGCHCSPKA-----
fugu Coco (76) CGARVQ-----EKRTSERSRGDLEHNDNNDDDDGDDGGDDDDVAG---
xCerberus (67) CTGSKN-----VVKVVMVVEECCEAHKSNFHTAQFNMDTSTTLHH---
hCerberus (68) CTGLSS-----VVKVVMVVEECQCKVTEHEDGHILHAGSQDSFIPG---
cCerberus (68) CTSEPT-----VVKVVMVVEECQCKVTERGEERLLLAGSQGSFIPG---
cCaronte (68) CTSSVP-----VVKVVMVVEECNCETQKIEDP--LLGS-LQSDPLGNVPE

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Figure 3

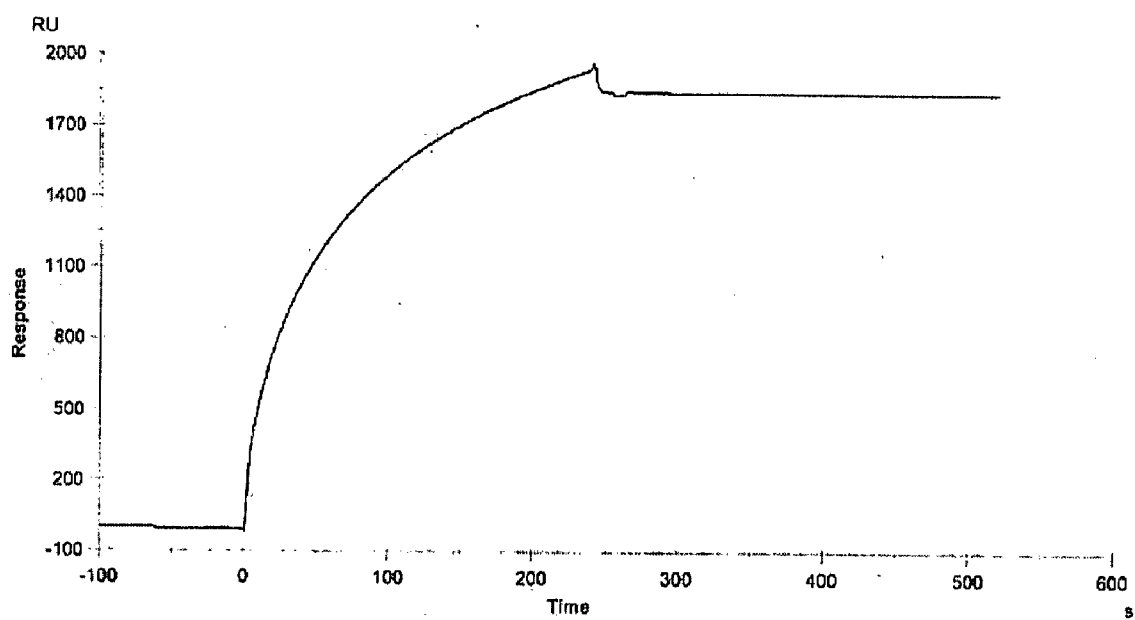


Figure 4

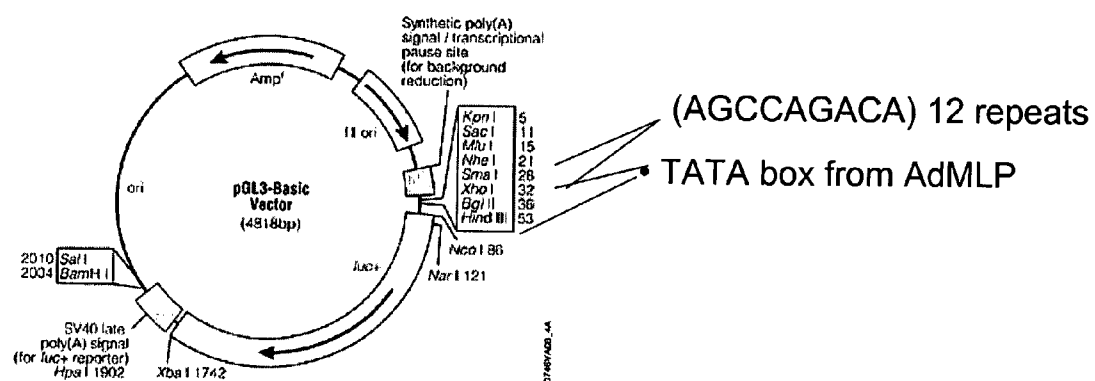


Figure 5

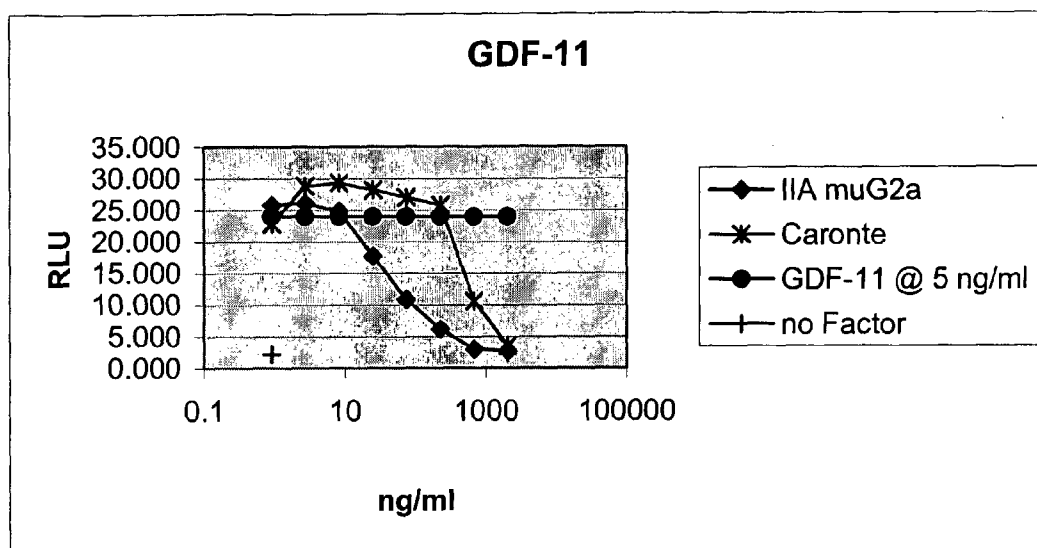


Figure 6

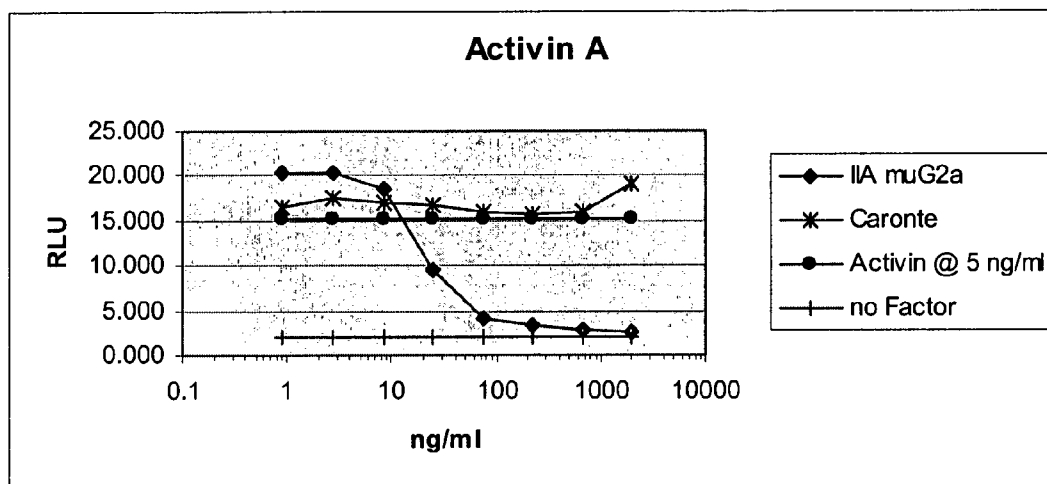


Figure 7

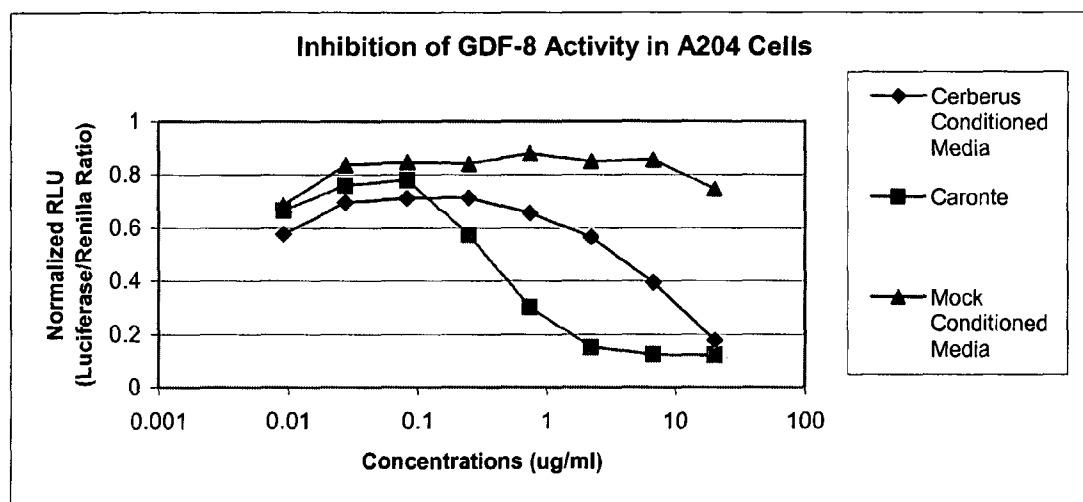


Figure 8

CERBERUS/COCO DERIVATIVES AND USES THEREOF

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application Ser. No. 60/575,062, filed May 27, 2004. All the teachings of the above-referenced application is incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] Transforming growth factor- β superfamily proteins represent a large family of cytokines that includes the TGF- β s, activins, inhibins, bone morphogenetic proteins (BMPs) and Mullerian-inhibiting substance (MIS) (for review, see Massague et al., *Trends Cell Biol.* 7:187-192, 1997). These proteins contain a conserved C-terminal cystine-knot motif, and serve as ligands for diverse families of plasma membrane receptors. Members of the TGF- β family exert a wide range of biological effects on a large variety of cell types. For example, they regulate cell growth, differentiation, matrix production and apoptosis. Many of them have important functions during embryonal development in pattern formation and tissue specification; in the adult, they are involved in processes such as tissue repair and modulation of the immune system.

[0003] Activities of the TGF- β superfamily proteins are regulated through various means. One of the negative regulations for the BMP subfamily of proteins is through a relatively large family of so-called Bone Morphogenetic Protein (BMP) antagonists/repressors. These BMP repressors represent a subgroup of proteins that bind BMPs, and interfere with BMP binding to their membrane receptors, thereby antagonizing their actions during development and morphogenesis.

[0004] The BMP repressors can be further divided into three groups of proteins based on structural analysis, especially the number of structurally conserved Cys residues in their C-terminal characteristic "Cys-knot" structures: the 8-, 9-, or 10-member ring Cys-knot BMP repressors. The 8-member ring (CAN subfamily) repressors can be divided further into four subgroups based on a conserved arrangement of additional cysteine residues—gremlin and PRDC, Cerberus and coco, and DAN, together with USAG-1 and sclerostin. Orthologs of these human BMP antagonists in the genomes of several model organisms have also been identified, and their phylogenetic relationship has been analyzed (Avsian-Kretschmer and Hsueh, *Mol Endocrinol.* 18(1): 1-12, 2004. Epub 2003 October 02, incorporated herein by reference).

[0005] Myostatin, or growth/differentiation factor 8 (GDF-8), also belongs to the transforming growth factor- β (TGF- β) superfamily (McPherron et al., *Nature* 387:83-90 (1997)). The human myostatin gene has been cloned (Nestor et al. *Proc. Natl. Acad. Sci.* 95:14938-43 (1998)), and it has been reported that myostatin immunoreactivity is detectable in human skeletal muscle in both type 1 and type 2 fibers. With respect to function, myostatin may play a role in negatively regulating the growth and development of skeletal muscle (Nestor et al., *supra*).

[0006] A study with myostatin knock-out mice provided the first evidence that myostatin is a key negative regulator of muscle development (McPherron et al., *Nature* 387:83-90 (1997)). In the myostatin null mice, the animals were significantly larger than wild-type mice and had a large and wide-

spread increase in skeletal muscle mass. Furthermore, two breeds of cattle, characterized by increased muscle mass, have mutations in the myostatin coding sequence (McPherron et al., *Proc. Natl. Acad. Sci.* 94:12457-61 (1997)). A naturally occurring myostatin reduced-function mutation in a human child is associated with gross muscle hypertrophy and a family history of exceptional strength. (Williams M S, *N Engl J. Med.* 2004 Sep. 2; 351(10):1030-1; author reply 1030-1.)

[0007] Additionally, it should be noted that the serum and intramuscular concentrations of immunoreactive myostatin are increased in HIV-infected men with muscle wasting compared with healthy men, and correlate inversely with the fat-free mass index. These data support the hypothesis that myostatin is a negative regulator of skeletal muscle growth in adult men and contributes to muscle wasting in HIV-infected men (Nestor et al., *supra*).

[0008] In view of the above findings, a need exists for a manner of regulating myostatin activity, particularly in individuals who experience muscle wasting as a result of a condition or disease state such as, for example, aging, Autoimmune Deficiency Syndrome (AIDS), Multiple Sclerosis, and cancer. The present invention provides methods and compositions which may be utilized to help individuals with such muscle wasting conditions and provides further insight into the regulation of myostatin gene expression.

SUMMARY OF THE INVENTION

[0009] One aspect of the invention provides a pharmaceutical preparation of Cerberus, Coco or other polypeptide from the Cerberus/Dan/Gremlin superfamily (collectively herein "CDG proteins") for inhibiting the function/signaling of Nodal, myostatin and BMP. Exemplary preparations of the subject CDG polypeptides may include variant Cerberus or Coco sequences that substantially retain the binding affinity of the parent protein to Nodal, myostatin and/or another BMP (such as BMP-4). For instance, the present invention provides pharmaceutical preparations for inhibiting myostatin, comprising a myostatin antagonist protein that includes (at least) a myostatin binding domain of a Cerberus/Dan/Gremlin polypeptide or variant thereof. The myostatin antagonist protein binds to and neutralizes one or more of nodal and/or myostatin. Preferably, the pharmaceutical preparation is substantially free of pyrogenic materials so as to be suitable for administration as a human or veterinarian therapeutic.

[0010] These pharmaceutical preparations can be used to reduce the severity of a pathologic condition, which is characterized, at least in part, by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject. For instance, the pharmaceutical preparations of the present invention can be administered in an amount effective to prevent, ameliorate or reduce the severity of a wasting disorder, such as cachexia, anorexia, DMD syndrome, BMD syndrome, AIDS wasting syndrome, muscular dystrophies, neuromuscular diseases, motor neuron diseases, diseases of the neuromuscular junction, and inflammatory myopathies. These pharmaceutical preparations can also be used to reduce the severity of a pathologic condition, which is characterized, at least in part, by an abnormal amount, development or metabolic activity of heterotopic ossification in tissues such as of muscles, tendons, and ligaments; Osteoarthritis (OA), including the development of osteophytes and synovial thick-

ening; ovarian cancer; Fibrodysplasia ossificans progressiva (FOP); Atherosclerosis, especially inflammatory response in early steps of atherogenesis in lesion-prone areas; and craniosynostoses.

[0011] Another aspect of the invention provides a pharmaceutical preparation of CDG protein derivative for specifically inhibiting Nodal and/or myostatin function without substantially compromising BMP (such as BMP-4) signaling (e.g., does not substantially bind BMP-4 or other BMPs). Exemplary preparations of this aspect of the invention include polypeptides including the N-terminal truncated versions of Cerberus or Coco, or other fragments that include the cysteine-core. These so-called "N-terminally truncated CDG derivatives" can be used to reduce the severity of a pathologic condition, which is characterized, at least in part, by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject. For instance, the pharmaceutical preparations of the present invention can be administered in an amount effective to prevent, ameliorate or reduce the severity of a wasting disorder, such as cachexia, anorexia, DMD syndrome, BMD syndrome, AIDS wasting syndrome, muscular dystrophies, neuromuscular diseases, motor neuron diseases, diseases of the neuromuscular junction, and inflammatory myopathies.

[0012] In certain embodiments, the myostatin inhibitor is a polypeptide that includes a myostatin binding domain of a CDG protein. For instance, the Cerberus protein variant can be derived from a human, mouse, or other species of Cer-

[0014] For example, residues 106-119 of human Cerberus is:

PPGTQSLIQPIDGM

[0015] Residues 241-267 of human Cerberus is:

CKVKTEHEDGHILHAGSQDSFIPGVSA

[0016] Also included are Cerberus derived variant sequence, e.g., N-terminally truncated myostatin binding domain of Cerberus that retains myostatin binding activity but loses other BMP binding activity. Variant sequences may be desirable as a way to alter selectivity of the inhibitor (e.g., relative to GDF-8, GDF-11 or nodal binding), alter other binding characteristics with respect to myostatin (such as K_d , and/or K_{on} or K_{off} rates), or improve biodistribution or half life in vivo or on the shelf.

[0017] In certain preferred embodiments, the Cerberus polypeptide (full-length or N-terminally truncated) comprising the myostatin binding domain binds myostatin with a K_d of 1 μ M or less, and more preferably a K_d of 100 nM, 10 nM or even 1 nM or less.

[0018] In certain related embodiments, the myostatin inhibitor is a polypeptide that includes a myostatin binding domain of a Coco protein, such as the human Coco protein shown in FIG. 3 or in GenBank Accession number 22749329. An exemplary human Coco protein sequence is

1 MLGQLSTLL CLLSGALPTG SGRPEPQSPR PQSWAANQT WALGPGALPP LVPASALGSW
61 KAFLGLQKAR QLGMRLQRG QDEVAAVTLP LNPQEVIQGM CKAVPFVQVF SRPGCSAIRL
121 RNHLFCGHCS SLYIPGSDPT PLVLCNSCMP ARKRWAPVVL WCLTGSSASR RRVKISTMLI
181 EGCHCSPKA

berus, including a human or mouse Cerberus variant sequence sharing at least about 50%, 60%, 70%, 80%, 90%, 95%, or 99% or more sequence similarity or identity with the human or mouse Cerberus protein, and substantially retain the binding affinity of wild-type Cerberus for myostatin. Likewise, the Coco protein variant can be derived from a human, mouse, or other species of Coco, including a human or mouse Coco variant sequence sharing at least about 50%, 60%, 70%, 80%, 90%, 95%, or 99% or more sequence similarity or identity with the human or mouse Coco protein, and substantially retain the binding affinity of wild-type Coco for myostatin.

[0013] In certain related embodiments, the myostatin inhibitor is a polypeptide that includes a myostatin binding domain of a CDG protein, which polypeptide does not substantially bind to BMP-4 (and other BMPs). For instance, the myostatin binding domain can be derived from a human, mouse, or other species of N-terminally truncated Cerberus, including a human or mouse Cerberus derivative, with amino acid residues starting from any one of residues 106-119 of SEQ ID No. 2 or 8 of US 2002/0164682 A1 (see below), and ending at any residue after residue 241 of SEQ ID No. 2 or 8 of US 2002/0164682 A1, preferably ending at any residue between residues 241 and 267 of SEQ ID No. 2 or 8 of US 2002/0164682 A1 (all residue numbers inclusive).

[0019] In certain preferred embodiments, the Coco polypeptide (full-length or N-terminally truncated) comprising the myostatin binding domain binds myostatin with a K_d of 1 μ M or to less, and more preferably a K_d of 100 nM, 10 nM or even 1 nM or less.

[0020] In certain embodiments, the myostatin binding domain is part of a fusion protein including, in addition to the myostatin binding domain, one or more polypeptide portions that enhance one or more of in vivo stability, in vivo half life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. For instance, the fusion protein can include an immunoglobulin Fc domain. The fusion protein may include a purification subsequence, such as an epitope tag, a FLAG tag, a polyhistidine sequence, or as a GST fusion.

[0021] In certain embodiments, the myostatin binding domain is part of a protein that includes one or more modified amino acid residues, such as a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, or an amino acid conjugated to an organic derivatizing agent.

[0022] In certain embodiments, the subject variant CDG polypeptide is selective for binding and inhibition of myostatin, e.g., relative to GDF-11 and/or nodal. For instance, the variant CDG polypeptide can be one which has a dissociation

constant (K_d) for myostatin binding that is at least 2 times less than its K_d for binding GDF-11 and/or nodal, and even more preferably at least 5, 10, 100 or even 1000 times less. Whether by virtue of binding kinetics or biodistribution, the subject variant CDG polypeptide can also be selected based on relative in vivo potency, such as an inhibitor that has an EC_{50} for inhibiting myostatin activity, or a particular physiological consequence (such as promoting muscle growth, promoting bone density or inducing adipocytes differentiation) that is at least 2 times less than its EC_{50} for inhibiting GDF-11 and/or nodal activities, and even more preferably at least 5, 10, 100 or even 1000 times less.

[0023] In certain embodiments, the subject variant CDG polypeptide is selective for binding and inhibition of myostatin, e.g., relative to other BMP proteins such as BMP-4. For instance, the variant CDG polypeptide can be one which has a dissociation constant (K_d) for myostatin binding that is at least 2 times less than its K_d for binding BMP-4, and even more preferably at least 5, 10, 100 or even 1000 times less. Whether by virtue of binding kinetics or biodistribution, the subject variant CDG polypeptide can also be selected based on relative in vivo potency, such as an inhibitor that has an EC_{50} for inhibiting myostatin activity, or a particular physiological consequence (such as promoting muscle growth, promoting bone density or inducing adipocytes differentiation) that is at least 2 times less than its EC_{50} for inhibiting BMP-4 activities, and even more preferably at least 5, 10, 100 or even 1000 times less.

[0024] In certain preferred embodiments, the variant CDG polypeptide binding domain binds myostatin with a K_d of 1 μ M or less, and more preferably a K_d of 100 nM, 10 nM or even 1 nM or less.

[0025] In general, the subject myostatin inhibitor preparations are suitable for use in a human patients. In preferred embodiments, the subject preparations of variant CDG polypeptides will be substantially free of pyrogenic materials so as to be suitable for administration to a human patient.

[0026] In other embodiments, the subject variant CDG polypeptides can be administered to non-human animals, particularly other mammals. For example, the compounds of the present invention can be given to chickens, turkeys, livestock animals (such as sheep, pigs, horses, cattle, etc.), companion animals (e.g., cats and dogs) or may have utility in aquaculture to accelerate growth and improve the protein/fat ratio. To further illustrate, the subject variant Cerberus polypeptides can be used to stimulate growth or enhance feed efficiency of animals raised for meat production to improve carcass quality, or to increase milk production in dairy cattle.

[0027] Another aspect of the invention relates to packaged pharmaceuticals comprising a pharmaceutical preparation of a variant CDG polypeptide, as described herein, and a label or instructions for use in promoting growth of muscle tissue in a human patient.

[0028] Still another aspect of the invention relates to packaged pharmaceuticals comprising a pharmaceutical preparation of a variant CDG polypeptide, as described herein, and a label or instructions for veterinarian use in promoting growth of muscle tissue in a non-human mammal.

[0029] Another aspect of the invention relates to a method for inhibiting myostatin signal transduction in vivo by administering a pharmaceutical preparation of one or more of the subject variant CDG polypeptides. The subject method can be used to promote muscle growth, promote adipogenic differ-

entiation, and/or promote bone growth or mineralization in human patients or in non-human animals.

[0030] In certain embodiments, the treatment methods of the present invention can be used to reduce the severity of a pathologic condition, which is characterized, at least in part, by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject. For instance, the pharmaceutical preparations of the present invention can be administered in an amount effective to prevent, ameliorate or reduce the severity of a wasting disorder, such as cachexia, anorexia, DMD syndrome, BMD syndrome, AIDS wasting syndrome, muscular dystrophies, neuromuscular diseases, motor neuron diseases, diseases of the neuromuscular junction, and inflammatory myopathies.

[0031] Exemplary muscular dystrophies that can be treated with a regimen including the subject myostatin include: Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD), Emery-Dreifuss Muscular Dystrophy (EDMD), Limb-Girdle Muscular Dystrophy (LGMD), Facioscapulohumeral Muscular Dystrophy (FSH or FSHD) (Also known as Landouzy-Dejerine), Myotonic Dystrophy (MD) (Also known as Steinert's Disease), Oculopharyngeal Muscular Dystrophy (OPMD), Distal Muscular Dystrophy (DD), and Congenital Muscular Dystrophy (CMD).

[0032] Exemplary motor neuron diseases that can be treated with a regimen including the subject myostatin include: Amyotrophic Lateral Sclerosis (ALS) (Also known as Lou Gehrig's Disease), Infantile Progressive Spinal Muscular Atrophy (SMA, SMA1 or WH) (Also known as SMA Type 1, Werdnig-Hoffman), Intermediate Spinal Muscular Atrophy (SMA or SMA2) (Also known as SMA Type 2), Juvenile Spinal Muscular Atrophy (SMA, SMA3 or KW) (Also known as SMA Type 3, Kugelberg-Welander), Spinal Bulbar Muscular Atrophy (SBMA) (Also known as Kennedy's Disease and X-Linked SBMA), and Adult Spinal Muscular Atrophy (SMA).

[0033] Exemplary inflammatory myopathies that can be treated with a regimen including the subject myostatin include: Dermatomyositis (PM/DM), Polymyositis (PM/DM), and Inclusion Body Myositis (IBM).

[0034] Exemplary diseases of the neuromuscular junction that can be treated with a regimen including the subject myostatin include: Myasthenia Gravis (MG), Lambert-Eaton Syndrome (LES), and Congenital Myasthenic Syndrome (CMS).

[0035] Exemplary myopathies due to endocrine abnormalities that can be treated with a regimen including the subject myostatin include: Hyperthyroid Myopathy (HYPTM) and Hypothyroid Myopathy (HYPOTM).

[0036] Exemplary diseases of peripheral nerve that can be treated with a regimen including the subject myostatin include: Charcot-Marie-Tooth Disease (CMT), Dejerine-Sottas Disease (DS), and Friedreich's Ataxia (FA).

[0037] Other exemplary myopathies that can be treated with a regimen including the subject myostatin include: Myotonia Congenita (MC), Paramyotonia Congenita (PC), Central Core Disease (CCD), Nemaline Myopathy (NM), Myotubular Myopathy (MTM or MM), and Periodic Paralysis (PP).

[0038] Exemplary metabolic diseases of muscle that can be treated with a regimen including the subject myostatin include: Phosphorylase Deficiency (MPD or PYGM), Acid Maltase Deficiency (AMD), Phosphofructokinase Deficiency (PFKM), Debrancher Enzyme Deficiency (DBD),

Mitochondrial Myopathy (MITO), Carnitine Deficiency (CD), Carnitine Palmitoyl Transferase Deficiency (CPT), Phosphoglycerate Kinase Deficiency (PGK), Phosphoglycerate Mutase Deficiency (PGAM or PGAMM), Lactate Dehydrogenase Deficiency (LDHA), and Myoadenylate Deaminase Deficiency (MAD).

[0039] The subject method can also be used to prevent, ameliorate or reduce the severity of a metabolic disorder, such as in the treatment of obesity or type II diabetes. To further illustrate, the subject variant CDG polypeptide preparations can be used to decrease body fat proportion in a subject.

[0040] In still other embodiments, the variant CDG polypeptide preparations can be used as part of such methods as: treating or preventing congestive heart failure; for reducing frailty associated with aging; increasing bone density (such as for treating osteoporosis) or accelerating bone fracture repair; treating growth retardation, treatment of physiological short stature, attenuating protein catabolic response such as after a major operation; reducing protein loss due to chronic illness; accelerating wound healing; accelerating the recovery of burn patients or patients having undergone major surgery; maintenance of skin thickness; metabolic homeostasis and renal homeostasis. Still other uses of the subject variant CDG polypeptides include: treating growth hormone deficient adults and preventing catabolic side effects of glucocorticoids.

[0041] The subject pharmaceutical composition can also be used as myostatin antagonist to treat a number of neuronal system disease conditions, including CNS injuries/disease such as spinal cord injury and stroke, and PNS injuries/diseases.

[0042] The present invention also contemplates the use of the subject myostatin formulations conjointly with one or more other compounds useful in an effort to treat the diseases or therapeutic indications enumerated above. In these combinations, the therapeutic agents and the variant CDG polypeptides of this invention may be independently and sequentially administered or co-administered. Combined therapy to inhibit bone resorption, prevent osteoporosis, reduce skeletal fracture, enhance the healing of bone fractures, stimulate bone formation and increase bone mineral density can be effectuated by combinations of bisphosphonates and the variant CDG polypeptides of this invention. Bisphosphonates with these utilities include but are not limited to alendronate, tiludronate, dimethyl-APD, risedronate, etidronate, YM-175, clodronate, pamidronate, and BM-210995 (ibandronate).

[0043] The subject myostatin inhibitors may be combined with a mammalian estrogen agonist/antagonist. The term estrogen agonist/antagonist refers to compounds which bind with the estrogen receptor, inhibit bone turnover and prevent bone loss. In particular, estrogen agonists are herein defined as chemical compounds capable of binding to the estrogen receptor sites in mammalian tissue, and mimicking the actions of estrogen in one or more tissue. Estrogen antagonists are herein defined as chemical compounds capable of binding to the estrogen receptor sites in mammalian tissue, and blocking the actions of estrogen in one or more tissues. A variety of these compounds are described and referenced below, however, other estrogen agonists/antagonists will be known to those skilled in the art. Exemplary estrogen agonist/antagonists include droloxifene and associated compounds (see U.S. Pat. No. 5,047,431), tamoxifen and associated compounds (see U.S. Pat. No. 4,536,516), 4-hydroxy tamoxifen

(see U.S. Pat. No. 4,623,660), raloxifene and associated compounds (see U.S. Pat. No. 4,418,068), and idoxifene and associated compounds (see U.S. Pat. No. 4,839,155).

[0044] The subject myostatin inhibitors may also be combined with one or more of the following agents: glutamate antagonists (including partial antagonists) such as riluzole and topiramate; polypeptide growth factors, such as growth hormone (GH) and insulin-like growth factor 1 (IGF-1), or drugs that increases the body's own production of neurotrophic factors, such as xaliproden; anti-inflammatory agents, such as, celecoxib (Celebrex) and other COX-2 inhibitors; antibiotics, such as minocycline (Minocin, Dynacin) or other agents that inhibit caspase enzymes; Protein kinase C inhibitors such as tamoxifen (Nolvadex); and various over-the-counter substances, including vitamin E, coenzyme Q10 and creatine.

BRIEF DESCRIPTION OF THE DRAWINGS

[0045] FIG. 1 is a schematic drawing of a full-length and an N-terminally truncated version of Cerberus, showing the intact C-terminal Cys-knot domain. The lower panel shows the predicted position of the N-terminal deletion of Cerberus, based on the crystal structure of Noggin bound to BMP-7.

[0046] FIG. 2 shows a schematic drawing of where Wnt, Nodal and BMP bind to Cerberus. BMP-2 and the highly related BMP-4 competitively bind Cerberus, likely in the same region. Other more distantly related or unrelated proteins, such as TGF- β 1, EGF, and PDGF, do not compete with BMP-4. The N-terminally truncated version of Cerberus still binds Xnr-1 (Xenopus homolog of mouse Nodal).

[0047] FIG. 3 Coco, a BMP inhibitor. (A) Nucleotide sequence of Xenopus Coco, showing the nucleotide sequence, including the coding region, and the corresponding translated amino acid sequence. (B) Alignment at the amino acid level of Xenopus, Fugu, human and mouse Coco and other family members, Cerberus and Caronte. See Bell et al. (2003) *Development* 130(7): 1381-1389 which is incorporated by reference herein.

[0048] FIG. 4 Binding of Caronte to GDF-11. The tracing shows that Caronte binds to GDF-11 on a BiaCore chip. GDF-11 was immobilized on a BiaCore CM5 chip using standard amine coupling procedure. Trace: Caronte (200 μ g/ml; R&D Systems) was injected on the GDF-11 coupled chip.

[0049] FIG. 5 A-204 Reporter Gene Assay. The figure shows the Reporter vector: pGL3(CAGA)12 (described in Dennler et al, 1998, EMBO 17: 3091-3100.) The CAGA12 motif is present in TGF- β responsive genes (PM-1 gene), so this vector is of general use for factors signaling through Smad2 and 3.

[0050] FIG. 6 Caronte inhibits GDF-11 signaling in the A-204 Reporter Gene Assay. An ActRIIA-Fc ("IIA muG2a") fusion also inhibits GDF-11 signaling.

[0051] FIG. 7 Caronte does not inhibit Activin A in the A-204 Reporter Gene Assay. An ActRIIA-Fc fusion ("IIA muG2a"), as expected, does inhibit Activin A signaling.

[0052] FIG. 8 Cerberus and Caronte both inhibit GDF-8 signaling in the A-204 Reporter Gene Assay.

DETAILED DESCRIPTION OF THE INVENTION

I. Overview

[0053] Cerberus is expressed in the anterior endomesoderm (Bouwmeester et al., *Nature* 382: 595-601, 1996; Piccolo et

al., Nature 397: 707-10, 1999; Rodriguez et al., Nature 401: 243-51, 1999) during development. Caronte, a chick ortholog, is involved in left-right asymmetry in the chick embryo (Rodriguez, supra). Cerberus functions as a multivalent growth factor antagonist in the extracellular space and inhibits signaling by BMP-4, nodal, and Wnt (Belo et al., Genesis 26: 265-70, 2000). Mouse Cerberus binds to BMP proteins and nodal via independent sites (Piccolo, supra), whereas the *Xenopus* Cerberus also binds Wnt proteins and inhibits their actions (Belo, supra). Cerberus has the unique property of inducing ectopic heads in the absence of trunk structures (Piccolo, supra). The expression of Cerberus during gastrulation is activated by nodal-related signals in endoderm and by Spemann-organizer factors (Yamamoto et al., Dev Biol 257: 190-204, 2003).

[0054] Orthologs for Cerberus can be found in *Xenopus tropicalis* and *Fugu rubripes*, but are missing in invertebrates. In *Fugu rubripes*, there is only one ortholog for Cerberus. All orthologous genes for Cerberus have two exons; the first eight amino acids of the cystine-knot domain are encoded by the 3' end of the first exon and the remainder of the motif by the second exon. In some orthologs, a predicted proteolytic cleavage site can be found upstream of the beginning of the cystine-knot domain.

[0055] Coco is another member of the Cerberus/Dan family of proteins that inhibits Nodal signaling.

[0056] In part, the present invention provides Coco or Cerberus derivatives for inhibiting Nodal, GDF-11 and/or myostatin function. In certain embodiments, the Coco and Cerberus derivatives inhibit Nodal, GDF-11 and/or myostatin function without substantially compromising BMP (such as BMP-4) signaling (e.g., does not substantially bind BMP-4 or other BMPs). The subject Cerberus derivatives may also be used to inhibit BMP (such as BMP-4) signaling.

[0057] Exemplary preparations of the subject invention include Cerberus polypeptide derivatives, including the N-terminal truncated versions of Cerberus. These so-called "Cerberus derivatives" can be used to reduce the severity of a pathologic condition, which is characterized, at least in part, by an abnormal amount, development or metabolic activity of muscle or adipose tissue in a subject. For instance, the pharmaceutical preparations of the present invention can be administered in an amount effective to prevent, ameliorate or reduce the severity of a wasting disorder, such as cachexia, anorexia, DMD syndrome, BMD syndrome, AIDS wasting syndrome, muscular dystrophies, neuromuscular diseases, motor neuron diseases, diseases of the neuromuscular junction, and inflammatory myopathies.

II. Definitions

[0058] The terms used in this specification generally have their ordinary meanings in the art, within the context of this invention and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the invention and how to make and use them. The scope of a meaning of any use of a term will be apparent from the specific context in which the term is used.

[0059] "About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Typically, exem-

plary degrees of error are within 20 percent (%), preferably within 10%, and more preferably within 5% of a given value or range of values.

[0060] Alternatively, and particularly in biological systems, the terms "about" and "approximately" may mean values that are within an order of magnitude, preferably within 5-fold and more preferably within 2-fold of a given value. Numerical quantities given herein are approximate unless stated otherwise, meaning that the term "about" or "approximately" can be inferred when not expressly stated.

[0061] The methods of the invention may include steps of comparing sequences to each other, including wild-type sequence to one or more mutants/sequence variants. Such comparisons typically comprise alignments of polymer sequences, e.g., using sequence alignment programs and/or algorithms that are well known in the art (for example, BLAST, FASTA and MEGALIGN, to name a few). The skilled artisan can readily appreciate that, in such alignments, where a mutation contains a residue insertion or deletion, the sequence alignment will introduce a "gap" (typically represented by a dash, or "A") in the polymer sequence not containing the inserted or deleted residue.

[0062] "Homologous," in all its grammatical forms and spelling variations, refers to the relationship between two proteins that possess a "common evolutionary origin," including proteins from superfamilies in the same species of organism, as well as homologous proteins from different species of organism. Such proteins (and their encoding nucleic acids) have sequence homology, as reflected by their sequence similarity, whether in terms of percent identity or by the presence of specific residues or motifs and conserved positions.

[0063] The term "sequence similarity," in all its grammatical forms, refers to the degree of identity or correspondence between nucleic acid or amino acid sequences that may or may not share a common evolutionary origin.

[0064] However, in common usage and in the instant application, the term "homologous," when modified with an adverb such as "highly," may refer to sequence similarity and may or may not relate to a common evolutionary origin.

[0065] A nucleic acid molecule is "hybridizable" to another nucleic acid molecule, such as a cDNA, genomic DNA, or RNA, when a single stranded form of the nucleic acid molecule can anneal to the other nucleic acid molecule under the appropriate conditions of temperature and solution ionic strength (see Sambrook et al. *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.). The conditions of temperature and ionic strength determine the "stringency" of the hybridization. For preliminary screening for homologous nucleic acids, low stringency hybridization conditions, corresponding to a T_m (melting temperature) of 55° C., can be used, e.g., 5×SSC, 0.1% SDS, 0.25% milk, and no formamide; or 30% formamide, 5×SSC, 0.5% SDS).

[0066] Moderate stringency hybridization conditions correspond to a higher T_m , e.g., 40% formamide, with 5× or 6×SSC. High stringency hybridization conditions correspond to the highest T_m , e.g., 50% formamide, 5× or 6×SSC. SSC is 0.15 M NaCl, 0.015 M Na-citrate.

[0067] "High stringent condition" is well understood in the art to encompass conditions of hybridization which allow hybridization of structurally related, but not structurally dissimilar, nucleic acids. The term "stringent" is a term of art which is understood by the skilled artisan to describe any of a

number of alternative hybridization and wash conditions which allow annealing of only highly complementary nucleic acids.

[0068] Exemplary high stringent hybridization conditions is equivalent to about 20-27° C. below the melting temperature (T_m) of the DNA duplex formed in about 1 M salt. Many equivalent procedures exist and several popular molecular cloning manuals describe suitable conditions for stringent hybridization and, furthermore, provide formulas for calculating the length of hybrids expected to be stable under these conditions (see e.g. Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1-6 or 13.3.6; or pages 9.47-9.57 of Sambrook, et al. (1989) *Molecular Cloning*, 2nd ed., Cold Spring Harbor Press).

[0069] Hybridization requires that the two nucleic acids contain complementary sequences, although depending on the stringency of the hybridization, mismatches between bases are possible. The appropriate stringency for hybridizing nucleic acids depends on the length of the nucleic acids and the degree of complementation, variables well known in the art. The greater the degree of similarity or homology between two nucleotide sequences, the greater the value of T_m , for hybrids of nucleic acids having those sequences. The relative stability (corresponding to higher T_m) of nucleic acid hybridizations decreases in the following order: RNA:RNA, DNA:RNA, DNA:DNA. For hybrids of greater than 100 nucleotides in length, equations for calculating T_m have been derived (see Sambrook et al., supra, 9.51). For hybridization with shorter nucleic acids, i.e., oligonucleotides, the position of mismatches becomes more important, and the length of the oligonucleotide determines its specificity (see Sambrook et al., supra, 11.8). A minimum length for a hybridizable nucleic acid is at least about 10 nucleotides; preferably at least about 15 nucleotides; and more preferably the length is at least about 20 nucleotides.

[0070] Unless specified, the term “standard hybridization conditions” refers to a T_m of about 55° C., and utilizes conditions as set forth above. In a preferred embodiment, the T_m is 60° C.; in a more preferred embodiment, the T_m is 65° C. In a specific embodiment, “high stringency” refers to hybridiza-

tion and/or washing conditions at 68° C. in 0.2×SSC, at 42° C. in 50% formamide, 4×SSC, or under conditions that afford levels of hybridization equivalent to those observed under either of these two conditions.

[0071] Suitable hybridization conditions for oligonucleotides (e.g., for oligonucleotide probes or primers) are typically somewhat different than for full-length nucleic acids (e.g., full-length cDNA), because of the oligonucleotides' lower melting temperature. Because the melting temperature of oligonucleotides will depend on the length of the oligonucleotide sequences involved, suitable hybridization temperatures will vary depending upon the oligonucleotide molecules used. Exemplary temperatures may be 37° C. (for 14-base oligonucleotides), 48° C. (for 17-base oligonucleotides), 55° C. (for 20-base oligonucleotides) and 60° C. (for 23-base oligonucleotides). Exemplary suitable hybridization conditions for oligonucleotides include washing in 6×SSC, 0.05% sodium pyrophosphate, or other conditions that afford equivalent levels of hybridization.

[0072] “Polypeptide,” “peptide” or “protein” are used interchangeably to describe a chain of amino acids that are linked together by chemical bonds called “peptide bonds.” A protein or polypeptide, including an enzyme, may be a “native” or “wild-type,” meaning that it occurs in nature; or it may be a “mutant,” “variant,” or “modified,” meaning that it has been made, altered, derived, or is in some way different or changed from a native protein or from another mutant.

[0073] As used herein, the terms “Cerberus/Dan/Gremlin superfamily protein” and “CDG protein” are both used to signify the protein family which comprises Cerberus, Coco and other related proteins.

[0074] “Cerberus or Cerberus-like protein” refers to mammalian Cerberus and Cerberus-like proteins, such as the murine (NCBI RefSeq ID NP_034017) or human (NCBI RefSeq ID NP_005445) Cerberus proteins (also see SEQ ID Nos. 2 and 8, respectively, of US 2002/0164682 A1, the entire contents of which is incorporated herein by reference), and other proteins which share sequence homology to the highly conserved cysteine pattern of the C-terminal portion of the mammalian Cerberus proteins. Exemplary amino acid sequences for Cerberus proteins include

Murine Cerberus protein (NCBI RefSeq ID NP_034017):

```
1 mhl1lvqllv llplgkadc vdgcqsgsl sfp11lgrrr dlhvanheea edkpd1fvav
61 phlmgtslag egqrqrgkml srlgrfwkpk etefyprdv esdhvssgmq avtqpadgrk
121 versplqeea krfrhfrfmr kgpafggvil pikshevhwe tcrtpvfnqt iahedcqvkv
181 vqnnlcfgkc ssirfpgega dahsfcschcs ptkfttvhlml lncstptpvv kmvmqveecq
241 cmvktergee rlllagsgqs fipglpaskt np
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Human Cerberus protein (NCBI RefSeq ID NP_005445):

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1 mhl1lvqllv llplgktrrh qdgrqngsl spvllprnqr elptgnheea eekpd1fvav
61 phlvatpaga egqrqrgkml srlgrfwkpk eremhpsrds dsefpfpgtg sliqpidgmk
121 meksplreea kkfwhhfrfmr ktpasggvil pikshevhwe tcrtpvfnqt ithegcekvv
181 vqnnlcfgkc gsvhfpgaag hshtscshcl pakfttmhlp lncelssvi kvvmlveecq
241 ckvktehedg hilhagsqds fipqvsa
```

[0075] The mouse and human Cerberus are as disclosed in US 2002/0164682 A1, as SEQ ID NOs. 1 and 7 (incorporated herein by reference).

NCBI RefSeq ID NM_005454.1 (human Cerberus mRNA):

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1 atgcattctcc tcttatttca gctgctggtg ctctgcctc taggaaagac cacacggcac
61 caggatggcc gccagaatca gattctctt tcccccgtag tctgccaag gaatcaaga
121 gagcttccca caggcaacca tgaggaagct gaggagaagc cagatctgtt tgtcgcagtg
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181 ccacacacctg tagccaccag cctgcaggg gaaggccaga ggcagagaga gaagatgctg
241 tccagatttg gcaggttctg gaagaagcct gagagagaaa tgcattccatc cagggactca
301 gatagtgagc ccttcccacc tgggaccacg tccctcatcc agccgataga tggaatgaaa
361 atggagaaat ctctctctcg ggaagaagcc aagaaattct ggcaccactt catgttcaga
421 aaaactccgg cttctcaggg ggtcatcttg cccatcaaaa gccatgaagt acattggggag
481 acctgcagga cagtgcctct cagccagact ataaccacg aaggctgtga aaaagtgtt
541 gttcagaaca acccttgctt tgggaaatgc ggtctgttct atttctctgg agccgcgcag
601 cactcccata cctctgtctc tcaactgttg cctgccaaat tcaccacgat gcacttgcca
661 ctgaactgca ctgaactttc ctccgtgatc aagggtgtga tgcctgtgga ggagtgcacg
721 tgcaagggtga agcggagaca tgaagatgga cacatcctac atgtcggctc ccaggattcc
781 tttatcccag gagtttcagc ttga

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NM_009887.1 (mouse *Cerberus* mRNA).

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1 gggggggggg ggggtcagag ggagctttct tttaggcccg tccatctgtg aatctaacct
61 cagtttctgg gaatcaggaa gcatgcatct cctcttagtt cagctgcttg ttctcttgcc
121 tctggggaag gcagacctat gtgtggatgg ctgccagagt cagggctctt tatcctttcc
181 tctctagaaa aggggtcgca gagatctcca cgtggccaac cagcaggagg cagaagacaa
241 gccggatctg tttgtggcgg tgcacacact catgggcacc agcctggctg ggggaaggcca
301 gaggoagaga ggggaagatgc tgtccaggct tggaagattc tggaagaaac ctgagaccga
361 attttaccce ccaagggatg tggaaagcga tcatgtctca tcggggatgc aggcctgtac
421 tcagccagca gatgggagga aagtggagag atcacctcta caggaggaag ccaagaggtt
481 ctggcatcgg ttcattgttc gaaagggccc ggcgttccag ggagtcattc tgcccatcaa
541 aagccacgaa gtacactggg agacctgcag gactgtgccc tcaaccaga ccattgccc
601 tgaagactgt caaaaagtgc ttgtccagaa caacctttgc tttggcaaat gcagttccat
661 tcgtttttcc gggaaggggg cagatgcccc cagctctctg tccactgct cgccaccacaa
721 attcaccacc gtgcacttga tgcagaactg caccagcccc acccccgtgg tcaagatggt
781 gatgcaagta gaagagtgtc agtgcattgt gaagacggaa cgtggagagg agccctcct
841 actggctggt tcccagggtt cctcatccc tggacttcca gcttcaaaaa caaacccatg
901 aattacctca acagaaagca aaacctcaac agaataagtg aggggtattc aatctggaaa
961 tgttatgtga gttatatata gatcagtggg aaatatcttt ctctctccct ctctccctct
1021 ctctctctcc tctattttct ctctctctct ctctctctct ctctctctca
1081 cacacacaca cacacacaca cacacacaca catgtttgtg tttagacagg gtcttatgta
1141 ttctcagctg gcctcaaaact cacaatgtgg ctggggatga ttttaaaact ctgatccaat
1201 tccctgagtg ctcagattca gacatgctcc ataanacata gctcccagaa ggatttttaa
1261 aagagatttt gcatgtttca aagttgcctt tgagactcag aaatatattg atntattgaa
1321 tggccttgcc acagatgtgg gaggcagctt gcttggtggc ccaagtattt ttttttgtt
1381 cgttcagaaat tctccacatg aagtttttac tgttggttat ctggcggtga agaaggaata
1441 gtgaaggtac ttttaacagt ttacacgtgg aaggggctca ggcactagga accaaccttt
1501 tcccggataa tgaggaaaat acatgaacag tatttagatc acttgaggaa gttactagga
1561 aacgccataa gtcctccaag acattgtgag tcattttgaa ggacaatcgt gtatatagac
1621 gaaatcttct actcgtatgc ttttgaatct tctagcaagt taggtttcta tgtttgggct
1681 tcttctattt gtctaagagt atgtgtgaca aattcaacct gacaaatacc tcaatggcaa
1741 attctgaccg tg

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It is also expected that Cerberus related proteins also exist in other species, including family members in *Xenopus*, and *Drosophila*, *C. elegans*, zebrafish, as well as in all mammals, for example, rats, mice and humans. "Cerberus or Cerberus-like proteins" also includes variants of the Cerberus proteins, such as allelic variants or variants induced by mutagenesis or deletions, and fragments of Cerberus proteins which variants and fragments retain myostatin binding activity. "Cerberus-like" proteins is also used to signify the family of proteins sharing structural and/or functional similarity, including those proteins which are described further herein. Such proteins may have amino acid sequences sharing significant sequence identity (e.g., at least about 50%, 60%, 70%, 80%, 90%, 95%, 99% or more) with the human or mouse Cerberus proteins, over the full-length, or at least within the myostatin

binding domain of the human or mouse Cerberus. Cerberus-like proteins also include proteins that have amino acid sequences that are encoded by nucleic acid sequences that hybridize under stringent conditions with the coding sequences for human or mouse Cerberus, particularly that portion of the coding sequence for the myostatin binding domain. A Cerberus derivative or variant sequence may or may not lack the N-terminal BMP binding domain.

[0076] "Coco or Coco-like protein" refers to mammalian Coco proteins and related homologs, such as the human Coco protein of GenBank Accession 22749329, and other proteins which share sequence homology to the highly conserved cysteine pattern of the C-terminal portion of the mammalian Coco proteins. An exemplary amino acid sequences for human Coco protein is

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1 MLLGQLSTLL CLLSGALPTG SGRPEPQSPR PQSWAAANQT WALGPGALPP LVPASALGSW
61 KAFLGLQKAR QLGMRLQRG QDEVAAVTLP LNPQEVIGQM CKAVPFVQVF SRPGCSAIRL
121 RNHLCFGHCS SLYIPGSDPT PLVLCNSCMP ARKRWAPVVL WCLTGSSASR RRVKISTMLI
181 EGCHCSPKA

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[0077] The human Coco coding sequence is disclosed in GenBank Accession 22749328 (incorporated herein by reference).

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1 agtccggaca gacagacagg cagacagacg caccggacaag.cagatgctcc ttggccagct
61 atccactctt ctgtgcctgc ttagcggggc cctgcctaca ggctcaggga ggccctgaacc
121 ccagtctcct cgacctcagt cctgggctgc agccaatcag acctgggctc tgggcccagg
181 ggccctgccc ccactggtgc cagcttctgc ccttgggagc tggaaggcct tcttgggcct
241 gcagaaagcc aggcagctgg ggatgggcag gctgcagczgt gggcaagacg aggtggctgc
301 tgtgactctg ccgctgaacc ctcaggaagt gatccagggg atgtgtaagg ctgtgccctt
361 cgttcaggtg ttctccggc ccggctgctc agccatacgc ctccgaaac atctgtgctt
421 tggtcattgc tcctctctct acatccctgg ctcggaaccc accccactag tcctgtgcaa
481 cagctgtatg cctgctcgca agcgttgggc acccgtggtc ctgtggtgtc tcaactggcag
541 ctcagcctcc cgtcgacggg tgaagatata caccatgctg atcgaggggt gtcaactgcag
601 cccaaaagca tgaactgagc atcgtggatg ggtgcacgga gacacgcacc ttggagaaat
661 gaggggagat ggaccaagaa agacgtggac ctggatgatg tactctgggt caagagacca
721 gggatgcagg gttaggcaga caggteccca gagtctcac cctgctcccc agacagtaga
781 cacagtgcct gtcctggagt tgcaccactg atagtcacag cacacaatga ttgacaactc
841 actttttttt ttttttttga gatggagtct cgtctctgctg ccagagctgg agtgcagtgg
901 cgcaatctca gctcaactga agctccacct cccgggttta tgccattctc ctgtctcagc
961 ctcccagata gctgggacta caggcacccg ccaacacgcc cggctaattt ttcgtatttt
1021 tagtaaagac agggtttcac cgtgttagcc aggatgggtc ctatctcctg acctcgtgat
1081 ctgctgcctt tggccttatt attttttttt ttttaaggaca gagtctctct ctgtcaccca
1141 ggctggagtg caatggcgcg atcttggtc actgtaactt ccacttgcca ggctcaagca
1201 gttctcctgc ctcagcctcc tgagtagctg ggactacagg caccgccac catgcccagc
1261 taatttttgt atttttagta gagacagagt ttcaccatat tagcctgggt ggtctcaaac
1321 tcctggcctc aggtgatctg ccacacctcg cctcccaaag tgetgggac aaatccactg
1381 ttaatcatta ggctgaactg tctcttatag aatgaggtca aagacactcc cagttgcagg
1441 gagggtagat gggcccccac agaccgagag acacagtgat gacctcagcc tagggacacc
1501 aaaaaaaaaa aaaaaaaaaa cccaaaccaa aaacgcaaac caaagcaggc aggcagacag
1561 ctgctggggg aaatcctggg gtcccttgaga cagaggcagg accctcgtgt tcccagctgc
1621 ctcttgctct gatagtgggt ctgtgtccct ctcagacccc ccacctgagt cccacagag
1681 cccacgcctt ggcatggcat tccacagaaa ccataaaggt tggtgagtc c

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It is also expected that Coco-related proteins also exist in other species, including family members in *Xenopus*, and *Drosophila*, *C. elegans*, zebrafish, as well as in all mammals, for example, rats, mice and non-human primates. “Coco or Coco-like proteins” also includes variants of the naturally occurring Coco proteins, such as allelic variants or variants induced by mutagenesis or deletions, and fragments of Coco proteins which variants and fragments retain myostatin binding activity. “Coco-like” proteins is also used to signify the family of proteins sharing structural and/or functional similarity, including those proteins which are described further herein. Such proteins may have amino acid sequences sharing significant sequence identity (e.g., at least about 50%, 60%,

70%, 80%, 90%, 95%, 99% or more) with the human Coco protein, over the full-length, or at least within the myostatin binding domain of the human Coco. Coco-like proteins also include proteins that have amino acid sequences that are encoded by nucleic acid sequences that hybridize under stringent conditions with the coding sequences for human Coco, particularly that portion of the coding sequence for the myostatin binding domain. A Coco derivative or variant sequence may or may not lack the N-terminal BMP binding domain.

[0078] Unless specifically stated otherwise, “Cerberus (derivative) therapeutics” or its grammatical variations include the full-length or the N-terminally truncated versions of Cerberus therapeutics.

[0079] As used herein, the term “Cerberus or Cerberus-like activity” refers to one or more of the activities which are exhibited by the mammalian Cerberus-like proteins of the present invention. In particular, “Cerberus or Cerberus-like activity” includes the ability to induce, enhance and/or inhibit the formation, growth, proliferation, differentiation, maintenance of neurons and/or related neural cells and tissues such as brain cells, Schwann cells, glial cells and astrocytes. “Cerberus or Cerberus-like” activity also includes the ability to induce molecular markers of neuroendocrine or ectoderm tissue, such as OTX2, N-CAM, MASH, chromagranin, and AP2, as well as the ability to induce the formation of neurons and/or related neural cells and tissues such as brain cells, Schwann cells, glial cells and astrocytes. “Cerberus or Cerberus-like activity” may also include the ability to regulate the interaction of ligands and their protein receptors. “Cerberus or Cerberus-like activity” may further include the ability to regulate the formation, differentiation, proliferation and/or maintenance of other cells and/or tissue, for example connective tissue, organs and wound healing. In particular, to “Cerberus or Cerberus-like activity” may include the ability to enhance and/or inhibit the formation, growth, proliferation, differentiation and/or maintenance of cardiac, spleen, liver, pancreas, stomach, kidney, lung and brain cells and tissue, as well as osteoblasts and bone, chondrocytes and cartilage, tendon, epidermis and muscle. “Cerberus and Cerberus-like activity” also includes the activities of Cerberus and Cerberus-like protein in the assays described in the examples and specification herein.

[0080] Cerberus and Cerberus-like nucleotide sequences in mouse and human are as disclosed in US 2002/0164682 A1, as SEQ ID NOs. 1 and 7 (incorporated herein by reference). Also see NCBI RefSeq ID NM_005454.1 (human) and NM 009887.1 (mouse).

[0081] In certain related embodiments, the myostatin inhibitor is a polypeptide that includes a myostatin binding domain of a Coco protein, such as the human Coco protein shown in FIG. 3 or in GenBank Accession number 22749329. An exemplary human Coco protein sequence is

```
1 MLLGQLSTLL CLLSGALPTG SGRPEPQSPR PQSWAAANQT WALGPGALPP LVPASALGSW
61 KAFGLGLQKAR QLGMGRLLRG QDEVAAVTLP LNPQEVIGQM CKAVPFVQVF SRPGCSAIRL
121 RNHLFCFHCSS SLYIPGSDPT PLVLCNSCMP ARKRWAPVVL WCLTGSSASR RRVKISTMLI
181 EGCHCSPKA
```

[0082] The terms “antibody” and “antibody agent” are used interchangeably herein, and refer to an immunoglobulin molecule obtained by in vitro or in vivo generation of the humoral response, and includes both polyclonal and monoclonal antibodies. The term also includes genetically engineered forms such as chimeric antibodies (e.g., humanized murine antibodies), heteroconjugate antibodies (e.g., bispecific antibodies), and recombinant single chain Fv fragments (scFv). The term “antibody” also includes antigen binding forms of antibodies (e.g., Fab', F(ab')₂, Fab, Fv, rIgG, and, inverted IgG).

[0083] The term “antigen binding fragment” includes any portion of an antibody that binds to a target epitope. An antigen binding fragment may be, for example, a polypeptide including a CDR3 region, or other fragment of an immunoglobulin molecule which retains the affinity and specificity of the myostatin epitope.

[0084] “Specifically binds” includes reference to the preferential association of a ligand, in whole or part, with a particular target molecule (i.e., “binding partner” or “binding moiety”) relative to compositions lacking that target molecule. It is, of course, recognized that a certain degree of non-specific interaction may occur between the subject myostatin neutralizing antibodies and a other proteins. Nevertheless, specific binding, may be distinguished as mediated through specific recognition of the myostatin protein. Typically specific binding results in a much stronger association between the antibody and myostatin protein than between the antibody and other proteins, e.g., GDF11. Specific binding by an antibody to myostatin under such conditions requires an antibody that is selected for its specificity for a particular protein. The affinity constant (K_a, as opposed to K_d) of the antibody binding site for its cognate monovalent antigen is at least 10⁷, usually at least 10⁸, preferably at least 10⁹, more preferably at least 10¹⁰, and most preferably at least 10¹¹ M. A variety of immunoassay formats are appropriate for selecting antibodies specifically reactive with myostatin. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically reactive with a protein. See Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific reactivity.

[0085] Immunoassays in the competitive binding format can be used to determine cross-reactivity of antibodies with myostatin, e.g., to identify whether a test antibody is a myostatin neutralizing antibody. For example, the myostatin protein, or a fragment thereof is immobilized to a solid support. Test antibodies are added to the assay compete with the binding of a TGF receptor, such as ActRII or ALK7, to the immobilized antigen. The ability of the test antibodies to compete with the binding of a TGF receptor to the immobilized myostatin antigen is compared.

[0086] Similarly, immunoassays in the competitive binding format can be used to determine cross-reactivity determinations, e.g., to determine the specificity of a myostatin neutral-

izing antibody. For example, the myostatin protein, or the myostatin epitope thereof is immobilized to a solid support. Epitopes from other proteins, such as GDF-11, Nodal or BMP-4 or other proteins having sequence homology with myostatin are added to the assay to compete with the binding of a potential myostatin neutralizing antibody to the immobilized antigen. The ability of the test peptides to compete with the binding of potential myostatin neutralizing antibody with the immobilized myostatin antigen is compared. The percent cross-reactivity of the potential myostatin neutralizing antibody for the other antigens is calculated, using standard calculations. In certain preferred embodiments, the subject myostatin neutralizing antibodies have less than 10% cross-reactivity with GDF-11. In other preferred embodiments, the subject myostatin neutralizing antibodies have less than 1%, 5%, or 10% cross-reactivity with BMP-4.

III. Exemplary Cerberus and Coco Derivatives

[0087] In certain embodiments, the myostatin inhibitor is a Cerberus polypeptide sharing at least about 50%, 60%, 70%, 80%, 90%, 95%, 99% or more sequence identity over the full-length of the human or mouse Cerberus protein (*infra*).

[0088] In certain other embodiments, the myostatin inhibitor is a polypeptide that includes a Cerberus sequence obtained from human, mouse, or other species, their variants or derivatives, including N-terminally truncated versions of Cerberus. The full-length mouse and human Cerberus proteins, disclosed as SEQ ID NOs. 2 and 8, respectively, in US 2002/0164682 A1, are also disclosed in NCBI RefSeq format below:

[0089] Human Cerberus full length protein:

```
1 MHLLLFQLLV LLPLGKTRH QDGRQNSL SPVLLPRNQR ELPTGNHEEA EEKPDFVAV
61 PHLVATSPAG EGQRQREKML SRFGRFWKKP EREMHPSRDS DSEPPPGTQ SLIQPIDGMK
121 MEKSPLREEA KKFVHHFMFR KTPASQGVIL PIKSHEVHWE TCRTVPFSQT ITHEGCEKVV
181 VQNNLCFGKC GSVHFPGAAQ HSHTSCSHCL PAKFTTMHLP LNCTELSSVI KVMMLVEECQ
241 CKVKTEHEDG HILHAGSQDS FIPGVSA
```

[0090] Residues 106-119 (from any one of which residues the subject Cerberus derivatives may begin), and residues 241-267 (to any one of which residues the subject Cerberus derivatives may end) are underlined.

[0091] Mouse Cerberus full length protein:

```
1 MHLLLVQLLV LLPLGKADLC VDGCQSQGSL SFPLLERGR DLHVNHEEA EDKPDFVAV
61 PHLMGTSLAG EGQRQRGKML SRLGRFWKKP ETEFYPPRDV ESDHVSSGMQ AVTQPADGRK
121 VERSPLQEEA KRFVHRFMFR KGPAFQGVIL PIKSHEVHWE TCRTVPFNQT IAHEDCQKVV
181 VQNNLCFGKC SSIRFPGEA DAHSFCSHCS PTKFTTVHLM LNCTSPTPVV KMVMQVEECQ
241 CMVKTERGEE RLLLAGSQGS FIPGLPASKT NP
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[0092] Residues 106-119 (from any one of which residues the subject Cerberus derivatives may begin), and residues 241-272 (to any one of which residues the subject Cerberus derivatives may end) are underlined. Note that the mouse protein is largely homologous to the human protein throughout the sequences, with the exception of 5 additional residues at the C-terminus. Therefore, whenever a non-human Cerberus derivative is used, the residue numbers refers to those corresponding to the human sequences.

[0093] As described above, in certain embodiments, preferred fragments of the human Cerberus derivative proteins are ones which begins anywhere from residues 106-119 (inclusive) at the N-terminus, and ends anywhere after residue 241.

[0094] Also included are Cerberus derived variant sequence, including mutants or variants of the wild-type myostatin binding domains that retain myostatin binding activity, optionally substantially loses BMP-4 binding. Variant sequences without BMP binding affinity may be desirable as a way to alter selectivity of the inhibitor (e.g., relative to GDF-11 or nodal binding, where preferential binding to one of the proteins occur. Also includes more preferential—

higher affinity than wild-type—binding to myostatin, or more discriminatory—lower affinity than wild-type truncated version—binding to BMP-4), alter other binding characteristics with respect to myostatin (such as K_d , and/or K_{on} or K_{off} rates), or improve biodistribution or half life in vivo or on the shelf.

[0095] Certain other Cerberus sequences are listed below based on homology search in databases of identified proteins, and the subject variant Cerberus polypeptides can be derived from those proteins as well. Since these sequences are retrieved from public databases available on the internet, additional homologs of the proteins in other species may be obtained as these databases are being updated. Furthermore, other species of Cerberus proteins, especially those of mam-

mals, can be readily obtained by standard molecular biology protocols, such as PCR, low stringency hybridization, Ab-mediated screening of expression libraries using antibodies cross-reacting with identified Cerberus homologs in target species, etc.

[0096] For example, sequence alignments using softwares such as DNASTar's MegaAlign (*supra*) can identify the most conserved regions in the known members of a protein family. PCR can then be carried out using degenerate oligos covering such most conserved regions, and templates DNA from the target organism. In preferred embodiments, such conserved regions include the kinase domain, and/or the ligand binding domain.

[0097] These same conserved regions may be used to generate probes for screening nucleic acid libraries at moderate to low stringency hybridization conditions (see definition section).

[0098] Xenopus homolog: gil1513088.

[0099] Fugu rubripes: FuguGenscan_32561/SIN-FRUP00000076662.

[0100] In certain embodiments, the myostatin inhibitor is a Cerberus polypeptide sharing at least about 50%, 60%, 70%, 80%, 90%, 95%, 99% or more sequence identity over the full-length of the human or mouse Cerberus protein (*infra*).

[0101] In certain other embodiments, the myostatin inhibitor is a polypeptide that includes a Coco sequence obtained from human, mouse, or other species, their variants or deriva-

tives, including N-terminally truncated versions of Coco. The full-length human Coco protein is disclosed above.

[0102] The various Cerberus and Coco polypeptides may be prepared as fusion proteins. A fusion protein may include one or more additional polypeptide portion that enhance one or more of in vivo stability, in vivo half life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. For example, a fusion protein may include an immunoglobulin Fc domain and/or a purification subsequence selected from: an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion. The myostatin antagonist protein may include one or more modified amino acid residues selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

[0103] A fusion protein or coupled protein system (e.g. non-fusion covalent linkage by crosslinking) may also include a second myostatin inhibitor domain, which is a polypeptide affinity reagent that selectively binds to myostatin and competes with the binding of an ALK7 or ALK4 receptor. The affinity reagent may be an antibody agent. An antibody agent may be, for example, a recombinant antibody; a monoclonal antibody; a VH domain; a VL domain; an scFv; an Fab fragment; an Fab' fragment; an F(ab')₂; an Fv; or a disulfide linked Fv, a fully human antibody or a humanized chimeric antibody, or an antigen binding fragment thereof. An affinity reagent is a peptide or scaffolded peptide that selectively binds to myostatin and competes with the binding of an ALK7 or ALK4 receptor. An affinity reagent may include a myostatin binding domain of ALK7 or ALK4. For example, an extracellular domain of ALK7 or ALK4 (preferably human ALK7 or ALK4) may be used. The affinity reagent may be a small organic molecule that selectively binds to myostatin and competes with the binding of an ALK7 or ALK4 receptor.

[0104] An example of a human ALK7 myostatin binding domain is shown below:

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LKCVCLLCDSNNFTCQTGACWASVMLTNGKEQVIKSCVSLPELNAQVFC
HSSNNVTKTECCFTDFCNITLHLP
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[0105] An example of a human ALK4 myostatin binding domain is shown below:

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ALLCACTSLQANYTCETDGACMVSIFNLDGMEHHVRTCIPKVELVPAGK
PFYCLSSDLRNTGCCYTDY
```

[0106] As shown herein, Caronte, and therefore human Cerberus and presumably Coco also, does not substantially inhibit Activin A signaling in an A204 Reporter Gene Assay. Thus, such myostatin antagonists will preferably exhibit little or no interaction with Activin A-mediated signaling.

IV. Exemplary Therapeutic Uses

[0107] The subject variant Coco and Cerberus polypeptides, such as the full-length and the N-terminally truncated Cerberus derivatives or Coco derivatives, can be used in a number of therapeutic settings to treat a number of diseases resulting from or exacerbated by the presence of myostatin.

[0108] In certain embodiments, the subject polypeptides and derivatives thereof are used as part of a treatment for a muscular dystrophy. The term "muscular dystrophy" refers to a group of degenerative muscle diseases characterized by gradual weakening and deterioration to of skeletal muscles and sometimes the heart and respiratory muscles. Muscular dystrophies are genetic disorders characterized by progressive muscle wasting and weakness that begin with microscopic changes in the muscle. As muscles degenerate over time, the person's muscle strength declines. Exemplary muscular dystrophies that can be treated with a regimen including the subject myostatin include: Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD), Emery-Dreifuss Muscular Dystrophy (EDMD), Limb-Girdle Muscular Dystrophy (LGMD), Facioscapulohumeral Muscular Dystrophy (FSH or FSHD) (Also known as Landouzy-Dejerine), Myotonic Dystrophy (MMD) (Also known as Steinert's Disease), Oculopharyngeal Muscular Dystrophy (OPMD), Distal Muscular Dystrophy (DD), Congenital Muscular Dystrophy (CMD).

[0109] Duchenne Muscular Dystrophy (DMD) was first described by the French neurologist Guillaume Benjamin Amand Duchenne in the 1860s. Becker Muscular Dystrophy (BMD) is named after the German doctor Peter Emil Becker, who first described this variant of DMD in the 1950s. DMD is one of the most frequent inherited diseases in males, affecting one in 3,500 boys. DMD occurs when the dystrophin gene, located on the short arm of the X chromosome, is broken. Since males only carry one copy of the X chromosome, they only have one copy of the dystrophin gene. Without the dystrophin protein, muscle is easily damaged during cycles of contraction and relaxation. While early in the disease muscle compensates by regeneration, later on muscle progenitor cells cannot keep up with the ongoing damage and healthy muscle is replaced by non-functional fibro-fatty tissue.

[0110] In DMD, boys begin to show signs of muscle weakness as early as age 3. The disease gradually weakens the skeletal or voluntary muscles, those in the arms, legs and trunk. By the early teens or even earlier, the boy's heart and respiratory muscles may also be affected. BMD is a much milder version of DMD. Its onset is usually in the teens or early adulthood, and the course is slower and far less predictable than that of DMD. (Though DMD and BMD affect boys almost exclusively, in rare cases they can affect girls.

[0111] Until the 1980s, little was known about the cause of any kind of muscular dystrophy. In 1986, the dystrophin gene deficiency was identified as the cause of DMD. BMD results from different mutations in the same gene. BMD patients have some dystrophin, but it's either insufficient in quantity or poor in quality. Having some dystrophin protects the muscles of those with BMD from degenerating as badly or as quickly as those of people with DMD.

[0112] Recent researches demonstrate that blocking or eliminating Myostatin function in vivo can effectively treat at least certain symptoms in DMD and BMD patients (Bogdanovich et al., supra; Wagner et al., supra). Thus, the subject Cerberus derivatives, especially the N-terminally truncated versions thereof, constitute an alternative means of blocking the function of Myostatin in vivo in DMD and BMD patients.

[0113] Similarly, the subject Coco or Cerberus derivatives, especially the N-terminally truncated versions thereof, provide an effective means to increase muscle mass in other disease conditions that are in need of muscle growth. For

example, Gonzalez-Cadavid et al. (supra) reported that that Myostatin expression correlates inversely with fat-free mass in humans and that increased expression of the Myostatin gene is associated with weight loss in men with AIDS wasting syndrome. By inhibiting the function of Myostatin in AIDS patients, at least certain symptoms of AIDS may be alleviated, if not completely eliminated, thus significantly improving quality of life in AIDS patients.

[0114] Since loss of Myostatin function is also associated with fat loss without diminution of nutrient intake (Zimmers et al., supra; McPherron and Lee, supra), the subject Coco or Cerberus derivatives, especially the N-terminally truncated versions thereof, may further be used as a therapeutic agent for slowing or preventing the development of obesity and type II diabetes.

[0115] The cancer anorexia-cachexia syndrome is among the most debilitating and life-threatening aspects of cancer. Progressive weight loss in cancer anorexia-cachexia syndrome is a common feature of many types of cancer and is responsible not only for a poor quality of life and poor response to chemotherapy, but also a shorter survival time than is found in patients with comparable tumors without weight loss. Associated with anorexia, fat and muscle tissue wasting, psychological distress, and a lower quality of life, cachexia arises from a complex interaction between the cancer and the host. It is one of the most common causes of death among cancer patients and is present in 80% at death. It is a complex example of metabolic chaos effecting protein, carbohydrate, and fat metabolism. Tumors produce both direct and indirect abnormalities, resulting in anorexia and weight loss. Currently, there is no treatment to control or reverse the process.

[0116] Cancer anorexia-cachexia syndrome affects cytokine production, release of lipid-mobilizing and proteolysis-inducing factors, and alterations in intermediary metabolism. Although anorexia is common, a decreased food intake alone is unable to account for the changes in body composition seen in cancer patients, and increasing nutrient intake is unable to reverse the wasting syndrome. Cachexia should be suspected in patients with cancer if an involuntary weight loss of greater than five percent of premorbid weight occurs within a six-month period.

[0117] Since systemic overexpression of Myostatin in adult mice was found to induce profound muscle and fat loss analogous to that seen in human cachexia syndromes (Zimmers et al., supra), the subject Coco or Cerberus derivatives, especially the N-terminally truncated versions thereof as a pharmaceutical composition can be beneficially used as a Myostatin antagonist/blocker to prevent, treat, or alleviate the symptoms of the cachexia syndrome, where muscle growth is desired.

[0118] In certain embodiments, the subject variant Coco or Cerberus polypeptides, particularly the N-terminally truncated Cerberus derivatives, can be used to form pharmaceutical compositions that can be beneficially used to prevent, treat, or alleviate symptoms of a host of diseases involving neurodegeneration. While not wishing to be bound by any particular theory, the subject Cerberus derivatives may antagonize the inhibitory feedback mechanism mediated through the wild-type ALK7 receptor, thus allowing new neuronal growth and differentiation. The subject Cerberus derivative as a pharmaceutical composition can be beneficially used to prevent, treat, or alleviate symptoms of diseases with neurodegeneration, including Alzheimer's Disease

(AD), Parkinson's Disease (PD), Amyotrophic Lateral Sclerosis (ALS), Huntington's disease, etc.

[0119] Alzheimer's disease (AD) is a chronic, incurable, and unstoppable central nervous system (CNS) disorder that occurs gradually, resulting in memory loss, unusual behavior, personality changes, and a decline in thinking abilities. These losses are related to the death of specific types of brain cells and the breakdown of connections between them.

[0120] AD has been described as childhood development in reverse. In most people with AD, symptoms appear after the age 60. The earliest symptoms include loss of recent memory, faulty judgment, and changes in personality. Later in the disease, those with AD may forget how to do simple tasks like washing their hands. Eventually people with AD lose all reasoning abilities and become dependent on other people for their everyday care. Finally, the disease becomes so debilitating that patients are bedridden and typically develop coexisting illnesses. AD patients most commonly die from pneumonia, 8 to 20 years from disease onset.

[0121] Parkinson's disease (PD) is a chronic, incurable, and unstoppable CNS disorder that occurs gradually and results in uncontrolled body movements, rigidity, tremor, and gait difficulties. These motor system problems are related to the death of brain cells in an area of the brain that produces dopamine—a chemical that helps control muscle activity.

[0122] In most people with PD, symptoms appear after age 50. The initial symptoms of PD are a pronounced tremor affecting the extremities, notably in the hands or lips. Subsequent characteristic symptoms of PD are stiffness or slowness of movement, a shuffling walk, stooped posture, and impaired balance. There are wide ranging secondary symptoms such as memory loss, dementia, depression, emotional changes, swallowing difficulties, abnormal speech, sexual dysfunction, and bladder and bowel problems. These symptoms will begin to interfere with routine activities, such as holding a fork or reading a newspaper. Finally, people with PD become so profoundly disabled that they are bedridden. People with PD usually die from pneumonia.

[0123] Amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease; motor neuron disease) is a chronic, incurable, and unstoppable CNS disorder that attacks the motor neurons, components of the CNS that connect the brain to the skeletal muscles. In ALS, the motor neurons deteriorate and eventually die, and though a person's brain normally remains fully functioning and alert, the command to move never reaches the muscles.

[0124] Most people who get ALS are between 40 and 70 years old. The first motor neurons that weaken are those leading to the arms or legs. Those with ALS may have trouble walking, they may drop things, fall, slur their speech, and laugh or cry uncontrollably. Eventually the muscles in the limbs begin to atrophy from disuse. This muscle weakness will become debilitating and a person will need a wheel chair or become unable to function out of bed. Most ALS patients die from respiratory failure or from complications of ventilator assistance like pneumonia, 3-5 years from disease onset.

[0125] The causes of these neurological diseases has remained largely unknown. They are conventionally defined as distinct diseases, yet clearly show extraordinary similarities in basic processes and commonly demonstrate overlapping symptoms far greater than would be expected by chance alone. Current disease definitions fail to properly deal with the issue of overlap and a new classification of the neurodegenerative disorders has been called for.

[0126] Huntington's disease (HD) is another neurodegenerative disease resulting from genetically programmed degeneration of neurons in certain areas of the brain. This degeneration causes uncontrolled movements, loss of intellectual faculties, and emotional disturbance. HD is a familial disease, passed from parent to child through a dominant mutation in the wild-type gene. Some early symptoms of HD are mood swings, depression, irritability or trouble driving, learning new things, remembering a fact, or making a decision. As the disease progresses, concentration on intellectual tasks becomes increasingly difficult and the patient may have difficulty feeding himself or herself and swallowing. The rate of disease progression and the age of onset vary from person to person.

[0127] Tay-Sachs disease and Sandhoff disease are glycolipid storage diseases caused by the lack of lysosomal β -hexosaminidase (Gravel et al., in *The Metabolic Basis of Inherited Disease*, eds. Scriver et al., McGraw-Hill, New York, pp. 2839-2879, 1995). In both disorders, G_{M2} ganglioside and related glycolipid substrates for β -hexosaminidase accumulate in the nervous system and trigger acute neurodegeneration. In the most severe forms, the onset of symptoms begins in early infancy. A precipitous neurodegenerative course then ensues, with affected infants exhibiting motor dysfunction, seizure, visual loss, and deafness. Death usually occurs by 2-5 years of age. Neuronal loss through an apoptotic mechanism has been demonstrated (Huang et al., *Hum. Mol. Genet.* 6: 1879-1885, 1997).

[0128] It is well-known that apoptosis plays a role in AIDS pathogenesis in the immune system. However, HIV-1 also induces neurological disease. Shi et al. (*J. Clin. Invest.* 98: 1979-1990, 1996) examined apoptosis induced by HIV-1 infection of the central nervous system (CNS) in an in vitro model and in brain tissue from AIDS patients, and found that HIV-1 infection of primary brain cultures induced apoptosis in neurons and astrocytes in vitro. Apoptosis of neurons and astrocytes was also detected in brain tissue from 10/11 AIDS patients, including 5/5 patients with HIV-1 dementia and 4/5 nondemented patients.

[0129] Neuronal loss is also a salient feature of prion diseases, such as Creutzfeldt-Jakob disease in human, BSE in cattle (mad cow disease), Scrapie Disease in sheep and goats, and feline spongiform encephalopathy (FSE) in cats.

[0130] The subject Cerberus derivatives, including the N-terminally truncated Cerberus derivatives are also useful to prevent, treat, and alleviate symptoms of various PNS disorders, such as the ones described below. The PNS is composed of the nerves that lead to or branch off from the CNS. The peripheral nerves handle a diverse array of functions in the body, including sensory, motor, and autonomic functions. When an individual has a peripheral neuropathy, nerves of the PNS have been damaged. Nerve damage can arise from a number of causes, such as disease, physical injury, poisoning, or malnutrition. These agents may affect either afferent or efferent nerves. Depending on the cause of damage, the nerve cell axon, its protective myelin sheath, or both may be injured or destroyed.

[0131] The term peripheral neuropathy encompasses a wide range of disorders in which the nerves outside of the brain and spinal cord—peripheral nerves—have been damaged. Peripheral neuropathy may also be referred to as peripheral neuritis, or if many nerves are involved, the terms polyneuropathy or polyneuritis may be used.

[0132] Peripheral neuropathy is a widespread disorder, and there are many underlying causes. Some of these causes are common, such as diabetes, and others are extremely rare, such as acrylamide poisoning and certain inherited disorders. The most common worldwide cause of peripheral neuropathy is leprosy. Leprosy is caused by the bacterium *Mycobacterium leprae*, which attacks the peripheral nerves of affected people. According to statistics gathered by the World Health Organization, an estimated 1.15 million people have leprosy worldwide.

[0133] Leprosy is extremely rare in the United States, where diabetes is the most commonly known cause of peripheral neuropathy. It has been estimated that more than 17 million people in the United States and Europe have diabetes-related polyneuropathy. Many neuropathies are idiopathic—no known cause can be found. The most common of the inherited peripheral neuropathies in the United States is Charcot-Marie-Tooth disease, which affects approximately 125,000 persons.

[0134] Another of the better known peripheral neuropathies is Guillain-Barré syndrome, which arises from complications associated with viral illnesses, such as cytomegalovirus, is Epstein-Barr virus, and human immunodeficiency virus (HIV), or bacterial infection, including *Campylobacter jejuni* and Lyme disease. The worldwide incidence rate is approximately 1.7 cases per 100,000 people annually. Other well-known causes of peripheral neuropathies include chronic alcoholism, infection of the varicella-zoster virus, botulism, and poliomyelitis. Peripheral neuropathy may develop as a primary symptom, or it may be due to another disease. For example, peripheral neuropathy is only one symptom of diseases such as amyloid neuropathy, certain cancers, or inherited neurologic disorders. Such diseases may affect the peripheral nervous system (PNS) and the central nervous system (CNS), as well as other body tissues.

[0135] Other PNS diseases treatable with the subject Cerberus derivatives, especially the N-terminally truncated Cerberus derivatives include: Brachial Plexus Neuropathies (Diseases of the cervical and first thoracic roots, nerve trunks, cords, and peripheral nerve components of the brachial plexus. Clinical manifestations include regional pain, paresthesia; muscle weakness, and decreased sensation in the upper extremity. These disorders may be associated with trauma, including birth injuries; thoracic outlet syndrome; neoplasms, neuritis, radiotherapy; and other conditions. See Adams et al. *Principles of Neurology*, 6th ed, pp 1351-2); *Diabetic Neuropathies* (Peripheral, autonomic, and cranial nerve disorders that are associated with diabetes mellitus. These conditions usually result from diabetic microvascular injury involving small blood vessels that supply nerves (vasa nervorum). Relatively common conditions which may be associated with diabetic neuropathy include third nerve palsy; mononeuropathy; mononeuropathy multiplex; diabetic amyotrophy; a painful polyneuropathy; autonomic neuropathy; and thoracoabdominal neuropathy. See Adams et al., *Principles of Neurology*, 6th ed, p 1325); *Mononeuropathies* (Disease or trauma involving a single peripheral nerve in isolation, or out of proportion to evidence of diffuse peripheral nerve dysfunction. Mononeuropathy multiplex refers to a condition characterized by multiple isolated nerve injuries. Mononeuropathies may result from a wide variety of causes, including ischemia; traumatic injury; compression; connective tissue diseases; cumulative trauma disorders; and other conditions); *Neuralgia* (Intense or aching pain that occurs

along the course or distribution of a peripheral or cranial nerve); *Peripheral Nervous System Neoplasms* (Neoplasms which arise from peripheral nerve tissue. This includes neurofibromas; Schwannomas; granular cell tumors; and malignant peripheral nerve sheath tumors. See DeVita Jr et al., *Cancer: Principles and Practice of Oncology*, 5th ed, pp 1750-1); *Nerve Compression Syndromes* (Mechanical compression of nerves or nerve roots from internal or external causes. These may result in a conduction block to nerve impulses, due to, for example, myelin sheath dysfunction, or axonal loss. The nerve and nerve sheath injuries may be caused by ischemia; inflammation; or a direct mechanical effect); Neuritis (A general term indicating inflammation of a peripheral or cranial nerve. Clinical manifestation may include pain; paresthesias; paresis; or hyperesthesia); *Polyneuropathies* (Diseases of multiple peripheral nerves. The various forms are categorized by the type of nerve affected (e.g., sensory, motor, or autonomic), by the distribution of nerve injury (e.g., distal vs. proximal), by nerve component primarily affected (e.g., demyelinating vs. axonal), by etiology, or by pattern of inheritance).

[0136] In certain embodiments, the subject full-length Coco or Cerberus polypeptides or variants thereof are used as part of a treatment for diseases or conditions characterized by excessive or undesirable levels of BMP, such as the ones described below.

[0137] The heterotopic ossification of muscles, tendons, and ligaments is a common problem faced by orthopaedic surgeons. Hannallah et al. (J Bone Joint Surg Am. 2004 January; 86-A(1):80-91) investigated the ability of Noggin (a BMP [bone morphogenetic protein] antagonist) to inhibit heterotopic ossification. Three varying doses of Noggin-expressing muscle-derived stem cells inhibited the heterotopic ossification elicited by BMP-4-expressing muscle-derived stem cells. Each of three varying doses of Noggin-expressing muscle-derived stem cells also significantly inhibited the heterotopic ossification elicited by demineralized bone matrix. All eleven animals that underwent Achilles tenotomy developed heterotopic ossification at the site of the injury in the control limbs. In contrast, the limbs treated with the Noggin-expressing muscle-derived stem cells had a reduction in the formation of heterotopic ossification of 83% and eight of the eleven animals had no radiographic evidence of heterotopic ossification ($p < 0.05$). Thus, delivery of Noggin mediated by muscle-derived stem cells can inhibit heterotopic ossification caused by BMP-4, demineralized bone matrix, and trauma in an animal model, indicating that gene therapy to deliver BMP inhibitors (Noggin or Cerberus) may become a powerful method to inhibit heterotopic ossification in targeted areas of the body. See also Glaser et al. (J Bone Joint Surg Am. 2003 December; 85-A(12):2332-42).

[0138] Osteoarthritis (OA) is a joint disease characterized by osteophyte development, fibrosis, and articular cartilage damage. Effects of exogenous transforming growth factor beta (TGF-beta) isoforms and bone morphogenetic proteins (BMPs) suggest a role for these growth factors in the pathogenesis of OA. Scharstuhl et al. (Arthritis Rheum. 2003 December; 48(12):3442-51) used adenoviral overexpression of TGF-beta and BMP antagonists to block the signaling of TGF-beta and BMP. The inhibitors studied include a secreted, pan-specific TGF-beta antagonist called murine latency-associated peptide 1 (mLAP-1), intracellular inhibitory Smad6 (a BMP antagonist), and Smad7 (a TGF-beta/BMP inhibitor). Intraarticular injection of papain caused increased protein

expression of several TGF-beta and BMP isoforms in synovium and cartilage. Adenovirus transfection into the joint resulted in a strong expression of the transgenes in the synovial lining. Overexpression of mLAP-1, Smad6, and Smad7 led to a significant reduction in osteophyte formation compared with that in controls. Smad6 and Smad7 overexpression also significantly decreased synovial thickening. Furthermore, the secreted TGF-beta inhibitor mLAP-1 increased articular cartilage PG loss. These results indicate a pivotal role of excessive endogenous TGF-beta and BMP in the development of osteophytes and synovial thickening, implicating excessive endogenous TGF-beta and BMP in the pathogenesis of OA. In contrast, the prevention of cartilage damage by endogenous TGF-beta signifies the protective role of TGF-beta in articular cartilage. Thus the subject Coco or Cerberus pharmaceutical compositions can be used as BMP antagonists to treat OA, including the development of osteophytes and synovial thickening.

[0139] In an analysis of normal ovarian surface epithelium (OSE) and ovarian cancer (OC) cells, Shepherd and Nachtigal (Endocrinology. 2003 August; 144(8):3306-14) observed BMP4 mRNA expression and found that primary OC cells produce mature BMP4. In addition, each member of the downstream signaling pathway was expressed in primary OSE and OC cells. Smad1 was phosphorylated and underwent nuclear translocation in normal OSE and OC cells upon treatment with BMP4. Interestingly, the BMP target genes ID1 and ID3 were up-regulated 10- to 15-fold in primary OC cells, compared with a 2- to 3-fold increase in normal OSE. The growth of several primary OC cells was relatively unaltered by BMP4 treatment; however, long-term BMP4 treatment of primary OC cells resulted in decreased cell density as well as increased cell spreading and adherence. These data demonstrate the existence and putative function of BMP signaling in normal OSE and OC cells, and thus the subject Cerberus pharmaceutical preparations can be used to regulate BMP4 signaling in OC pathogenesis.

[0140] Fibrodysplasia ossificans progressiva (FOP), a rare genetic disabling disease characterized by heterotopic bone formation, is of special interest for general medicine since the bone morphogenetic proteins (especially BMP-4) involved in its pathogenesis are known to play a role in skeletal morphogenesis, and the gene antagonist to BMP-4 (such as noggin) might be useful in preventing lamellar bone formation. See Blaszczyk et al. (Eur J. Dermatol. 2003 May-June; 13(3):234-7). Thus the subject Cerberus therapeutics may also be used to treat FOP.

[0141] Atherosclerosis is now viewed as an inflammatory disease occurring preferentially in to arterial regions exposed to disturbed flow conditions, including oscillatory shear stress (OS), in branched arteries. Sorescu et al. (J Biol Chem. 278(33):31128-35, 2003) suggest that BMP4 is a mechanosensitive, inflammatory factor playing a critical role in early steps of atherogenesis in the lesion-prone areas. Thus the subject Cerberus therapeutics may be used to control BMP-4 induced inflammatory response in early steps of atherogenesis in those areas.

[0142] During skull development, the cranial connective tissue framework undergoes intramembranous ossification to form skull bones (calvaria). As the calvarial bones advance to envelop the brain, fibrous sutures form between the calvarial plates. Expansion of the brain is coupled with calvarial growth through a series of tissue interactions within the cranial suture complex. Craniosynostosis, or premature cranial

suture fusion, results in an abnormal skull shape, blindness and mental retardation. Recent studies have demonstrated that gain-of-function mutations in fibroblast growth factor receptors (fgfr) are associated with syndromic forms of craniosynostosis. Noggin, an antagonist of bone morphogenetic proteins (BMPs), is required for embryonic neural tube, somites and skeleton patterning. Warren et al. (Nature. 2003 Apr. 10; 422(6932):625-9) show that noggin is expressed postnatally in the suture mesenchyme of patent, but not fusing, cranial sutures, and that noggin expression is suppressed by FGF2 and syndromic fgfr signalling. Since noggin misexpression prevents cranial suture fusion in vitro and in vivo, it is suggested that syndromic fgfr-mediated craniosynostoses may be the result of inappropriate downregulation of noggin expression, leading to abnormally high BMP activity. Thus the subject Cerberus therapeutics may be used to down-regulate BMP activity to prevent or treat such conditions.

V. Exemplary Formulations

[0143] The subject compositions may be used alone, or as part of a conjoint therapy with other compounds/pharmaceutical compositions.

[0144] The soluble Coco or Cerberus derivative therapeutics, including the N-terminally truncated Cerberus derivative therapeutics for use in the subject methods may be conveniently formulated for administration with a biologically acceptable medium, such as water, buffered saline, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like) or suitable mixtures thereof. The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures well known to medicinal chemists. As used herein, "biologically acceptable medium" includes any and all solvents, dispersion media, and the like which may be appropriate for the desired route of administration of the pharmaceutical preparation. The use of such media for to pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the activity of the therapeutics, its use in the pharmaceutical preparation of the invention is contemplated. Suitable vehicles and their formulation inclusive of other proteins are described, for example, in the book *Remington's Pharmaceutical Sciences* (Remington's *Pharmaceutical Sciences*. Mack Publishing Co., Easton, Pa., USA 1985). These vehicles include injectable "deposit formulations."

[0145] Pharmaceutical formulations of the present invention can also include veterinary compositions, e.g., pharmaceutical preparations of the Coco or Cerberus derivative therapeutics suitable for veterinary uses, e.g., for the treatment of live stock (cow, sheep, goat, pig, and horse, etc.) or domestic animals, e.g., cats and dogs.

[0146] Methods of invention may also be provided by rechargeable or biodegradable devices. Various slow release polymeric devices have been developed and tested in vivo in recent years for the controlled delivery of drugs, including proteinacious biopharmaceuticals. A variety of biocompatible polymers (including hydrogels), including both biodegradable and non-degradable polymers, can be used to form an implant for the sustained release of a therapeutic at a particular target site.

[0147] The pharmaceutical compositions according to the present invention may be administered as either a single dose or in multiple doses. The pharmaceutical compositions of the present invention may be administered either as individual

therapeutic agents or in combination with other therapeutic agents. The treatments of the present invention may be combined with conventional therapies, which may be administered sequentially or simultaneously. The pharmaceutical compositions of the present invention may be administered by any means that enables the Coco or Cerberus derivatives to reach the targeted cells/tissues/organs. In some embodiments, routes of administration include those selected from the group consisting of oral, intravesically, intravenous, intraarterial, intraperitoneal, local administration into the blood supply of the organ in which the targeted cells reside or directly into the cells. Intravenous administration is the preferred mode of administration. It may be accomplished with the aid of an infusion pump.

[0148] The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

[0149] The phrases "systemic administration," "administered systemically," "peripheral administration" and "administered peripherally" as used herein mean the administration of a compound, drug or other material other than directly into the central nervous system, such that it enters the patient's system and, thus, is subject to metabolism and other like processes, for example, subcutaneous administration.

[0150] These compounds may be administered to humans and other animals for therapy by any suitable route of administration, including orally, intravesically, nasally, as by, for example, a spray, rectally, intravaginally, parenterally, intracisternally and topically, as by powders, ointments or drops, including buccally and sublingually.

[0151] Regardless of the route of administration selected, the compounds of the present invention, which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically acceptable dosage forms such as described below or by other conventional methods known to those of skill in the art.

[0152] Actual dosage levels of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient.

[0153] The selected dosage level will depend upon a variety of factors including the activity of the particular compound of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular therapeutic employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[0154] A physician or veterinarian having ordinary skill in the art can readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the compounds of the invention employed in the pharmaceutical

composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved.

[0155] In general, a suitable daily dose of a compound of the invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally depend upon the factors described above. Generally, intravenous, intracerebroventricular and subcutaneous doses of the compounds of this invention for a patient will range from about 0.0001 to about 100 mg per kilogram of body weight per day.

[0156] If desired, the effective daily dose of the active compound may be administered as two, three, four, five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms.

[0157] The term "treatment" is intended to encompass also prophylaxis, therapy and cure.

[0158] The patient receiving this treatment is any animal in need, including primates, in particular humans, and other non-human mammals such as equines, cattle, swine and sheep; and poultry and pets in general.

[0159] The compound of the invention can be administered as such or in admixtures with pharmaceutically acceptable carriers and can also be administered in conjunction with other antimicrobial agents such as penicillins, cephalosporins, aminoglycosides and glycopeptides. Conjunctive therapy, thus includes sequential, simultaneous and separate administration of the active compound in a way that the therapeutic effects of the first administered one is not entirely disappeared when the subsequent is administered.

[0160] Combined with certain formulations, the subject Coco or Cerberus derivatives can be effective soluble agents. The therapeutic polypeptide can be provided a fusion peptide along with a second peptide which promotes solubility. To illustrate, the Cerberus derivatives of the present invention can be provided as part of a fusion polypeptide with all or a fragment of the hinge or Fc portion of the immunoglobulin, which can promote solubility and/or serum stability.

[0161] The present invention also contemplates a peptidomimetic sequence of the subject polypeptide derivatives as described herein.

[0162] Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Md. (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes Cellis, J. E., ed. (1994); "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, Conn. (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W.H. Freeman and Co., New York (1980); available immunoassays are extensively described in

the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, Calif. (1990); Marshak et al., "Strategies for Protein Purification and Characterization—A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

Exemplification

[0163] The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain embodiments and embodiments of the present invention, and are not intended to limit the invention.

Example 1

Sources of Caronte and Human Cerberus Protein

[0164] A human Cerberus sequence was cloned into a human CMV derived expression vector. This construct was transiently transfected in HEK293 cells using polyethylenimine (PEI). After culturing, cells were harvested and conditioned media was collected for purification.

[0165] Caronte (from Gallus gallus) was ordered from R&D Systems (Minneapolis, Minn.).

Example 2

Caronte Binds GDF-11

[0166] GDF-11 is a close homolog of myostatin that regulates neurological processes. GDF-11 was immobilized on a BioCore CM5 chip using standard amine coupling procedure. Trace: Caronte (200 µg/ml; R&D Systems) was injected on the GDF-11 coupled chip. The tracing in FIG. 4 shows binding of Caronte to GDF-11.

Example 3

Caronte and Human Cerberus Inhibit GDF-11 and Myostatin-Mediated Signaling

[0167] An A-204 Reporter Gene Assay was used to evaluate the effects of Caronte and Cerberus on signaling by GDF-11, myostatin and Activin A. Cell line: Human Rhabdomyosarcoma (derived from muscle). Reporter vector: pGL3 (CAGA)12 (Described in Dennler et al, 1998, EMBO 17: 3091-3100.) See FIG. 5. The CAGA12 motif is present in TGF-Beta responsive genes (PAI-1 gene), so this vector is of general use for factors signaling through Smad2 and 3.

[0168] Day 1: Split A-204 cells into 48-well plate.

[0169] Day 2: A-204 cells transfected with 10 ug pGL3 (CAGA)₁₂ or pGL3(CAGA)₁₂(10 ug)+pRLCMV (1 ug) and Eugene.

[0170] Day 3: Add factors (diluted into medium+0.1% BSA). Inhibitors need to be preincubated with Factors for 1 hr before adding to cells. 6 hrs later, cells rinsed with PBS, and lyse cells.

[0171] This is followed by a Luciferase assay. In the absence of any inhibitors, Activin A showed 10 fold stimulation of reporter gene expression and an ED50~2 ng/ml. GDF-8: ED50: ~5 ng/ml, 15 fold stimulation. GDF-11: 16 fold stimulation, ED50: ~1.5 ng/ml.

[0172] As shown in FIG. 6, Caronte inhibits GDF-11 signaling in the A-204 Reporter Gene Assay. An ActRIIA-Fc ("IIA muG2a") fusion also inhibits GDF-11 signaling. As

shown in FIG. 7, Caronte does not inhibit Activin A in the A-204 Reporter Gene Assay. An ActRIIA-Fc fusion ("IIA muG2a"), as expected, does inhibit Activin A signaling. Thus, Caronte is a selective inhibitor of GDF-11/myostatin while not affecting Activin A signaling. This type of selectivity suggests that Caronte, Cerberus and Coco will have relatively few side effects when used as a therapeutic. As expected, Cerberus behaved much like Caronte, and inhibited myostatin signaling. See FIG. 8.

EQUIVALENTS

[0173] A skilled artisan will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

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Pro Pro Leu Val Pro Ala Ser Ala Leu Gly Ser Trp Lys Ala Phe Leu
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Gln Asp Glu Val Ala Ala Val Thr Leu Pro Leu Asn Pro Gln Glu Val
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Cys Asn Ser Cys Met Pro Ala Arg Lys Arg Trp Ala Pro Val Val Leu
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 <211> LENGTH: 1731
 <212> TYPE: DNA
 <213> ORGANISM: Homo sapiens

<400> SEQUENCE: 8

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<210> SEQ ID NO 9
<211> LENGTH: 75
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 9

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                20             25             30
Gln Val Ile Lys Ser Cys Val Ser Leu Pro Glu Leu Asn Ala Gln Val
                35             40             45
Phe Cys His Ser Ser Asn Asn Val Thr Lys Thr Glu Cys Cys Phe Thr
 50             55             60
Asp Phe Cys Asn Asn Ile Thr Leu His Leu Pro
65             70             75

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<210> SEQ ID NO 10
<211> LENGTH: 70
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

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<400> SEQUENCE: 10

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                20             25             30
Glu His His Val Arg Thr Cys Ile Pro Lys Val Glu Leu Val Pro Ala
                35             40             45
Gly Lys Pro Phe Tyr Cys Leu Ser Ser Glu Asp Leu Arg Asn Thr His
 50             55             60
Cys Cys Tyr Thr Asp Tyr
65             70

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1-21. (canceled)

22. A method for inhibiting myostatin signal transduction in a muscle cell or an adipose tissue cell in an animal, comprising administering by intravenous, intramuscular, intraperitoneal, intracerebroventricular or subcutaneous route a pharmaceutical preparation to an animal in need thereof, wherein the pharmaceutical preparation comprises the myostatin binding domain of Cerberus and is substantially free of pyrogenic materials so as to be suitable for administration as a human or veterinarian therapeutic.

23-54. (canceled)

55. The method of claim 22, wherein the myostatin binding domain of Cerberus is an N-terminally truncated derivative of wild-type Cerberus protein, said derivative comprising:

(a) a sequence beginning at a position corresponding to any one of residues 106-119 of human Cerberus (SEQ ID NO: 5); and,

(b) a sequence ending at a position corresponding to any residue after residue 240 of human Cerberus (SEQ ID NO: 5),

wherein said derivative binds myostatin, GDF-11, and/or Nodal, but does not substantially bind BMP-4.

56. A method for inhibiting myostatin signal transduction in a muscle cell or an adipose tissue cell in an animal comprising administering by intravenous, intramuscular, intraperitoneal, intracerebroventricular or subcutaneous route a pharmaceutical preparation to an animal in need thereof, wherein the pharmaceutical preparation comprises the myostatin binding domain of Coco and is substantially free of pyrogenic materials so as to be suitable for administration as a human or veterinarian therapeutic.

57. The method of claim 56, wherein the myostatin binding domain of Coco comprises the amino acid of SEQ ID NO: 15.

58. A sustained release implant comprising the myostatin binding domain of Cerberus.

59. A sustained release implant comprising the myostatin binding domain of Coco.

60. A method for inhibiting myostatin signal transduction in an animal comprising administering a pharmaceutical preparation to an animal in need thereof, wherein the pharmaceutical preparation comprises the myostatin binding domain of Cerberus and is substantially free of pyrogenic materials so as to be suitable for administration as a human or veterinarian therapeutic, wherein the animal has a disease or disorder selected from the group consisting of: a neurodegenerative disease, Fibrodysplasia ossificans progressiva (FOP), atherosclerosis, osteoporosis, obesity or congestive heart failure.

61. A method for inhibiting myostatin signal transduction in an animal comprising administering a pharmaceutical preparation to an animal in need thereof, wherein the pharmaceutical preparation comprises the myostatin binding domain of Coco and is substantially free of pyrogenic materials so as to be suitable for administration as a human or veterinarian therapeutic, wherein the animal has a disease or disorder selected from the group consisting of: a neurodegenerative disease, Fibrodysplasia ossificans progressiva (FOP), atherosclerosis, osteoporosis, obesity or congestive heart failure.

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