patients on hemodialysis and Acceleron Pharma's ACE-031, an ActRIIB-Fc fusion protein has been clinically evaluated (Ph1, Ph2) for therapeutic efficacy in Duchenne muscle dystrophy (DMD) (ClinicalTrials.gov).

[007] Unfortunately, while appearing effective, the clinical potential of ACE-011 for treating osteoporosis was hampered by safety concerns (high RBC growth) and ACE-031, while demonstrating a significant muscle gain which was more pronounced than that caused by a myostatin-selective inhibitor, such as myostatin antibody, peptibody or propeptide, has been hampered by the adverse event in nose and gum bleeding seen in DMD patients, thus there was a clinical hold put on ACE-031 (Smith R.C. and Lin B.K., Curr Opin Support Palliat Care. 7(4): 352–360, 2013). Consequently, ACE-031 was discontinued in DMD Phase2 trial due to the side effect of nose and gum bleeding despite its promising sign of efficacy to improve muscle mass and function in DMD patients. It thus appears that the wild-type ActRIIB-Fc is capable of blocking BMP9 mediated Smad1/3/8 signaling that is critical in maintaining blood vessel homeostasis, and this functional blockade of BMP9 by the wild-type ActRIIB is believed to be the underlying cause of the bleeding side effect, as compelling new genetic evidence has linked loss-of-function mutations of BMP9 to vascular anomaly and bleeding syndrome in humans (Wooderchak-Donahue et. al., Am J Hum Genet., 93(3): 530–537, Sep 2013).

[008] So while there have been important advancements, there still exists a critical need to provide novel therapeutics, which are both highly effective and safe, for the treatment of muscle wasting and/or bone disease associated with the development of a number of chronic, neurological, genetic, inflammatory, fibrotic or infectious pathologies.

Incorporation by Reference

[009] All references disclosed herein are hereby incorporated by reference in their entirety for all purposes.

Summary

[009a] In one aspect, the present disclosure refers to an isolated hybrid activin IIB receptor (ActRIIB) ligand trap protein comprising a hybrid soluble activin IIB receptor-extracellular domain (ActRIIB-ECD) polypeptide, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 16, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID

NO: 9, SEQ ID NO: 10, SEQ ID NO: 13, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 37.

[009b] In another aspect, the present disclosure refers to an isolated hybrid activin IIB receptor (ActRIIB) ligand trap protein comprising a hybrid soluble activin IIB receptor-extracellular domain (ActRIIB-ECD) polypeptide, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises an amino acid sequence that is at least 98% identical to a sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 27, and SEQ ID NO: 29, wherein at least amino acid residues corresponding to positions F58 and E70 of SEQ ID NO: 1 are substituted with another amino acid relative to SEQ ID NO: 1, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for bone morphogenic protein 9 (BMP9) relative to a wild-type ActRIIB-ECD polypeptide.

[009c] In another aspect, the present disclosure refers to an isolated hybrid activin IIB receptor (ActRIIB) ligand trap protein comprising a hybrid soluble activin IIB receptor-extracellular domain (ActRIIB-ECD) polypeptide, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises an amino acid sequence that is at least 99% identical to a sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 27, and SEQ ID NO: 29, and wherein said hybrid soluble ActRIIB-ECD polypeptide comprises at least amino acid substitutions F58I and E70K relative to SEQ ID NO: 1, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for bone morphogenic protein 9 (BMP9) relative to a wild-type ActRIIB-ECD polypeptide.

[009d] In another aspect, the present disclosure refers to a pharmaceutical composition comprising a therapeutically effective amount of the hybrid ActRIIB ligand trap protein of any one of the disclosure in admixture with a pharmaceutically acceptable carrier.

[009e] In another aspect, the present disclosure refers to a use of a hybrid ActRIIB ligand trap protein of the disclosure, in the manufacture of a medicament for use in combination therapy for treating or preventing muscle wasting in a subject in need thereof, and wherein said medicament is to be administered to a subject in need thereof in combination with a second agent, wherein said second agent is selected from the group consisting of: growth hormone, ghrelin, IGF1, insulin, prednisone, corticosteroid therapy, androgen deprivation therapy, anabolic steroids, antagonists to angiotensin or angiotensin receptor, antagonists to inflammatory cytokines such as TNF-alpha, IL-6, IL-1 and their receptors, and other antagonists to myostatin, activin A or another member of the TGF-beta family and to their receptors,

bisphosphonates, RANKL inhibitors, agonists of peroxisome proliferator-activated receptors, β2 agonists, activator of PGC-1alpha, proteasome inhibitors, cancer therapeutics, chemotherapeutic agents, cell therapy, stem cell therapy, gene therapy, gene targeting therapy, and antisense oligonucleotide therapy.

[009f] In another aspect, the present disclosure refers to a use of a hybrid ActRIIB ligand trap protein of the disclosure in the manufacture of a medicament for treating or preventing muscle wasting in a subject in need thereof, wherein the medicament is to be administered to a subject in need thereof, and wherein the muscle wasting is associated with a condition selected from the group consisting of muscular dystrophy, myositis, myopathy, motorneuron disease, muscle atrophy, spinal muscular atrophy, neuromuscular junction disease, peripheral nerve disease, spinal cord injury, stroke, neurodegenerative disease, anorexia, cachexia, metabolic disorder, cancer, organ failure, trauma, disuse, infection, chronic obstructive pulmonary disease (COPD), cardiovascular disease, chronic kidney disease, sarcopenia, sarcopenic obesity, osteoarthritis, an aging condition, fibrosis and autoimmune disorder.

[009g] In another aspect, the present disclosure refers to a use of a hybrid ActRIIB ligand trap protein of the disclosure, in the manufacture of a medicament for treating or preventing bone disease in a subject in need thereof, wherein the medicament is to be administered to a subject in need thereof, and wherein the bone disease is selected from the group consisting of: osteoporosis, renal osteodystrophy, osteomalacia, osteogenesis imperfecta, fibrodysplasia ossificans progressiva, corticosteroid-induced bone loss, androgen-deprivation therapy-induced bone loss, bone fracture, cancer-induced bone loss, bone metastasis, Paget’s disease of the bone, Rickets, Perthes' disease and fibrous dysplasia.

[009h] In another aspect, the present disclosure refers to a polynucleotide encoding a polypeptide comprising:

a. a hybrid soluble ActRIIB-ECD polypeptide sequence selected from the group consisting of SEQ ID NO: 16 and SEQ ID NO: 29, and

b. a human Fc domain sequence.

[009i] In another aspect, the present disclosure refers to vector comprising the polynucleotide of the disclosure.

[009j] In another aspect, the present disclosure refers to a host cell comprising the polynucleotide of the disclosure.

[009k] In another aspect, the present disclosure refers to method of producing a hybrid ActRIIB protein comprising culturing the host cell of the disclosure under conditions promoting the expression of the protein, and recovering the protein.

[009l] In another aspect, the present disclosure refers to a hybrid ActRIIB protein produced by the method of the disclosure.

Disclosure of the Invention

[010] In one aspect, the present disclosure provides a method of treating or preventing a muscle wasting in a subject, comprising administering to the subject a therapeutically effective amount (either as monotherapy or in a combination therapy regimen) of a hybrid ActRIIB ligand trap of the present disclosure in admixture with a pharmaceutically acceptable carrier, wherein such administration attenuates the loss of muscle mass and/or loss of muscle function. In

What is claimed is:

1. An isolated hybrid activin IIB receptor (ActRIIB) ligand trap protein comprising a hybrid soluble activin IIB receptor-extracellular domain (ActRIIB-ECD) polypeptide, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 29, SEQ ID NO: 16, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 13, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NO: 31 and SEQ ID NO: 37.

2. An isolated hybrid activin IIB receptor (ActRIIB) ligand trap protein comprising a hybrid soluble activin IIB receptor-extracellular domain (ActRIIB-ECD) polypeptide, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises an amino acid sequence that is at least 98% identical to a sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 27, and SEQ ID NO: 29, wherein at least amino acid residues corresponding to positions F58 and E70 of SEQ ID NO: 1 are substituted with another amino acid relative to SEQ ID NO: 1, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for bone morphogenic protein 9 (BMP9) relative to a wild-type ActRIIB-ECD polypeptide.

3. The isolated hybrid ActRIIB ligand trap protein of claim 2, wherein the hybrid soluble ActRIIB-ECD polypeptide comprises an amino acid sequence that is at least 99% identical to a sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 27, and SEQ ID NO: 29.

4. An isolated hybrid activin IIB receptor (ActRIIB) ligand trap protein comprising a hybrid soluble activin IIB receptor-extracellular domain (ActRIIB-ECD) polypeptide, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises an amino acid sequence that is at least 99% identical to a sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 27, and SEQ ID NO: 29, and wherein said hybrid soluble ActRIIB-ECD polypeptide comprises at least amino acid substitutions F58I and E70K relative to SEQ ID NO: 1, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for bone morphogenic protein 9 (BMP9) relative to a wild-type ActRIIB-ECD polypeptide.

5. The isolated hybrid ActRIIB ligand trap protein of any one of claims 1 to 4, wherein said hybrid ActRIIB ligand trap protein comprises a human Fc domain linked to the hybrid soluble

ActRIIB-ECD polypeptide, wherein the human Fc domain is selected from the group consisting of the Fc domain of a human IgG1 immunoglobulin, the Fc domain of a human IgG2 immunoglobulin, and the Fc domain of a human IgG4 immunoglobulin.

6. The isolated hybrid ActRIIB ligand trap protein of claim 5, wherein the human Fc domain is linked to the hybrid soluble ActRIIB-ECD polypeptide by a linker comprising an amino acid sequence selected from the group consisting of:

a) the peptide linker sequence set forth in SEQ ID NO: 44;

b) the hinge linker sequence set forth in SEQ ID NO: 118; and

c) both the peptide linker sequence set forth in SEQ ID NO: 44 and the hinge linker sequence set forth in SEQ ID NO: 118.

7. The isolated hybrid ActRIIB ligand trap protein of claim 5, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 16 and SEQ ID NO: 29.

8. The isolated hybrid ActRIIB ligand trap protein of claim 7, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 16, wherein said hybrid soluble ActRIIB-ECD polypeptide is fused to a peptide linker comprising the amino acid sequence set forth in SEQ ID NO: 44, wherein said peptide linker is fused to a hinge linker comprising the amino acid sequence set forth in SEQ ID NO: 118, and wherein said hinge linker is fused to a human Fc domain comprising the amino acid sequence of SEQ ID NO: 43.

9. The isolated hybrid ActRIIB ligand trap protein of claim 7, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence of SEQ ID NO: 29, wherein said hybrid soluble ActRIIB-ECD polypeptide is fused to a peptide linker comprising the amino acid sequence set forth in SEQ ID NO: 44, wherein said peptide linker is fused to a hinge linker comprising the amino acid sequence set forth in SEQ ID NO: 118, and wherein said hinge linker is fused to a human Fc domain comprising the amino acid sequence of SEQ ID NO: 43.

10. The hybrid ActRIIB ligand trap protein of claim 5, wherein the human Fc domain comprises an amino acid sequence selected from the group consisting of: SEQ ID NO: 39, SEQ ID NO: 41, and SEQ ID NO: 43.

11. A pharmaceutical composition comprising a therapeutically effective amount of the hybrid ActRIIB ligand trap protein of any one of claims 1-10 in admixture with a pharmaceutically acceptable carrier.

12. Use of a hybrid ActRIIB ligand trap protein of any one of claims 1-10, in the manufacture of a medicament for use in combination therapy for treating or preventing muscle wasting in a subject in need thereof, and wherein said medicament is to be administered to a subject in need thereof in combination with a second agent, wherein said second agent is selected from the group consisting of: growth hormone, ghrelin, IGF1, insulin, prednisone, corticosteroid therapy, androgen deprivation therapy, anabolic steroids, antagonists to angiotensin or angiotensin receptor, antagonists to inflammatory cytokines such as TNF-alpha, IL-6, IL-1 and their receptors, and other antagonists to myostatin, activin A or another member of the TGF-beta family and to their receptors, bisphosphonates, RANKL inhibitors, agonists of peroxisome proliferator-activated receptors, β2 agonists, activator of PGC-1alpha, proteasome inhibitors, cancer therapeutics, chemotherapeutic agents, cell therapy, stem cell therapy, gene therapy, gene targeting therapy, and antisense oligonucleotide therapy.

13. The use of claim 12, wherein said second agent is an antisense oligonucleotide therapy.

14. The use of claim 12, wherein said hybrid ActRIIB ligand trap protein and said second agent are provided in separate dosage forms.

15. Use of a hybrid ActRIIB ligand trap protein of any one of claims 1-10 in the manufacture of a medicament for treating or preventing muscle wasting in a subject in need thereof, wherein the medicament is to be administered to a subject in need thereof, and wherein the muscle wasting is associated with a condition selected from the group consisting of muscular dystrophy, myositis, myopathy, motorneuron disease, muscle atrophy, spinal muscular atrophy, neuromuscular junction disease, peripheral nerve disease, spinal cord injury, stroke, neurodegenerative disease, anorexia, cachexia, metabolic disorder, cancer, organ failure, trauma, disuse, infection, chronic obstructive pulmonary disease (COPD), cardiovascular disease, chronic kidney disease, sarcopenia, sarcopenic obesity, osteroarthritis, an aging condition, fibrosis and autoimmune disorder.

16. The use of claim 15, wherein said spinal muscular atrophy is selected from the group consisting of infantile progressive spinal muscular atrophy, intermediate spinal muscular atrophy, juvenile spinal muscular atrophy and adult spinal muscular atrophy.

17. The use of claim 15, wherein said peripheral nerve disease is selected from the group consisting of Charcot-Marie Tooth disease, Dejerine-Sottas disease and Friedreich's ataxia.

18. The use of claim 15, wherein said neurodegenerative disease is selected from the group consisting of Parkinson's disease, Huntington's disease, Alzheimer's disease and Creutzfeldt-Jakob disease.

19. The use according to claim 15, wherein the aging condition is selected from the group consisting of: frailty of the elderly, age-related sarcopenia, and osteoarthritis.

20. The use of claim 15, wherein said motorneuron disease is amyotrophic lateral sclerosis (ALS or Lou Gehrig's Disease).

21. Use of a hybrid ActRIIB ligand trap protein of any one of claims 1-10, in the manufacture of a medicament for treating or preventing bone disease in a subject in need thereof, wherein the medicament is to be administered to a subject in need thereof, and wherein the bone disease is selected from the group consisting of: osteoporosis, renal osteodystrophy, osteomalacia, osteogenesis imperfecta, fibrodysplasia ossificans progressiva, corticosteroid-induced bone loss, androgen-deprivation therapy-induced bone loss, bone fracture, cancer-induced bone loss, bone metastasis, Paget's disease of the bone, Rickets, Perthes' disease and fibrous dysplasia.

22. A polynucleotide encoding a polypeptide comprising:

a. a hybrid soluble ActRIIB-ECD polypeptide sequence selected from the group consisting of SEQ ID NO: 16 and SEQ ID NO: 29, and

b. a human Fc domain sequence.

23. The polynucleotide of claim 22, wherein the polypeptide encoded by the polynucleotide further comprises a linker comprising an amino acid sequence selected from the group consisting of:

a. The peptide linker sequence set forth in SEQ ID NO: 44;

b. The hinge linker sequence set forth in SEQ ID NO: 118; or

c. both the peptide linker sequence set forth in SEQ ID NO: 44 and the hinge linker sequence set forth in SEQ ID NO: 118.

24. The polynucleotide of claim 22 or claim 23, wherein the polypeptide encoded by the polynucleotide further comprises a signal peptide leader sequence.

25. A vector comprising the polynucleotide of any one of claims 22-24.

26. A host cell comprising the polynucleotide of any one of claims 22-24.

27. The host cell of claim 26, wherein the host cell is a mammalian cell.

28. A method of producing a hybrid ActRIIB protein comprising culturing the host cell of claim 26 or claim 27 under conditions promoting the expression of the protein, and recovering the protein.

29. The use according to claim 15, wherein the muscular dystrophy is selected from the group consisting of: Duchenne's Muscular Dystrophy (DMD), Becker Muscular Dystrophy, Limb-Girdle Muscular Dystrophy, Myotonic Muscular Dystrophy and Facioscapulohumeral Muscular Dystrophy (FSHD).

30. The use according to claim 15, wherein the medicament to be administered is suitable for administration by a method selected from the group consisting of: subcutaneous, intramuscular, intravenous, or intrathecal administration.

31. The use according to claim 15, wherein the medicament comprising a hybrid ActRIIB ligand trap is to be administered to the subject in a combination therapy regimen, wherein the combination therapy regimen comprises administering to the subject the medicament comprising a hybrid ActRIIB ligand trap and a separate composition comprising a second therapeutic agent, wherein the second therapeutic agent is selected from the group consisting of gene therapy, gene targeting therapy, and antisense oligonucleotide therapy.

32. A hybrid ActRIIB protein produced by the method of claim 28.