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**Compositions and methods for increasing muscle mass and muscle strength by specifically antagonizing GDF8 and/or Activin A**

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(54) **Title:** COMPOSITIONS AND METHODS FOR INCREASING MUSCLE MASS AND MUSCLE STRENGTH BY SPECIFICALLY ANTAGONIZING GDF8 AND/OR ACTIVIN A

(57) **Abstract:** The present invention provides compositions and methods which involve specifically antagonizing GDF8 and Activin A. In certain embodiments, compositions are provided which comprise a GDF8-specific binding protein and an Activin A-specific binding protein. For example, the invention includes compositions comprising an anti-GDF8 antibody and an anti-Activin A antibody, in other embodiments, antigen-binding molecules are provided which comprise a GDF8-specific binding domain and an Activin A-specific binding domain. For example, the invention includes bispecific antibodies that bind GDF 8 and Activin A. The compositions of the present invention are useful for the treatment of diseases and conditions characterized by reduced muscle mass or strength, as well as other conditions which are treatable by antagonizing GDF8 and/or Activin A activity.



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## COMPOSITIONS AND METHODS FOR INCREASING MUSCLE MASS AND MUSCLE STRENGTH BY SPECIFICALLY ANTAGONIZING GDF8 AND/OR ACTIVIN A

### FIELD OF THE INVENTION

[0001] The present invention relates to compositions and methods for increasing muscle mass and muscle strength in a subject. More specifically, the invention relates to compositions that specifically bind GDF8 and Activin A and the use of such compositions to treat diseases and disorders characterized by decreased muscle mass or strength.

### BACKGROUND

[0002] Growth and differentiation factor-8 (GDF8, also known as myostatin), is a secreted ligand belonging to the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily of growth factors. GDF8 plays a central role in the development and maintenance of skeletal muscle, acting as a negative regulator of muscle mass. While the myostatin null mouse phenotype demonstrates the importance of GDF8 in the control of muscle size during development, muscle hypertrophy can also be elicited in adult muscle through inhibition of GDF8 with neutralizing antibodies, decoy receptors, or other antagonists. Administration of GDF8 neutralizing antibodies has been reported to result in muscle mass increases of between 10 and 30%. The increased muscle mass seen is due to increased fiber diameter as opposed to myofiber hyperplasia (fiber number). A number of studies have also reported increases in muscle strength or performance commensurate with increased size including twitch and tetanic force. Use of a cleavage resistant version of the GDF8 propeptide also leads to increased muscle size.

[0003] Other GDF8 antagonists have been used in adult mice with significant effects on skeletal muscle mass. These include the extracellular portion of the Type II GDF8 receptor, ActRIIB, stabilized by fusion to an IgG Fc domain ("ActRIIB-Fc"). The clinical molecule "ACE-031" is an example of an ActRIIB-Fc molecule.

[0004] Although ActRIIB-Fc has been shown to increase muscle mass in experimental animals, in human clinical trials this molecule was shown to cause various adverse side effects. For example, administration of ACE-031 to postmenopausal women in a Phase Ib ascending dose study was shown to cause undesired increases in hemoglobin and decreases in FSH levels. In addition, a Phase II study of ACE-031 in pediatric patients with muscular dystrophy was discontinued due to adverse effects including nose and gum bleeding. Dilated blood vessels are also observed in patients treated with ActRIIB-Fc.

[0005] Experiments have shown that the muscle growth-inducing effects of ActRIIB-Fc are attenuated but not eliminated in myostatin null mice, suggesting that ActRIIB-Fc exerts its muscle mass-inducing effects by antagonizing other ActRIIB ligand(s) in addition to GDF8.

Other ligands that bind ActRIIB include Activin A, Activin B, Activin AB, Inhibin A, Inhibin B, GDF3, GDF11, Nodal, BMP2, BMP4, and BMP7.

#### **BRIEF SUMMARY OF THE INVENTION**

[0006] The present inventors hypothesized that the enhanced muscle growth effects of ActRIIB-Fc, as well as its unwanted side effects, are due to the binding of this molecule to additional ligands beside GDF8. Thus, the inventors sought to determine if it was possible to specifically antagonize only certain ActRIIB ligands but not others in order to produce the enhanced muscle growth effects of ActRIIB-Fc while at the same time avoiding the unwanted adverse side effects associated with this molecule. Through the experimentation set out in the Examples herein, it was surprisingly discovered that significant muscle growth enhancement could be achieved by specifically antagonizing Activin A. Importantly, it was also determined that the desired therapeutic effects of ActRIIB-Fc (e.g., enhanced skeletal muscle growth) could be achieved without unwanted side effects by specifically antagonizing GDF8 and Activin A but not antagonizing other ActRIIB ligands (e.g., GDF11, BMP9, BMP10, etc.).

[0007] Thus, according to one aspect of the present invention, there is provided a composition comprising a GDF8-specific binding protein and an Activin A-specific binding protein, wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A-specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A. According to another aspect, there is provided a composition comprising a GDF8-specific binding protein and an Activin A specific binding protein for use in a method of treating a disease or disorder characterized by decreased muscle mass or strength, wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A-specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A. According to a related aspect of the invention, a GDF8-specific binding protein when used in a method of treating a disease or disorder characterized by decreased muscle mass or strength, wherein the method comprises administering to a subject the GDF8-specific binding protein and an Activin A specific binding protein and wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A. According to another related aspect, there is provided an Activin A-specific binding protein when used in a method of treating a disease or disorder characterized by decreased muscle mass or strength, wherein the method comprises administering to a subject the Activin A-

specific binding protein and a GDF8-specific binding protein and wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A. In yet another related aspect, there is provided an antigen-binding molecule comprising an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8 and an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A. In yet another related aspect, there is provided an antigen-binding molecule comprising an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8 and an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A for use in a method of treating a disease or disorder characterized by decreased muscle mass or strength. In one embodiment of the invention, the antigen-binding molecule is a bispecific antibody comprising a first variable domain that specifically binds GDF8 and a second variable domain that specifically binds Activin A.

**[0008]** The present invention provides a method of increasing muscle mass or strength in a subject, the method comprising administering to the subject an Activin A-specific binding protein and a GDF8 specific binding protein, wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A-specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A. The present invention also provides a method of increasing muscle mass or strength in a subject, the method comprising administering to the subject an antigen-binding molecule comprising an anti-GDF8-specific antibody or antigen-binding fragment thereof that inhibits GDF8 and an anti-Activin A-specific antibody or antigen-binding fragment thereof that inhibits Activin A. The methods according to this aspect of the invention are useful for treating diseases or disorders associated with decreased muscle mass, strength or power, including, e.g., cachexia, sarcopenia and other muscle-wasting conditions.

**[0008a]** In yet another aspect, the invention provides the use of a GDF8-specific binding protein and an Activin A-specific binding protein in the manufacture of a medicament for treating a disease or disorder characterized by decreased muscle mass or strength, wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A-specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A. In yet another aspect, the invention provides the use of an antigen-binding molecule in the manufacture of a medicament for treating a disease or disorder characterized by decreased muscle mass or strength, wherein the antigen binding molecule comprises an

anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8 and an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A.

**[0009]** Other embodiments of the present invention will become apparent from a review of the ensuing detailed description.

## DETAILED DESCRIPTION

[0010] Before the present invention is described, it is to be understood that this invention is not limited to particular methods and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0011] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. As used herein, the term "about," when used in reference to a particular recited numerical value, means that the value may vary from the recited value by no more than 1%. For example, as used herein, the expression "about 100" includes 99 and 101 and all values in between (e.g., 99.1, 99.2, 99.3, 99.4, etc.).

### Antigen-Specific Binding Proteins

[0012] The present invention relates to compositions comprising antigen-specific binding proteins. More specifically, the present invention provides a composition comprising a GDF8-specific binding protein and an Activin A-specific binding protein.

[0013] As used herein, the expression "antigen-specific binding protein" means a protein comprising at least one domain which specifically binds a particular antigen. Exemplary categories of antigen-specific binding proteins include antibodies, antigen-binding portions of antibodies, peptides that specifically interact with a particular antigen (e.g., peptibodies), receptor molecules that specifically interact with a particular antigen, and proteins comprising a ligand-binding portion of a receptor that specifically binds a particular antigen.

[0014] The present invention includes antigen-specific binding proteins that specifically bind GDF8, *i.e.*, "GDF8-specific binding proteins". The term "GDF8" (also referred to as "growth and differentiation factor-8" and "myostatin") means the protein having the amino acid sequence of SEQ ID NO:25 (mature protein). According to the present invention, GDF8-specific binding proteins specifically bind GDF8 but do not bind other ActRIIB ligands such as GDF3, BMP2, BMP4, BMP7, BMP9, BMP10, GDF11, Activin A, Activin B, Activin AB, Nodal, etc.

[0015] The present invention also includes antigen-specific binding proteins that specifically bind Activin A, *i.e.*, "Activin A-specific binding proteins". Activins are homo- and heter-dimeric molecules comprising  $\beta$ A and/or  $\beta$ B subunits. The  $\beta$ A subunit has the amino acid sequence of SEQ ID NO:26 and the  $\beta$ B subunit has the amino acid sequence of SEQ ID NO:28. Activin A is a homodimer of two  $\beta$ A subunits; Activin B is a homodimer of two  $\beta$ B subunits; and Activin AB is a heterodimer of one  $\beta$ A subunit and one  $\beta$ B subunit. An Activin A-specific binding protein may be an antigen-specific binding protein that specifically binds the  $\beta$ A subunit. Since the  $\beta$ A subunit is found in both Activin A and Activin AB molecules, an "Activin A-specific binding

protein" can be an antigen-specific binding protein that specifically binds Activin A as well as Activin AB (by virtue of its interaction with the  $\beta$ A subunit). Therefore, according to the present invention, an Activin A-specific binding protein specifically binds Activin A, or Activin A and Activin AB, but does not bind other ActRIIB ligands such as Activin B, GDF3, GDF8, BMP2, BMP4, BMP7, BMP9, BMP10, GDF11, Nodal, etc.

[0016] In the context of the present invention, molecules such as ActRIIB-Fc (e.g., "ACE-031"), which comprise the ligand-binding portion of the ActRIIB receptor, are not considered "GDF8-specific binding proteins" or "Activin A-specific binding proteins" because such molecules bind multiple ligands besides GDF8, Activin A and Activin AB.

#### **Antigen-Binding Molecules with Two Different Antigen-Specific Binding Domains**

[0017] The present invention also includes antigen-binding molecules comprising two different antigen-specific binding domains. In particular, the present invention includes antigen-binding molecules comprising a GDF8-specific binding domain and an Activin A-specific binding domain. The term "antigen-specific binding domain," as used herein, includes polypeptides comprising or consisting of: (i) an antigen-binding fragment of an antibody molecule, (ii) a peptide that specifically interacts with a particular antigen (e.g., a peptibody), and/or (iii) a ligand-binding portion of a receptor that specifically binds a particular antigen. For example, the present invention includes bispecific antibodies with one arm comprising a first heavy chain variable region/light chain variable region (HCVR/LCVR) pair that specifically binds GDF8 and another arm comprising a second HCVR/LCVR pair that specifically binds Activin A.

#### **Specific Binding**

[0018] The term "specifically binds" or the like, as used herein, means that an antigen-specific binding protein, or an antigen-specific binding domain, forms a complex with a particular antigen characterized by a dissociation constant ( $K_D$ ) of 500 pM or less, and does not bind other unrelated antigens under ordinary test conditions. "Unrelated antigens" are proteins, peptides or polypeptides that have less than 95% amino acid identity to one another. Methods for determining whether two molecules specifically bind one another are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. For example, an antigen-specific binding protein or an antigen-specific binding domain, as used in the context of the present invention, includes molecules that bind a particular antigen (e.g., GDF8, or Activin A and/or AB) or a portion thereof with a  $K_D$  of less than about 500 pM, less than about 400 pM, less than about 300 pM, less than about 200 pM, less than about 100 pM, less than about 90 pM, less than about 80 pM, less than about 70 pM, less than about 60 pM, less than about 50 pM, less than about 40 pM, less than about 30 pM, less than about 20 pM, less than about 10 pM, less than about 5 pM, less than about 4 pM, less than about 2 pM, less



than about 1 pM, less than about 0.5 pM, less than about 0.2 pM, less than about 0.1 pM, or less than about 0.05 pM, as measured in a surface plasmon resonance assay.

[0019] As used herein, an antigen-specific binding protein or antigen-specific binding domain "does not bind" to a specified molecule (e.g., "does not bind GDF11", "does not bind BMP9", "does not bind BMP10", etc.) if the protein or binding domain, when tested for binding to the molecule at 25°C in a surface plasmon resonance assay, exhibits a  $K_D$  of greater than 1000 pM, or fails to exhibit any binding in such an assay or equivalent thereof.

[0020] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore™ system (Biacore Life Sciences division of GE Healthcare, Piscataway, NJ).

[0021] The term " $K_D$ ", as used herein, means the equilibrium dissociation constant of a particular protein-protein interaction (e.g., antibody-antigen interaction). Unless indicated otherwise, the  $K_D$  values disclosed herein refer to  $K_D$  values determined by surface plasmon resonance assay at 25°C.

#### **Antibodies and Antigen-Binding Fragments of Antibodies**

[0022] As indicated above, an antigen-specific binding protein can comprise or consist of an antibody or antigen-binding fragment of an antibody. Furthermore, in the case of antigen-binding molecules comprising two different antigen-specific binding domains, one or both of the antigen-specific binding domains may comprise or consist of an antigen-binding fragment of an antibody.

[0023] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprising four polypeptide chains, two heavy (H) chains and two light (L) chains interconnected by disulfide bonds, as well as multimers thereof (e.g., IgM). Each heavy chain comprises a heavy chain variable region (abbreviated herein as HCVR or  $V_H$ ) and a heavy chain constant region. The heavy chain constant region comprises three domains,  $C_{H1}$ ,  $C_{H2}$  and  $C_{H3}$ . Each light chain comprises a light chain variable region (abbreviated herein as LCVR or  $V_L$ ) and a light chain constant region. The light chain constant region comprises one domain ( $C_{L1}$ ). The  $V_H$  and  $V_L$  regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each  $V_H$  and  $V_L$  is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In different embodiments of the invention, the FRs of the antibodies of the invention (or antigen-binding portion thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[0024] The term "antibody," as used herein, also includes antigen-binding fragments of full antibody molecules. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. Antigen-binding fragments of an antibody may be derived, e.g., from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and optionally constant domains. Such DNA is known and/or is readily available from, e.g., commercial sources, DNA libraries (including, e.g., phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

[0025] Non-limiting examples of antigen-binding fragments include: (i) Fab fragments; (ii) F(ab')<sub>2</sub> fragments; (iii) Fd fragments; (iv) Fv fragments; (v) single-chain Fv (scFv) molecules; (vi) dAb fragments; and (vii) minimal recognition units consisting of the amino acid residues that mimic the hypervariable region of an antibody (e.g., an isolated complementarity determining region (CDR) such as a CDR3 peptide), or a constrained FR3-CDR3-FR4 peptide. Other engineered molecules, such as domain-specific antibodies, single domain antibodies, domain-deleted antibodies, chimeric antibodies, CDR-grafted antibodies, diabodies, triabodies, tetrabodies, minibodies, nanobodies (e.g. monovalent nanobodies, bivalent nanobodies, etc.), small modular immunopharmaceuticals (SMIPs), and shark variable IgNAR domains, are also encompassed within the expression "antigen-binding fragment," as used herein.

[0026] An antigen-binding fragment of an antibody will typically comprise at least one variable domain. The variable domain may be of any size or amino acid composition and will generally comprise at least one CDR which is adjacent to or in frame with one or more framework sequences. In antigen-binding fragments having a V<sub>H</sub> domain associated with a V<sub>L</sub> domain, the V<sub>H</sub> and V<sub>L</sub> domains may be situated relative to one another in any suitable arrangement. For example, the variable region may be dimeric and contain V<sub>H</sub>-V<sub>H</sub>, V<sub>H</sub>-V<sub>L</sub> or V<sub>L</sub>-V<sub>L</sub> dimers. Alternatively, the antigen-binding fragment of an antibody may contain a monomeric V<sub>H</sub> or V<sub>L</sub> domain.

[0027] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V<sub>H</sub>-C<sub>H</sub>1; (ii) V<sub>H</sub>-C<sub>H</sub>2; (iii) V<sub>H</sub>-C<sub>H</sub>3; (iv) V<sub>H</sub>-C<sub>H</sub>1-C<sub>H</sub>2; (v) V<sub>H</sub>-C<sub>H</sub>1-C<sub>H</sub>2-C<sub>H</sub>3; (vi) V<sub>H</sub>-C<sub>H</sub>2-C<sub>H</sub>3; (vii) V<sub>H</sub>-C<sub>L</sub>; (viii) V<sub>L</sub>-C<sub>H</sub>1; (ix) V<sub>L</sub>-C<sub>H</sub>2; (x) V<sub>L</sub>-C<sub>H</sub>3; (xi) V<sub>L</sub>-C<sub>H</sub>1-C<sub>H</sub>2; (xii) V<sub>L</sub>-C<sub>H</sub>1-C<sub>H</sub>2-C<sub>H</sub>3; (xiii) V<sub>L</sub>-C<sub>H</sub>2-C<sub>H</sub>3; and (xiv) V<sub>L</sub>-C<sub>L</sub>. In any

configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (e.g., 5, 10, 15, 20, 40, 60 or more) amino acids which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric  $V_H$  or  $V_L$  domain (e.g., by disulfide bond(s)).

[0028] The molecules of the present invention may comprise or consist of human antibodies and/or recombinant human antibodies, or fragments thereof. The term "human antibody", as used herein, includes antibodies having variable and constant regions derived from human germline immunoglobulin sequences. Human antibodies may nonetheless include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

[0029] The molecules of the present invention may comprise or consist of recombinant human antibodies or antigen-binding fragments thereof. The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further below), antibodies isolated from a recombinant, combinatorial human antibody library (described further below), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor et al. (1992) Nucl. Acids Res. 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the  $V_H$  and  $V_L$  regions of the recombinant antibodies are sequences that, while derived from and related to human germline  $V_H$  and  $V_L$  sequences, may not naturally exist within the human antibody germline repertoire *in vivo*.

#### **Anti-GDF8 Antibodies and Antigen-Binding Fragments Thereof**

[0030] In certain specific embodiments of the present invention, the GDF8-specific binding

protein, or the GDF8-specific binding domain, comprises or consists of an anti-GDF8 antibody or antigen-binding fragment thereof. Anti-GDF8 antibodies are mentioned in, *e.g.*, US Patent Nos. 6,096,506; 7,320,789; 7,261,893; 7,807,159; 7,888,486; 7,635,760; 7,632,499; in US Patent Appl. Publ. Nos. 2007/0178095; 2010/0166764; 2009/0148436; and International Patent Appl. Publ. No. WO 2010/070094. Anti-GDF8 antibodies are also described in US Patent Appl. No. 13/115,170, filed on May 25, 2011, and published as US 2011/0293630, including the antibodies designated 8D12, H4H1657N2, and H4H1669P. Any of the anti-GDF8 antibodies mentioned and/or described in any of the foregoing patents or publications, or antigen-binding fragments thereof, can be used in the context of the present invention, so long as such antibodies and/or antigen-binding fragments "specifically bind" GDF8, as that expression is defined herein.

**[0031]** Table 1 sets forth the sequence identifiers of the HCVRs, LCVRs, and CDRs of certain non-limiting, exemplary anti-GDF8 antibodies that can be used in the context of the present invention.

**Table 1**

Antibody	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
8D12	1	2	3	4	5	6	7	8
H4H1657N2	9	10	11	12	13	14	15	16
H4H1669P	17	18	19	20	21	22	23	24

#### **Anti-Activin A Antibodies and Antigen-Binding Fragments Thereof**

**[0032]** In certain specific embodiments of the present invention, the Activin A-specific binding protein, or the Activin A-specific binding domain, comprises or consists of an antibody or antigen-binding fragment thereof that specifically binds Activin A. In certain embodiments, the Activin A-specific binding protein specifically binds the  $\beta$ A subunit. An antigen-specific binding protein that specifically binds the  $\beta$ A subunit may recognize both Activin A ( $\beta$ A/ $\beta$ A homodimer) and Activin AB ( $\beta$ A/ $\beta$ B heterodimer). Thus, according to the present invention, an Activin A-specific binding protein may bind both Activin A and Activin AB (but not Activin B). Anti-Activin A antibodies are mentioned in, *e.g.*, US Patent Appl. Publ. No 2009/0234106. A particular anti-Activin A antibody is designated "MAB3381," and is available commercially from R&D Systems, Inc, Minneapolis, MN. MAB3381 specifically binds Activin A (homodimer) as well as Activin AB (heterodimer). Any of the aforementioned anti-Activin A antibodies, or antigen-binding fragments thereof, can be used in the context of the present invention, so long as such antibodies and/or antigen-binding fragments "specifically bind" Activin A and/or Activin AB, as defined herein.

#### **Pharmaceutical Compositions and Methods of Administration**

The present invention includes pharmaceutical compositions comprising a GDF8-specific binding protein and an Activin A-specific binding protein. The present invention also includes pharmaceutical compositions comprising an antigen-binding molecule comprising a

GDF8-specific binding domain and an Activin A-specific binding domain. The pharmaceutical compositions of the invention are formulated with suitable carriers, excipients, and other agents that provide suitable transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in, *e.g.*, Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. Suitable formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN™), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. Additional suitable formulations are also described in Powell et al. "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0034] Various delivery systems are known and can be used to administer the pharmaceutical compositions of the present invention, *e.g.*, encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu et al., 1987, J. Biol. Chem. 262:4429-4432). Methods of administration include, but are not limited to, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents.

[0035] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0036] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but are not limited to AUTOPEN™ (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Bergdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG™ pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN),

NOVOPEN™ I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD™ pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN™, OPTIPEN PRO™, OPTIPEN STARLET™, and OPTICLIK™ (sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but are not limited to the SOLOSTAR™ pen (sanofi-aventis), the FLEXPEN™ (Novo Nordisk), and the KWIKPEN™ (Eli Lilly), the SURECLICK™ Autoinjector (Amgen, Thousand Oaks, CA), the PENLET™ (Haselmeier, Stuttgart, Germany), the EPIPEN (Dey, L.P.), and the HUMIRA™ Pen (Abbott Labs, Abbott Park IL), to name only a few.

[0037] In certain situations, the pharmaceutical compositions of the present invention can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, 1987, *CRC Crit. Rev. Biomed. Eng.* 14:201). In another embodiment, polymeric materials can be used; see, *Medical Applications of Controlled Release*, Langer and Wise (eds.), 1974, CRC Pres., Boca Raton, Florida. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, 1984, in *Medical Applications of Controlled Release*, *supra*, vol. 2, pp. 115-138). Other controlled release systems are discussed in the review by Langer, 1990, *Science* 249:1527-1533.

[0038] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by known methods. For example, the injectable preparations may be prepared, e.g., by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (e.g., ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, e.g., sesame oil, soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0039] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc.

## Dosage

[0040] The amount of active ingredient (e.g., anti-GDF8 antibodies and anti-Activin A antibodies) that can be administered to a subject is, generally, a therapeutically effective amount. As used herein, the phrase "therapeutically effective amount" means a dose of antigen-specific binding proteins and/or antigen-binding molecules that results in a detectable increase in one or more of the following parameters: body weight, muscle mass (e.g., tibialis anterior [TA] muscle mass, gastrocnemius [GA] muscle mass, quadriceps [Quad] muscle mass, etc.), muscle strength/power, and/or muscle function. For example, a "therapeutically effective amount" of a GDF8-specific binding protein and/or an Activin A-specific binding protein includes, e.g., an amount of GDF8-specific binding protein and/or Activin A-specific binding protein that, when administered to a test subject, causes an increase in TA or GA muscle mass of at least 2%, 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60% or more, compared to control treated subjects, e.g., as illustrated in Example 1, herein.

[0041] In the case of antibodies of the present invention (e.g., anti-GDF8 antibodies, anti-Activin A antibodies, or bispecific antibodies that specifically bind GDF8 and Activin A), a therapeutically effective amount can be from about 0.05 mg to about 600 mg; e.g., about 0.05 mg, about 0.1 mg, about 1.0 mg, about 1.5 mg, about 2.0 mg, about 10 mg, about 20 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, about 200 mg, about 210 mg, about 220 mg, about 230 mg, about 240 mg, about 250 mg, about 260 mg, about 270 mg, about 280 mg, about 290 mg, about 300 mg, about 310 mg, about 320 mg, about 330 mg, about 340 mg, about 350 mg, about 360 mg, about 370 mg, about 380 mg, about 390 mg, about 400 mg, about 410 mg, about 420 mg, about 430 mg, about 440 mg, about 450 mg, about 460 mg, about 470 mg, about 480 mg, about 490 mg, about 500 mg, about 510 mg, about 520 mg, about 530 mg, about 540 mg, about 550 mg, about 560 mg, about 570 mg, about 580 mg, about 590 mg, or about 600 mg, of the respective antibody.

[0042] The amount of antibody of the present invention (e.g., anti-GDF8 antibodies, anti-Activin A antibodies, or bispecific antibodies that specifically bind GDF8 and Activin A) contained within the individual doses may be expressed in terms of milligrams of antibody per kilogram of patient body weight (i.e., mg/kg). For example, the anti-GDF8, anti-Activin A and/or anti-GDF8/anti-Activin A bispecific antibodies of the present invention may be administered to a patient at a dose of about 0.0001 to about 50 mg/kg of patient body weight (e.g. 0.5 mg/kg, 1.0 mg/kg, 1.5 mg/kg, 2.0 mg/kg, 2.5 mg/kg, 3.0 mg/kg, 3.5 mg/kg, 4.0 mg/kg, 4.5 mg/kg, 5.0 mg/kg, 5.5 mg/kg, 6.0 mg/kg, 6.5 mg/kg, 7.0 mg/kg, 7.5 mg/kg, 8.0 mg/kg, 8.5 mg/kg, 9.0 mg/kg, 9.5 mg/kg, 10.0 mg/kg, 10.5 mg/kg, 11.0 mg/kg, 11.5 mg/kg, etc.).

[0043] The compositions of the present invention may comprise equal amounts of GDF8-specific binding protein and Activin A-specific binding protein. Alternatively, the amount of

GDF8-specific binding protein in the composition may be less than or greater than the amount of Activin A-specific binding protein. A person of ordinary skill in the art, using routine experimentation, will be able to determine the appropriate amounts of the individual components in the compositions of the present invention necessary to produce a desired therapeutic effect.

### **Therapeutic Methods**

[0044] The present invention includes methods of treating conditions or afflictions which can be cured, alleviated or improved by increasing muscle strength/power and/or muscle mass and/or muscle function in an individual, or by favorably altering metabolism (carbohydrate, lipid and protein processing) by specifically binding GDF8, and/or Activin A, and/or Activin AB, and not binding other ActRIIB ligands. For example, the present invention includes methods for increasing muscle strength/power and/or muscle mass and/or muscle function in a subject, or for treating a disease or disorder characterized by decreased muscle mass or strength in a subject, by administering to the subject an Activin A-specific binding protein. The present invention also includes methods for increasing muscle strength/power and/or muscle mass and/or muscle function in a subject, or for treating a disease or disorder characterized by decreased muscle mass or strength in a subject, by administering to the subject a GDF8-specific binding protein and an Activin A-specific binding protein. Any of the GDF8-specific binding proteins and/or Activin A-specific binding proteins disclosed or referred to herein can be used in the context of these aspects of the invention. For example, the therapeutic methods of the present invention include administering to a subject an anti-GDF8 antibody and/or an anti-Activin A antibody.

[0045] In methods which comprise administering a GDF8-specific binding protein and an Activin A-specific binding protein to a subject, the GDF8-specific binding protein and the Activin A-specific binding protein may be administered to the subject at the same or substantially the same time, e.g., in a single therapeutic dosage, or in two separate dosages which are administered simultaneously or within less than about 5 minutes of one another. Alternatively, the GDF8-specific binding protein and the Activin A-specific binding protein may be administered to the subject sequentially, e.g., in separate therapeutic dosages separated in time from one another by more than about 5 minutes.

[0046] The present invention also includes methods for increasing muscle strength/power and/or muscle mass and/or muscle function in a subject, or for treating a disease or disorder characterized by decreased muscle mass or strength in a subject, by administering to the subject an antigen-binding molecule comprising a GDF8-specific binding domain and an Activin A-specific binding domain. Any of the antigen-binding molecules disclosed or referred to herein can be used in the context of this aspect of the invention. For example, the therapeutic methods of the present invention include administering to a subject a bispecific antibody comprising a first variable domain comprising a HCVR/LCVR pair that specifically binds GDF8 and a second



variable domain comprising a HCVR/LCVR pair that specifically binds Activin A.

[0047] The compositions of the present invention may be administered to a subject along with one or more additional therapeutic agents, including, *e.g.*, growth factor inhibitors, immunosuppressants, anti-inflammatory agents, metabolic inhibitors, enzyme inhibitors, and cytotoxic/cytostatic agents. The additional therapeutic agent(s) may be administered prior to, concurrent with, or after the administration of the GDF8- and Activin A-specific binding proteins of the present invention.

[0048] Exemplary diseases, disorders and conditions that can be treated with the compositions of the present invention include, but are not limited to, sarcopenia, cachexia (either idiopathic or secondary to other conditions, *e.g.*, cancer, chronic renal failure, or chronic obstructive pulmonary disease), muscle injury, muscle wasting and muscle atrophy, *e.g.*, muscle atrophy or wasting caused by or associated with disuse, immobilization, bed rest, injury, medical treatment or surgical intervention (*e.g.*, hip fracture, hip replacement, knee replacement, etc.) or by necessity of mechanical ventilation. The compositions of the invention may also be used to treat, prevent or ameliorate diseases such as cancer, obesity, diabetes, arthritis, multiple sclerosis, muscular dystrophy, amyotrophic lateral sclerosis, Parkinson's disease, osteoporosis, osteoarthritis, osteopenia, metabolic syndromes (including, but not limited to diabetes, obesity, nutritional disorders, organ atrophy, chronic obstructive pulmonary disease, and anorexia).

#### **Avoidance of Side Effects**

[0049] The present invention includes methods for increasing muscle strength/power and/or muscle mass and/or muscle function in a subject, or for treating a disease or disorder characterized by decreased muscle mass or strength in a subject, without causing adverse side effects associated with the administration of molecules which bind multiple (*e.g.*, 3 or more) ActRIIB ligands. For example, the clinical molecule referred to as ACE-031 (Accelaron Pharma, Inc., Cambridge, MA) is a multimer consisting of the extracellular portion of ActRIIB fused to an IgG Fc domain (this molecule is also referred to herein as "ActRIIB-Fc"). ActRIIB-Fc binds GDF8 as well as other ActRIIB ligands such as, *e.g.*, Activin A, Activin B, GDF11, BMP9, BMP10, and TGF $\beta$ , and is known to cause various adverse side effects when administered to human patients. Significantly, the present inventors have unexpectedly discovered that specifically inhibiting GDF8 and Activin A (*e.g.*, by administering an anti-GDF8 antibody and an anti-Activin A antibody), while not inhibiting other ActRIIB ligands such as Activin B, GDF11, BMP9, BMP10, and TGF $\beta$ , results in an increase in muscle mass that is at least equivalent to that observed by administration of ActRIIB-Fc, without causing the adverse side effects associated with non-specific Activin binding agents such as ActRIIB-Fc.

**Administration Regimens**

[0050] According to certain embodiments of the present invention, multiple doses of the compositions of the present invention (e.g., compositions comprising GDF8- and/or Activin A-specific binding proteins or antigen-binding molecules comprising a GDF8-specific binding domain and an Activin A-specific binding domain), may be administered to a subject over a defined time course. The methods according to this aspect of the invention comprise sequentially administering to a subject multiple doses of the compositions of the present invention. As used herein, "sequentially administering" means that each dose of the compositions of the present invention are administered to the subject at a different point in time, e.g., on different days separated by a predetermined interval (e.g., hours, days, weeks or months). The present invention includes methods which comprise sequentially administering to the patient an initial dose of a composition of the present invention, followed by one or more secondary doses of the composition, and optionally followed by one or more tertiary doses of the composition.

[0051] The terms "initial dose," "secondary doses," and "tertiary doses," refer to the temporal sequence of administration of the compositions of the present invention. Thus, the "initial dose" is the dose which is administered at the beginning of the treatment regimen (also referred to as the "baseline dose"); the "secondary doses" are the doses which are administered after the initial dose; and the "tertiary doses" are the doses which are administered after the secondary doses. The initial, secondary, and tertiary doses may all contain the same amount of active ingredient(s), but will generally differ from one another in terms of frequency of administration. In certain embodiments, however, the amount of active ingredient(s) contained in the initial, secondary and/or tertiary doses will vary from one another (e.g., adjusted up or down as appropriate) during the course of treatment.

[0052] In one exemplary embodiment of the present invention, each secondary and/or tertiary dose is administered 1 to 30 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more) days after the immediately preceding dose. The phrase "the immediately preceding dose," as used herein, means, in a sequence of multiple administrations, the dose(s) of the compositions of the present invention which are administered to a subject prior to the administration of the very next dose in the sequence with no intervening doses.

[0053] The methods according to this aspect of the invention may comprise administering to a patient any number of secondary and/or tertiary doses of the compositions of the present invention. For example, in certain embodiments, only a single secondary dose is administered to the patient. In other embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, or more) secondary doses are administered to the patient. Likewise, in certain embodiments, only a single tertiary

dose is administered to the patient. In other embodiments, two or more (e.g., 2, 3, 4, 5, 6, 7, 8, or more) tertiary doses are administered to the patient.

[0054] In embodiments involving multiple secondary doses, each secondary dose may be administered at the same frequency as the other secondary doses. For example, each secondary dose may be administered to the patient 1 to 29 days after the immediately preceding dose. Similarly, in embodiments involving multiple tertiary doses, each tertiary dose may be administered at the same frequency as the other tertiary doses. For example, each tertiary dose may be administered to the patient 1 to 60 days after the immediately preceding dose. Alternatively, the frequency at which the secondary and/or tertiary doses are administered to a patient can vary over the course of the treatment regimen. The frequency of administration may also be adjusted during the course of treatment by a physician depending on the needs of the individual patient following clinical examination.

### EXAMPLES

[0055] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

#### **Example 1. Specific Inhibition of GDF8 and Activin A Causes Synergistic Increases in Skeletal Muscle Mass**

##### **Introduction**

[0056] ActRIIB-Fc is a GDF8 antagonist consisting of the extracellular portion of the ActRIIB receptor, stabilized by fusion to an IgG Fc domain. ActRIIB-Fc has been shown to increase muscle mass in mice to a greater extent than anti-GDF8 antibodies. The present inventors hypothesized that the enhanced activity of ActRIIB-Fc could potentially be due to its ability to bind additional ActRIIB ligands besides GDF8. In particular, it was proposed that antagonism of Activin A, in addition to antagonism of GDF8, might cause greater increases in skeletal muscle mass than what has been observed in animals treated with anti-GDF8 antibodies alone. Thus, the present Example was designed to determine whether specific inhibition of GDF8 and Activin A can increase skeletal muscle mass to an extent that is at least equivalent to the increase observed using ActRIIB-Fc.

## Results and Discussion

[0057] The extent of skeletal muscle hypertrophy induced by administration of ActRIIB-Fc was compared to the effect of administration of a GDF8-specific antibody, an Activin A specific antibody, or a combination of an anti-GDF8 + anti-Activin A antibody. The ActRIIB-Fc construct used in this Example has the amino acid sequence of SEQ ID NO:27. The anti-GDF8 antibody used in this Example is the antibody designated H4H1657N2 (see Table 1). The anti-Activin A antibody used in this Example is the antibody designated MAB3381 (available from R&D Systems, Inc., Minneapolis, MN). An isotype-matched (hlgG4) antibody was used as negative control.

[0058] Briefly, 25 male CB17 SCID mice at approximately 10 weeks of age, were divided evenly according to body weight into 5 groups based on treatment (Isotype Control mAb, ActRIIB-Fc, H4H1657N2, MAB3381, or H4H1657N2 + MAB3381). Reagents were administered subcutaneously at a dose of 10 mg/kg twice for the first week (on day 0 and day 3) and once a week for the following three weeks (on day 7, day 14 and day 21). On day 28, mice were euthanized and weighed, and the tibialis anterior (TA) muscles, and the gastrocnemius (GA) muscles, were dissected and weighed. Tissues were normalized to starting body weight, and percent change in weight over the isotype-matched (hlgG4) control antibody was calculated. Results are summarized in Table 2 and are expressed as percent increase over negative control  $\pm$  standard error of the mean.

Table 2A

	Isotype Control	ActRIIB-Fc	H4H1657N2 (anti-GDF8)	MAB3381 (Anti-Activin A)	H4H1657N2 + MAB3381
<b>Dose</b>	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg (each)
<b>Body Weight</b>	0.00 $\pm$ 1.18	14.83 $\pm$ 4.36	7.88 $\pm$ 2.10	4.52 $\pm$ 1.02	16.08 $\pm$ 1.91
<b>TA Muscle</b>	0.00 $\pm$ 2.90	44.88 $\pm$ 5.35	22.42 $\pm$ 1.65	19.09 $\pm$ 2.04	55.13 $\pm$ 5.16
<b>GA Muscle</b>	0.00 $\pm$ 2.13	34.25 $\pm$ 6.97	24.17 $\pm$ 1.84	14.02 $\pm$ 0.91	41.72 $\pm$ 3.63

[0059] In order to confirm that muscle hypertrophy was the result of an increase in muscle fiber size, the tibialis anterior (TA) muscle was embedded in OCT and isopentane-frozen for histological examination and immunohistochemical labeling. Cross-sections of the TA muscle were stained with anti-laminin antibody to outline the muscle fiber, and the average cross-sectional-area (CSA) was determined by using an imaging analysis system. Results of two independent experiments (Exp#1 and Exp#2) are summarized in Table 2B. All data are expressed as means  $\pm$  the standard error of the mean.

Table 2B

	Isotype Control	ActRIIB-Fc	H4H1657N2 (anti-GDF8)	MAB3381 (Anti-Activin A)	H4H1657N2 + MAB3381
CSA ( $\mu\text{m}^2$ ) Exp#1	1800.1 $\pm$ 78.3	2488.7 $\pm$ 116.6	1987.2 $\pm$ 72.1	1962.6 $\pm$ 157.1	2435.4 $\pm$ 119.7
CSA ( $\mu\text{m}^2$ ) Exp#2	1702.7 $\pm$ 50.9	2571.9 $\pm$ 123.3	2006.3 $\pm$ 133.9	1690.9 $\pm$ 78.9	2452.6 $\pm$ 110.3

[0060] As shown in Table 2A, ActRIIB-Fc induced significant hypertrophy in all muscles examined, with increases of  $44.88 \pm 5.35\%$  in TA muscle mass, and  $34.25 \pm 6.97\%$  in GA muscle mass. Treatment with H4H1657N2 (anti-GDF8), or MAB3381 (anti-Activin A) alone also induced significant hypertrophy in TA muscle mass ( $22.42 \pm 1.65\%$  and  $19.09 \pm 2.04\%$ , respectively) and GA muscle mass ( $24.17 \pm 1.84$  and  $14.02 \pm 0.91\%$ , respectively) but not as pronounced as ActRIIB-Fc. However, the combination of H4H1657N2 and MAB3381 induced increases in TA ( $55.13 \pm 5.16\%$ ) and GA ( $41.72 \pm 3.63\%$ ), that were even greater than what was observed in ActRIIB-hFc-treated animals. Furthermore, it was confirmed that the muscle hypertrophy observed was the result of an increase in muscle fiber size (see Table 2B).

[0061] Importantly, the extent of increases in body weight, TA muscle, and GA muscle for the anti-GDF8 + anti-Activin A combination were substantially greater than the sums of the increases in these parameters observed in the anti-GDF8 plus anti-Activin A monotherapy subjects. Thus, combined inhibition of GDF8 and Activin A produces synergistic increases in body weight and skeletal muscle mass, and these increases are more pronounced than what is observed in ActRIIB-Fc-treated animals. Moreover, as demonstrated in the following Example, the increases in body weight and skeletal muscle mass in animals that are treated with GDF8- and Activin A-specific binding agents, can be achieved without causing the adverse side effects observed with molecules such as ActRIIB-Fc.

#### **Example 2: Specific Antagonism of GDF8 and Activin A Does Not Cause Adverse Side Effects Associated with Non-Specific Activin Ligand Binding Agents**

##### **Background**

[0062] ActRIIB-Fc binds multiple ActRIIB ligands and causes significant side effects. The present Example demonstrates that the adverse side effects associated with ActRIIB-Fc can be avoided by selectively antagonizing only certain ActRIIB ligands, namely GDF8 and/or Activin A. In particular, biomarker, protein expression studies, and *in vivo* red blood cell characteristics (*i.e.*, elevated endoglin levels and increased red cell distribution width), which appear to be linked to ActRIIB-Fc side effects in humans, were only seen in animals treated with ActRIIB-Fc, but not in animals treated with anti-GDF8 antibody, anti-Activin A antibody or a combination of anti-GDF8 and anti-Activin A antibodies. Thus, taken together, the results below show that specific antagonism of GDF8 and/or Activin A, without antagonizing other ActRIIB ligands such

as Activin B, GDF11, BMP9, BMP10, and/or TGF $\beta$ , does not cause the undesired phenotypes associated with ActRIIB-Fc.

### Results and Discussion

[0063] Hematology studies were conducted using mice treated with ActRIIB-Fc (SEQ ID NO:27), H4H1657N2 (anti-GDF8), MAB3381 (anti-Activin A), or a combination of H4H1657N2 + MAB3381 according to the dosing schedule described in Example 1 (*i.e.*, 10 mg/kg twice for the first week [on day 0 and day 3] and once a week for the following three weeks [on day 7, day 14 and day 21]). Specifically, hemoglobin levels and red blood cell distribution width (RDW) (an indicator of certain blood disorders such as anemia) were measured from blood samples taken from mice treated with the various agents. RDW results (normalized to Isotype Control values) are summarized in Table 3.

Table 3

	Isotype Control	ActRIIB-Fc	H4H1657N2 (anti-GDF8)	MAB3381 (Anti-Activin A)	H4H1657N2 + MAB3381
Dose	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg (each)
% RDW	0.0 $\pm$ 1.8	19.1 $\pm$ 2.1	-1.6 $\pm$ 0.7	4.4 $\pm$ 0.5	-1.9 $\pm$ 1.2

[0064] After 28 days of treatment, none of the groups had a significant increase in Hb levels. However, as shown in Table 3, mice treated with ActRIIB-Fc showed a significant increase in red blood cell distribution width (RDW), which reflects the extent of size variation of red blood cells in a sample. Surprisingly, mice treated with anti-GDF8 antibody, anti-Activin A antibody, or the combination of anti-GDF8 + anti-Activin A antibodies, did not exhibit an appreciable degree of increase in % RDW as compared to isotype control-treated mice. These experiments therefore demonstrate that antagonism of GDF8 or Activin A alone, or the combination of anti-GDF8 antibody + anti-Activin A antibodies, do not cause the hematological phenotypes that are observed with ActRIIB-Fc treatment.

[0065] To further investigate the differences in side effects between ActRIIB-Fc-treated subjects and anti-GDF8 + anti-Activin A combination-treated subjects, microarray and protein expression studies were conducted.

[0066] Microarray analysis was conducted on skeletal muscle samples from mice treated with isotype control, ActRIIB-Fc, and H4H1657N2 (anti-GDF8). From these experiments, a set of genes was identified that is uniquely affected by ActRIIB-Fc. Of particular interest was the up-regulation of Endoglin mRNA levels in skeletal muscle in samples from ActRIIB-Fc-treated subjects. Endoglin is a transmembrane protein expressed primarily in endothelial cells and interacts and promotes signaling through receptors of the TGF $\beta$  family (ALK1) in response to TGF $\beta$ , BMP9, or BMP10. Signaling mediated by Alk1 and Endoglin in endothelial cells is

required for maintaining normal vascular structures. Mutations in the Alk1 and Endoglin genes in humans causes Hereditary Haemorrhagic Telangiectasia (HHT). Patients suffering HHT display a vascular phenotype including dilated blood vessels, and bleeding in the nasal, oral, and gastrointestinal mucosa. Thus, elevated Endoglin levels caused by ActRIIB-Fc potentially reflect at least some of the adverse side effects observed in patients treated with this therapeutic agent.

[0067] Next, experiments were conducted to confirm that the changes observed in Endoglin mRNA levels were also reflected at the protein expression level using muscle samples from the previous experiment. Quantitative Western blot analysis of Endoglin protein levels was conducted on samples from mice treated with isotype control, ActRIIB-Fc, H4H1657N2 (anti-GDF8), MAB3381 (anti-Activin A), and the H4H1657N2 + MAB3381 combination. Expression of Endoglin was normalized by the endothelial cell marker CD31 to confirm that ActRIIB-hFc treatment does not increase the endothelial compartment. Results (normalized to Isotype Control values) are summarized in Table 4.

Table 4

	Isotype Control	ActRIIB-Fc	H4H1657N2 (anti-GDF8)	MAB3381 (Anti-Activin A)	H4H1657N2 + MAB3381
<b>Dose</b>	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg	10 mg/kg (each)
<b>Endoglin O.D.</b>	0.0 ± 1.4	88.4 ± 2.9	5.5 ± 2.1	4.7 ± 5.0	1.4 ± 7.6
<b>CD31 O.D.</b>	0.0 ± 4.2	2.2 ± 6.2	-6.8 ± 4.0	-2.1 ± 9.3	-19.3 ± 6.7
<b>O.D. Ratio</b>	0.0 ± 5.6	84.5 ± 8.3	13.1 ± 2.6	7.1 ± 5.1	25.5 ± 1.0

[0068] As shown in Table 4, levels of Endoglin protein were significantly elevated in the ActRIIB-hFc group, but not in the anti-GDF8 or anti-Activin A-treated groups. Interestingly, Endoglin levels were also not elevated in the anti-GDF8 + anti-Activin A combination-treated group.

### Summary and Conclusions

[0069] The results presented in the prior Example (Example 1) show that the combination of anti-GDF8 + anti-Activin A treatment can produce muscle hypertrophy effects that are at least equivalent to those observed with ActRIIB-Fc treatment. The present Example 2 shows that indicators of the adverse side effects of ActRIIB-Fc treatment, such as increased RDW and elevated Endoglin expression, are not observed with anti-GDF8, anti-Activin A, or anti-GDF8 + anti-Activin A combination treatment. Thus, the present inventors have surprisingly discovered that treatment with a GDF8-specific binding protein, or an Activin A-specific binding protein, or a combination of a GDF8-specific binding protein and an Activin A-specific binding protein provide

highly efficacious methods for increasing muscle mass and strength that avoid the adverse side effects caused by ActRIIB-Fc.

### Example 3: Effects of GDF8 and Activin A Antagonists on Wound Healing

[0070] Pharmaceutical agents which function to increase muscle mass and strength, such as GDF8 antagonists and Activin A antagonists, have utility in settings in which patients have undergone surgery (or will undergo surgery), e.g., for joint replacement or repair, etc. As such, agents that are administered to promote the rescue of muscle mass would ideally not interfere with other aspects of surgical recovery such as wound healing.

[0071] Accordingly, experiments were conducted to determine the effects of GDF8 blockade, Activin A blockade, and combinations thereof, on wound healing, as compared to the effects of ActRIIB-Fc treatment. These studies were carried out in SCID mice. In particular, the effects of H4H1657N2 (anti-GDF8) and MAB3381 (anti-Activin A) administration on wound healing, as single treatments or in combination with one another, were compared to the wound healing effects of the more broadly-acting decoy receptor ActRIIB-hFc (SEQ ID NO:27). Briefly, circular skin excisional wounds were made on the left abdominal flank of 30 male SCID mice approximately 7-8 weeks. The animals were divided into five treatment groups (n=6 per group) each receiving five subcutaneous injections of an isotype control antibody, ActRIIB-hFc, H4H1657N2, MAB3381, or H4H1657N2 + MAB3381. All reagents were administered at 10 mg/kg every 3-4 days. The first dose was given the day before wounding the animals, and the last one was given two days before terminating the study on day 14. Digital images of the wound were taken on day 0 (day of wounding), 6, 9, 12, and 14, and the excision wound size change was measured and compared to the isotype control group. Results are summarized in Table 5. All data are expressed as mean total wound size  $\pm$  the standard error of the mean.

**Table 5**

Treatment (10 mg/kg every 3-4 days)	Days After Wounding (total wound size mm <sup>2</sup> )				
	Day 0	Day 6	Day 9	Day 12	Day 14
Isotype control	59.95 $\pm$ 2.25	31.92 $\pm$ 3.43	18.75 $\pm$ 3.67	9.46 $\pm$ 2.25	7.66 $\pm$ 1.51
ActRIIB-hFc	61.30 $\pm$ 1.78	49.07 $\pm$ 2.74	30.50 $\pm$ 2.74	17.60 $\pm$ 1.53	14.90 $\pm$ 1.25
H4H1657N2 (anti-GDF8)	64.98 $\pm$ 2.56	30.69 $\pm$ 5.3	16.36 $\pm$ 3.27	7.71 $\pm$ 1.93	6.98 $\pm$ 1.32
MAB3381 (anti-Activin A)	62.07 $\pm$ 2.94	35.58 $\pm$ 4.96	23.38 $\pm$ 3.3	13.67 $\pm$ 2.19	11.01 $\pm$ 1.45
H4H1657N2 + MAB3381	61.08 $\pm$ 2.54	31.85 $\pm$ 2.83	17.68 $\pm$ 2.17	10.89 $\pm$ 1.74	9.09 $\pm$ 1.4



[0072] An analysis of wound size at the end of the experiment, as shown in Table 5, revealed that treatment with H4H1657N2, MAB3381, or H4H1657N2 + MAB3381 resulted in no significant difference in wound size at any time after the initial excision as compared to the isotype control group. By contrast, ActRIIB-hFc significantly delayed wound closure as indicated by the larger wound size at days 6, 9, 12, and 14 as compared to the wound size in mice treated with H4H1657N2, MAB3381, H4H1657N2 + MAB3381, or the isotype control.

[0073] This experiment demonstrates that therapeutic treatments involving GDF8 antagonism, Activin A antagonism, or GDF8 + Activin A dual antagonism, do not significantly impair wound healing, whereas the less specific antagonist ActRIIB-hFc does significantly impair wound healing. Accordingly, the present Example provides further support for the notion that specific antagonism of GDF8 and Activin A can produce enhanced muscle mass and function, similar to what is seen with ActRIIB-hFc treatment, but without the adverse side effects associated with ActRIIB-hFc treatment.

[0074] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and the accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. A composition comprising a GDF8-specific binding protein and an Activin A-specific binding protein, wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A-specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A.
2. A composition comprising a GDF8-specific binding protein and an Activin A specific binding protein for use in a method of treating a disease or disorder characterized by decreased muscle mass or strength, wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A-specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A.
3. A GDF8-specific binding protein when used in a method of treating a disease or disorder characterized by decreased muscle mass or strength, wherein the method comprises administering to a subject the GDF8-specific binding protein and an Activin A specific binding protein and wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A.
4. An Activin A-specific binding protein when used in a method of treating a disease or disorder characterized by decreased muscle mass or strength, wherein the method comprises administering to a subject the Activin A-specific binding protein and a GDF8-specific binding protein and wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A.
5. A method of increasing muscle mass or strength in a subject, the method comprising administering to the subject an Activin A-specific binding protein and a GDF8 specific binding protein, wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A-specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A.

6. A method of increasing muscle mass or strength in a subject, the method comprising administering to the subject an antigen-binding molecule comprising an anti-GDF8-specific antibody or antigen-binding fragment thereof that inhibits GDF8 and an anti-Activin A-specific antibody or antigen-binding fragment thereof that inhibits Activin A.

7. The composition according to claim 1 or 2, the GDF8-specific binding protein according to claim 3, the Activin A-specific binding protein according to claim 4, or the method according to claim 5 or 6, wherein the anti-GDF8 antibody or antigen-binding fragment thereof comprises three HCDRs comprising SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, and three LCDRs comprising SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16.

8. The composition according to any one of claims 1, 2 or 7, the GDF8-specific binding protein according to claim 3 or 7, the Activin A-specific binding protein according to claim 4 or 7, or the method according to any one of claims 5 to 7, wherein the anti-GDF8 antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising SEQ ID NO:9, and a light chain variable region (LCVR) comprising SEQ ID NO:13.

9. An antigen-binding molecule comprising an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8 and an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A.

10. An antigen-binding molecule comprising an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8 and an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A for use in a method of treating a disease or disorder characterized by decreased muscle mass or strength.

11. The antigen-binding molecule according to claim 9 or 10, wherein the anti-GDF8 antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) and a light chain variable region (LCVR).

12. The antigen-binding molecule according to any one of claims 9 to 11, wherein the HCVR comprises three heavy chain complementarity determining regions (HCDRs) comprising SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, and wherein

the LCVR comprises three light chain complementarity determining regions (LCDRs) comprising SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16.

13. The antigen-binding molecule or antigen binding molecule according to any one of claims 9 to 12, wherein the anti-GDF8 antibody or antigen-binding fragment thereof comprises a heavy chain variable region (HCVR) comprising SEQ ID NO:9, and a light chain variable region (LCVR) comprising SEQ ID NO:13.

14. The antigen-binding molecule or antigen binding molecule according to any one of claims 9 to 13, wherein the antigen-binding molecule is a bispecific antibody.

15. The use of a GDF8-specific binding protein and an Activin A-specific binding protein in the manufacture of a medicament for treating a disease or disorder characterized by decreased muscle mass or strength, wherein the GDF8-specific binding protein is an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8, and wherein the Activin A-specific binding protein is an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A.

16. The use according to claim 15, wherein the anti-GDF8 antibody or antigen-binding fragment thereof comprises three HCDRs comprising SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, and three LCDRs comprising SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16.

17. The use of an antigen-binding molecule in the manufacture of a medicament for treating a disease or disorder characterized by decreased muscle mass or strength, wherein the antigen binding molecule comprises an anti-GDF8 antibody or antigen-binding fragment thereof that inhibits GDF8 and an anti-Activin A antibody or antigen-binding fragment thereof that inhibits Activin A.

18. The use according to claim 17, wherein the anti-GDF8 antibody or antigen-binding fragment thereof comprises three HCDRs comprising SEQ ID NO:10, SEQ ID NO:11, and SEQ ID NO:12, and three LCDRs comprising SEQ ID NO:14, SEQ ID NO:15, and SEQ ID NO:16.

19. The use according to claim 17 or 18, wherein the antigen-binding molecule is a bispecific antibody.

20. The composition according to any one of claims 2, 7 or 8, the GDF8-specific binding protein according to any one of claims 3, 7 or 8, the Activin A-specific binding protein according to any one of claims 4, 7 or 8, the antigen binding molecule according to any one of claims 10 to 14, or the use of any one of claims 15 to 18, wherein the disease or disorder is selected from the group consisting of sarcopenia, cachexia, muscle injury, muscle wasting, and muscle atrophy.

21. The composition, the GDF8-specific binding protein, the Activin A-specific binding protein, the antigen binding molecule, or the use according to claim 20, wherein the cachexia is idiopathic or secondary to other conditions selected from cancer, chronic renal failure, or chronic obstructive pulmonary disease.

22. The composition, the GDF8-specific binding protein, the Activin A-specific binding protein, the antigen binding molecule, or the use according to claim 20, wherein the muscle atrophy or wasting is caused by or associated with disuse, immobilization, bed rest, injury, medical treatment, surgical intervention, or by necessity of mechanical ventilation.

23. The composition, the GDF8-specific binding protein, the Activin A-specific binding protein, the antigen binding molecule, or the use according to claim 22, wherein the surgical intervention is selected from hip fracture, hip replacement, or knee replacement.

## **REGENERON PHARMACEUTICALS**

WATERMARK INTELLECTUAL PROPERTY PTY LTD

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# SEQUENCE LISTING

<110> Regeneron Pharmaceuticals, Inc.

<120> Compositions and Methods for Increasing Muscle Mass and Muscle Strength by Specifically Antagonizing GDF8 and/or Activin A

<130> 6074A-WO

<140> To be assigned

<141> Filed herewith

<150> 61/559,175

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<151> 2012-06-19

<160> 28

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Lys	Val	Val	Asp	Ala	Arg	Lys	Ser	Thr	Trp	His	Val	Phe	Pro	Val	Ser		
	210					215					220						
Ser	Ser	Ile	Gln	Arg	Leu	Leu	Asp	Gln	Gly	Lys	Ser	Ser	Leu	Asp	Val		
225					230					235					240		
Arg	Ile	Ala	Cys	Glu	Gln	Cys	Gln	Glu	Ser	Gly	Ala	Ser	Leu	Val	Leu		
			245						250					255			
Leu	Gly	Lys	Lys	Lys	Lys	Lys	Glu	Glu	Glu	Gly	Glu	Gly	Lys	Lys	Lys		
		260					265						270				
Gly	Gly	Gly	Glu	Gly	Gly	Ala	Gly	Ala	Asp	Glu	Glu	Lys	Glu	Gln	Ser		
	275						280					285					
His	Arg	Pro	Phe	Leu	Met	Leu	Gln	Ala	Arg	Gln	Ser	Glu	Asp	His	Pro		
	290					295					300						
His	Arg	Arg	Arg	Arg	Arg	Gly	Leu	Glu	Cys	Asp	Gly	Lys	Val	Asn	Ile		
305					310					315				320			
Cys	Cys	Lys	Lys	Gln	Phe	Phe	Val	Ser	Phe	Lys	Asp	Ile	Gly	Trp	Asn		
			325						330				335				
Asp	Trp	Ile	Ile	Ala	Pro	Ser	Gly	Tyr	His	Ala	Asn	Tyr	Cys	Glu	Gly		
		340					345					350					
Glu	Cys	Pro	Ser	His	Ile	Ala	Gly	Thr	Ser	Gly	Ser	Ser	Leu	Ser	Phe		
	355						360					365					
His	Ser	Thr	Val	Ile	Asn	His	Tyr	Arg	Met	Arg	Gly	His	Ser	Pro	Phe		
	370					375					380						
Ala	Asn	Leu	Lys	Ser	Cys	Cys	Val	Pro	Thr	Lys	Leu	Arg	Pro	Met	Ser		
385					390					395				400			
Met	Leu	Tyr	Tyr	Asp	Asp	Gly	Gln	Asn	Ile	Ile	Lys	Lys	Asp	Ile	Gln		
			405						410					415			
Asn	Met	Ile	Val	Glu	Glu	Cys	Gly	Cys	Ser								
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 <211> 340  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Synthetic

<400> 27																	
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		20					25					30					
Lys	Arg	Leu	His	Cys	Tyr	Ala	Ser	Trp	Ala	Asn	Ser	Ser	Gly	Thr	Ile		
	35					40					45						
Glu	Leu	Val	Lys	Lys	Gly	Cys	Trp	Leu	Asp	Asp	Phe	Asn	Cys	Tyr	Asp		
50					55					60							
Arg	Gln	Glu	Cys	Val	Ala	Thr	Glu	Glu	Asn	Pro	Gln	Val	Tyr	Phe	Cys		
65				70					75				80				
Cys	Cys	Glu	Gly	Asn	Phe	Cys	Asn	Glu	Arg	Phe	Thr	His	Leu	Pro	Glu		
		85					90					95					
Ala	Gly	Gly	Pro	Glu	Val	Thr	Tyr	Glu	Pro	Pro	Pro	Thr	Ala	Pro	Ser		

				100					105					110			
Gly	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu		
				115				120					125				
Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu		
				130			135					140					
Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser		
145					150					155					160		
His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu		
				165				170						175			
Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr		
				180				185					190				
Tyr	Arg	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn			
				195			200					205					
Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro		
				210		215					220						
Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln		
225					230					235					240		
Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Asp	Glu	Leu	Thr	Lys	Asn	Gln	Val		
				245				250						255			
Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val		
			260					265					270				
Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro		
			275				280					285					
Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr		
			290			295					300						
Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val		
305					310					315					320		
Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu		
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Ser	Pro	Gly	Lys														
				340													

<210> 28  
 <211> 407  
 <212> PRT  
 <213> Homo Sapiens

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Leu	Ala	Ala	Gly	Trp	Leu	Gly	Pro	Glu	Ala	Trp	Gly	Ser	Pro	Thr	Pro		
			20					25				30					
Pro	Pro	Thr	Pro	Ala	Ala	Gln	Pro	Pro	Pro	Pro	Pro	Pro	Gly	Ser	Pro		
			35				40					45					
Gly	Gly	Ser	Gln	Asp	Thr	Cys	Thr	Ser	Cys	Gly	Gly	Phe	Arg	Arg	Pro		
			50			55				60							
Glu	Glu	Leu	Gly	Arg	Val	Asp	Gly	Asp	Phe	Leu	Glu	Ala	Val	Lys	Arg		
65					70				75					80			
His	Ile	Leu	Ser	Arg	Leu	Gln	Met	Arg	Gly	Arg	Pro	Asn	Ile	Thr	His		
				85				90					95				
Ala	Val	Pro	Lys	Ala	Ala	Met	Val	Thr	Ala	Leu	Arg	Lys	Leu	His	Ala		
			100					105					110				
Gly	Lys	Val	Arg	Glu	Asp	Gly	Arg	Val	Glu	Ile	Pro	His	Leu	Asp	Gly		
			115				120					125					
His	Ala	Ser	Pro	Gly	Ala	Asp	Gly	Gln	Glu	Arg	Val	Ser	Glu	Ile	Ile		
	130					135					140						
Ser	Phe	Ala	Glu	Thr	Asp	Gly	Leu	Ala	Ser	Ser	Arg	Val	Arg	Leu	Tyr		

145					150				155				160
Phe	Phe	Ile	Ser	Asn	Glu	Gly	Asn	Gln	Asn	Leu	Phe	Val	Val
				165					170				175
Ser	Leu	Trp	Leu	Tyr	Leu	Lys	Leu	Leu	Pro	Tyr	Val	Leu	Glu
			180					185				190	
Ser	Arg	Arg	Lys	Val	Arg	Val	Lys	Val	Tyr	Phe	Gln	Glu	Gln
			195				200				205		
Gly	Asp	Arg	Trp	Asn	Met	Val	Glu	Lys	Arg	Val	Asp	Leu	Lys
	210					215				220			Arg
Gly	Trp	His	Thr	Phe	Pro	Leu	Thr	Glu	Ala	Ile	Gln	Ala	Leu
	225				230				235				Phe
Arg	Gly	Glu	Arg	Arg	Leu	Asn	Leu	Asp	Val	Gln	Cys	Asp	Ser
			245					250				255	Cys
Glu	Leu	Ala	Val	Val	Pro	Val	Phe	Val	Asp	Pro	Gly	Glu	Glu
		260					265					270	Ser
Arg	Pro	Phe	Val	Val	Val	Gln	Ala	Arg	Leu	Gly	Asp	Ser	Arg
	275						280				285		His
Ile	Arg	Lys	Arg	Gly	Leu	Gln	Cys	Asp	Gly	Arg	Thr	Asn	Leu
	290					295				300			Cys
Arg	Gln	Gln	Phe	Phe	Ile	Asp	Phe	Arg	Leu	Ile	Gly	Trp	Asn
	305				310				315				Asp
Ile	Ile	Ala	Pro	Thr	Ser	Tyr	Tyr	Gly	Asn	Tyr	Cys	Glu	Gly
			325					330				335	Ser
Pro	Ala	Tyr	Leu	Ala	Gly	Val	Pro	Gly	Ser	Ala	Ser	Ser	Phe
		340					345					350	His
Ala	Val	Val	Asn	Gln	Tyr	Arg	Met	Arg	Gly	Leu	Asn	Pro	Gly
	355						360				365		Thr
Asn	Ser	Cys	Cys	Ile	Pro	Thr	Lys	Leu	Ser	Thr	Met	Ser	Met
	370					375				380			Leu
Phe	Asp	Asp	Glu	Tyr	Asn	Ile	Val	Lys	Arg	Asp	Val	Pro	Asn
	385				390				395				Met
Val	Glu	Glu	Cys	Gly	Cys	Ala							Ile
				405									