ActRIIB-Fc fusion protein (STM 434) is set to be evaluated (Ph1 recruitment in progress) in patients with ovarian cancer or other advanced solid tumors (ClinicalTrials.gov).

[007] Despite these advancements, there clearly still exists a critical need to provide novel therapeutics, which are both highly effective and safe, for the treatment of muscle wasting diseases.

Summary

[007a] In one aspect, there is provided an isolated protein comprising a hybrid soluble activin IIB receptor-extracellular domain (ActRIIB-ECD) polypeptide, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 3-37, 51-104, and 110-117, wherein said 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. In another aspect, there is provided a pharmaceutical composition comprising a therapeutically effective amount of the isolated protein as described herein in admixture with a pharmaceutically acceptable carrier. In another aspect, there is provided the use of the isolated protein as described herein in the manufacture of a medicament for treating myostatin-related or activin A-related disorders in a subject in need thereof, wherein said myostatin-related or activin A-related disorder is selected from the group consisting of: a bone disorder, a muscle wasting disease, a cardiovascular disease, a metabolic disorder, a renal disease, an inflammatory/autoimmune disease, a fibrosis disease, anemia, pain, and aging. In another aspect, there is provided a pharmaceutical composition comprising a therapeutically effective amount of the isolated protein as described herein in admixture with a pharmaceutically acceptable carrier. In another aspect, there is provided a pharmaceutical composition comprising a therapeutically effective amount of the isolated protein as described herein in admixture with a pharmaceutically acceptable carrier.

Disclosure of the Disclosure

[008] In one aspect, the present disclosure provides isolated novel hybrid ActRIIB ligand trap proteins comprising novel hybrid soluble ActRIIB-ECD polypeptides which retain myostatin- and activin A-neutralizing activities, but demonstrate dramatically reduced BMP9-neutralization. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a

hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least one of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least two of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58, Q64, E65, A68, T69, E70, E71, N72, Q74, F84, R88, T90, H91, L92, E94, A95, G96, G97, P98, E99, V100, Y102, E103, P105, P106, T107, A108, or T110 is substituted with another amino acid, and wherein the hybrid ActRIIB-ECD polypeptide is capable of binding myostatin and activin A, but demonstrates a decreased binding affinity for BMP9 relative to a wild-type ActRIIB-ECD polypeptide. In various embodiments, the hybrid ActRIIB ligand trap proteins comprise a hybrid soluble ActRIIB-ECD polypeptide having the amino acid sequence of SEQ ID NO: 1 wherein at least three of amino acid residues R3, I6, Y7, Y8, L14, E15, S20, L22, R24, E26, E28, Q29, L33, L48, Y36, S38, R40, S42, T45, K51, F58,

Claims

1. An isolated protein comprising a hybrid soluble activin IIB receptor-extracellular domain (ActRIIB-ECD) polypeptide, wherein said hybrid soluble ActRIIB-ECD polypeptide comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 3-37, 51-104, and 110-117, wherein said 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.

2. The isolated protein of claim 1, wherein the hybrid soluble ActRIIB-ECD polypeptide is fused to at least one heterologous protein.

3. The isolated protein of claim 2, wherein the heterologous protein comprises a constant domain of an immunoglobulin.

4. The isolated protein of claim 3, wherein the heterologous protein comprises an Fc domain of an immunoglobulin.

5. The isolated protein of claim 4, wherein the Fc domain is selected from the group consisting of the Fc domain of a human immunoglobulin gamma-1 (IgG1), the Fc domain of a human immunoglobulin gamma-2 (IgG2), and the Fc domain of a human immunoglobulin gamma-4 (IgG4) IgG4.

6. The isolated protein of claim 2, wherein the hybrid soluble ActRIIB-ECD polypeptide is fused to the heterologous protein by a peptide linker sequence.

7. The isolated protein of claim 6, wherein the heterologous protein comprises a human Fc domain comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 39, SEQ ID NO: 41, and SEQ ID NO: 43.

8. The isolated protein of claim 7, wherein a linker comprising the amino acid sequence set forth in SEQ ID NO: 44 is used with a hinge linker comprising the amino acid sequence set forth in SEQ ID NO: 118 to link the human Fc domain to the hybrid soluble ActRIIB-ECD polypeptide.

9. The isolated protein of claim 6, wherein the isolated protein comprises a hybrid soluble ActRIIB-ECD polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 16 and SEQ ID NO: 29 attached to a human Fc domain comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 39, SEQ ID NO: 41, and SEQ ID NO: 43.

10. The isolated protein of claim 9, wherein the isolated protein comprises a hybrid soluble ActRIIB-ECD polypeptide comprising the amino acid sequence of SEQ ID NO: 16 attached to a human Fc domain comprising the amino acid sequence of SEQ ID NO: 43.

11. The isolated protein of claim 9, wherein the isolated protein comprises a hybrid soluble ActRIIB-ECD polypeptide comprising the amino acid sequence of SEQ ID NO: 29 attached to a human Fc domain comprising the amino acid sequence of SEQ ID NO: 43.

12. A pharmaceutical composition comprising a therapeutically effective amount of the isolated protein of claim 6 in admixture with a pharmaceutically acceptable carrier.

13. Use of the isolated protein of claim 1 in the manufacture of a medicament for treating myostatin-related or activin A-related disorders in a subject in need thereof, wherein said myostatin-related or activin A-related disorder is selected from the group consisting of: a bone disorder, a muscle wasting disease, a cardiovascular disease, a metabolic disorder, a renal disease, an inflammatory/autoimmune disease, a fibrosis disease, anemia, pain, and aging.

14. The use of claim 13, wherein the muscle wasting disease is selected from the group consisting of muscular dystrophy, amyotrophic lateral sclerosis, myositis, Intensive Care Unit (ICU) myopathy, drug-induced myopathy, androgen deprivation, congestive obstructive pulmonary disease, emphysema, cystic fibrosis, chronic heart failure, cardiac atrophy, cancer cachexia, renal failure, uremia, protein energy wasting, anorexia, malnutrition, sarcopenia, Acquired Immunodeficiency Syndrome (AIDS), sepsis, burn injury, diabetes, Huntington's disease, Parkinson's disease, Alzheimer's disease, carpal tunnel syndrome, and muscle wasting due to prolonged bed rest, spinal cord injury, stroke, bone fracture, aging and exposure to microgravity.

15. The use of claim 13, wherein the metabolic disorder is selected from the group consisting of insulin resistance, diabetes, obesity, sarcopenic obesity, dyslipidemia and fatty liver disease.

16. The use of claim 13, wherein the renal disease is selected from the group consisting of chronic kidney disease, end-stage renal disease, uremia, protein energy wasting, and kidney transplantation.

17. The use of claim 13, wherein the fibrosis disease is selected from the group consisting of pulmonary fibrosis, liver cirrhosis, cardiac fibrosis, renal fibrosis, myelofibrosis, idiopathic retroperitoneal fibrosis, nephrogenic fibrosing dermopathy, Crohn's Disease, keloid, scleroderma, systemic sclerosis, and arthrofibrosis.

18. The use of claim 13, wherein the bone disorder is selected from the group consisting of osteomalacia, osteoporosis, osteogenesis imperfecta, fibrodysplasia ossificans progressive, corticosteroid-induced bone loss, bone fracture, and bone metastasis.

19. The isolated protein of claim 8, wherein said hybrid soluble ActRIIB-ECD polypeptide consists of the amino acid sequence of SEQ ID NO: 16, wherein said hybrid soluble ActRIIB-ECD polypeptide is fused to a peptide linker consisting of the amino acid sequence set forth in SEQ ID NO: 44, wherein said peptide linker is fused to a hinge linker consisting of the amino

acid sequence set forth in SEQ ID NO: 118, and wherein said hinge linker is fused to an Fc domain consisting of the amino acid sequence of SEQ ID NO: 43.

20. The isolated protein of claim 8, wherein said hybrid soluble ActRIIB-ECD polypeptide consists of the amino acid sequence of SEQ ID NO: 29, wherein said hybrid soluble ActRIIB-ECD polypeptide is fused to a peptide linker consisting of the amino acid sequence set forth in SEQ ID NO: 44, wherein said peptide linker is fused to a hinge linker consisting of the amino acid sequence set forth in SEQ ID NO: 118, and wherein said hinge linker is fused to an Fc domain consisting of the amino acid sequence of SEQ ID NO: 43.

21. A pharmaceutical composition comprising a therapeutically effective amount of the isolated protein of claim 19 in admixture with a pharmaceutically acceptable carrier.

22. A pharmaceutical composition comprising a therapeutically effective amount of the isolated protein of claim 20 in admixture with a pharmaceutically acceptable carrier.