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(54) Title: METHODS FOR TREATING MYELODYSPLASTIC SYNDROMES AND SIDEROBLASTIC ANEMIAS

(57) Abstract: In certain aspects, the present disclosure provides compositions and methods for increasing red blood cell and/or hemoglobin levels in vertebrates, including rodents and primates, and particularly in humans. In some embodiments, the compositions of the disclosure may be used to treat or prevent sideroblastic anemias and myelodysplasia syndromes or one or more complications associated sideroblastic anemias and myelodysplasia syndromes.

METHODS FOR TREATING MYELODYSPLASTIC SYNDROMES AND SIDEROBLASTIC ANEMIAS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to United States provisional application serial number 62/086,977, filed December 3, 2014; United States provisional application serial number 62/088,087, filed December 5, 2014; and United States provisional application serial number 62/155,395, filed April 30, 2015. The disclosures of each of the foregoing applications are hereby incorporated by reference in their entirety.

BACKGROUND OF THE INVENTION

The present disclosure relates to treatments for dysregulated production of blood cellular components, including red blood cells, neutrophils, and platelets. Hematopoiesis is the formation of cellular components of the blood from self-renewing hematopoietic stem cells located mainly in the bone marrow, spleen, or lymph nodes during postnatal life. Blood cells can be classified as belonging to the lymphocytic lineage, myelocytic lineage, or erythroid lineage. By a process known as lymphopoiesis, common lymphoid progenitor cells give rise to T-cells, B-cells, natural killer cells, and dendritic cells. By a process termed myelopoiesis, common myeloid progenitor cells give rise to macrophages, granulocytes (basophils, neutrophils, eosinophils, and mast cells), and thrombocytes (platelets). Finally, by a process known as erythropoiesis, erythroid progenitor cells give rise to red blood cells (RBC, erythrocytes).

Postnatal erythropoiesis occurs primarily in the bone marrow and in the red pulp of the spleen. The coordinated action of various signaling pathways controls the balance of cell proliferation, differentiation, survival, and death. Under normal conditions, red blood cells are produced at a rate that maintains a constant red cell mass in the body, and production may increase or decrease in response to various stimuli, including increased or decreased oxygen tension or tissue demand. The process of erythropoiesis begins with the formation of lineage-committed precursor cells and proceeds through a series of distinct precursor cell types. The final stages of erythropoiesis occur as reticulocytes are released into the bloodstream and lose their mitochondria and ribosomes while assuming the morphology of mature red blood cell. An elevated level of reticulocytes, or an elevated reticulocyte:erythrocyte ratio, in the blood is indicative of increased red blood cell production rates. The mature red blood cell (RBC) is responsible for oxygen transport in the circulatory systems of vertebrates. Red blood cells

contain high concentrations of hemoglobin, a protein that binds to oxygen in the lungs at relatively high partial pressure of oxygen (pO_2) and delivers oxygen to areas of the body with relatively low pO_2.

Erythropoietin (EPO) is widely recognized as a significant positive regulator of postnatal erythropoiesis in vertebrates. EPO regulates the compensatory erythropoietic response to reduced tissue oxygen tension (hypoxia) and low red blood cell levels or low hemoglobin levels. In humans, elevated EPO levels promote red blood cell formation by stimulating the generation of erythroid progenitors in the bone marrow and spleen. In the mouse, EPO enhances erythropoiesis primarily in the spleen.

Effects of EPO are mediated by a cell-surface receptor belonging to the cytokine receptor superfamily. The human EPO receptor gene encodes a 483 amino acid transmembrane protein; however, the active EPO receptor is thought to exist as a multimeric complex even in the absence of ligand (see, *e.g.*, U.S. Pat. No. 6,319,499). The cloned full-length EPO receptor expressed in mammalian cells binds EPO with an affinity similar to that of the native receptor on erythroid progenitor cells. Binding of EPO to its receptor causes a conformational change resulting in receptor activation and biological effects including increased proliferation of immature erythroblasts, increased differentiation of immature erythroblasts, and decreased apoptosis in erythroid progenitor cells [see, *e.g.*, Liboi *et al.* (1993) Proc Natl Acad Sci USA 90:11351-11355; Koury *et al.* (1990) Science 248:378-381].

Various forms of recombinant EPO are used by physicians to increase red blood cell levels in a variety of clinical settings, particularly in the treatment of anemia. Anemia is a broadly-defined condition characterized by lower than normal levels of hemoglobin or red blood cells in the blood. In some instances, anemia is caused by a primary disorder in the production or survival of red blood cells (*e.g.,* myelodysplastic syndromes). More commonly, anemia is secondary to diseases of other systems [see, *e.g.*, Weatherall & Provan (2000) Lancet 355, 1169-1175]. Anemia may result from a reduced rate of production or increased rate of destruction of red blood cells or by loss of red blood cells due to bleeding. Anemia may result from a variety of disorders that include, for example, acute or chronic renal failure or end stage renal disease, chemotherapy treatment, a myelodysplastic syndrome, rheumatoid arthritis, and bone marrow transplantation.

Treatment with EPO typically causes a rise in hemoglobin by about 1-3 g/dL in healthy humans over a period of weeks. When administered to anemic individuals, this

treatment regimen often provides substantial increases in hemoglobin and red blood cell levels and leads to improvements in quality of life and prolonged survival. However, EPO is not uniformly effective, and many individuals are refractory to even high doses [see, *e.g.*, Horl *et al.* (2000) Nephrol Dial Transplant 15, 43-50]. For example, over 50% of patients with cancer have an inadequate response to EPO, and approximately 10% with end-stage renal disease are hyporesponsive to EPO [see, *e.g.*, Glaspy *et al.* (1997) J Clin Oncol 15, 1218-1234; Demetri *et al.* (1998) J Clin Oncol 16, 3412-3425]. Although the molecular mechanisms of resistance to EPO are as yet unclear, several factors, including inflammation, iron and vitamin deficiency, inadequate dialysis, aluminum toxicity, and hyperparathyroidism may predict a poor therapeutic response. In addition, recent evidence suggests that higher doses of EPO may be associated with an increased risk of cardiovascular morbidity, tumor growth, and mortality in some patient populations [see, *e.g.*, Krapf *et al.* (2009) Clin J Am Soc Nephrol 4:470-480; Glaspy (2009) Annu Rev Med 60:181-192]. Therefore, it has been recommended that EPO-based therapeutic compounds (*e.g.*, erythropoietin-stimulating agents, ESAs) be administered at the lowest dose that allows a patient to avoid red blood cell transfusions [see, *e.g.*, Jelkmann *et al.* (2008) Crit Rev Oncol. Hematol 67:39-61].

Sideroblastic anemia, which occurs in both inherited and acquired forms, is characterized by the presence of "ring sideroblasts" in bone marrow. These distinctive red blood cell precursors (erythroblasts) can be identified by the presence of perinuclear siderotic granules, which are revealed by histologic staining with Prussian blue and are indicative of pathologic iron deposits in mitochondria [see, *e.g.*, Mufti et al. (2008) Haematologica 93:1712-1717; Bottomley et al. (2014) Hematol Oncol Clin N Am 28:653-670]. Acquired sideroblastic anemia occurs most frequently in the context of myelodysplastic syndromes (MDS), a heterogeneous group of hematopoietic stem-cell disorders estimated to affect between 30,000 and 40,000 patients per year in the United States [Bejar et al. (2014) Blood 124:2793-2803]. These disorders are characterized by ineffective hematopoiesis, abnormal "dysplastic" cell morphology, and the potential for clonal evolution to acute myeloid leukemia. As discussed below, recent advances in the genetic basis of MDS have the potential to greatly improve its diagnosis and treatment.

There is high unmet need for effective therapies for MDS, sideroblastic anemia and complications of those disorders. Endogenous EPO levels are commonly elevated in subsets of patients with MDS, thus suggesting that EPO has diminished effectiveness in these patients. It has been estimated that fewer than 10% of patients with MDS respond favorably

to EPO [Estey (2003) Curr Opin Hematol 10, 60-67], while a more recent meta-analysis found that EPO response rates range from 30% to 60% depending on the study [Moyo et al (2008) Ann Hematol 87:527-536]. Compared to other MDS patients, those with ring sideroblasts tend to be at substantially lower risk of developing acute myeloid leukemia and would therefore stand to benefit for an extended period from anti-anemia therapeutic agents that do not contribute to systemic iron burden and that instead help to reduce the iron overload frequently present in such patients [see, e.g., Temraz et al., 2014, Crit Rev Oncol Hematol 91:64-73].

Thus, it is an object of the present disclosure to provide methods treating patients with MDS and sideroblastic anemias with ActRII antagonists disclosed herein and, in particular, to guide selection of MDS patients that are most likely to show therapeutically beneficial increases in red blood cells, neutrophils, and other blood cells as a result of treatment.

SUMMARY OF THE INVENTION

In part, the disclosure provides methods of treating MDS and sideroblastic anemias, particularly treating or preventing one or more complications or subtypes of MDS, including MDS patients characterized by the presence of sideroblasts in the bone marrow, with one or more ActRII antagonists.

In part the disclosure provides methods for treating or preventing disorders or complications of a disorder that is associated with germ line or somatic mutations in *SF3B1*, such as myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML) as well as in breast cancer, pancreatic cancer, gastric cancer, prostate cancer, and uveal melanoma with one or more ActRII antagonists. In certain aspects the disorder may be in a subject that has bone marrow cells that test positive for an *SF3B1* mutation, particularly myelodysplastic syndrome, CLL and AML. Optionally a mutation in the *SF3B1* gene is in an exon, intron or 5' and/or 3' untranslated region. For example, in some embodiments, a mutation in the *SF3B1* gene is in exon 14, 15, and/or 16 of *SF31B*. Optionally a mutation in *SF3B1* causes a change in the amino acid sequence or does not cause a change in the amino acid sequence of the protein encoded by the gene. Optionally a mutation in the *SF3B1* gene causes a change in the amino acid of the protein encoded by the gene selected from the following changes: K182E, E491G, R590K, E592K, R625C, R625G, N626D, N626S, H662Y, T663A, K666M, K666Q, K666R, Q670E, G676D, V701I, I704N,

I704V, G740R, A744P, D781G, A1188V, N619K, N626H, N626Y, R630S, I704T, G740E, K741N, G742D, D894G, Q903R, R1041H, I1241T, G347V, E622D, Y623C, R625H, R625L, H662D, H662Q, T663I, K666E, K666N, K666T, K700E, E783K, and V701F.

In certain aspects, the disclosure provides methods for treating or preventing sideroblastic anemia in a human subject, comprising administering to a subject in need thereof a polypeptide comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1, wherein the polypeptide comprises an acidic amino acid [a naturally occurring amino acid (e.g., D or E) or an artificial amino acid] at position 79 with respect to SEQ ID NO: 1, wherein the subject is on a dosing schedule that comprising administering from 0.125 to 1.75 mg/kg (e.g., 0.75 to 1.75 mg/kg) of the polypeptide to the subject. In other aspects, the disclosure provides methods for treating or preventing sideroblastic anemia in a human subject, comprising administering to a subject in need thereof a polypeptide comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-125 of SEQ ID NO: 1, wherein the polypeptide comprises an acidic amino acid [a naturally occurring amino acid (e.g., D or E) or an artificial amino acid] at position 79 with respect to SEQ ID NO: 1, wherein the subject is on a dosing schedule that comprising administering from 0.125 to 1.75 mg/kg (e.g., 0.75 to 1.75 mg/kg) of the polypeptide to the subject. In even other aspects, the disclosure provides methods for treating or preventing sideroblastic anemia in a human subject, comprising administering to a subject in need thereof a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 44, wherein the polypeptide comprises an acidic amino acid [a naturally occurring amino acid (e.g., D or E) or an artificial amino acid] at position 79 with respect to SEQ ID NO: 1, wherein the subject is on a dosing schedule that comprising administering from 0.125 to 1.75 mg/kg (e.g., 0.75 to 1.75 mg/kg) of the polypeptide to the subject. In certain aspects, the disclosure provides methods for treating or preventing one or more complications of sideroblastic anemia in a human subject, comprising administering to a subject in need thereof a polypeptide comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1, wherein the polypeptide comprises an acidic amino acid [a naturally occurring amino

acid (e.g., D or E) or an artificial amino acid] at position 79 with respect to SEQ ID NO: 1, wherein the subject is on a dosing schedule that comprising administering from 0.125 to 1.75 mg/kg (e.g., 0.75 to 1.75 mg/kg) of the polypeptide to the subject. In other aspects, the disclosure provides methods for treating or preventing and/or one or more complications of sideroblastic anemia in a human subject, comprising administering to a subject in need thereof a polypeptide comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 25-125 of SEQ ID NO: 1, wherein the polypeptide comprises an acidic amino acid [a naturally occurring amino acid (e.g., D or E) or an artificial amino acid] at position 79 with respect to SEQ ID NO: 1, wherein the subject is on a dosing schedule that comprising administering from 0.125 to 1.75 mg/kg (e.g., 0.75 to 1.75 mg/kg) of the polypeptide to the subject. In even other aspects, the disclosure provides methods for treating or preventing and/or one or more complications of sideroblastic anemia in a human subject, comprising administering to a subject in need thereof a polypeptide comprising, consisting essentially of, or consisting of an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence of SEQ ID NO: 44, wherein the polypeptide comprises an acidic amino acid [a naturally occurring amino acid (e.g., D or E) or an artificial amino acid] at position 79 with respect to SEQ ID NO: 1, wherein the subject is on a dosing schedule that comprising administering from 0.125 to 1.75 mg/kg (e.g., 0.75 to 1.75 mg/kg) of the polypeptide to the subject. In certain preferred embodiments, polypeptides to be used in accordance with the methods described herein are dimers (e.g., a homodimer comprising two polypeptides corresponding to the amino acid sequence of SEQ ID NO: 44 associated by covalent or non-covalent interactions). Optionally the polypeptide may bind to one or more ligand of the TGFβ superfamily. For example, in some embodiments, polypeptides described herein (e.g., ActRIIA and ActRIIB polypeptides as well as variant thereof such as GDF traps) may bind to GDF11. In other embodiments, polypeptides described herein (e.g., ActRIIA and ActRIIB polypeptides as well as variant thereof such as GDF traps) may bind to GDF8. In still other embodiments, polypeptides described herein (e.g., ActRIIA and ActRIIB polypeptides as well as variant thereof such as GDF traps) may bind to GDF11 and GDF8. Optionally the polypeptide may comprises one or more amino acid modifications selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, and an amino acid conjugated to a lipid moiety. In certain preferred embodiments the polypeptide is glycosylated and has a mammalian

glycosylation pattern. Optionally the polypeptide has a glycosylation pattern obtainable from a Chinese hamster ovary cell line. Optionally the methods comprises subcutaneously administered the polypeptide to the subject. Optionally the dosing schedule further comprises administering the polypeptide to the patient twice every week, once every week, once every 3 weeks, once every 4 weeks, once every 5 weeks, once every 6 weeks, once every 7 weeks, once every 8 weeks, once every 9 weeks, once every 10 weeks, once every 12 weeks, once every 14 weeks, once every 16 weeks, once every 18 weeks, once every 20 weeks, once every 24 weeks, once every 26 weeks, once every 28 weeks, once every 30 weeks, once every 32 weeks, once every 34 weeks, or once every 36 weeks In certain preferred embodiments the dosing schedule further comprises administering the polypeptide to the patient once every three weeks. Optionally the subject has undesirably high levels of endogenous EPO. Optionally the subject has previously been treated with one or more EPO receptor agonists. Optionally the subject has an inadequate response to the EPO receptor agonist. Optionally the subject is no longer responsive to the EPO receptor agonist. Optionally the EPO receptor agonist is EPO. Optionally the treatment increases red blood cell levels. Optionally the treatment increases hemoglobin levels. Optionally wherein the treatment results in a hemoglobin increase of $\geq$ 1.5 g/dL for $\geq$ two weeks. Optionally the treatment results in a hemoglobin increase of $\geq$ 1.5 g/dL for $\geq$ eight weeks. Optionally the subject has been administered one or more blood cell transfusions prior to the start of treatment. Optionally wherein the subject is a low transfusion burden subject. Optionally the subject is a high transfusion burden subject. Optionally the treatment decreases blood cell transfusion burden. Optionally the treatment decreases blood cell transfusion by $\geq$ 50% for at least four weeks relative to the equal time prior to start of treatment. Optionally the treatment decreases blood cell transfusion by $\geq$ 50% for at least eight weeks relative to the equal time prior to start of treatment. Optionally the patient has myelodysplastic syndrome. Optionally the patient has an International Prognostic Scoring System (IPSS) or IPSS-R score of low or intermediate. Optionally the sideroblastic anemia subject has at least 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% ring blasts as a percentage of bone marrow erythroid precursors in his or her bone marrow. Optionally the treatment increases neutrophil levels. Optionally the subject has bone marrow cells that test positive for one or more mutations in SF3B1. Optionally the subject has bone marrow cells that test positive for one or more mutations in DNMT3A. Optionally the subject has bone marrow cells that test positive for one or more mutations in TET2. Optionally the treatment decreases

iron overload. In some embodiments, the treatment decrease tissue iron overload (e.g., iron overload in the kidney, liver, and/or spleen). In some embodiments, the treatment decrease serum iron overload.

In part the disclosure provides methods for treating or preventing disorders or complications of a disorder that is associated with germ line or somatic mutations in *DNMT3A*, such as myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML) with one or more ActRII antagonists. In certain aspects the disorder may be in a subject that has bone marrow cells that test positive for a *DNMT3A* mutation, particularly myelodysplastic syndrome, CLL and AML. Optionally a mutation in the *DNMT3A* gene is in an exon, intron and/or 5' or 3' untranslated region. For example, in some embodiments, a mutation in the *DNMT3A* gene is in exon 10, 18, and/or 22 of *DNMT3A*. Optionally a mutation in *DNMT3A* causes a change in the amino acid sequence or does not cause a change in the amino acid sequence of the protein encoded by the gene. Optionally a mutation in the *DNMT3A* gene causes a change in the amino acid of the protein encoded by the gene selected from the following changes: R882C, R882H, P904L, and P905P. Optionally a mutation in the *DNMT3A* gene introduces a premature stop codon. For example, in some embodiments, a mutation in the *DNMT3A* gene that introduces a premature stop codon is selected from the following positions: Y436X and W893X.

In part the disclosure provides methods for treating or preventing disorders or complications of a disorder that is associated with germ line or somatic mutations in *TET2*, such as myelodysplastic syndrome (MDS), chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML) with one or more ActRII antagonists. In certain aspects the disorder may be in a subject that has bone marrow cells that test positive for a *TET2* mutation, particularly myelodysplastic syndrome, CLL and AML. Optionally a mutation in the *TET2* gene is in an exon, intron and/or 5' or 3' untranslated region. For example, in some embodiments, a mutation in the *TET2* gene is in exon 1, exon 4, exon 5, exon 6, exon 7, exon 8, and/or exon 9 of *TET2*. Optionally a mutation in *TET2* causes a change in the amino acid sequence or does not cause a change in the amino acid sequence of the protein encoded by the gene. Optionally a mutation in the *TET2* gene causes a change in the amino acid of the protein encoded by the gene selected from the following changes: E47Q, Q1274R, W1291R, G1370R, N1387S, and Y1724H. Optionally a mutation in the *TET2* gene introduces a premature stop codon. For example, in some embodiments, a mutation in the *TET2* gene that

introduces a premature stop codon is selected from the following positions: R550X, Q1009X, Y1337X, R1404X, R1516X, and Q1652X.

In certain aspects, the disclosure provides methods for treating or preventing a bone marrow disorder in a subject, comprising administering to a subject in need thereof an effective amount of an ActRII antagonist, wherein the subject has bone marrow cells that test positive for one or more mutations in a gene selected from the group consisting of: SF3B1, DNMT3A, and TET2. In some embodiments, the subject tests positive for one or more SF3B1 mutations. Optionally one or more of the SF3B1 mutations are in a SF3B1 exon. Optionally one or more of the SF3B1 mutations are in a SF3B1 intron. Optionally one or more of the SF3B1 mutations are in a SF3B1 5' and/or 3' region. Optionally one or more of the SF3B1 mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated SF3B1 gene. Optionally one or more SF3B1 mutations causes a substitution of one or more amino acid selected from the group consisting of: K182E, E491G, R590K, E592K, R625C, R625G, N626D, N626S, H662Y, T663A, K666M, K666Q, K666R, Q670E, G676D, V701I, I704N, I704V, G740R, A744P, D781G, A1188V, N619K, N626H, N626Y, R630S, I704T, G740E, K741N, G742D, D894G, Q903R, R1041H, I1241T, G347V, E622D, Y623C, R625H, R625L, H662D, H662Q, T663I, K666E, K666N, K666T, K700E, V701F, and E783K. Optionally one or more SF3B1 mutations are in a SF3B1 exon selected from the group consisting of: exon 14, exon 15 and exon 16. In some embodiment, the subject tests positive for one or more DNMT3A mutations. Optionally one or more of the DNMT3A mutations are in a DNMT3A exon. Optionally one or more of the DNMT3A mutations are in a DNMT3A intron. Optionally one or more of the DNMT3A mutations are in a DNMT3A 5' and/or 3' region. Optionally one or more of the DNMT3A mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated DNMT3A gene. Optionally one or more DNMT3A mutations causes a substitution of one or more amino acid selected from the group consisting of: R882C, R882H, P904L, and P905P. Optionally the one or more DNMT3A mutations are in a DNMT3A exon selected from the group consisting of: exon 10, exon 18 and exon 22. Optionally one or more DNMT3A mutations introduces a premature stop codon. Optionally one or more DNMT3A mutations is selected from the group consisting of Y436X and W893X. In some embodiments, subject tests positive for one or more TET2 mutations. Optionally one or more of the TET2 mutations are in a TET2 exon. Optionally one or more of the TET2 mutations are in a TET2 intron. Optionally one or more of the TET2 mutations are in a TET2 5' and/or

3' region. Optionally one or more of the TET2 mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated TET2 gene. Optionally one or more TET2 mutations causes a substitution of one or more amino acid selected from the group consisting of: E47Q, Q1274R, W1291R, G1370R, G1370E, N1387S, and Y1724H. Optionally one or more TET2 mutations are in a TET2 exon selected from the group consisting of: exon 1, exon 4, exon 5, exon 6, exon 7, exon 8, and exon 9. Optionally one or more TET2 mutations introduces a premature stop codon. Optionally one or more TET2 mutations is selected from the group consisting of: R550X, Q1009X, Y1337X, R1404X, R1516X, and Q1652X. Optionally the subject has anemia. Optionally the subject has undesirably high levels of endogenous EPO. Optionally the subject has previously been treated with one or more EPO receptor agonists. Optionally the subject has an inadequate response to the EPO receptor agonist. Optionally the subject is no longer responsive to the EPO receptor agonist. Optionally the EPO receptor agonist is EPO. Optionally the treatment delays conversion to leukemia. Optionally the treatment delays conversion to acute myeloid leukemia. Optionally the subject is a pre-leukemia patient. Optionally the treatment increases red blood cell levels and/or hemoglobin levels in the subject. Optionally the subject has been administered one or more blood cell transfusions prior to the start of the ActRII antagonist treatment. Optionally the treatment decreases blood cell transfusion burden. Optionally the treatment decreases blood cell transfusion by greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% for 4 to 8 weeks relative to the equal time prior to the start of the ActRII antagonist treatment. Optionally the treatment decreases blood cell transfusion by greater than about 50% for 4 to 8 weeks relative to the equal time prior to the start of the ActRII antagonist treatment. Optionally the treatment decreases iron overload. Optionally the treatment decrease iron content in the liver and/or spleen.

In certain aspects, the disclosure provides methods for treating or preventing myelodysplastic syndrome (MDS) and/or one or more complications of MDS, comprising administering to a subject in need thereof an effective amount of an ActRII antagonist, wherein the subject has bone marrow cells that test positive for one or more mutations in a gene selected from the group consisting of: SF3B1, DNMT3A, and TET2. In some embodiments, the subject tests positive for one or more SF3B1 mutations. Optionally one or more of the mutations are in a SF3B1 exon. Optionally one or more of the SF3B1 mutations are in a SF3B1 intron. Optionally one or more of the SF3B1 mutations are in a SF3B1 5' and/or 3' region. Optionally one or more of the SF3B1 mutations causes a deletion, addition,

and/or substitution of an amino acid in the protein encoded by the mutated SF3B1 gene. Optionally one or more SF3B1 mutations causes a substitution of one or more amino acid selected from the group consisting of: K182E, E491G, R590K, E592K, R625C, R625G, N626D, N626S, H662Y, T663A, K666M, K666Q, K666R, Q670E, G676D, V701I, I704N, I704V, G740R, A744P, D781G, A1188V, N619K, N626H, N626Y, R630S, I704T, G740E, K741N, G742D, D894G, Q903R, R1041H, I1241T, G347V, E622D, Y623C, R625H, R625L, H662D, H662Q, T663I, K666E, K666N, K666T, K700E, V701F, and E783K. Optionally one or more SF3B1 mutations are in a SF3B1 exon selected from the group consisting of: exon 14, exon 14 and exon 16. In some embodiments, the subject tests positive for one or more DNMT3A mutations. Optionally one or more of the DNMT3A mutations are in a DNMT3A exon. Optionally one or more of the DNMT3A mutations are in a DNMT3A intron. Optionally one or more of the DNMT3A mutations are in a DNMT3A 5' and/or 3' region. Optionally one or more of the DNMT3A mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated DNMT3A gene. Optionally one or more DNMT3A mutations causes a substitution of one or more amino acid selected from the group consisting of: R882C, R882H, P904L, and P905P. Optionally one or more DNMT3A mutations are in a DNMT3A exon selected from the group consisting of: exon 10, exon 18, and exon 22. Optionally one or more DNMT3A mutations introduces a premature stop codon. Optionally one or more DNMT3A mutations are selected from the group consisting of Y436X and W893X. In some embodiments, the subject tests positive for one or more TET2 mutations. Optionally one or more of the TET2 mutations are in a TET2 exon. Optionally one or more of the TET2 mutations are in a TET2 intron. Optionally one or more of the TET2 mutations are in a TET2 5' and/or 3' region. Optionally one or more of the TET2 mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated TET2 gene. Optionally one or more TET2 mutations causes a substitution of one or more amino acid selected from the group consisting of: E47Q, Q1274R, W1291R, G1370R, G1370E, N1387S, and Y1724H. Optionally one or more TET2 mutations are in a TET2exon selected from the group consisting of: exon 1, exon 4, exon 5, exon 6, exon 7, exon 8, and exon 9. Optionally one or more TET2 mutations introduces a premature stop codon. Optionally one or more TET2 mutations is selected from the group consisting of: R550X, Q1009X, Y1337X, R1404X, R1516X, and Q1652X. Optionally the subject has anemia. Optionally the subject has undesirably high levels of endogenous EPO. Optionally the subject has previously been treated with one or more EPO receptor agonists. Optionally the subject has an inadequate response to the EPO receptor agonist. Optionally

the subject is no longer responsive to the EPO receptor agonist. Optionally the EPO receptor agonist is EPO. Optionally the treatment delays conversion to leukemia. Optionally the treatment delays conversion to acute myeloid leukemia. Optionally the treatment increases red blood cell levels and/or hemoglobin levels in the subject. Optionally the subject has been administered one or more blood cell transfusions prior to the start of the ActRII antagonist treatment. Optionally the treatment decreases blood cell transfusion burden. Optionally the treatment decreases blood cell transfusion by greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% for 4 to 8 weeks relative to the equal time prior to the start of the ActRII antagonist treatment. Optionally the treatment decreases blood cell transfusion by greater than about 50% for 4 to 8 weeks relative to the equal time prior to the start of the ActRII antagonist treatment. Optionally the treatment decreases iron overload. Optionally the treatment decrease iron content in the liver and/or spleen. Optionally the subject has a subtype of MDS selected from: MDS with refractory anemia with ring sideroblasts (RARS); MDS with refractory anemia with ring sideroblasts and thrombocytosis (RARS-T); MDS with refractory cytopenia with unilineage dysplasia (RCUD); MDS with refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS); MDS with a somatic mutation in one or more of *SF3B1*, *SRSF2*, *DNMT3A*, and *TET2*; MDS without a somatic mutation in *ASXL1* or *ZRSR2*; MDS with iron overload; and MDS with neutropenia. Optionally the subject has an International Prognostic Scoring System (IPSS) score selected from: low, intermediate 1, or intermediate 2. Optionally the subject has sideroblasts. Optionally the subject has at least 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% sideroblasts as a percentage of bone marrow erythroid precursors in his or her bone marrow.

ActRII antagonists of the disclosure include, for example, agents that can inhibit ActRII receptor (*e.g.*, an ActRIIA and/or ActRIIB receptor) mediated activation of a signal transduction pathway (*e.g.*, activation of signal transduction via intracellular mediators, such as SMAD 1, 2, 3, 5, and/or 8); agents that can inhibit one or more ActRII ligands (*e.g.*, activin A, activin B, activin AB, activin C, activin E, GDF11, GDF8, BMP6, BMP7, Nodal, *etc.*) from, *e.g.*, binding to and/or activating an ActRII receptor; agents that inhibit expression (*e.g.*, transcription, translation, cellular secretion, or combinations thereof) of an ActRII ligand and/or an ActRII receptor; and agents that can inhibit one or more intracellular mediators of the ActRII signaling pathway (*e.g.*, SMADs 1, 2, 3, 5, and/or 8).

In particular, the disclosure provides methods for using an ActRII antagonist, or combination of ActRII antagonists, to treat or prevent one or more complications of MDS and sideroblastic anemias including, for example, anemia, neutropenia, splenomegaly, blood transfusion requirement, development of acute myeloid leukemia, iron overload, and complications of iron overload, among which are congestive heart failure, cardiac arrhythmia, myocardial infarction, other forms of cardiac disease, diabetes mellitus, dyspnea, hepatic disease, and adverse effects of iron chelation therapy and as other examples, to increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with *SF3B1, DNMT3A,* and/or *TET2* mutations in a subject in need thereof..

In particular, the disclosure provides methods for using an ActRII antagonist, or combination of ActRII antagonists, to treat or prevent complications in a subtype of MDS, including MDS patients with elevated numbers of erythroblasts (hypercellularity) in bone marrow; in MDS patients with more than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% sideroblasts, as a percentage of erythroid precursors in bone marrow; in MDS patients with refractory anemia with ring sideroblasts (RARS); in MDS patients with refractory anemia with ring sideroblasts and thrombocytosis (RARS-T); in MDS patients with refractory cytopenia with unilineage dysplasia (RCUD); in MDS patients with refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS); in MDS patients with a somatic mutation in *SF3B1*, *SRSF2*, *DNMT3A*, *TET2*, or *SETBP1*; in MDS patients without a somatic mutation in *ASXL1* or *ZRSR2*; in MDS patients with iron overload; and in MDS patients with neutropenia.

Also in particular, the disclosure provides methods for using an ActRII antagonist, or combination of ActRII antagonists, to treat or prevent complications of a sideroblastic anemia, including refractory anemia with ring sideroblasts (RARS); refractory anemia with ring sideroblasts and thrombocytosis (RARS-T); refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS); sideroblastic anemia associated with alcoholism; drug-induced sideroblastic anemia; sideroblastic anemia resulting from copper deficiency (zinc

toxicity); sideroblastic anemia resulting from hypothermia; X-linked sideroblastic anemia (XLSA); SLC25A38 deficiency; glutaredoxin 5 deficiency; erythropoietic protoporphyria; X-linked sideroblastic anemia with ataxia (XLSA/A); sideroblastic anemia with B-cell immunodeficiency, fevers, and developmental delay (SIFD); Pearson marrow-pancreas syndrome; myopathy, lactic acidosis, and sideroblastic anemia (MLASA); thiamine-responsive megaloblastic anemia (TRMA); and syndromic/nonsyndromic sideroblastic anemia of unknown cause.

In certain embodiments, preferred ActRII antagonists to be used in accordance with the methods disclosed herein are agents that bind to and/or inhibit GDF11 and/or GDF8 (*e.g.*, an agent that inhibits GDF11- and/or GDF8-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). Such agents are referred to collectively as GDF-ActRII antagonists. Optionally, such GDF-ActRII antagonists may further inhibit one or more of activin A, activin B, activin AB, activin C, activin E, GDF11, GDF8, BMP6, BMP7, and Nodal. Therefore, in some embodiments, the disclosure provides methods of using one or more ActRII antagonists, including, for example, soluble ActRIIA polypeptides, soluble ActRIIB polypeptides, GDF trap polypeptides, anti-ActRIIA antibodies, anti-ActRIIB antibodies, anti-ActRII ligand antibodies (*e.g.* anti-GDF11 antibodies, anti-GDF8 antibodies, anti-activin A antibodies, anti-activin B antibodies, anti-activin AB antibodies, anti-activin C antibodies, anti-activin E antibodies, anti-BMP6 antibodies, anti-BMP7 antibodies, and anti-Nodal antibodies), small-molecule inhibitors of ActRIIA, small-molecule inhibitors of ActRIIB, small-molecule inhibitors of one or more ActRII ligands (*e.g.*, activin A, activin B, activin AB, activin C, activin E, GDF11, GDF8, BMP6, BMP7, Nodal, *etc.*), inhibitor nucleotides (nucleotide-based inhibitors) of ActRIIA, inhibitor nucleotides of ActRIIB, inhibitor nucleotides of one or more ActRII ligands (*e.g.*, activin A, activin B, activin AB, activin C, activin E, GDF11, GDF8, BMP6, BMP7, Nodal, *etc.*), or combinations thereof, to increase red blood cell levels and/or hemoglobin levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof, treat sideroblastic anemia or MDS in a subject in need thereof, or treat one or more complications of sideroblastic anemia or MDS in a subject in need thereof and as other examples, to increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction,

hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with *SF3B1, DNMT3A,* and/or *TET2* mutations in a subject in need thereof. In certain embodiments, ActRII antagonists to be used in accordance with the methods disclosed herein do not substantially bind to and/or inhibit activin A (*e.g.*, activin A-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling).

In part, the present disclosure demonstrates that an ActRII antagonist comprising a variant, extracellular (soluble) ActRIIB domain that binds to and inhibits GDF11 activity (*e.g.*, GDF11-mediated ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling) may be used to increase red blood cell levels *in vivo*, treat anemia resulting from various conditions/disorders, and treat sideroblastic anemia or MDS. Therefore, in certain embodiments, preferred ActRII antagonists to be used in accordance with the methods disclosed herein (*e.g.*, methods of increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associatd with *SF3B1* mutations in a subject in need thereof, *etc.*) are soluble ActRII polypeptides (*e.g.* soluble ActRIIA or ActRIIB polypeptides) that bind to and/or inhibit GDF11 (*e.g.*, GDF11-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). While soluble ActRIIA and soluble ActRIIB ActRII antagonists may affect red blood cell formation and/or morphology through a mechanism other than GDF11 antagonism, the disclosure nonetheless demonstrates that desirable therapeutic agents, with respect to the methods disclosed herein, may be selected on the basis of GDF11 antagonism or ActRII antagonism or both. Optionally, such soluble ActRII polypeptide antagonist may further bind to and/or inhibit GDF8 (*e.g.* inhibit GDF8-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). In some embodiments, soluble ActRIIA and ActRIIB polypeptides of the disclosure that bind to and/or inhibit GDF11 and/or GDF8 may further bind to and/or inhibit one or more additional ActRII ligands selected from: activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, and Nodal.

In certain aspects, the present disclosure provides GDF traps that are variant ActRII polypeptides (*e.g.*, ActRIIA and ActRIIB polypeptides), including ActRII polypeptides having amino- and carboxy-terminal truncations and/or other sequence alterations (one or more amino acid substitutions, additions, deletions, or combinations thereof). Optionally, GDF traps of the invention may be designed to preferentially antagonize one or more ligands of ActRII receptors, such as GDF8 (also called myostatin), GDF11, Nodal, BMP6, and BMP7 (also called OP-1). As disclosed herein, examples of GDF traps include a set of variants derived from ActRIIB that have greatly diminished affinity for activin, particularly activin A. These variants exhibit desirable effects on red blood cells while reducing effects on other tissues. Examples of such variants include those having an acidic amino acid [*e.g.*, aspartic acid (D) or glutamic acid (E)] at the position corresponding to position 79 of SEQ ID NO:1. In certain embodiments, preferred GDF traps to be used in accordance with the methods disclosed herein (*e.g.*, methods to increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with *SF3B1, DNMT3A,* and/or *TET2* mutations in a subject in need thereof, *etc.*) bind to and/or inhibit GDF11. Optionally, such GDF traps may further bind to and/or inhibit GDF8. In some embodiments, GDF traps that bind to and/or inhibit GDF11 and/or GDF8 may further bind to and/or inhibit one or more additional ActRII ligands (*e.g.*, activin B, activin E, BMP6, BMP7, and Nodal). In some embodiments, GDF traps to be used in accordance with the methods disclosed herein to not substantially bind to and/or inhibit activin A (*e.g.*, activin A-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). In certain embodiments, a GDF trap polypeptide comprises an amino acid sequence that comprises, consists of, or consists essentially of, the amino acid sequence of SEQ ID NOs: 36, 37, 41, 44, 45, 50 or 51, and polypeptides that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to any of the foregoing. In other embodiments, a GDF trap polypeptide comprises an amino acid sequence that comprises, consists of, or consists essentially of the amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6, 10, 11, 22, 26, 28, 29, 31, or 49, and polypeptides that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to any of the foregoing. In still other embodiments, a GDF trap polypeptide comprises an amino acid sequence that

comprises of the amino acid sequence of SEQ ID NOs: 2, 3, 4, 5, 6, 29, 31, or 49, and polypeptides that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to any of the foregoing, wherein the position corresponding to 79 in SEQ ID NO: 1, 4, or 50 is an acidic amino acid. A GDF trap may include a functional fragment of a natural ActRII polypeptide, such as one comprising at least 10, 20, or 30 amino acids of a sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 9, 10, 11, or 49 or a sequence of SEQ ID NO: 2, 5, 10, 11, or 49 lacking the C-terminal 1, 2, 3, 4, 5 or 10 to 15 amino acids and lacking 1, 2, 3, 4 or 5 amino acids at the N-terminus. A preferred polypeptide will comprise a truncation relative to SEQ ID NO: 2 or 5 of between 2 and 5 amino acids at the N-terminus and no more than 3 amino acids at the C-terminus. A preferred GDF trap for use in such a preparation consists of, or consists essentially of, the amino acid sequence of SEQ ID NO: 36.

Optionally, a GDF trap comprising an altered ActRII ligand-binding domain has a ratio of K_d for activin A binding to K_d for GDF11 and/or GDF8 binding that is at least 2-, 5-, 10-, 20, 50-, 100-, or even 1000-fold greater relative to the ratio for the wild-type ligand-binding domain. Optionally, the GDF trap comprising an altered ligand-binding domain has a ratio of IC_{50} for inhibiting activin A to IC_{50} for inhibiting GDF11 and/or GDF8 that is at least 2-, 5-, 10-, 20-, 25- 50-, 100-, or even 1000-fold greater relative to the wild-type ActRII ligand-binding domain. Optionally, the GDF trap comprising an altered ligand-binding domain inhibits GDF11 and/or GDF8 with an IC_{50} at least 2, 5, 10, 20, 50, or even 100 times less than the IC_{50} for inhibiting activin A. These GDF traps can be fusion proteins that include an immunoglobulin Fc domain (either wild-type or mutant). In certain cases, the subject soluble GDF traps are antagonists (inhibitors) of GDF8 and/or GDF11.

In certain aspects, the disclosure provides GDF traps which are soluble ActRIIB polypeptides comprising an altered ligand-binding (*e.g.*, GDF11-binding) domain. GDF traps with altered ligand-binding domains may comprise, for example, one or more mutations at amino acid residues such as E37, E39, R40, K55, R56, Y60, A64, K74, W78, L79, D80, F82 and F101 of human ActRIIB (numbering is relative to SEQ ID NO: 1). Optionally, the altered ligand-binding domain can have increased selectivity for a ligand such as GDF8/GDF11 relative to a wild-type ligand-binding domain of an ActRIIB receptor. To illustrate, these mutations are demonstrated herein to increase the selectivity of the altered ligand-binding domain for GDF11 (and therefore, presumably, GDF8) over activin: K74Y, K74F, K74I, L79D, L79E, and D80I. The following mutations have the reverse effect, increasing the ratio of activin binding over GDF11: D54A, K55A, L79A and F82A. The

overall (GDF11 and activin) binding activity can be increased by inclusion of the "tail" region or, presumably, an unstructured linker region, and also by use of a K74A mutation. Other mutations that caused an overall decrease in ligand binding affinity, include: R40A, E37A, R56A, W78A, D80K, D80R, D80A, D80G, D80F, D80M and D80N. Mutations may be combined to achieve desired effects. For example, many of the mutations that affect the ratio of GDF11:activin binding have an overall negative effect on ligand binding, and therefore, these may be combined with mutations that generally increase ligand binding to produce an improved binding protein with ligand selectivity. In an exemplary embodiment, a GDF trap is an ActRIIB polypeptide comprising an L79D or L79E mutation, optionally in combination with additional amino acid substitutions, additions, or deletions.

In certain embodiments, ActRII antagonists to be used in accordance with the methods disclosed herein are ActRIIB polypeptides or ActRIIB-based GDF trap polypeptides. In general such ActRIIB polypeptides and ActRIIB-based GDF trap polypeptides are soluble polypeptides that comprise a portion/domain derived from the ActRIIB sequence of SEQ ID NO:1, 4, or 49, particularly an extracellular, ligand-binding portion/domain derived from the ActRIIB sequence of SEQ ID NO:1, 4, or 49. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any one of amino acids 21-29 (*e.g.*, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO:1 or 4 [optionally beginning at 22-25 (*e.g.*, 22, 23, 24, or 25) of SEQ ID NO:1 or 4] and ending at any one of amino acids 109-134 (*e.g.*, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1 or 4. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any one of amino acids 20-29 (*e.g.*, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 or 4 [optionally beginning at 22-25 (*e.g.*, 22, 23, 24, or 25) of SEQ ID NO:1 or 4] and ending at any one of amino acids 109-133 (*e.g.*, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133) of SEQ ID NO: 1 or 4. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any one of amino acids 20-24 (*e.g.*, 20, 21, 22, 23, or 24) of SEQ ID NO: 1 or 4 [optionally beginning at 22-25 (*e.g.*, 22, 23, 24, or 25) of SEQ ID NO:1 or 4] and ending at any one of amino acids 109-133 (*e.g.*, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133) of SEQ ID NO: 1 or 4. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any one of amino acids 21-24 (*e.g.*, 21, 22, 23, or 24) of SEQ ID NO: 1 or 4 and ending at

any of amino acids 109-134 (*e.g.*, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1 or 4. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any one of amino acids 20-24 (*e.g.*, 20, 21, 22, 23, or 24) of SEQ ID NO: 1 or 4 and ending at any one of amino acids 118-133 (*e.g.*, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133) of SEQ ID NO: 1 or 4 In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any one of amino acids 21-24 (*e.g.*, 21, 22, 23, or 24) of SEQ ID NO: 1 or 4 and ending at any one of amino acids 118-134 (*e.g.*, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1 or 4. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any one of amino acids 20-24 (*e.g.*, 20, 21, 22, 23, or 24) of SEQ ID NO: 1 or 4 and ending at any one of amino acids 128-133 (*e.g.*, 128, 129, 130, 131, 132, or 133) of SEQ ID NO: 1 or 4. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any of amino acids 20-24 (*e.g.*, 20, 21, 22, 23, or 24) of SEQ ID NO: 1 or 39 and ending at any of amino acids 128-133 (*e.g.*, 128, 129, 130, 131, 132, or 133) of SEQ ID NO: 1 or 39. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any one of amino acids 21-29 (*e.g.*, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 or 4 and ending at any one of amino acids 118-134 (*e.g.*, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1 or 4. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any one of amino acids 20-29 (*e.g.*, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 or 4 and ending at any one of amino acids 118-133 (*e.g.*, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133) of SEQ ID NO: 1 or 4. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at one any of amino acids 21-29 (*e.g.*, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 or 4 and ending at any one of amino acids 128-134 (*e.g.*, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1 or 4. In some embodiments, the portion derived from ActRIIB corresponds to a sequence beginning at any one of amino acids 20-29 (*e.g.*, 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 or 4 and ending at any one of amino acids 128-133 (*e.g.*, 128, 129, 130, 131, 132, or 133) of SEQ ID NO: 1 or 4. Surprisingly, ActRIIB and ActRIIB-based GDF trap constructs beginning at 22-25 (*e.g.*, 22, 23, 24, or 25) of SEQ ID NO: 1 or 4 have activity levels greater than proteins having the full extracellular domain of human ActRIIB. In a preferred embodiment, the ActRIIB polypeptides and ActRIIB-based GDF trap polypeptides comprise, consist essentially of, or consist of, an

amino acid sequence beginning at amino acid position 25 of SEQ ID NO: 1 or 4 and ending at amino acid position 131 of SEQ ID NO: 1 or 4. Any of the foregoing ActRIIB polypeptides or ActRIIB-based GDF trap polypeptides may be produced as a homodimer. Any of the foregoing ActRIIB polypeptides or ActRIIB-based GDF trap polypeptides may further comprise a heterologous portion that comprises a constant region from an IgG heavy chain, such as an Fc domain. Any of the above ActRIIB-based GDF trap polypeptides may comprise an acidic amino acid at the position corresponding to position 79 of SEQ ID NO: 1, optionally in combination with one or more additional amino acid substitutions, deletions, or insertions relative to SEQ ID NO: 1. Any of the above ActRIIB polypeptides or ActRIIB-based GDF trap polypeptides, including homodimer and/or fusion proteins thereof, may bind to and/or inhibit signaling by activin (*e.g.*, activin A, activin B, activin C, or activin AB) in a cell-based assay. Any of the above ActRIIB polypeptides or ActRIIB-based GDF trap polypeptides, including homodimer and/or fusion proteins thereof, may bind to and/or inhibit signaling by GDF11 and/or GDF8 in a cell based assay. Optionally, any of the above ActRIIB polypeptides or ActRIIB-based GDF trap polypeptides, including homodimer and/or fusion proteins thereof, may bind to and/or inhibit signaling of one or more of activin B, activin C, activin E, BMP6, BMP7, and Nodal in a cell-based assay.

Other ActRIIB polypeptides and ActRIIB-based GDF Trap polypeptides are contemplated, such as the following. An ActRIIB polypeptide or GDF trap polypeptide comprising an amino acid sequence that is at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the sequence of amino acids 29-109 of SEQ ID NO: 1 or 4, wherein the position corresponding to 64 of SEQ ID NO: 1 is an R or K, and wherein the ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide inhibits signaling by activin, GDF8, and/or GDF11 in a cell-based assay. The ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide as above, wherein at least one alteration with respect to the sequence of SEQ ID NO: 1 or 4 is positioned outside of the ligand-binding pocket. The ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide as above, wherein at least one alteration with respect to the sequence of SEQ ID NO: 1 or 4 is a conservative alteration positioned within the ligand-binding pocket. The ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide as above, wherein at least one alteration with respect to the sequence of SEQ ID NO: 1 or 4 is an alteration at one or more positions selected from the group consisting of K74, R40, Q53, K55, F82, and L79.

Other ActRIIB polypeptides and ActRIIB-based GDF trap polypeptides are contemplated, such as the following. An ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide comprising an amino acid sequence that is at least 80% (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100%) identical to the sequence of amino acids 29-109 of SEQ ID NO: 1 or 4, and wherein the protein comprises at least one N-X-S/T sequence at a position other than an endogenous N-X-S/T sequence of ActRIIB, and at a position outside of the ligand binding pocket. The ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide as above, wherein the ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide comprises an N at the position corresponding to position 24 of SEQ ID NO: 1 or 4 and an S or T at the position corresponding to position 26 of SEQ ID NO: 1 or 4, and wherein the ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide inhibits signaling by activin, GDF8, and/or GDF11 in a cell-based assay. The ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide as above, wherein the ActRIIB polypeptide or ActRIIB-based GDF Trap polypeptide comprises an R or K at the position corresponding to position 64 of SEQ ID NO: 1 or 4. The ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide as above, wherein ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide comprises a D or E at the position corresponding to position 79 of SEQ ID NO: 1 or 4, and wherein the ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide inhibits signaling by activin, GDF8, and/or GDF11 in a cell-based assay. The ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide as above, wherein at least one alteration with respect to the sequence of SEQ ID NO: 1 or 4 is a conservative alteration positioned within the ligand-binding pocket. The ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide as above, wherein at least one alteration with respect to the sequence of SEQ ID NO: 1 or 4 is an alteration at one or more positions selected from the group consisting of K74, R40, Q53, K55, F82, and L79. The ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide above, wherein the ActRIIB polypeptide or ActRIIB-based GDF trap polypeptide is a fusion protein further comprising one or more heterologous portion. Any of the above ActRIIB polypeptides or ActRIIB-based GDF trap polypeptides, or fusion proteins thereof, may be produced as a homodimer. Any of the above ActRIIB fusion proteins or ActRIIB-based GDF trap fusion proteins may have a heterologous portion that comprises a constant region from an IgG heavy chain, such as an Fc domain.

In certain embodiments, a preferred ActRIIB polypeptide, for use in accordance with the methods disclosed herein, comprises an amino acid sequence that comprises, consists of,

or consists essentially of, the amino acid sequence of SEQ ID NOs: 2, 3, 5, 6, 29, 31, or 49, and polypeptides that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to any of the foregoing. An ActRIIB polypeptide may include a functional fragment of a natural ActRIIB polypeptide, such as one comprising at least 10, 20 or 30 amino acids of a sequence selected from SEQ ID NOs: 2, 3, 5, 6, 29, 31, or 49 or a sequence of SEQ ID NO: 2 or 5, lacking the C-terminal 1, 2, 3, 4, 5 or 10 to 15 amino acids and lacking 1, 2, 3, 4 or 5 amino acids at the N-terminus. A preferred polypeptide will comprise a truncation relative to SEQ ID NO: 2 or 5 of between 2 and 5 amino acids at the N-terminus and no more than 3 amino acids at the C-terminus. A preferred GDF trap for use in accordance with the methods described herein consists of, or consists essentially of, the amino acid sequence of SEQ ID NO:29.

A general formula for an active (*e.g.*, ligand binding) ActRIIA polypeptide is one that comprises a polypeptide that starts at amino acid 30 and ends at amino acid 110 of SEQ ID NO:9. Accordingly, ActRIIA polypeptides and ActRIIA-based GDF traps of the present disclosure may comprise, consist, or consist essentially of a polypeptide that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO:9. Optionally, ActRIIA polypeptides and ActRIIA-based GDF trap polypeptides of the present disclosure comprise, consists, or consist essentially of a polypeptide that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids amino acids 12-82 of SEQ ID NO:9 optionally beginning at a position ranging from 1-5 (*e.g.*, 1, 2, 3, 4, or 5) or 3-5 (*e.g.*, 3, 4, or 5) and ending at a position ranging from 110-116 (*e.g.*, 110, 111, 112, 113, 114, 115, or 116) or 110-115 (*e.g.*, 110, 111, 112, 113, 114, or 115) or SEQ ID NO:9, respectively, and comprising no more than 1, 2, 5, 10 or 15 conservative amino acid changes in the ligand binding pocket, and zero, one or more non-conservative alterations at positions 40, 53, 55, 74, 79 and/or 82 in the ligand-binding pocket with respect to SEQ ID NO:9. Any of the foregoing ActRIIA polypeptides or ActRIIA-based GDF trap polypeptides may be produced as a homodimer. Any of the foregoing ActRIIA polypeptides or ActRIIA-based GDF trap polypeptides may further comprise a heterologous portion that comprises a constant region from an IgG heavy chain, such as an Fc domain. Any of the above ActRIIA polypeptides or ActRIIA-based GDF trap polypeptides, including homodimer and/or fusion proteins thereof, may bind to and/or inhibit signaling by activin (*e.g.*, activin A, activin B, activin C, or activin AB) in a cell-based assay. Any of the above ActRIIA polypeptides or ActRIIA-based GDF trap polypeptides, including homodimer and/or fusion proteins thereof,

may bind to and/or inhibit signaling by GDF11 and/or GDF8 in a cell-based assay. Optionally, any of the above ActRIIA polypeptides or ActRIIB-based GDF trap polypeptides, including homodimer and/or fusion proteins thereof, may bind to and/or inhibit signaling of one or more of activin B, activin C, activin E, GDF7, and Nodal in a cell-based assay.

In certain embodiments, preferred ActRIIA polypeptides and ActRIIA-based GDF-trap polypeptides, for use in accordance with the methods disclosed herein, comprise an amino acid sequence that comprises, consists of, or consists essentially of, the amino acid sequence of SEQ ID NOs: 9, 10, 22, 26, or 28, and polypeptides that are at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to any of the foregoing. An ActRIIA polypeptide or ActRIIA-based GDF-trap polypeptide may include a functional fragment of a natural ActRIIA polypeptide, such as one comprising at least 10, 20 or 30 amino acids of a sequence selected from SEQ ID NOs: 9, 10, 22, 26, or 28 or a sequence of SEQ ID NO:10, lacking the C-terminal 1, 2, 3, 4, 5 or 10 to 15 amino acids and lacking 1, 2, 3, 4 or 5 amino acids at the N-terminus. A preferred polypeptide will comprise a truncation relative to SEQ ID NO:10 of between 2 and 5 amino acids at the N-terminus and no more than 3 amino acids at the C-terminus. A preferred ActRIIA polypeptide for use in the methods described herein consists of, or consists essentially of, the amino acid sequence of SEQ ID NO: 26 or 28.

An ActRII polypeptide (*e.g.* an ActRIIA or ActRIIB polypeptide) or GDF trap polypeptide of the disclosure may include one or more alterations (*e.g.*, amino acid additions, deletions, substitutions, or combinations thereof) in the amino acid sequence of an ActRII polypeptide (*e.g.*, in the ligand-binding domain) relative to a naturally occurring ActRII polypeptide. The alteration in the amino acid sequence may, for example, alter glycosylation of the polypeptide when produced in a mammalian, insect, or other eukaryotic cell or alter proteolytic cleavage of the polypeptide relative to the naturally occurring ActRII polypeptide.

Optionally, ActRII polypeptides (*e.g.* an ActRIIA or ActRIIB polypeptides) and GDF trap polypeptides of the disclosure comprise one or more modified amino acid residues selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent.

In some embodiments, an ActRII polypeptide (*e.g.* an ActRIIA or ActRIIB polypeptide) or GDF trap polypeptide of the disclosure may be a fusion protein that has, as one domain, an ActRII polypeptide or GDF trap polypeptide (*e.g.*, a ligand-binding domain

of an ActRII receptor, optionally with one or more sequence variations) and one or more additional heterologous domains that provide a desirable property, such as improved pharmacokinetics, easier purification, targeting to particular tissues, *etc*. For example, a domain of a fusion protein may enhance one or more of *in vivo* stability, *in vivo* half-life, uptake/administration, tissue localization or distribution, formation of protein complexes, multimerization of the fusion protein, and/or purification. ActRII polypeptide and GDF trap fusion proteins may include an immunoglobulin Fc domain (wild-type or mutant) or a serum albumin. In certain embodiments, an ActRII polypeptide and GDF trap fusion protein comprises a relatively unstructured linker positioned between the Fc domain and the ActRII or GDF trap domain. This unstructured linker may correspond to the roughly 15 amino acid unstructured region at the C-terminal end of the extracellular domain of ActRII or GDF trap (the "tail"), or it may be an artificial sequence of between 3 and 5, 15, 20, 30, 50 or more amino acids that are relatively free of secondary structure. A linker may be rich in glycine and proline residues and may, for example, contain repeating sequences of threonine/serine and glycines [*e.g.*, TG_4 (SEQ ID NO:52), TG_3 (SEQ ID NO:53), or SG_4 (SEQ ID NO:54) singlets or repeats] or a series of three glycines. A fusion protein may include a purification subsequence, such as an epitope tag, a FLAG tag, a polyhistidine sequence, and a GST fusion. In certain embodiments, an ActRII fusion protein or GDF trap fusion comprises a leader sequence. The leader sequence may be a native ActRII leader sequence (*e.g.*, a native ActRIIA or ActRIIB leader sequence) or a heterologous leader sequence. In certain embodiments, the leader sequence is a tissue plasminogen activator (TPA) leader sequence. In some embodiment, an ActRII fusion protein or GDF trap fusion protein comprises an amino acid sequence as set forth in the formula A-B-C. The B portion is an N- and C-terminally truncated ActRII or GDF trap polypeptide as described herein. The A and C portions may be independently zero, one, or more than one amino acids, and both A and C portions are heterologous to B. The A and/or C portions may be attached to the B portion via a linker sequence.

Optionally, ActRII polypeptides (*e.g.*, ActRIIA and ActRIIB polypeptides) or GDF trap polypeptides, including variants and fusion proteins thereof, to be used in accordance with the methods disclosed herein bind to one or more ActRIIB ligands (*e.g.*, activin A, activin B, activin AB, activin C, activin E, GDF11, GDF8, BMP6, BMP7, and/or Nodal) with a Kd less than 10 micromolar, less than 1 micromolar, less than 100 nanomolar, less than 10 nanomolar, or less than 1 nanomolar. Optionally, such ActRII polypeptides or GDF trap

polypeptides inhibit ActRII signaling, such as ActRIIA and/or ActRIIB intracellular signal transduction events triggered by an ActRII ligand (*e.g.*, SMAD 2/3 and/or SMAD 1/5/8 signaling).

In certain aspects, the disclosure provides pharmaceutical preparations comprising an ActRII antagonist of the present disclosure (*e.g.*, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap polypeptide) and a pharmaceutically acceptable carrier. A pharmaceutical preparation may also include one or more additional compounds such as a compound that is used to treat a disorder or condition described herein (*e.g.*, an additional compound that increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associatd with *SF3B1* mutations in a subject in need thereof). Preferably, a pharmaceutical preparation of the disclosure is substantially pyrogen-free. In general, it is preferable that an ActRIIA polypeptide, an ActRIIB polypeptide, or a GDF trap polypeptide be expressed in a mammalian cell line that mediates suitably natural glycosylation of the polypeptide so as to diminish the likelihood of an unfavorable immune response in a patient. Human and CHO cell lines have been used successfully, and it is expected that other common mammalian expression vectors will be useful. In some embodiments, preferable ActRIIA polypeptides, ActRIIB polypeptides, and GDF trap polypeptides are glycosylated and have a glycosylation pattern that is obtainable from a mammalian cell, preferably a CHO cell. In certain embodiments, the disclosure provides packaged pharmaceuticals comprising a pharmaceutical preparation described herein and labeled for use in one or more of increasing red blood cell levels and/or hemoglobin in a mammal (preferably a human), treating or preventing anemia in a mammal (preferably a human), treating sideroblastic anemia or MDS in a mamamal (preferably a human), and/or treating or preventing one or more complications of sideroblastic anemia or MDS (*e.g.,* anemia, vaso-occlusive crisis, *etc.*) in a mammal (preferably a human) or treat or prevent a disorder associatd with *SF3B1* mutations in a mammal (preferably a human).

In certain aspects, the disclosure provides nucleic acids encoding an ActRII polypeptide (*e.g.*, an ActRIIA or ActRIIB polypeptide) or GDF trap polypeptide. An isolated

polynucleotide may comprise a coding sequence for a soluble ActRII polypeptide or GDF trap polypeptide, such as described herein. For example, an isolated nucleic acid may include a sequence coding for an ActRII polypeptide or GDF trap comprising an extracellular domain (*e.g.*, ligand-binding domain) of an ActRII polypeptide having one or more sequence variations and a sequence that would code for part or all of the transmembrane domain and/or the cytoplasmic domain of an ActRII polypeptide, but for a stop codon positioned within the transmembrane domain or the cytoplasmic domain, or positioned between the extracellular domain and the transmembrane domain or cytoplasmic domain. For example, an isolated polynucleotide coding for a GDF trap may comprise a full-length ActRII polynucleotide sequence such as SEQ ID NO: 1, 4, or 9 or having one or more variations, or a partially truncated version, said isolated polynucleotide further comprising a transcription termination codon at least six hundred nucleotides before the 3'-terminus or otherwise positioned such that translation of the polynucleotide gives rise to an extracellular domain optionally fused to a truncated portion of a full-length ActRII. Nucleic acids disclosed herein may be operably linked to a promoter for expression, and the disclosure provides cells transformed with such recombinant polynucleotides. Preferably the cell is a mammalian cell, such as a CHO cell.

In certain aspects, the disclosure provides methods for making an ActRII polypeptide or a GDF trap. Such a method may include expressing any of the nucleic acids disclosed herein (*e.g.*, SEQ ID NO: 8, 13, 27, 32, 39, 42, 46, or 48) in a suitable cell, such as a Chinese hamster ovary (CHO) cell. Such a method may comprise: a) culturing a cell under conditions suitable for expression of the GDF trap polypeptide, wherein said cell is transformed with a GDF trap expression construct; and b) recovering the GDF trap polypeptide so expressed. GDF trap polypeptides may be recovered as crude, partially purified or highly purified fractions using any of the well-known techniques for obtaining protein from cell cultures.

In certain aspects, the present disclosure relates to an antibody, or combination of antibodies, that antagonize ActRII activity (*e.g.*, inhibition of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 and/or SMAD 1/5/8 signaling). In particular, the disclosure provides methods of using an antibody ActRII antagonist, or combination of antibody ActRII antagonists, to, *e.g.*, increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction,

hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with *SF3B1, DNMT3A,* and/or *TET2* mutations in a subject in need thereof.

In certain embodiments, a preferred antibody ActRII antagonist of the disclosure is an antibody, or combination of antibodies, that binds to and/or inhibits activity of at least GDF11 (*e.g.*, GDF11-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). Optionally, the antibody, or combination of antibodies, further binds to and/or inhibits activity of GDF8 (*e.g.*, GDF8-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling), particularly in the case of a multispecific antibody that has binding affinity for both GDF11 and GDF8 or in the context of a combination of one or more anti-GDF11 antibody and one or more anti-GDF8 antibody. Optionally, an antibody, or combination of antibodies, of the disclosure does not substantially bind to and/or inhibit activity of activin A (*e.g.*, activin A-mediated activation of ActRIIA or ActRIIB signaling transduction, such as SMAD 2/3 signaling). In some embodiments, an antibody, or combination of antibodies, of the disclosure that binds to and/or inhibits the activity of GDF11 and/or GDF8 further binds to and/or inhibits activity of one of more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, and Nodal (*e.g.*, activation of ActRIIA or ActRIIB signaling transduction, such as SMAD 2/3 and/or SMAD 1/5/8 signaling), particularly in the case of a multispecific antibody that has binding affinity for multiple ActRII ligands or in the context of a combination multiple antibodies – each having binding affinity for a different ActRII ligand.

In part, the disclosure demonstrates that ActRII antagonists may be used in combination (*e.g.*, administered at the same time or different times, but generally in such a manner as to achieve overlapping pharmacological effects) with EPO receptor activators to increase red blood cell levels (erythropoiesis) or, as examples, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with *SF3B1, DNMT3A,* and/or *TET2* mutations in a subject in need thereof. In part, the disclosure demonstrates that a GDF trap can be administered in combination with an EPO receptor activator to synergistically increase formation of red blood cells in a patient,

particularly in sideroblastic anemia or MDS patients. Thus, the effect of this combined treatment can be significantly greater than the sum of the effects of the ActRII antagonists and the EPO receptor activator when administered separately at their respective doses. In certain embodiments, this synergism may be advantageous since it enables target levels of red blood cells to be attained with lower doses of an EPO receptor activator, thereby avoiding potential adverse effects or other problems associated with higher levels of EPO receptor activation. Accordingly, in certain embodiments, the methods of the present disclosure (*e.g.*, methods of increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with *SF3B1, DNMT3A,* and/or *TET2* mutations in a subject in need thereof) comprise administering a patient in need thereof one or more ActRII antagonists (*e.g.*, ActRIIA polypeptides, ActRIIB polypeptides, and/or GDF trap polypeptides) in combination with one or more EPO receptor activators.

An EPO receptor activator may stimulate erythropoiesis by directly contacting and activating EPO receptor. In certain embodiments, the EPO receptor activator is one of a class of compounds based on the 165 amino-acid sequence of native EPO and generally known as erythropoiesis-stimulating agents (ESAs), examples of which are epoetin alfa, epoetin beta, epoetin delta, and epoetin omega. In other embodiments, ESAs include synthetic EPO proteins (SEPs) and EPO derivatives with nonpeptidic modifications conferring desirable pharmacokinetic properties (lengthened circulating half-life), examples of which are darbepoetin alfa and methoxy-polyethylene-glycol epoetin beta. In certain embodiments, an EPO receptor activator may be an EPO receptor agonist that does not incorporate the EPO polypeptide backbone or is not generally classified as an ESA. Such EPO receptor agonists may include, but are not limited to, peptidic and nonpeptidic mimetics of EPO, agonistic antibodies targeting EPO receptor, fusion proteins comprising an EPO mimetic domain, and erythropoietin receptor extended-duration limited agonists (EREDLA).

In certain embodiments, an EPO receptor activator may stimulate erythropoiesis indirectly, without contacting EPO receptor itself, by enhancing production of endogenous EPO. For example, hypoxia-inducible transcription factors (HIFs) are endogenous

stimulators of EPO gene expression that are suppressed (destabilized) under normoxic conditions by cellular regulatory mechanisms. In part, the disclosure provides increased erythropoiesis in a patient by combined treatment with a GDF trap and an indirect EPO receptor activator with HIF stabilizing properties, such as a prolyl hydroxylase inhibitor.

In certain instances, when administering a GDF trap polypeptide for these other therapeutic indications, it may be desirable to monitor the effects on red blood cells during administration of the ActRII antagonist, or to determine or adjust the dosing of the ActRII antagonist, in order to reduce undesired effects on red blood cells. For example, increases in red blood cell levels, hemoglobin levels, or hematocrit levels may cause increases in blood pressure.

BRIEF DESCRIPTION OF THE DRAWINGS

This patent or patent application filed contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

Figure 1 shows an alignment of extracellular domains of human ActRIIA (SEQ ID NO: 56) and human ActRIIB (SEQ ID NO: 2) with the residues that are deduced herein, based on composite analysis of multiple ActRIIB and ActRIIA crystal structures, to directly contact ligand indicated with boxes.

Figure 2 shows a multiple sequence alignment of various vertebrate ActRIIB proteins and human ActRIIA (SEQ ID NOs: 57-64).

Figure 3 shows the purification of ActRIIA-hFc expressed in CHO cells. The protein purifies as a single, well-defined peak as visualized by sizing column (top panel) and Coomassie stained SDS-PAGE (bottom panel) (left lane: molecular weight standards; right lane: ActRIIA-hFc).

Figure 4 shows the binding of ActRIIA-hFc to activin and GDF-11, as measured by Biacore™ assay.

Figures 5A and 5B shows the effects of ActRIIA-hFc on red blood cell counts in female non-human primates (NHPs). Female cynomolgus monkeys (four groups of five monkeys each) were treated with placebo or 1 mg/kg, 10 mg/kg or 30 mg/kg of ActRIIA-hFc

on day 0, day 7, day 14, and day 21. Figure 5A shows red blood cell (RBC) counts. Figure 5B shows hemoglobin levels. Statistical significance is relative to baseline for each treatment group. At day 57, two monkeys remained in each group.

Figures 6A and 6B shows the effects of ActRIIA-hFc on red blood cell counts in male non-human primates. Male cynomolgus monkeys (four groups of five monkeys each) were treated with placebo or 1 mg/kg, 10 mg/kg, or 30 mg/kg of ActRIIA-hFc on day 0, day 7, day 14, and day 21. Figure 6A shows red blood cell (RBC) counts. Figure 6B shows hemoglobin levels. Statistical significance is relative to baseline for each treatment group. At day 57, two monkeys remained in each group.

Figures 7A and 7B shows the effects of ActRIIA-hFc on reticulocyte counts in female non-human primates. Cynomolgus monkeys (four groups of five monkeys each) were treated with placebo or 1 mg/kg, 10 mg/kg, or 30 mg/kg of ActRIIA-hFc on day 0, day 7, day 14, and day 21. Figure 7A shows absolute reticulocyte counts. Figure 7B shows the percentage of reticulocytes relative to RBCs. Statistical significance is relative to baseline for each group. At day 57, two monkeys remained in each group.

Figures 8A and 8B shows the effects of ActRIIA-hFc on reticulocyte counts in male non-human primates. Cynomolgus monkeys (four groups of five monkeys each) were treated with placebo or 1 mg/kg, 10 mg/kg, or 30 mg/kg of ActRIIA-hFc on day 0, day 7, day 14, and day 21. Figure 8A shows absolute reticulocyte counts. Figure 8B shows the percentage of reticulocytes relative to RBCs. Statistical significance is relative to baseline for each group. At day 57, two monkeys remained in each group.

Figure 9 shows results from the human clinical trial described in Example 5, where the area-under-curve (AUC) and administered dose of ActRIIA-hFc have a linear correlation, regardless of whether ActRIIA-hFc was administered intravenously (IV) or subcutaneously (SC).

Figure 10 shows a comparison of serum levels of ActRIIA-hFc in patients administered IV or SC.

Figure 11 shows bone alkaline phosphatase (BAP) levels in response to different dose levels of ActRIIA-hFc. BAP is a marker for anabolic bone growth.

Figure 12 depicts the median change from baseline of hematocrit levels from the human clinical trial described in Example 5. ActRIIA-hFc was administered intravenously (IV) at the indicated dosage.

Figure 13 depicts the median change from baseline of hemoglobin levels from the human clinical trial described in Example 5. ActRIIA-hFc was administered intravenously (IV) at the indicated dosage.

Figure 14 depicts the median change from baseline of RBC (red blood cell) count from the human clinical trial described in Example 5. ActRIIA-hFc was administered intravenously (IV) at the indicated dosage.

Figure 15 depicts the median change from baseline of reticulocyte count from the human clinical trial described in Example 5. ActRIIA-hFc was administered intravenously (IV) at the indicated dosage.

Figure 16 shows the full amino acid sequence for the GDF trap ActRIIB(L79D 20-134)-hFc (SEQ ID NO:38), including the TPA leader sequence (double underlined), ActRIIB extracellular domain (residues 20-134 in SEQ ID NO: 1; underlined), and hFc domain. The aspartate substituted at position 79 in the native sequence is double underlined and highlighted, as is the glycine revealed by sequencing to be the N-terminal residue in the mature fusion protein.

Figures 17A and 17B show a nucleotide sequence encoding ActRIIB(L79D 20-134)-hFc. SEQ ID NO:39 corresponds to the sense strand, and SEQ ID NO:40 corresponds to the antisense strand. The TPA leader (nucleotides 1-66) is double underlined, and the ActRIIB extracellular domain (nucleotides 76-420) is underlined.

Figure 18 shows the full amino acid sequence for the truncated GDF trap ActRIIB(L79D 25-131)-hFc (SEQ ID NO:41), including the TPA leader (double underlined), truncated ActRIIB extracellular domain (residues 25-131 in SEQ ID NO:1; underlined), and hFc domain. The aspartate substituted at position 79 in the native sequence is double underlined and highlighted, as is the glutamate revealed by sequencing to be the N-terminal residue in the mature fusion protein.

Figures 19A and 19B shows a nucleotide sequence encoding ActRIIB(L79D 25-131)-hFc. SEQ ID NO:42 corresponds to the sense strand, and SEQ ID NO:43 corresponds to the antisense strand. The TPA leader (nucleotides 1-66) is double underlined, and the truncated

ActRIIB extracellular domain (nucleotides 76-396) is underlined. The amino acid sequence for the ActRIIB extracellular domain (residues 25-131 in SEQ ID NO: 1) is also shown.

Figure 20 shows the amino acid sequence for the truncated GDF trap ActRIIB(L79D 25-131)-hFc without a leader (SEQ ID NO:44). The truncated ActRIIB extracellular domain (residues 25-131 in SEQ ID NO:1) is underlined. The aspartate substituted at position 79 in the native sequence is double underlined and highlighted, as is the glutamate revealed by sequencing to be the N-terminal residue in the mature fusion protein.

Figure 21 shows the amino acid sequence for the truncated GDF trap ActRIIB(L79D 25-131) without the leader, hFc domain, and linker (SEQ ID NO:45). The aspartate substituted at position 79 in the native sequence is underlined and highlighted, as is the glutamate revealed by sequencing to be the N-terminal residue in the mature fusion protein.

Figures 22A and 22B shows an alternative nucleotide sequence encoding ActRIIB(L79D 25-131)-hFc. SEQ ID NO:46 corresponds to the sense strand, and SEQ ID NO:47 corresponds to the antisense strand. The TPA leader (nucleotides 1-66) is double underlined, the truncated ActRIIB extracellular domain (nucleotides 76-396) is underlined, and substitutions in the wild-type nucleotide sequence of the extracellular domain are double underlined and highlighted (compare with SEQ ID NO:42, Figures 19A and 19B). The amino acid sequence for the ActRIIB extracellular domain (residues 25-131 in SEQ ID NO:1) is also shown.

Figure 23 shows nucleotides 76-396 (SEQ ID NO:48) of the alternative nucleotide sequence shown in Figures 22A and 22B (SEQ ID NO:46). The same nucleotide substitutions indicated in Figures 22A and 22B are also underlined and highlighted here. SEQ ID NO:48 encodes only the truncated ActRIIB extracellular domain (corresponding to residues 25-131 in SEQ ID NO:1) with a L79D substitution, *e.g.*, ActRIIB(L79D 25-131).

Figure 24 shows the effect of treatment with ActRIIB(L79D 20-134)-hFc (gray) or ActRIIB(L79D 25-131)-hFc (black) on the absolute change in red blood cell concentration from baseline in cynomolgus monkey. VEH = vehicle. Data are means $\pm$ SEM. n = 4-8 per group.

Figure 25 shows the effect of treatment with ActRIIB(L79D 20-134)-hFc (gray) or ActRIIB(L79D 25-131)-hFc (black) on the absolute change in hematocrit from baseline in cynomolgus monkey. VEH = vehicle. Data are means $\pm$ SEM. n = 4-8 per group.

Figure 26 shows the effect of treatment with ActRIIB(L79D 20-134)-hFc (gray) or ActRIIB(L79D 25-131)-hFc (black) on the absolute change in hemoglobin concentration from baseline in cynomolgus monkey. VEH = vehicle. Data are means ± SEM. n = 4-8 per group.

Figure 27 shows the effect of treatment with ActRIIB(L79D 20-134)-hFc (gray) or ActRIIB(L79D 25-131)-hFc (black) on the absolute change in circulating reticulocyte concentration from baseline in cynomolgus monkey. VEH = vehicle. Data are means ± SEM. n = 4-8 per group.

Figure 28 shows the effect of combined treatment with erythropoietin (EPO) and ActRIIB(L79D 25-131)-hFc for 72 hours on hematocrit in mice. Data are means ± SEM (n = 4 per group), and means that are significantly different from each other ($p < 0.05$, unpaired t-test) are designated by different letters. Combined treatment increased hematocrit by 23% compared to vehicle, a synergistic increase greater than the sum of the separate effects of EPO and ActRIIB(L79D 25-131)-hFc.

Figure 29 shows the effect of combined treatment with EPO and ActRIIB(L79D 25-131)-hFc for 72 hours on hemoglobin concentrations in mice. Data are means ± SEM (n = 4 per group), and means that are significantly different from each other ($p < 0.05$) are designated by different letters. Combined treatment increased hemoglobin concentrations by 23% compared to vehicle, which was also a synergistic effect.

Figure 30 shows the effect of combined treatment with EPO and ActRIIB(L79D 25-131)-hFc for 72 hours on red blood cell concentrations in mice. Data are means ± SEM (n = 4 per group), and means that are significantly different from each other ($p < 0.05$) are designated by different letters. Combined treatment increased red blood cell concentrations by 20% compared to vehicle, which was also a synergistic effect.

Figure 31 shows the effect of combined treatment with EPO and ActRIIB(L79D 25-131)-hFc for 72 hours on numbers of erythropoietic precursor cells in mouse spleen. Data are means ± SEM (n = 4 per group), and means that are significantly different from each other ($p < 0.01$) are designated by different letters. Whereas EPO alone increased the number of basophilic erythroblasts (BasoE) dramatically at the expense of late-stage precursor maturation, combined treatment increased BasoE numbers to a lesser but still significant extent while supporting undiminished maturation of late-stage precursors.

Figure 32 shows that a GDF trap can mitigate ineffective erythropoiesis and ameliorate anemia at multiple stages of disease severity in a mouse model of MDS. (A) RBC numbers and hemoglobin concentrations (top) and morphological enumeration of hematopoietic precursors in bone marrow (bottom) in wild-type (Wt) mice treated with vehicle (Tris-bufered saline, TBS, n = 5), MDS mice treated with TBS (n = 5), and MDS mice treated with ActRIIB(L79D 25-131)-mFc (RAP-536, 10 mg/kg, n = 6) twice weekly for 8 weeks ending at approximately 6 months of age (early stage). *P < 0.05, **P < 0.01, vs. TBS-treated MDS mice; ### P < 0.001 vs. wild-type mice. (B) Same endpoints as in panel A in MDS mice treated with RAP-536 (10 mg/kg, twice weekly, n = 5) or TBS (n = 4) for 7 weeks ending at approximately 12 months of age (late stage). *P < 0.05 vs. TBS-treated MDS mice. Data are means ± SEM.

DETAIL DESCRIPTION OF THE INVENTION

1. Overview

The transforming growth factor-beta (TGF-beta) superfamily contains a variety of growth factors that share common sequence elements and structural motifs. These proteins are known to exert biological effects on a large variety of cell types in both vertebrates and invertebrates. Members of the superfamily perform important functions during embryonic development in pattern formation and tissue specification and can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis, cardiogenesis, hematopoiesis, neurogenesis, and epithelial cell differentiation. By manipulating the activity of a member of the TGF-beta family, it is often possible to cause significant physiological changes in an organism. For example, the Piedmontese and Belgian Blue cattle breeds carry a loss-of-function mutation in the GDF8 (also called myostatin) gene that causes a marked increase in muscle mass [see, *e.g.*, Grobet *et al.* (1997) Nat Genet. 17(1):71-4]. Furthermore, in humans, inactive alleles of GDF8 are associated with increased muscle mass and, reportedly, exceptional strength [see, *e.g.*, Schuelke *et al.* (2004) N Engl J Med, 350:2682-8].

TGF-β signals are mediated by heteromeric complexes of type I and type II serine/threonine kinase receptors, which phosphorylate and activate downstream SMAD proteins (*e.g.*, SMAD proteins 1, 2, 3, 5, and 8) upon ligand stimulation [see, *e.g.*, Massagué (2000) Nat. Rev. Mol. Cell Biol. 1:169-178]. These type I and type II receptors are transmembrane proteins, composed of a ligand-binding extracellular domain with cysteine-

rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine specificity. Type I receptors are essential for signaling. Type II receptors are required for binding ligands and for activation of type I receptors. Type I and II activin receptors form a stable complex after ligand binding, resulting in phosphorylation of type I receptors by type II receptors.

Two related type II receptors (ActRII), ActRIIA and ActRIIB, have been identified as the type II receptors for activins [see, *e.g.*, Mathews and Vale (1991) Cell 65:973-982; and Attisano *et al.* (1992) Cell 68: 97-108]. Besides activins, ActRIIA and ActRIIB can biochemically interact with several other TGF-β family proteins including, for example, BMP6, BMP7, Nodal, GDF8, and GDF11 [see, *e.g.*, Yamashita *et al.* (1995) J. Cell Biol. 130:217-226; Lee and McPherron (2001) Proc. Natl. Acad. Sci. USA 98:9306-9311; Yeo and Whitman (2001) Mol. Cell 7: 949-957; and Oh *et al.* (2002) Genes Dev. 16:2749-54]. ALK4 is the primary type I receptor for activins, particularly for activin A, and ALK-7 may serve as a receptor for other activins as well, particularly for activin B. In certain embodiments, the present disclosure relates to antagonizing a ligand of an ActRII receptor (also referred to as an ActRII ligand) with one or more inhibitor agents disclosed herein, particularly inhibitor agents that can antagonize one or more of activin A, activin B, activin C, activin E, GDF11 and/or GDF8.

Activins are dimeric polypeptide growth factors that belong to the TGF-beta superfamily. There are three principal activin forms (A, B, and AB) that are homo/heterodimers of two closely related β subunits ($\beta_A\beta_A$, $\beta_B\beta_B$, and $\beta_A\beta_B$, respectively). The human genome also encodes an activin C and an activin E, which are primarily expressed in the liver, and heterodimeric forms containing β_C or β_E are also known.

In the TGF-beta superfamily, activins are unique and multifunctional factors that can stimulate hormone production in ovarian and placental cells, support neuronal cell survival, influence cell-cycle progress positively or negatively depending on cell type, and induce mesodermal differentiation at least in amphibian embryos [DePaolo *et al.* (1991) Proc Soc Ep Biol Med. 198:500-512; Dyson *et al.* (1997) Curr Biol. 7:81-84; and Woodruff (1998) Biochem Pharmacol. 55:953-963]. Moreover, erythroid differentiation factor (EDF) isolated from the stimulated human monocytic leukemic cells was found to be identical to activin A [Murata *et al.* (1988) PNAS, 85:2434]. It has been suggested that activin A promotes erythropoiesis in the bone marrow. In several tissues, activin signaling is antagonized by its related heterodimer, inhibin. For example, during the release of follicle-stimulating hormone

(FSH) from the pituitary, activin promotes FSH secretion and synthesis, while inhibin prevents FSH secretion and synthesis. Other proteins that may regulate activin bioactivity and/or bind to activin include follistatin (FS), follistatin-related protein (FSRP, also known as FLRG or FSTL3), and α_2-macroglobulin.

As described herein, agents that bind to "activin A" are agents that specifically bind to the β_A subunit, whether in the context of an isolated β_A subunit or as a dimeric complex (*e.g.*, a $\beta_A\beta_A$ homodimer or a $\beta_A\beta_B$ heterodimer). In the case of a heterodimer complex (*e.g.*, a $\beta_A\beta_B$ heterodimer), agents that bind to "activin A" are specific for epitopes present within the β_A subunit, but do not bind to epitopes present within the non-β_A subunit of the complex (*e.g.*, the β_B subunit of the complex). Similarly, agents disclosed herein that antagonize (inhibit) "activin A" are agents that inhibit one or more activities as mediated by a β_A subunit, whether in the context of an isolated β_A subunit or as a dimeric complex (*e.g.*, a $\beta_A\beta_A$ homodimer or a $\beta_A\beta_B$ heterodimer). In the case of $\beta_A\beta_B$ heterodimers, agents that inhibit "activin A" are agents that specifically inhibit one or more activities of the β_A subunit, but do not inhibit the activity of the non-β_A subunit of the complex (*e.g.*, the β_B subunit of the complex). This principle applies also to agents that *bind to* and/or *inhibit* "activin B", "activin C", and "activin E". Agents disclosed herein that antagonize "activin AB" are agents that inhibit one or more activities as mediated by the β_A subunit and one or more activities as mediated by the β_B subunit.

Nodal proteins have functions in mesoderm and endoderm induction and formation, as well as subsequent organization of axial structures such as heart and stomach in early embryogenesis. It has been demonstrated that dorsal tissue in a developing vertebrate embryo contributes predominantly to the axial structures of the notochord and pre-chordal plate while it recruits surrounding cells to form non-axial embryonic structures. Nodal appears to signal through both type I and type II receptors and intracellular effectors known as SMAD proteins. Studies support the idea that ActRIIA and ActRIIB serve as type II receptors for Nodal [see, *e.g.*, Sakuma *et al.* (2002) Genes Cells. 2002, 7:401-12]. It is suggested that Nodal ligands interact with their co-factors (*e.g.*, cripto) to activate activin type I and type II receptors, which phosphorylate SMAD2. Nodal proteins are implicated in many events critical to the early vertebrate embryo, including mesoderm formation, anterior patterning, and left-right axis specification. Experimental evidence has demonstrated that Nodal signaling activates pAR3-Lux, a luciferase reporter previously shown to respond specifically to activin and TGF-beta. However, Nodal is unable to induce pTlx2-Lux, a

reporter specifically responsive to bone morphogenetic proteins. Recent results provide direct biochemical evidence that Nodal signaling is mediated by both activin-TGF-beta pathway SMADs, SMAD2 and SMAD3. Further evidence has shown that the extracellular cripto protein is required for Nodal signaling, making it distinct from activin or TGF-beta signaling.

Growth and differentiation factor-8 (GDF8) is also known as myostatin. GDF8 is a negative regulator of skeletal muscle mass. GDF8 is highly expressed in the developing and adult skeletal muscle. The GDF8 null mutation in transgenic mice is characterized by a marked hypertrophy and hyperplasia of the skeletal muscle [McPherron *et al.*, Nature (1997) 387:83-90]. Similar increases in skeletal muscle mass are evident in naturally occurring mutations of GDF8 in cattle [see, *e.g.*, Ashmore *et al.* (1974) Growth, 38:501-507; Swatland and Kieffer (1994) J. Anim. Sci. 38:752-757; McPherron and Lee (1997) Proc. Natl. Acad. Sci. USA 94:12457-12461; and Kambadur *et al.* (1997) Genome Res. 7:910-915] and, strikingly, in humans [see, *e.g.*, Schuelke *et a*l. (2004) N Engl J Med 350:2682-8]. Studies have also shown that muscle wasting associated with HIV-infection in humans is accompanied by increases in GDF8 protein expression [see, *e.g.*, Gonzalez-Cadavid *et al.* (1998) PNAS 95:14938-43]. In addition, GDF8 can modulate the production of muscle-specific enzymes (*e.g.*, creatine kinase) and modulate myoblast cell proliferation [see, *e.g.* international patent application publication no. WO 00/43781]. The GDF8 propeptide can noncovalently bind to the mature GDF8 domain dimer, inactivating its biological activity [see, *e.g.*, Miyazono *et al.* (1988) J. Biol. Chem., 263: 6407-6415; Wakefield *et al.* (1988) J. Biol. Chem., 263: 7646-7654; and Brown *et al.* (1990) Growth Factors, 3: 35-43]. Other proteins which bind to GDF8 or structurally related proteins and inhibit their biological activity include follistatin, and potentially, follistatin-related proteins [see, *e.g.*, Gamer *et al.* (1999) Dev. Biol., 208: 222-232].

Growth and differentiation factor-11 (GDF11), also known as BMP11, is a secreted protein [McPherron *et al.* (1999) Nat. Genet. 22: 260-264]. GDF11 is expressed in the tail bud, limb bud, maxillary and mandibular arches, and dorsal root ganglia during mouse development [see, *e.g.*, Nakashima *et al.* (1999) Mech. Dev. 80: 185-189]. GDF11 plays a unique role in patterning both mesodermal and neural tissues [see, *e.g.*, Gamer *et al.* (1999) Dev Biol., 208:222-32]. GDF11 was shown to be a negative regulator of chondrogenesis and myogenesis in developing chick limb [see, *e.g.*, Gamer *et al.* (2001) Dev Biol. 229:407-20]. The expression of GDF11 in muscle also suggests its role in regulating muscle growth in a

similar way to GDF8. In addition, the expression of GDF11 in brain suggests that GDF11 may also possess activities that relate to the function of the nervous system. Interestingly, GDF11 was found to inhibit neurogenesis in the olfactory epithelium [see, *e.g.*, Wu *et al.* (2003) Neuron. 37:197-207].

Bone morphogenetic protein (BMP7), also called osteogenic protein-1 (OP-1), is well known to induce cartilage and bone formation. In addition, BMP7 regulates a wide array of physiological processes. For example, BMP7 may be the osteoinductive factor responsible for the phenomenon of epithelial osteogenesis. It is also found that BMP7 plays a role in calcium regulation and bone homeostasis. Like activin, BMP7 binds to type II receptors, ActRIIA and ActRIIB. However, BMP7 and activin recruit distinct type I receptors into heteromeric receptor complexes. The major BMP7 type I receptor observed was ALK2, while activin bound exclusively to ALK4 (ActRIIB). BMP7 and activin elicited distinct biological responses and activated different SMAD pathways [see, *e.g.*, Macias-Silva *et al.* (1998) J Biol Chem. 273:25628-36].

As demonstrated herein, ActRII polypeptides (*e.g.*, ActRIIA and ActRIIB polypeptides) can be used to increase red blood cell levels *in vivo*. In certain examples, it is shown that a GDF trap polypeptide (specifically a variant ActRIIB polypeptide) is characterized by unique biological properties in comparison to a corresponding sample of a wild-type (unmodified) ActRII polypeptide. This GDF trap is characterized, in part, by substantial loss of binding affinity for activin A, and therefore significantly diminished capacity to antagonize activin A activity, but retains near wild-type levels of binding and inhibition of GDF11. *In vivo*, the GDF trap is more effective at increasing red blood cell levels as compared to the wild-type ActRIIB polypeptide and has beneficial effects in a variety of models for anemia. It should be noted that hematopoiesis is a complex process, regulated by a variety of factors, including erythropoietin, G-CSF, and iron homeostasis. The terms "increase red blood cell levels" and "promote red blood cell formation" refer to clinically observable metrics, such as hematocrit, red blood cell counts, and hemoglobin measurements, and are intended to be neutral as to the mechanism by which such changes occur.

The data of the present disclosure therefore indicate that the observed biological activity of an ActRII polypeptide, with respect to red blood cell levels, is not dependent on activin A inhibition. However, it is to be noted that the unmodified ActRIIB polypeptide, which retains activin A binding, still demonstrates the capacity to increase red blood cells *in*

vivo. Furthermore, an ActRIIB or ActRIIA polypeptide that retains activin A inhibition may be more desirable in some applications, in comparison to a GDF trap having diminished binding affinity for activin A, where more modest gains in red blood cell levels are desirable and/or where some level of off-target activity is acceptable (or even desirable).

Accordingly, the methods of the present disclosure, in general, are directed to the use of one or more ActRII antagonist agents described herein, optionally in combination with one or more supportive therapies, to increase red blood cell formation in a subject in need thereof, treat or prevent an anemia in a subject in need thereof, to treat myelodysplastic syndrome, to treat sideroblastic anemia in a subject in need thereof, and to treat or prevent one or more complications of sideroblastic anemia or myelodysplastic syndrome (*e.g.*, anemia, blood transfusion requirement, iron overload, neutropenia, splenomegaly, progression to acute myeloid leukemia), and, optionally, in a subgroup of patients with ring sideroblasts and/or one or more mutations in the *SF3B1, DNMT3A,* and/or *TET2* gene in bone marrow cells. Another subgroup of patients that are identified as being particularly likely to respond to an ActRII antagonist is patients that have failed prior treatment with EPO therapy or other EPO receptor activator therapy.

As evidenced herein, the ActRII antagonist agents described may be used in combination with an EPO receptor activator or in patients that have failed treatment with EPO receptor activators. EPO is a glycoprotein hormone involved in the growth and maturation of erythroid progenitor cells into erythrocytes. EPO is produced by the liver during fetal life and by the kidney in adults. Decreased production of EPO, which commonly occurs in adults as a consequence of renal failure, leads to anemia. EPO has been produced by genetic engineering techniques based on expression and secretion of the protein from a host cell transfected with the EPO gene. Administration of such recombinant EPO has been effective in the treatment of anemia. For example, Eschbach *et al.* (1987, N Engl J Med 316:73) describe the use of EPO to correct anemia caused by chronic renal failure.

Effects of EPO are mediated through its binding to, and activation of, a cell surface receptor belonging to the cytokine receptor superfamily and designated the EPO receptor. The human and murine EPO receptors have been cloned and expressed [see, *e.g.*, D'Andrea *et al.* (1989) Cell 57:277; Jones *et al.* (1990) Blood 76:31; Winkelman *et al.* (1990) Blood 76:24; and U.S. Pat. No. 5,278,065]. The human EPO receptor gene encodes a 483-amino-acid transmembrane protein comprising an extracellular domain of approximately 224 amino acids and exhibits approximately 82% amino acid sequence identity with the murine EPO

receptor (see, *e.g.*, U.S. Pat. No. 6,319,499). The cloned, full-length EPO receptor expressed in mammalian cells (66-72 kDa) binds EPO with an affinity (K_D = 100-300 nM) similar to that of the native receptor on erythroid progenitor cells. Thus, this form is thought to contain the main EPO binding determinant and is referred to as the EPO receptor. By analogy with other closely related cytokine receptors, the EPO receptor is thought to dimerize upon agonist binding. Nevertheless, the detailed structure of the EPO receptor, which may be a multimeric complex, and its specific mechanism of activation are not completely understood (see, *e.g.*, U.S. Pat. No. 6,319,499).

Activation of the EPO receptor results in several biological effects. These include increased proliferation of immature erythroblasts, increased differentiation of immature erythroblasts, and decreased apoptosis in erythroid progenitor cells [see, *e.g.*, Liboi *et al.* (1993) Proc Natl Acad Sci USA 90:11351-11355; Koury *et al.* (1990) Science 248:378-381]. The EPO receptor signal transduction pathways mediating proliferation and differentiation appear to be distinct [see, *e.g.*, Noguchi *et al.* (1988) Mol Cell Biol 8:2604; Patel *et al.* (1992) J Biol Chem, 267:21300; and Liboi et al. (1993) Proc Natl Acad Sci USA 90:11351-11355]. Some results suggest that an accessory protein may be required for mediation of the differentiation signal [see, *e.g.*, Chiba *et al.* (1993) Nature 362:646; and Chiba *et al.* (1993) Proc Natl Acad Sci USA 90:11593]. However, there is controversy regarding the role of accessory proteins in differentiation since a constitutively activated form of the receptor can stimulate both proliferation and differentiation [see, *e.g.*, Pharr *et al.* (1993) Proc Natl Acad Sci USA 90:938].

EPO receptor activators include small molecule erythropoiesis-stimulating agents (ESAs) as well as EPO-based compounds. An example of the former is a dimeric peptide-based agonist covalently linked to polyethylene glycol (proprietary names Hematide™ and Omontys®), which has shown erythropoiesis-stimulating properties in healthy volunteers and in patients with both chronic kidney disease and endogenous anti-EPO antibodies [see, *e.g.*, Stead *et al.* (2006) Blood 108:1830-1834; and Macdougall *et al.* (2009) N Engl J Med 361:1848-1855]. Other examples include nonpeptide-based ESAs [see, *e.g.*, Qureshi *et al.* (1999) Proc Natl Acad Sci USA 96:12156-12161].

EPO receptor activators also include compounds that stimulate erythropoiesis indirectly, without contacting EPO receptor itself, by enhancing production of endogenous EPO. For example, hypoxia-inducible transcription factors (HIFs) are endogenous stimulators of EPO gene expression that are suppressed (destabilized) under normoxic

conditions by cellular regulatory mechanisms. Therefore, inhibitors of HIF prolyl hydroxylase enzymes are being investigated for EPO-inducing activity *in vivo*. Other indirect activators of EPO receptor include inhibitors of GATA-2 transcription factor [see, *e.g.*, Nakano *et al.* (2004) Blood 104:4300-4307], which tonically inhibits EPO gene expression, and inhibitors of hemopoietic cell phosphatase (HCP or SHP-1), which functions as a negative regulator of EPO receptor signal transduction [see, *e.g.*, Klingmuller *et al.* (1995) Cell 80:729-738].

As described herein, patients that exhibit ring sideroblasts may be particularly suited to treatment with ActRII antagonists. Sideroblastic anemias can be classified broadly into congenital (inherited) and acquired forms, which can be further subdivided as shown in Table 1.

Table 1. Classification of Sideroblastic Anemias*

Class	Gene	Anemia Severity	Iron Homeostasis
Congenital			
Nonsyndromic			
X-linked	*ALAS2*	Mild to severe	Iron overload
SLC25A38 deficiency	*SLC25A38*	Severe	Iron overload
Glutaredoxin 5 deficiency	*GLRX5*	Mild to severe	Iron overload
Erythropoietic protoporphyria	*FECH*	Mild	---
Syndromic			
X-linked with ataxia	*ABCB7*	Mild to moderate	---
SIFD	Unknown	Severe	Iron overload
Pearson marrow- pancreas Syndrome	mtDNA	Severe	---
Myopathy, lactic acidosis, and sideroblastic anemia (MLASA)	*PUS1/YARS2*	Mild to severe	---
Thiamine-responsive megaloblastic anemia (TRMA)	*SLC19A2*	Severe	---
Syndromic/nonsyndromic of unknown cause	Unknown	Variable	---
Acquired			
Clonal / Neoplastic			
MDS**	Variable	Mild to severe	Iron overload
Metabolic			

Alcoholism	---	Variable	---
Drug-induced	---	Variable	---
Copper deficiency (zinc toxicity)	---	Variable	---
Hypothermia	---	Variable	---

* See Bottomley et al., 2014, Hematol Oncol Clin N Am 28:653-670.

** See table below for MDS subclassifications according to the World Health Organization.

MDS represent the most common class of acquired bone-marrow-failure syndromes in adults. Although MDS are increasingly well understood from a biological standpoint, improved pathologic insight has not yet translated into highly effective or curative therapies for most patients suffering from these disorders. Increasing failure of cellular differentiation in MDS is associated with evolution to secondary acute myeloid leukemia (AML), which is currently defined by the WHO as having at least 20% myeloblasts in the blood or marrow, or the presence of one of several AML-defining karyotypic abnormalities regardless of blast proportion [see, e.g., Vardiman et al. (2009) Blood 114:937-951]. AML is ultimately diagnosed in as many as 30% of MDS patients. Since the biological heterogeneity that underlies MDS translates into wide variations in clinical outcomes, prognostic classification schemes have been developed to predict the natural course of MDS and to counsel patients [Zeidan et al (2013) Curr Hematol Malig Rep 8:351-360]. The International Prognostic Scoring System (IPSS) is an example of one such classification that categorizes patients according to risk profile, ranging from Low (median survival of 5.7 years) to Intermediate 1 (median survival of 3.5 years) to Intermediate 2 (median survival of 1.2 years) to High (median survival of 0.4 years). An alternate system referred to as IPSS-R may also be used for patient stratification. From a patient's perspective, the prognosis helps define the severity of disease and sets expectations as to how it is likely to impact them. From a physician's standpoint, the prognosis provides a means of staging the disease in a manner that can be used to help direct therapy. Notably, such schemes do not typically take patient comorbidities into account and are not intended to predict clinical benefit in relation to a specific therapy.

Additional MDS classification systems have been proposed to facilitate appropriate treatment and management of patients with MDS. Classification has evolved over several decades to incorporate progress in understanding these complex syndromes. The French-American-British (FAB) classification scheme for MDS was proposed in 1982 [Bennett et al.

(1982) Br J Haematol 51:189-199] and served as the basis for a modified classification system established by the WHO in 2001. As summarized in Table 2 below, the current version of the WHO classification (revised in 2008) is based on (1) the percentage of myeloblasts in the bone marrow and peripheral blood, (2) the type and degree of dysplasia, (3) the presence of ring sideroblasts, and (4) the presence of cytogenetic abnormalities. Thus, ring sideroblasts are characteristic of RARS but can also be present in other subtypes of MDS [see, *e.g.*, Juneja et al. (1983) J Clin Pathol 36:566-569; Malcovati et al. (2013) Best Pract Res Clin Haematol 26:377-385]. Depending on the MDS subtype, anemia can occur, for example, in the presence or absence of ring sideroblasts, alone or in combination with abnormally low numbers of neutrophils (neutropenia), low numbers of platelets (thrombocytopenia), or elevated levels of platelets (thrombocytosis). Refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T) is currently included as a provisional entry in the WHO classification (Table 2) within the group of MDS neoplasms unclassifiable (MDS-U). RARS-T is defined as anemia with dysplastic ineffective erythropoiesis and ring sideroblasts ≥ 15% of erythroid precursors, no blasts in peripheral blood and < 5% in the bone marrow, and thrombocytosis with a platelet count ≥ 450 x 10^9/L [Malcovati et al. (2013) Best Pract Res Clin Haematol 26:377-385]. Due to a compromised immune system, patients with neutropenia may be at serious risk of infection and even sepsis, and it is therefore important to treat this condition. Patients with thrombocytopenia are at increased risk of internal hemorrhage, and depending on severity it may also be beneficial to treat this condition.

Table 2. 2008 WHO Classification System for MDS*

MDS Subtype	Blood Findings	Bone Marrow Findings
Refractory cytopenia with unilineage dysplasia (RCUD): (a) refractory anemia, (b) refractory neutropenia, or (c) refractory thrombocytopenia	Predominantly unicytopenia	Unilineage dysplasia: ≥ 10% of cells in one myeloid lineage < 5% blasts < 15% of erythroid precursors are ring sideroblasts
Refractory anemia with ring sideroblasts (RARS)	Anemia No blasts	≥ 15% of erythroid precursors are ring sideroblasts Erythroid dysplasia only < 5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s) No or rare blasts (< 1%) No Auer rods < 1 x 10^9 per L monocytes	Dysplasia in ≥ 10% of cells in ≥ 2 myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes) < 5% blasts No Auer rods ± 15% ring sideroblasts

Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenia(s) < 5% blasts No Auer rods < 1 x 10^9 per L monocytes	Unilineage or multilineage dysplasia 5% – 9% blasts No Auer rods
Refractory anemia with excess blasts-1 (RAEB-2)	Cytopenia(s) 5% – 19% blasts Auer rods ± < 1 x 10^9 per L monocytes	Unilineage or multilineage dysplasia 10% – 19% blasts Auer rods ±
Myelodysplastic syndrome–unclassified (MDS-U) **	Cytopenias < 1% blasts	Unequivocal dysplasia in < 10% of cells in one or more myeloid lineages when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS < 5% blasts
MDS associated with isolated del(5q)	Anemia Usually normal or increased platelet count No or rare blasts (< 1%)	Normal-to-increased megakaryocytes with hypolobated nuclei < 5% blasts Isolated del(5q) cytogenetic abnormality No Auer rods

* From Vardiman et al (2009) Blood 114:937-951

** Includes refractory anemia with ring sideroblasts associated with marked thrombocytosis (RARS-T)

In one embodiment of the disclosure, ActRII antagonists are useful for treating anemia in patients, including MDS patients or patients with sideroblastic anemia, in whom more than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% of erythroid precursors are ring sideroblasts, e.g., in refractory anemia with ring sideroblasts (RARS), RARS associated with marked thrombocytosis (RARS-T), or refractory cytopenia with multilineage dysplasia (RCMD, also known as RCMD-RS in patients where ring sideroblasts are prominent).

Anemia occurs frequently in MDS. Approximately 80% of MDS patients present with anemia, and a substantial percentage of them become dependent on blood transfusions during the course of their disease [Steensma et al. (2006) Mayo Clin Proc 81:104-130]. Some MDS subtypes are characterized by "ineffective erythropoiesis", in which there is impaired differentiation (maturation) of late-stage erythroid precursor cells despite EPO-stimulated hyperproliferation of early-stage erythroid progenitor cells in response to tissue hypoxia. Thus, a key sign of ineffective erythropoiesis is persistent anemia in spite of elevated levels of endogenous EPO. Ineffective erythropoiesis occurs frequently in the RARS subtype of MDS but not the RAEB subtype, which is characterized by relative *hypo*proliferation of the erythroid marrow [Cazzola et al. (1982) Br J Haematol 50:55-62].

Circulating levels of hepcidin, a critical regulator of iron homeostasis, are about an order of magnitude lower in RARS than RAEB [Santini et al. (2011) PLoS One 6:e23109]. Since low levels of hepcidin promote iron absorption, the inappropriately low levels of hepcidin measured in disorders such as RARS and thalassemia are thought to account for iron overload observed in these disorders even in the absence of blood transfusions.

Chronic red blood cell transfusions alleviate anemia but expose patients to multiple risks, including infectious disease, allergic or hemolytic reactions, and exacerbation of iron overload [Rawn (2008) Curr Opin Anaesthesiol 21:664-668; Özcan et al. (2013) Expert Rev Hematol 6:165-189]. As systemic iron levels increase, the body increases ferritin production for iron storage and reduces transferrin receptor production to reduce iron entry into cells. When the iron-binding capacity of circulating transferrin is exceeded, iron is found in the plasma as non-transferrin bound iron (NTBI). In MDS, levels of non-transferrin bound iron are higher in low-risk than high-risk subtypes and highest in RARS [Santini et al. (2011) PLoS One 6:e23109]. Since iron cannot be actively secreted from the body, it initially accumulates in the reticuloendothelial macrophages and is later deposited primarily in parenchymal cells of the heart, liver, and endocrine glands [Siah et al. (2006) Clin Biochem Rev 27:5-16]. Under conditions of iron overload, non-transferrin bound iron changes to its redox-active form known as labile plasma iron, which is transported into cells where it promotes formation of reactive oxygen species. These highly toxic molecules adversely impact hematopoiesis and particularly damage cardiac, hepatic, and endocrine tissues.

The MDS patient population consists mainly of elderly with comorbid conditions – including a propensity for cardiac failure, infection, hemorrhage, and hepatic cirrhosis – and iron overload may rapidly exacerbate such pre-existing conditions. Compared to MDS patients at high risk of developing AML, patients at low or intermediate-1 risk may be more prone to iron overload due to their longer life expectancy. For these reasons, iron chelation therapy to reduce iron burden is considered advisable in patients with low- or intermediate-1 risk MDS subtypes who have a long life expectancy and are anticipated to receive more than 20 RBC transfusions [Temraz et al. (2014) Crit Rev Oncol Hematol 91:64-73].

Novel sequencing techniques have led in the past few years to identification of dozens of genes that are recurrently mutated in MDS. A 2013 list of such genes classified by type is shown in Table 3. One or more such mutations can be found in almost all patients with MDS, and knowing the nature of the genes involved has improved understanding of how MDS develops and evolves, although it has not yet had an impact on treatment. Whole-genome

sequencing applied to MDS patient samples has identified an entirely novel class of cancer-associated genes encoding mRNA splicing (spliceosome) factors. The first such gene identified in MDS was *SF3B1*, which is mutated particularly frequently in patients with RARS [Papaemmanuil et al. (2011) N Engl J Med 365:1384-1395]. Other major categories of mutated genes are epigenetic (DNA methylation) regulators, transcription factors, and signaling molecules [Cazzola et al. (2013) Blood 122:4021-4034; Bejar et al. (2014) Blood 124:2793-2803]. The extent to which these mutations co-occur in MDS patients seems to vary with gene type. For example, approximately 50% of MDS patients possess one of ten genes identified to date encoding mutant splicing factors, but these mutant genes rarely co-occur in the same patient [Bejar et al. (2014) Blood 124:2793-2803]. Thus, these mutant genes are seldom redundant markers for the same individuals. Genes encoding mutant epigenetic regulators co-occur more frequently with each other and with mutant splicing factor genes in the same patient. As disclosed herein, the differential occurrence of mutant genes such as those listed in Table 3 provides a genetic signature that can assist in predicting which patients with MDS or sideroblastic anemia are likely to be either responsive or nonresponsive therapeutically to an ActRII antagonist.

Table 3. MDS-Associated Somatic Mutations*

Gene	Frequency in MDS (% cases)
RNA Splicing	
SF3B1	14-28
SRSF2	15
U2AF1	8
ZRSR2	6
PRPF40B	1
SF3A1	1
SF1	1
U2AF65	< 1
LUC7L2	Rare
PRPF8	Rare
Epigenetic Regulators	
TET2	19-26
ASXL1	10-20
DNMT3A	10
IDH1 / IDH2	4-12
EZH2	6
UTX	1
ATRX	< 1
Transcription Factors	

RUNX1	10-20
TP53	4-14
ETV6	1-3
PHF6	Rare
WT1	Rare
Signaling	
NRAS	10
CBL	3
JAK2	3
FLT3	2-3
KRAS	1-2
c-KIT	1
BRAF	< 1
CDKN2A	< 1
GNAS	< 1
PTEN	< 1
PTPN11	< 1
CBLB	Rare
MPL, CSF1R	Rare
Others	
NPM1	2-3

* From Tothova et al. (2013) Clin Cancer Res 19:1637-1643.

Among the genes listed in Table 3, the gene encoding splicing factor 3B1 (*SF3B1*) has been implicated recently as critical in MDS, particularly in the RARS, RARS-T, and RCMD-RS subtypes [Malcovati et al. (2011) Blood 118:6239-6246; Dolatshad et al. (2014) Leukemia doi: 10.1038/leu.2014.331 epub ahead of print]. Somatic mutations in SF3B1 also occur in hematologic cancers including chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML) as well as in breast cancer, pancreatic cancer, gastric cancer, prostate cancer, and uveal melanoma [Malcovati et al. (2011) Blood 118:6239-6246; Wang et al. (2011) N Engl J Med 365:2497-2506; The Cancer Genome Atlas Network (2012) Nature 490:61-70; Biankin et al. (2012) Nature 491:399-405; Chesnais et al. (2012) Oncotarget 3:1284-1293; Furney et al. (2013) Cancer Discov 3:1122-1129; Je et al. (2013) Int J Cancer 133:260-266]. A spectrum of *SF3B1* mutations, many clustered at a few locations in the protein, have been identified in clinical samples or in cell lines exposed to high concentrations of pladienolide [Webb et al. (2013) Drug Discov Today 18:43-49]. *SF3B1* mutations identified in MDS include, for example, K182E, E491G, R590K, E592K, R625C, R625G, N626D, N626S, H662Y, T663A, K666M, K666Q, K666R, Q670E, G676D, V701I, I704N, I704V, G740R, A744P, D781G, and A1188V. *SF3B1* mutations identified in cancer

include, for example, N619K, N626H, N626Y, R630S, I704T, G740E, K741N, G742D, D894G, Q903R, R1041H, and I1241T. Finally, *SF3B1* mutations found in both MDS and cancer include, for example, G347V, E622D, Y623C, R625H, R625L, H662D, H662Q, T663I, K666E, K666N, K666T, K700E, and V701F.

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

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

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

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

"Percent (%) sequence identity" with respect to a reference polypeptide (or nucleotide) sequence is defined as the percentage of amino acid residues (or nucleic acids) in a candidate sequence that are identical to the amino acid residues (or nucleic acids) in the reference polypeptide (nucleotide) sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to

achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid (nucleic acid) sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

"Agonize", in all its grammatical forms, refers to the process of activating a protein and/or gene (*e.g.*, by activating or amplifying that protein's gene expression or by inducing an inactive protein to enter an active state) or increasing a protein's and/or gene's activity.

"Antagonize", in all its grammatical forms, refers to the process of inhibiting a protein and/or gene (e.g., by inhibiting or decreasing that protein's gene expression or by inducing an active protein to enter an inactive state) or decreasing a protein's and/or gene's activity.

As used herein, unless otherwise stated, "does not substantially bind to *X*" is intended to mean that an agent has a K_D that is greater than about 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, or greater (*e.g.*, no detectable binding by the assay used to determine the K_D) for "X" or has relatively modest binding for "X", e.g., about 1 x 10^{-8} M or about 1 x 10^{-9} M.

The terms "about" and "approximately" as used in connection with a numerical value throughout the specification and the claims denotes an interval of accuracy, familiar and acceptable to a person skilled in the art. In general, such interval of accuracy is ± 10%. Alternatively, and particularly in biological systems, the terms "about" and "approximately" may mean values that are within an order of magnitude, preferably ≤ 5 -fold and more preferably ≤ 2-fold of a given value.

Numeric ranges disclosed herein are inclusive of the numbers defining the ranges.

The terms "a" and "an" include plural referents unless the context in which the term is used clearly dictates otherwise. The terms "a" (or "an"), as well as the terms "one or more," and "at least one" can be used interchangeably herein. Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two or more specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone).

Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

Throughout this specification, the word "comprise" or variations such as "comprises" or "comprising" will be understood to imply the inclusion of a stated integer or groups of integers but not the exclusion of any other integer or group of integers.

2. ActRII Antagonists

The data presented herein demonstrates that antagonists (inhibitors) of ActRII (*e.g.*, antagonist of ActRIIA and/or ActRIIB SMAD 2/3 and/or SMAD 1/5/8 signaling) can be used to increase red blood cell levels *in vivo* and provide other benefits to patients. In particular, such ActRII antagonists are shown herein to be effective in treating various anemias as well as various complications (*e.g.*, disorders/conditions) of MDS and sideroblastic anemias. Accordingly, the present disclosure provides, in part, various ActRII antagonist agents that can be used, alone or in combination with one or more erythropoiesis stimulating agents (*e.g.*, EPO) or other supportive therapies [*e.g.*, hematopoietic growth factors (*e.g.*, G-CSF or GM-CSF), transfusion of red blood cells or whole blood, iron chelation therapy], increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with SF3B1, *DNMT3A,* and/or *TET2* mutations in a subject in need thereof.

In certain embodiments, preferred ActRII antagonists to be used in accordance with the methods disclosed herein are GDF-ActRII antagonists (*e.g.*, antagonists of GDF-mediated ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling), particularly GDF11- and/or GDF8-mediated ActRII signaling. In some embodiments, preferred ActRII antagonists of the present disclosure are soluble ActRII polypeptides (*e.g.*, soluble ActRIIA and ActRIIB polypeptides) and GDF trap polypeptides, such as ActRIIA-Fc fusion proteins, ActRIIB-Fc fusion proteins, and GDF trap-Fc fusion proteins.

Although soluble ActRII polypeptides and GDF trap polypeptides of the disclosure may affect red blood cell levels and/or various complications of MDS and sideroblastic anemia through a mechanism other than GDF (*e.g.* GDF11 and/or GDF8) antagonism [*e.g.*, GDF11 and/or GDF8 inhibition may be an indicator of the tendency of an agent to inhibit the activities of a spectrum of additional agents, including, perhaps, other members of the TGF-beta superfamily (*e.g.*, activin B, activin C, activin E, BMP6, BMP7, and/or Nodal) and such collective inhibition may lead to the desired effect on, *e.g.*, hematopoiesis], other types of GDF-ActRII antagonist are expected to be useful including, for example, anti-GDF11 antibodies; anti-GDF8 antibodies; anti-activin A, B, C and/or E antibodies, anti-ActRIIA antibodies; anti-ActRIIB antibodies; anti-ActRIIA/IIB antibodies, antisense, RNAi, or ribozyme nucleic acids that inhibit the production of one or more of GDF11, GDF8, ActRIIA, and/or ActRIIB; and other inhibitors (*e.g.*, small-molecule inhibitors) of one or more of GDF11, GDF8, ActRIIA, and/or ActRIIB, particularly agents that disrupt GDF11- and/or GDF8-ActRIIA binding and/or GDF11- and/or GDF8-ActRIIB binding as well as agents that inhibit expression of one or more of GDF11, GDF8, ActRIIA, and/or ActRIIB. Optionally, GDF-ActRII antagonists of the present disclosure may bind to and/or inhibit the activity (or expression) of other ActRII ligands including, for example, activin A, activin AB, activin B, activin C, activin E, BMP6, BMP7, and/or Nodal. Optionally, a GDF-ActRII antagonist of the present disclosure may be used in combination with at least one additional ActRII antagonist agent that binds to and/or inhibits the activity (or expression) of one or more additional ActRII ligands including, for example, activin A, activin AB, activin B, activin C, activin E, BMP6, BMP7, and/or Nodal. In some embodiments, ActRII antagonists to be used in accordance with the methods disclosed herein do not substantially bind to and/or inhibit activin A (*e.g.*, activin A-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling).

A. ActRII polypeptides and GDF traps

In certain aspects, the present disclosure relates to ActRII polypeptides. In particular, the disclosure provides methods of using ActRII polypeptides, alone or in combination with one or more erythropoiesis stimulating agents (*e.g.*, EPO) or other supportive therapies [*e.g.*, hematopoietic growth factors (*e.g.*, G-CSF or GM-CSF), transfusion of red blood cells or whole blood, iron chelation therapy], to, *e.g.*, increase red blood cell levels in a subject in

need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with SF3B1, *DNMT3A,* and/or *TET2* mutations in a subject in need thereof. As used herein the term "ActRII" refers to the family of type II activin receptors. This family includes both the activin receptor type IIA and the activin receptor type IIB.

As used herein, the term "ActRIIB" refers to a family of activin receptor type IIB (ActRIIB) proteins from any species and variants derived from such ActRIIB proteins by mutagenesis or other modification. Reference to ActRIIB herein is understood to be a reference to any one of the currently identified forms. Members of the ActRIIB family are generally transmembrane proteins, composed of a ligand-binding extracellular domain comprising a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ActRIIB polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ActRIIB family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Examples of such variant ActRIIA polypeptides are provided throughout the present disclosure as well as in International Patent Application Publication No. WO 2006/012627, which is incorporated herein by reference in its entirety. Optionally, ActRIIB polypeptides of the present disclosure can be used to increase red blood cell levels in a subject. Numbering of amino acids for all ActRIIB-related polypeptides described herein is based on the numbering of the human ActRIIB precursor protein sequence provided below (SEQ ID NO:1), unless specifically designated otherwise.

The human ActRIIB precursor protein sequence is as follows:

```
  1 MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE
 51 GEQDKRLHCY ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY
101 FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS
151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
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201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA

251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY

301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK

351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC

401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL

451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV

501 TNVDLPPKES SI (SEQ ID NO:1)

The signal peptide is indicated with single underline; the extracellular domain is indicated in **bold** font; and the potential, endogenous N-linked glycosylation sites are indicated with double underline.

The processed soluble (extracellular) human ActRIIB polypeptide sequence is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKK GCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPT APT (SEQ ID NO:2).

In some embodiments, the protein may be produced with an "SGR…" sequence at the N-terminus. The C-terminal "tail" of the extracellular domain is indicated by single underline. The sequence with the "tail" deleted (a Δ15 sequence) is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKK GCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO:3).

A form of ActRIIB with an alanine at position 64 of SEQ ID NO:1 (A64) is also reported in the literature [see, *e.g.*, Hilden *et al.* (1994) Blood, 83(8): 2163-2170]. Applicants have ascertained that an ActRIIB-Fc fusion protein comprising an extracellular domain of ActRIIB with the A64 substitution has a relatively low affinity for activin and GDF11. By contrast, the same ActRIIB-Fc fusion protein with an arginine at position 64 (R64) has an affinity for activin and GDF11 in the low nanomolar to high picomolar range. Therefore, sequences with an R64 are used as the "wild-type" reference sequence for human ActRIIB in this disclosure.

The form of ActRIIB with an alanine at position 64 is as follows:

1 MTAPWVALAL LWGSLCAGS**G RGEAETRECI YYNANWELER TNQSGLERCE**

51 **GEQDKRLHCY ASWANSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY**

101 **FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPT**LLTVLA YSLLPIGGLS

151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR

201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA

251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY

301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK

351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC

401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL

451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV

501 TNVDLPPKES SI (SEQ ID NO:4).

The signal peptide is indicated by single underline and the extracellular domain is indicated by **bold** font.

The processed soluble (extracellular) ActRIIB polypeptide sequence of the alternative A64 form is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKK GCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPT APT (SEQ ID NO:5).

In some embodiments, the protein may be produced with an "SGR…" sequence at the N-terminus. The C-terminal "tail" of the extracellular domain is indicated by single underline. The sequence with the "tail" deleted (a Δ15 sequence) is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKK GCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO:6).

The nucleic acid sequence encoding human ActRIIB precursor protein is shown below (SEQ ID NO: 7), consisting of nucleotides 25-1560 of Genbank Reference Sequence NM_001106.3, which encode amino acids 1-513 of the ActRIIB precursor. The sequence as shown provides an arginine at position 64 and may be modified to provide an alanine instead. The signal sequence is underlined.

1 ATGACGGCGC CCTGGGTGGC CCTCGCCCTC CTCTGGGGAT CGCTGTGCGC

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  51 CGGCTCTGGG CGTGGGGAGG CTGAGACACG GGAGTGCATC TACTACAACG
 101 CCAACTGGGA GCTGGAGCGC ACCAACCAGA GCGGCCTGGA GCGCTGCGAA
 151 GGCGAGCAGG ACAAGCGGCT GCACTGCTAC GCCTCCTGGC GCAACAGCTC
 201 TGGCACCATC GAGCTCGTGA AGAAGGGCTG CTGGCTAGAT GACTTCAACT
 251 GCTACGATAG GCAGGAGTGT GTGGCCACTG AGGAGAACCC CCAGGTGTAC
 301 TTCTGCTGCT GTGAAGGCAA CTTCTGCAAC GAACGCTTCA CTCATTTGCC
 351 AGAGGCTGGG GGCCCGGAAG TCACGTACGA GCCACCCCCG ACAGCCCCCA
 401 CCCTGCTCAC GGTGCTGGCC TACTCACTGC TGCCCATCGG GGGCCTTTCC
 451 CTCATCGTCC TGCTGGCCTT TTGGATGTAC CGGCATCGCA AGCCCCCCTA
 501 CGGTCATGTG GACATCCATG AGGACCCTGG GCCTCCACCA CCATCCCCTC
 551 TGGTGGGCCT GAAGCCACTG CAGCTGCTGG AGATCAAGGC TCGGGGGCGC
 601 TTTGGCTGTG TCTGGAAGGC CCAGCTCATG AATGACTTTG TAGCTGTCAA
 651 GATCTTCCCA CTCCAGGACA AGCAGTCGTG GCAGAGTGAA CGGGAGATCT
 701 TCAGCACACC TGGCATGAAG CACGAGAACC TGCTACAGTT CATTGCTGCC
 751 GAGAAGCGAG GCTCCAACCT CGAAGTAGAG CTGTGGCTCA TCACGGCCTT
 801 CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT
 851 GGAACGAACT GTGTCATGTA GCAGAGACGA TGTCACGAGG CCTCTCATAC
 901 CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT
 951 TGCCCACAGG GACTTTAAAA GTAAGAATGT ATTGCTGAAG AGCGACCTCA
1001 CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTTGA GCCAGGGAAA
1051 CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC
1101 TGAGGTGCTC GAGGGAGCCA TCAACTTCCA GAGAGATGCC TTCCTGCGCA
1151 TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC
1201 AAGGCTGCAG ACGGACCCGT GGATGAGTAC ATGCTGCCCT TTGAGGAAGA
1251 GATTGGCCAG CACCCTTCGT TGGAGGAGCT GCAGGAGGTG GTGGTGCACA
1301 AGAAGATGAG GCCCACCATT AAAGATCACT GGTTGAAACA CCCGGGCCTG
1351 GCCCAGCTTT GTGTGACCAT CGAGGAGTGC TGGGACCATG ATGCAGAGGC
1401 TCGCTTGTCC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTCGGAGGT
1451 CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCCTGGT GACCTCTGTC
1501 ACCAATGTGG ACCTGCCCCC TAAAGAGTCA AGCATC
```

(SEQ ID NO: 7).

A nucleic acid sequence encoding processed soluble (extracellular) human ActRIIB polypeptide is as follows (SEQ ID NO: 8). The sequence as shown provides an arginine at position 64, and may be modified to provide an alanine instead.

```
  1  GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG
 51  GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC
101  AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC
151  ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA
201  TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT
251  GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT
301  GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACC
```

(SEQ ID NO:8).

In certain embodiments, the present disclosure relates to ActRIIA polypeptides. As used herein, the term "ActRIIA" refers to a family of activin receptor type IIA (ActRIIA) proteins from any species and variants derived from such ActRIIA proteins by mutagenesis or other modification. Reference to ActRIIA herein is understood to be a reference to any one of the currently identified forms. Members of the ActRIIA family are generally transmembrane proteins, composed of a ligand-binding extracellular domain comprising a cysteine-rich region, a transmembrane domain, and a cytoplasmic domain with predicted serine/threonine kinase activity.

The term "ActRIIA polypeptide" includes polypeptides comprising any naturally occurring polypeptide of an ActRIIA family member as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. Examples of such variant ActRIIA polypeptides are provided throughout the present disclosure as well as in International Patent Application Publication No. WO 2006/012627, which is incorporated herein by reference in its entirety. Optionally, ActRIIA polypeptides of the present disclosure can be used to increase red blood cell levels in a subject. Numbering of amino acids for all ActRIIA-related polypeptides described herein is based on the numbering of the human ActRIIA precursor protein sequence provided below (SEQ ID NO:9), unless specifically designated otherwise.

The human ActRIIA precursor protein sequence is as follows:

```
  1  MGAAAKLAFA VFLISCSSGA ILGRSETQEC LFFNANWEKD RTNQTGVEPC
 51  YGDKDKRRHC FATWKNISGS IEIVKQGCWL DDINCYDRTD CVEKKDSPEV
101  YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKPPYYNIL LYSLVPLMLI
151  AGIVICAFWV YRHHKMAYPP VLVPTQDPGP PPPSPLLGLK PLQLLEVKAR
201  GRFGCVWKAQ LLNEYVAVKI FPIQDKQSWQ NEYEVYSLPG MKHENILQFI
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251  GAEKRGTSVD VDLWLITAFH EKGSLSDFLK ANVVSWNELC HIAETMARGL
301  AYLHEDIPGL KDGHKPAISH RDIKSKNVLL KNNLTACIAD FGLALKFEAG
351  KSAGDTHGQV GTRRYMAPEV LEGAINFQRD AFLRIDMYAM GLVLWELASR
401  CTAADGPVDE YMLPFEEEIG QHPSLEDMQE VVVHKKKRPV LRDYWQKHAG
451  MAMLCETIEE CWDHDAEARL SAGCVGERIT QMQRLTNIIT TEDIVTVVTM
501  VTNVDFPPKE SSL          (SEQ ID NO:9)
```

The signal peptide is indicated by single underline; the extracellular domain is indicated in **bold** font; and the potential, endogenous N-linked glycosylation sites are indicated by double underline.

The processed soluble (extracellular) human ActRIIA polypeptide sequence is as follows:

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQG CWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPK PP (SEQ ID NO:10)

The C-terminal "tail" of the extracellular domain is indicated by single underline. The sequence with the "tail" deleted (a Δ15 sequence) is as follows:

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQG CWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEM (SEQ ID NO:11)

The nucleic acid sequence encoding human ActRIIA precursor protein is shown below (SEQ ID NO: 12), as follows nucleotides 159-1700 of Genbank Reference Sequence NM_001616.4. The signal sequence is underlined.

```
   1  atgggagctg ctgcaaagtt ggcgtttgcc gtctttctta tctcctgttc
  51  ttcaggtgct atacttggta gatcagaaac tcaggagtgt cttttcttta
 101  atgctaattg ggaaaaagac agaaccaatc aaactggtgt tgaaccgtgt
 151  tatggtgaca aagataaacg gcggcattgt tttgctacct ggaagaatat
 201  ttctggttcc attgaaatag tgaaacaagg ttgttggctg gatgatatca
 251  actgctatga caggactgat tgtgtagaaa aaaaagacag ccctgaagta
 301  tatttttgtt gctgtgaggg caatatgtgt aatgaaaagt tttcttattt
 351  tccggagatg gaagtcacac agcccacttc aaatccagtt acacctaagc
 401  caccctatta caacatcctg ctctattcct tggtgccact tatgttaatt
 451  gcggggattg tcatttgtgc attttgggtg tacaggcatc acaagatggc
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 501  ctaccctcct gtacttgttc caactcaaga cccaggacca cccccacctt
 551  ctccattact aggtttgaaa ccactgcagt tattagaagt gaaagcaagg
 601  ggaagatttg gttgtgtctg gaaagcccag ttgcttaacg aatatgtggc
 651  tgtcaaaata tttccaatac aggacaaaca gtcatggcaa aatgaatacg
 701  aagtctacag tttgcctgga atgaagcatg agaacatatt acagttcatt
 751  ggtgcagaaa aacgaggcac cagtgttgat gtggatcttt ggctgatcac
 801  agcatttcat gaaaagggtt cactatcaga ctttcttaag gctaatgtgg
 851  tctcttggaa tgaactgtgt catattgcag aaaccatggc tagaggattg
 901  gcatatttac atgaggatat acctggccta aaagatggcc acaaacctgc
 951  catatctcac agggacatca aaagtaaaaa tgtgctgttg aaaaacaacc
1001  tgacagcttg cattgctgac tttgggttgg ccttaaaatt tgaggctggc
1051  aagtctgcag gcgataccca tggacaggtt ggtacccgga ggtacatggc
1101  tccagaggta ttagagggtg ctataaactt ccaaagggat gcatttttga
1151  ggatagatat gtatgccatg ggattagtcc tatgggaact ggcttctcgc
1201  tgtactgctg cagatggacc tgtagatgaa tacatgttgc catttgagga
1251  ggaaattggc cagcatccat ctcttgaaga catgcaggaa gttgttgtgc
1301  ataaaaaaaa gaggcctgtt ttaagagatt attggcagaa acatgctgga
1351  atggcaatgc tctgtgaaac cattgaagaa tgttgggatc acgacgcaga
1401  agccaggtta tcagctggat gtgtaggtga aagaattacc cagatgcaga
1451  gactaacaaa tattattacc acagaggaca ttgtaacagt ggtcacaatg
1501  gtgacaaatg ttgactttcc tcccaaagaa tctagtcta
```

(SEQ ID NO:12)

The nucleic acid sequence encoding processed soluble (extracellular) human ActRIIA polypeptide is as follows:

```
   1  atacttggta gatcagaaac tcaggagtgt cttttcttta atgctaattg
  51  ggaaaaagac agaaccaatc aaactggtgt tgaaccgtgt tatggtgaca
 101  aagataaacg gcggcattgt tttgctacct ggaagaatat ttctggttcc
 151  attgaaatag tgaaacaagg ttgttggctg gatgatatca actgctatga
 201  caggactgat tgtgtagaaa aaaaagacag ccctgaagta tatttttgtt
 251  gctgtgaggg caatatgtgt aatgaaaagt tttcttattt tccggagatg
 301  gaagtcacac agcccacttc aaatccagtt acacctaagc caccc
```

(SEQ ID NO:13).

An alignment of the amino acid sequences of human ActRIIB soluble extracellular domain and human ActRIIA soluble extracellular domain are illustrated in Figure 1. This alignment indicates amino acid residues within both receptors that are believed to directly contact ActRII ligands. Figure 2 depicts a multiple-sequence alignment of various vertebrate ActRIIB proteins and human ActRIIA. From these alignments it is possible to predict key amino acid positions within the ligand-binding domain that are important for normal ActRII-ligand binding activities as well as to predict amino acid positions that are likely to be tolerant to substitution without significantly altering normal ActRII-ligand binding activities.

In other aspects, the present disclosure relates to GDF trap polypeptides (also referred to as "GDF traps") which may be used, for example, alone or in combination with one or more erythropoiesis stimulating agents (*e.g.*, EPO) or other supportive therapies [*e.g.*, hematopoietic growth factors (*e.g.*, G-CSF or GM-CSF), transfusion of red blood cells or whole blood, iron chelation therapy], to, *e.g.*, increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) in a subject in need thereof.

In some embodiments, GDF traps of the present disclosure are soluble, variant ActRII polypeptides (*e.g.*, ActRIIA and ActRIIB polypeptides) that comprise one or more mutations (*e.g.*, amino acid additions, deletions, substitutions, and combinations thereof) in the extracellular domain (also referred to as the ligand-binding domain) of an ActRII polypeptide (*e.g.*, a "wild-type" ActRII polypeptide) such that the variant ActRII polypeptide has one or more altered ligand-binding activities than the corresponding wild-type ActRII polypeptide. In preferred embodiments, GDF trap polypeptides of the present disclosure retain at least one similar activity as a corresponding wild-type ActRII polypeptide (*e.g.*, an ActRIIA or ActRIIB polypeptide). For example, a GDF trap may bind to and/or inhibit (*e.g.* antagonize) the function of one or more ActRII ligands (*e.g.*, inhibit ActRII ligand-mediated activation of the ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 and/or SMAD 1/5/8 signaling pathway). In some embodiments, GDF traps of the present disclosure bind to and/or inhibit one or more of activin A, activin B, activin AB, activin C, activin E, Nodal, GDF8, GDF11, BMP6 and/or BMP7).

In certain embodiments, GDF trap polypeptides of the disclosure have elevated binding affinity for one or more specific ActRII ligands (*e.g.*, GDF8, GDF11, BMP6, Nodal, and/or BMP7). In other embodiments, GDF trap polypeptides of the disclosure have decreased binding affinity for one or more specific ActRII ligands (*e.g.*, activin A, activin B, activin AB, activin C, and/or activin E). In still other embodiments, GDF trap polypeptides of the disclosure have elevated binding affinity for one or more specific ActRII ligands and decreased binding affinity for one or more different/other ActRII ligands. Accordingly, the present disclosure provides GDF trap polypeptides that have an altered binding specificity for one or more ActRII ligands.

In certain preferred embodiments, GDF traps of the present disclosure are designed to preferentially bind to and antagonize GDF11 and/or GDF8 (also known as myostatin), *e.g.*, in comparision to a wild-type ActRII polypeptide. Optionally, such GDF11 and/or GDF8-binding traps may further bind to and/or antagonize one or more of Nodal, GDF8, GDF11, BMP6 and/or BMP7. Optionally, such GDF11 and/or GDF8-binding traps may further bind to and/or antagonize one or more of activin B, activin C, activin E, Nodal, GDF8, GDF11, BMP6 and/or BMP7. Optionally, such GDF11 and/or GDF8-binding traps may further bind to and/or antagonize one or more of activin A, activin A/B, activin B, activin C, activin E, Nodal, GDF8, GDF11, BMP6 and/or BMP7. In certain embodiments, GDF traps of the present disclosure have diminished binding affinity for activins (*e.g.*, activin A, activin A/B, activin B, activin C, activin E), *e.g.*, in comparision to a wild-type ActRII polypeptide. In certain preferred embodiments, a GDF trap polypeptide of the present disclosure has diminished binding affinity for activin A.

For example, the disclosure provides GDF trap polypeptides that preferentially bind to and/or antagonize GDF8/GDF11 relative to activin A. As demonstrated by the Examples of the disclosure, such GDF trap polypeptides are more potent activators of erythropoiesis *in vivo* in comparision to ActRII polypeptides that retain high binding affinity for activin A. Furthermore, these non-activin-A-binding GDF trap polypeptides demonstrate decreased effects on other tissues. Therefore, such GDF traps may be useful for increasing red blood cell levels in a subject while reducing potential off-target effects associated with binding/antagonizing activin A. However, such selective GDF trap polypeptides may be less desirable in some applications wherein more modest gains in red blood cell levels may be needed for therapeutic effect and wherein some level of off-target effect is acceptable (or even desirable).

Amino acid residues of the ActRIIB proteins (*e.g.*, E39, K55, Y60, K74, W78, L79, D80, and F101) are in the ActRIIB ligand-binding pocket and help mediated binding to its ligands including, for example, activin A, GDF11, and GDF8. Thus the present disclosure provides GDF trap polypeptides comprising an altered-ligand binding domain (*e.g.*, a GDF8/GDF11-binding domain) of an ActRIIB receptor which comprises one or more mutations at those amino acid residues.

Optionally, the altered ligand-binding domain can have increased selectivity for a ligand such as GDF11 and/or GDF8 relative to a wild-type ligand-binding domain of an ActRIIB receptor. To illustrate, one or more mutations may be selected that increase the selectivity of the altered ligand-binding domain for GDF11 and/or GDF8 over one or more activins (activin A, activin B, activin AB, activin C, and/or activin A), particularly activin A. Optionally, the altered ligand-binding domain has a ratio of K_d for activin binding to K_d for GDF11 and/or GDF8 binding that is at least 2-, 5-, 10-, 20-, 50-, 100- or even 1000-fold greater relative to the ratio for the wild-type ligand-binding domain. Optionally, the altered ligand-binding domain has a ratio of IC_{50} for inhibiting activin to IC_{50} for inhibiting GDF11 and/or GDF8 that is at least 2-, 5-, 10-, 20-, 50-, 100- or even 1000-fold greater relative to the wild-type ligand-binding domain. Optionally, the altered ligand-binding domain inhibits GDF11 and/or GDF8 with an IC_{50} at least 2-, 5-, 10-, 20-, 50-, 100- or even 1000-times less than the IC_{50} for inhibiting activin.

As a specific example, the positively-charged amino acid residue Asp (D80) of the ligand-binding domain of ActRIIB can be mutated to a different amino acid residue to produce a GDF trap polypeptide that preferentially binds to GDF8, but not activin. Preferably, the D80 residue with respect to SEQ ID NO:1 is changed to an amino acid residue selected from the group consisting of: an uncharged amino acid residue, a negative amino acid residue, and a hydrophobic amino acid residue. As a further specific example, the hydrophobic residue L79 of SEQ ID NO:1 can be altered to confer altered activin-GDF11/GDF8 binding properties. For example, an L79P substitution reduces GDF11 binding to a greater extent than activin binding. In contrast, replacement of L79 with an acidic amino acid [an aspartic acid or glutamic acid; an L79D or an L79E substitution] greatly reduces activin A binding affinity while retaining GDF11 binding affinity. In exemplary embodiments, the methods described herein utilize a GDF trap polypeptide which is a variant ActRIIB polypeptide comprising an acidic amino acid (*e.g.*, D or E) at the

position corresponding to position 79 of SEQ ID NO: 1, optionally in combination with one or more additional amino acid substitutions, additions, or deletions.

As will be recognized by one of skill in the art, most of the described mutations, variants or modifications described herein may be made at the nucleic acid level or, in some cases, by post-translational modification or chemical synthesis. Such techniques are well known in the art and some of which are described herein.

In certain embodiments, the present disclosure relates to ActRII polypeptides (ActRIIA and ActRIIB polypeptides) which are soluble ActRII polypeptides. As described herein, the term "soluble ActRII polypeptide" generally refers to polypeptides comprising an extracellular domain of an ActRII protein. The term "soluble ActRII polypeptide," as used herein, includes any naturally occurring extracellular domain of an ActRII protein as well as any variants thereof (including mutants, fragments, and peptidomimetic forms) that retain a useful activity (*e.g.*, a GDF trap polypeptide as described herein). Other examples of soluble ActRII polypeptides comprise a signal sequence in addition to the extracellular domain of an ActRII or GDF trap protein. For example, the signal sequence can be a native signal sequence of an ActRIIA or ActRIIB protein, or a signal sequence from another protein including, for example, a tissue plasminogen activator (TPA) signal sequence or a honey bee melittin (HBM) signal sequence.

In part, the present disclosure identifies functionally active portions and variants of ActRII polypeptides that can be used as guidance for generating and using ActRIIA polypeptides, ActRIIB polypeptides, and GDF trap polypeptides within the scope of the methods described herein.

ActRII proteins have been characterized in the art in terms of structural and functional characteristics, particularly with respect to ligand binding [see, *e.g.*, Attisano *et al.* (1992) Cell 68(1):97-108; Greenwald *et al.* (1999) Nature Structural Biology 6(1): 18-22; Allendorph *et al.* (2006) PNAS 103(20: 7643-7648; Thompson *et al.* (2003) The EMBO Journal 22(7): 1555-1566; and U.S. Patent Nos: 7,709,605, 7,612,041, and 7,842,663].

For example, Attisano *et al.* showed that a deletion of the proline knot at the C-terminus of the extracellular domain of ActRIIB reduced the affinity of the receptor for activin. An ActRIIB-Fc fusion protein containing amino acids 20-119 of present SEQ ID NO:1, "ActRIIB(20-119)-Fc", has reduced binding to GDF-11 and activin relative to an ActRIIB(20-134)-Fc, which includes the proline knot region and the complete

juxtamembrane domain (see, *e.g.*, U.S. Patent No. 7,842,663). However, an ActRIIB(20-129)-Fc protein retains similar but somewhat reduced activity relative to the wild-type, even though the proline knot region is disrupted. Thus, ActRIIB extracellular domains that stop at amino acid 134, 133, 132, 131, 130 and 129 (with respect to present SEQ ID NO:1) are all expected to be active, but constructs stopping at 134 or 133 may be most active. Similarly, mutations at any of residues 129-134 (with respect to SEQ ID NO:1) are not expected to alter ligand-binding affinity by large margins. In support of this, mutations of P129 and P130 (with respect to SEQ ID NO:1) do not substantially decrease ligand binding. Therefore, an ActRIIB polypeptide or an ActRIIB-based GDF trap polypeptide of the present disclosure may end as early as amino acid 109 (the final cysteine), however, forms ending at or between 109 and 119 (*e.g.*, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, or 119) are expected to have reduced ligand binding. Amino acid 119 (with respect to present SEQ ID NO:1) is poorly conserved and so is readily altered or truncated. ActRIIB polypeptides and ActRIIB-based GDF traps ending at 128 (with respect to present SEQ ID NO:1) or later should retain ligand binding activity. ActRIIB polypeptides and ActRIIB-based GDF traps ending at or between 119 and 127 (*e.g.*, 119, 120, 121, 122, 123, 124, 125, 126, or 127),with respect to SEQ ID NO:1, will have an intermediate binding ability. Any of these forms may be desirable to use, depending on the clinical or experimental setting.

At the N-terminus of ActRIIB, it is expected that a protein beginning at amino acid 29 or before (with respect to present SEQ ID NO:1) will retain ligand-binding activity. Amino acid 29 represents the initial cysteine. An alanine-to-asparagine mutation at position 24 (with respect to present SEQ ID NO:1) introduces an N-linked glycosylation sequence without substantially affecting ligand binding (see, *e.g.*, U.S. Patent No. 7,842,663). This confirms that mutations in the region between the signal cleavage peptide and the cysteine cross-linked region, corresponding to amino acids 20-29, are well tolerated. In particular, ActRIIB polypeptides and ActRIIB-based GDF traps beginning at position 20, 21, 22, 23, and 24 (with respect to present SEQ ID NO:1) should retain general ligand-biding activity, and ActRIIB polypeptides and ActRIIB-based GDF traps beginning at positions 25, 26, 27, 28, and 29 (with respect to present SEQ ID NO:1) are also expected to retain ligand-biding activity. Data shown herein as well as in, *e.g.*, U.S. Patent No. 7,842,663 demonstrates that, surprisingly, an ActRIIB construct beginning at 22, 23, 24, or 25 will have the most activity.

Taken together, an active portion (*e.g.*, ligand-binding activity) of ActRIIB comprises amino acids 29-109 of SEQ ID NO:1. Therefore ActRIIB polypeptides and ActRIIB-based

GDF traps of the present disclosure may, for example, comprise an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to a portion of ActRIIB beginning at a residue corresponding to amino acids 20-29 (*e.g.*, beginning at amino acid 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) of SEQ ID NO: 1 and ending at a position corresponding to amino acids 109-134 (*e.g.,* ending at amino acid 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. In some embodiments, ActRIIB-based GDF trap polypeptides of the present disclosure do not comprise or consist of amino acids 29-109 of SEQ ID NO:1. Other examples include polypeptides that begin at a position from 20-29 (*e.g.*, position 20, 21, 22, 23, 24, 25, 26, 27, 28, or 29) or 21-29 (*e.g.*, position 21, 22, 23, 24, 25, 26, 27, 28, or 29) and end at a position from 119-134 (*e.g.,* 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-133 (*e.g.,* 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, or 133), 129-134 (*e.g.,* 129, 130, 131, 132, 133, or 134), or 129-133 (*e.g.,* 129, 130, 131, 132, or 133) of SEQ ID NO: 1. Other examples include constructs that begin at a position from 20-24 (*e.g.,* 20, 21, 22, 23, or 24), 21-24 (*e.g.,* 21, 22, 23, or 24), or 22-25 (*e.g.,* 22, 22, 23, or 25) and end at a position from 109-134 (*e.g.*, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134), 119-134 (*e.g.*, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, or 134) or 129-134 (*e.g.*, 129, 130, 131, 132, 133, or 134) of SEQ ID NO: 1. Variants within these ranges are also contemplated, particularly those having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the corresponding portion of SEQ ID NO: 1. In some embodiments, the ActRIIB polypeptides and ActRIIB-based GDF traps comprise a polypeptide having an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acid residues 25-131 of SEQ ID NO: 1. In certain embodiments, ActRIIB-based GDF trap polypeptides do not comprise or consist of amino acids 25-131 of SEQ ID NO: 1.

The disclosure includes the results of an analysis of composite ActRIIB structures, shown in Figure 1, demonstrating that the ligand-binding pocket is defined, in part, by residues Y31, N33, N35, L38 through T41, E47, E50, Q53 through K55, L57, H58, Y60, S62, K74, W78 through N83, Y85, R87, A92, and E94 through F101. At these positions, it is expected that conservative mutations will be tolerated, although a K74A mutation is well-tolerated, as are R40A, K55A, F82A and mutations at position L79. R40 is a K in Xenopus, indicating that basic amino acids at this position will be tolerated. Q53 is R in bovine

ActRIIB and K in Xenopus ActRIIB, and therefore amino acids including R, K, Q, N and H will be tolerated at this position. Thus, a general formula for an ActRIIB polypeptide and ActRIIB-based GDF trap polypeptide of the disclosure is one that comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 29-109 of SEQ ID NO: 1, optionally beginning at a position ranging from 20-24 (*e.g.*, 20, 21, 22, 23, or 24) or 22-25(*e.g.*, 22, 23, 24, or 25) and ending at a position ranging from 129-134 (*e.g.*, 129, 130, 131, 132, 133, or 134), and comprising no more than 1, 2, 5, 10 or 15 conservative amino acid changes in the ligand-binding pocket, and zero, one or more non-conservative alterations at positions 40, 53, 55, 74, 79 and/or 82 in the ligand-binding pocket. Sites outside the binding pocket, at which variability may be particularly well tolerated, include the amino and carboxy termini of the extracellular domain (as noted above), and positions 42-46 and 65-73 (with respect to SEQ ID NO:1). An asparagine-to-alanine alteration at position 65 (N65A) actually improves ligand binding in the A64 background, and is thus expected to have no detrimental effect on ligand binding in the R64 background (see, *e.g.*, U.S. Patent No. 7,842,663). This change probably eliminates glycosylation at N65 in the A64 background, thus demonstrating that a significant change in this region is likely to be tolerated. While an R64A change is poorly tolerated, R64K is well-tolerated, and thus another basic residue, such as H may be tolerated at position 64 (see, *e.g.*, U.S. Patent No. 7,842,663).

ActRIIB is well-conserved across nearly all vertebrates, with large stretches of the extracellular domain conserved completely. Many of the ligands that bind to ActRIIB are also highly conserved. Accordingly, comparisons of ActRIIB sequences from various vertebrate organisms provide insights into residues that may be altered. Therefore, an active, human ActRIIB variant polypeptide and ActRIIB-based GDF trap useful in accordance with the presently disclosed methods may include one or more amino acids at corresponding positions from the sequence of another vertebrate ActRIIB, or may include a residue that is similar to that in the human or other vertebrate sequence. The following examples illustrate this approach to defining an active ActRIIB variant. L46 is a valine in Xenopus ActRIIB, and so this position may be altered, and optionally may be altered to another hydrophobic residue, such as V, I or F, or a non-polar residue such as A. E52 is a K in Xenopus, indicating that this site may be tolerant of a wide variety of changes, including polar residues, such as E, D, K, R, H, S, T, P, G, Y and probably A. T93 is a K in Xenopus, indicating that a wide structural variation is tolerated at this position, with polar residues favored, such as S, K,

R, E, D, H, G, P, G and Y. F108 is a Y in Xenopus, and therefore Y or other hydrophobic group, such as I, V or L should be tolerated. E111 is K in Xenopus, indicating that charged residues will be tolerated at this position, including D, R, K and H, as well as Q and N. R112 is K in Xenopus, indicating that basic residues are tolerated at this position, including R and H. A at position 119 is relatively poorly conserved, and appears as P in rodents and V in Xenopus, thus essentially any amino acid should be tolerated at this position.

It has been previously demonstrated that the addition of a further N-linked glycosylation site (N-X-S/T) is well-tolerated relative to the ActRIIB(R64)-Fc form (see, *e.g.*, U.S. Patent No. 7,842,663). Therefore, N-X-S/T sequences may be generally introduced at positions outside the ligand binding pocket defined in Figure 1 in ActRIIB polypeptide and ActRIIB-based GDF traps of the present disclosure. Particularly suitable sites for the introduction of non-endogenous N-X-S/T sequences include amino acids 20-29, 20-24, 22-25, 109-134, 120-134 or 129-134 (with respect to SEQ ID NO:1). N-X-S/T sequences may also be introduced into the linker between the ActRIIB sequence and an Fc domain or other fusion component. Such a site may be introduced with minimal effort by introducing an N in the correct position with respect to a pre-existing S or T, or by introducing an S or T at a position corresponding to a pre-existing N. Thus, desirable alterations that would create an N-linked glycosylation site are: A24N, R64N, S67N (possibly combined with an N65A alteration), E105N, R112N, G120N, E123N, P129N, A132N, R112S and R112T (with respect to SEQ ID NO:1). Any S that is predicted to be glycosylated may be altered to a T without creating an immunogenic site, because of the protection afforded by the glycosylation. Likewise, any T that is predicted to be glycosylated may be altered to an S. Thus the alterations S67T and S44T (with respect to SEQ ID NO:1) are contemplated. Likewise, in an A24N variant, an S26T alteration may be used. Accordingly, an ActRIIB polypeptide and ActRIIB-based GDF trap polypeptide of the present disclosure may be a variant having one or more additional, non-endogenous N-linked glycosylation consensus sequences as described above.

The variations described herein may be combined in various ways. Additionally, the results of the mutagenesis program described herein indicate that there are amino acid positions in ActRIIB that are often beneficial to conserve. With respect to SEQ ID NO:1, these include position 64 (basic amino acid), position 80 (acidic or hydrophobic amino acid), position 78 (hydrophobic, and particularly tryptophan), position 37 (acidic, and particularly aspartic or glutamic acid), position 56 (basic amino acid), position 60 (hydrophobic amino acid, particularly phenylalanine or tyrosine). Thus, in the ActRIIB polypeptides and

ActRIIB-based GDF traps disclosed herein, the disclosure provides a framework of amino acids that may be conserved. Other positions that may be desirable to conserve are as follows: position 52 (acidic amino acid), position 55 (basic amino acid), position 81 (acidic), 98 (polar or charged, particularly E, D, R or K), all with respect to SEQ ID NO:1.

A general formula for an active (*e.g.*, ligand binding) ActRIIA polypeptide is one that comprises a polypeptide that starts at amino acid 30 and ends at amino acid 110 of SEQ ID NO:9. Accordingly, ActRIIA polypeptides and ActRIIA-based GDF traps of the present disclosure may comprise a polypeptide that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids 30-110 of SEQ ID NO:9. In some embodiments, ActRIIA-based GDF traps of the present disclosure do not comprise or consist of amino acids 30-110 of SEQ ID NO:9. Optionally, ActRIIA polypeptides and ActRIIA-based GDF trap polypeptides of the present disclosure comprise a polypeptide that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to amino acids amino acids 12-82 of SEQ ID NO:9 optionally beginning at a position ranging from 1-5 (*e.g.*, 1, 2, 3, 4, or 5) or 3-5 (*e.g.*, 3, 4, or 5) and ending at a position ranging from 110-116 (*e.g.*, 110, 111, 112, 113, 114, 115, or 116) or 110-115 (*e.g.*, 110, 111, 112, 113, 114, or 115), respectively, and comprising no more than 1, 2, 5, 10 or 15 conservative amino acid changes in the ligand binding pocket, and zero, one or more non-conservative alterations at positions 40, 53, 55, 74, 79 and/or 82 in the ligand-binding pocket with respect to SEQ ID NO:9.

In certain embodiments, functionally active fragments of ActRII polypeptides (*e.g.* ActRIIA and ActRIIB polypeptides) and GDF trap polypeptides of the present disclosure can be obtained by screening polypeptides recombinantly produced from the corresponding fragment of the nucleic acid encoding an ActRII polypeptide or GDF trap polypeptide (*e.g.*, SEQ ID NOs: 7, 8, 12, 13, 27, 32, 39, 40, 42, 46, and 48). In addition, fragments can be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-Moc or t-Boc chemistry. The fragments can be produced (recombinantly or by chemical synthesis) and tested to identify those peptidyl fragments that can function as antagonists (inhibitors) of ActRII receptors and/or one or more ActRII ligands (*e.g.*, GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, and/or Nodal).

In some embodiments, an ActRIIA polypeptide of the present disclosure is a polypeptide comprising an amino acid sequence that is at least 75% identical to an amino acid sequence selected from SEQ ID NOs: 9, 10, 11, 22, 26, and 28. In certain embodiments,

the ActRIIA polypeptide comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 9, 10, 11, 22, 26, and 28. In certain embodiments, the ActRIIA polypeptide consists essentially of, or consists of, an amino acid sequence that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 9, 10, 11, 22, 26, and 28.

In some embodiments, an ActRIIB polypeptide of the present disclosure is a polypeptide comprising an amino acid sequence that is at least 75% identical to an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 29, 31, and 49. In certain embodiments, the ActRIIB polypeptide comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 29, 31, and 49. In certain embodiments, the ActRIIB polypeptide consists essentially of, or consists of, an amino acid sequence that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 29, 31, and 49.

In some embodiments, a GDF trap polypeptide of the present disclosure is a variant ActRIIB polypeptide comprising an amino acid sequence that is at least 75% identical to an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 29, 30, 31, 36, 37, 38, 41, 44, 45, 49, 50, and 51. In certain embodiments, the GDF trap comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 29, 30, 31, 36, 37, 38, 41, 44, 45, 49, 50, and 51. In certain embodiments, the GDF trap comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 29, 30, 31, 36, 37, 38, 41, 44, 45, 49, 50, and 51, wherein the position corresponding to L79 of SEQ ID NO:1, 4, or 49 is an acidic amino acids (a D or E amino acid residue). In certain embodiments, the GDF trap consists essentially of, or consists of, an amino acid sequence that at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 36, 37, 38, 41, 44, 45, 50, and 51. In certain embodiments, the GDF trap does not comprise or consists of an amino acid sequence selected from SEQ ID NOs: 1, 2, 3, 4, 5, 6, 29, and 31.

In some embodiments, a GDF trap polypeptide of the present disclosure is a variant ActRIIA polypeptide comprising an amino acid sequence that is at least 75% identical to an

amino acid sequence selected from SEQ ID NOs: 9, 10, 11, 22, 26, 28, 29, and 31. In certain embodiments, the GDF trap comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 9, 10, 11, 22, 26, 28, 29, and 31. In certain embodiments, the GDF trap consists essentially of, or consists of, an amino acid sequence that at least 80%, 85%, 90%, 95%, 97%, 98%, 99% or 100% identical to an amino acid sequence selected from SEQ ID NOs: 9, 10, 11, 22, 26, 28, 29, and 31. In certain embodiments, the GDF trap does not comprise or consists of an amino acid sequence selected from SEQ ID NOs: 9, 10, 11, 22, 26, 28, 29, and 31.

In some embodiments, the present disclosure contemplates making functional variants by modifying the structure of an ActRII polypeptide (*e.g.* and ActRIIA or ActRIIB polypeptide) or a GDF trap for such purposes as enhancing therapeutic efficacy, or stability (*e.g.*, shelf-life and resistance to proteolytic degradation *in vivo*). Variants can be produced by amino acid substitution, deletion, addition, or combinations thereof. For instance, it is reasonable to expect that an isolated replacement of a leucine with an isoleucine or valine, an aspartate with a glutamate, a threonine with a serine, or a similar replacement of an amino acid with a structurally related amino acid (*e.g.*, conservative mutations) will not have a major effect on the biological activity of the resulting molecule. Conservative replacements are those that take place within a family of amino acids that are related in their side chains. Whether a change in the amino acid sequence of a polypeptide of the disclosure results in a functional homolog can be readily determined by assessing the ability of the variant polypeptide to produce a response in cells in a fashion similar to the wild-type polypeptide, or to bind to one or more ligands, such as GDF11, activin A, activin B, activin AB, activin C, activin E, GDF8, BMP6, and BMP7, as compared to the unmodified or a wild-type polypeptide.

In certain embodiments, the present disclosure contemplates specific mutations of ActRII polypeptides and GDF trap polypeptides of the present disclosure so as to alter the glycosylation of the polypeptide. Such mutations may be selected so as to introduce or eliminate one or more glycosylation sites, such as O-linked or N-linked glycosylation sites. Asparagine-linked glycosylation recognition sites generally comprise a tripeptide sequence, asparagine-X-threonine or asparagine-X-serine (where "X" is any amino acid) which is specifically recognized by appropriate cellular glycosylation enzymes. The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the polypeptide (for O-linked glycosylation sites). A variety of amino acid

substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second position) results in non-glycosylation at the modified tripeptide sequence. Another means of increasing the number of carbohydrate moieties on a polypeptide is by chemical or enzymatic coupling of glycosides to the polypeptide. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine; (b) free carboxyl groups; (c) free sulfhydryl groups such as those of cysteine; (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline; (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan; or (f) the amide group of glutamine. Removal of one or more carbohydrate moieties present on a polypeptide may be accomplished chemically and/or enzymatically. Chemical deglycosylation may involve, for example, exposure of a polypeptide to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the amino acid sequence intact. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.* [Meth. Enzymol. (1987) 138:350]. The sequence of a polypeptide may be adjusted, as appropriate, depending on the type of expression system used, as mammalian, yeast, insect, and plant cells may all introduce differing glycosylation patterns that can be affected by the amino acid sequence of the peptide. In general, ActRII polypeptides and GDF trap polypeptides of the present disclosure for use in humans may be expressed in a mammalian cell line that provides proper glycosylation, such as HEK293 or CHO cell lines, although other mammalian expression cell lines are expected to be useful as well.

This disclosure further contemplates a method of generating mutants, particularly sets of combinatorial mutants of ActRII polypeptides and GDF trap polypeptides of the present disclosure, as well as truncation mutants. Pools of combinatorial mutants are especially useful for identifying ActRII and GDF trap sequences. The purpose of screening such combinatorial libraries may be to generate, for example, polypeptides variants which have altered properties, such as altered pharmacokinetic or altered ligand binding. A variety of screening assays are provided below, and such assays may be used to evaluate variants. For example, ActRII polypeptides and GDF trap polypeptides may be screened for ability to bind to an ActRII receptor, to prevent binding of an ActRII ligand (*e.g.*, GDF11, GDF8, activin A,

activin B, activin AB, activin C, activin E, BMP7, BMP6, and/or Nodal) to an ActRII polypeptide, or to interfere with signaling caused by an ActRII ligand.

The activity of an ActRII polypeptides or GDF trap polypeptides may also be tested in a cell-based or *in vivo* assay. For example, the effect of an ActRII polypeptide or GDF trap polypeptide on the expression of genes involved in hematopoiesis may be assessed. This may, as needed, be performed in the presence of one or more recombinant ActRII ligand proteins (*e.g.*, GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP7, BMP6, and/or Nodal), and cells may be transfected so as to produce an ActRII polypeptide or GDF trap polypeptide, and optionally, an ActRII ligand. Likewise, an ActRII polypeptide or GDF trap polypeptide may be administered to a mouse or other animal, and one or more blood measurements, such as an RBC count, hemoglobin, or reticulocyte count may be assessed using art-recognized methods.

Combinatorial-derived variants can be generated which have a selective or generally increased potency relative to a reference ActRII polypeptide or GDF trap polypeptide. Such variants, when expressed from recombinant DNA constructs, can be used in gene therapy protocols. Likewise, mutagenesis can give rise to variants which have intracellular half-lives dramatically different than the corresponding unmodified ActRII polypeptide or GDF trap polypeptide. For example, the altered protein can be rendered either more stable or less stable to proteolytic degradation or other cellular processes which result in destruction, or otherwise inactivation, of an unmodified polypeptide. Such variants, and the genes which encode them, can be utilized to alter ActRII polypeptide or GDF trap polypeptide levels by modulating the half-life of the polypeptide. For instance, a short half-life can give rise to more transient biological effects and, when part of an inducible expression system, can allow tighter control of recombinant ActRII polypeptide or GDF trap polypeptide levels within the cell. In an Fc fusion protein, mutations may be made in the linker (if any) and/or the Fc portion to alter the half-life of the protein.

A combinatorial library may be produced by way of a degenerate library of genes encoding a library of polypeptides which each include at least a portion of potential ActRII or GDF trap sequences. For instance, a mixture of synthetic oligonucleotides can be enzymatically ligated into gene sequences such that the degenerate set of potential ActRII or GDF trap polypeptide encoding nucleotide sequences are expressible as individual polypeptides, or alternatively, as a set of larger fusion proteins (*e.g.*, for phage display).

There are many ways by which the library of potential homologs can be generated from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be carried out in an automatic DNA synthesizer, and the synthetic genes can then be ligated into an appropriate vector for expression. The synthesis of degenerate oligonucleotides is well known in the art. See, *e.g.*, Narang, SA (1983) Tetrahedron 39:3; Itakura *et al.* (1981) Recombinant DNA, Proc. 3rd Cleveland Sympos. Macromolecules, ed. AG Walton, Amsterdam: Elsevier pp273-289; Itakura *et al.* (1984) Annu. Rev. Biochem. 53:323; Itakura *et al.* (1984) Science 198:1056; Ike *et al.* (1983) Nucleic Acid Res. 11:477. Such techniques have been employed in the directed evolution of other proteins. See, *e.g.*, Scott *et al.*, (1990) Science 249:386-390; Roberts *et al.* (1992) PNAS USA 89:2429-2433; Devlin *et al.* (1990) Science 249: 404-406; Cwirla *et al.*, (1990) PNAS USA 87: 6378-6382; as well as U.S. Patent Nos: 5,223,409, 5,198,346, and 5,096,815.

Alternatively, other forms of mutagenesis can be utilized to generate a combinatorial library. For example, ActRII polypeptides or GDF trap polypeptides of the present disclosure can be generated and isolated from a library by screening using, for example, alanine scanning mutagenesis [see, *e.g.*, Ruf *et al.* (1994) Biochemistry 33:1565-1572; Wang *et al.* (1994) J. Biol. Chem. 269:3095-3099; Balint *et al.* (1993) Gene 137:109-118; Grodberg *et al.* (1993) Eur. J. Biochem. 218:597-601; Nagashima *et al.* (1993) J. Biol. Chem. 268:2888-2892; Lowman *et al.* (1991) Biochemistry 30:10832-10838; and Cunningham *et al.* (1989) Science 244:1081-1085], by linker scanning mutagenesis [see, *e.g.*, Gustin *et al.* (1993) Virology 193:653-660; and Brown *et al.* (1992) Mol. Cell Biol. 12:2644-2652; McKnight *et al.* (1982) Science 232:316], by saturation mutagenesis [see, *e.g.*, Meyers *et al.*, (1986) Science 232:613]; by PCR mutagenesis [see, *e.g.*, Leung *et al.* (1989) Method Cell Mol Biol 1:11-19]; or by random mutagenesis, including chemical mutagenesis [*see*, *e.g.*, Miller *et al.* (1992) A Short Course in Bacterial Genetics, CSHL Press, Cold Spring Harbor, NY; and Greener *et al.* (1994) Strategies in Mol Biol 7:32-34]. Linker scanning mutagenesis, particularly in a combinatorial setting, is an attractive method for identifying truncated (bioactive) forms of ActRII polypeptides.

A wide range of techniques are known in the art for screening gene products of combinatorial libraries made by point mutations and truncations, and, for that matter, for screening cDNA libraries for gene products having a certain property. Such techniques will be generally adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of ActRII polypeptides or GDF trap polypeptides of the disclosure.

The most widely used techniques for screening large gene libraries typically comprises cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates relatively easy isolation of the vector encoding the gene whose product was detected. Preferred assays include ActRII ligand (*e.g.*, GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP7, BMP6, and/or Nodal) binding assays and/or ActRII ligand-mediated cell signaling assays.

In certain embodiments, ActRII polypeptides or GDF trap polypeptides of the present disclosure may further comprise post-translational modifications in addition to any that are naturally present in the ActRII (*e.g.*, an ActRIIA or ActRIIB polypeptide) or GDF trap polypeptide. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. As a result, the ActRII polypeptide or GDF trap polypeptide may contain non-amino acid elements, such as polyethylene glycols, lipids, polysaccharide or monosaccharide, and phosphates. Effects of such non-amino acid elements on the functionality of a ligand trap polypeptide may be tested as described herein for other ActRII or GDF trap variants. When a polypeptide of the disclosure is produced in cells by cleaving a nascent form of the polypeptide, post-translational processing may also be important for correct folding and/or function of the protein. Different cells (*e.g.*, CHO, HeLa, MDCK, 293, WI38, NIH-3T3 or HEK293) have specific cellular machinery and characteristic mechanisms for such post-translational activities and may be chosen to ensure the correct modification and processing of the ActRII polypeptides or GDF trap polypeptides.

In certain aspects, ActRII polypeptides or GDF trap polypeptides of the present disclosure include fusion proteins having at least a portion (domain) of an ActRII polypeptide (*e.g.*, an ActRIIA or ActRIIB polypeptide) or GDF trap polypeptide and one or more heterologous portions (domains). Well-known examples of such fusion domains include, but are not limited to, polyhistidine, Glu-Glu, glutathione S-transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy-chain constant region (Fc), maltose binding protein (MBP), or human serum albumin. A fusion domain may be selected so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. For the purpose of affinity purification, relevant matrices for affinity chromatography, such as glutathione-, amylase-, and nickel- or cobalt- conjugated resins are used. Many of such matrices are available in "kit" form, such as the Pharmacia GST purification system and the QIAexpress^{TM} system (Qiagen) useful with

(HIS_6) fusion partners. As another example, a fusion domain may be selected so as to facilitate detection of the ligand trap polypeptides. Examples of such detection domains include the various fluorescent proteins (*e.g.*, GFP) as well as "epitope tags," which are usually short peptide sequences for which a specific antibody is available. Well-known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus haemagglutinin (HA), and c-myc tags. In some cases, the fusion domains have a protease cleavage site, such as for Factor Xa or thrombin, which allows the relevant protease to partially digest the fusion proteins and thereby liberate the recombinant proteins therefrom. The liberated proteins can then be isolated from the fusion domain by subsequent chromatographic separation. In certain preferred embodiments, an ActRII polypeptide or a GDF trap polypeptide is fused with a domain that stabilizes the polypeptide *in vivo* (a "stabilizer" domain). By "stabilizing" is meant anything that increases serum half-life, regardless of whether this is because of decreased destruction, decreased clearance by the kidney, or other pharmacokinetic effect. Fusions with the Fc portion of an immunoglobulin are known to confer desirable pharmacokinetic properties on a wide range of proteins. Likewise, fusions to human serum albumin can confer desirable properties. Other types of fusion domains that may be selected include multimerizing (*e.g.*, dimerizing, tetramerizing) domains and functional domains (that confer an additional biological function, such as further stimulation of muscle growth).

In certain embodiments, the present disclosure provides ActRII or GDF trap fusion proteins comprising the following IgG1 Fc domain sequence:

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  1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPVPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV
201 FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO:14).
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In other embodiments, the present disclosure provides ActRII or GDF trap fusion proteins comprising the following variant of the IgG1 Fc domain:

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  1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE
 51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK
101 VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF
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151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV

201 FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO:64)

In still other embodiments, the present disclosure provides ActRII or GDF trap fusion proteins comprising the following variant of the IgG1 Fc domain:

1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVD(A)VSHEDPE

51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK(A)

101 VSNKALPVPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF

151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG PFFLYSKLTV DKSRWQQGNV

201 FSCSVMHEAL HN(A)HYTQKSLS LSPGK (SEQ ID NO:15).

Optionally, the IgG1 Fc domain has one or more mutations at residues such as Asp-265, lysine 322, and Asn-434. In certain cases, the mutant IgG1 Fc domain having one or more of these mutations (e.g., Asp-265 mutation) has reduced ability of binding to the Fcγ receptor relative to a wild-type Fc domain. In other cases, the mutant Fc domain having one or more of these mutations (*e.g.*, Asn-434 mutation) has increased ability of binding to the MHC class I-related Fc-receptor (FcRN) relative to a wild-type IgG1 Fc domain.

In certain other embodiments, the present disclosure provides ActRII or GDF trap fusion proteins comprising variants of the IgG2 Fc domain, including the following:

1 VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ

51 FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS

101 NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP

151 SDIAVEWESN GQPENNYKTT PPMLDSDGSF FLYSKLTVDK SRWQQGNVFS

201 CSVMHEALHN HYTQKSLSLS PGK (SEQ ID NO:65)

It is understood that different elements of the fusion proteins may be arranged in any manner that is consistent with the desired functionality. For example, an ActRII polypeptide domain or GDF trap polypeptide domain may be placed C-terminal to a heterologous domain, or alternatively, a heterologous domain may be placed C-terminal to an ActRII polypeptide domain or GDF trap polypeptide domain. The ActRII polypeptide domain or GDF trap polypeptide domain and the heterologous domain need not be adjacent in a fusion protein,

and additional domains or amino acid sequences may be included C- or N-terminal to either domain or between the domains.

For example, an ActRII or GDF trap fusion protein may comprise an amino acid sequence as set forth in the formula A-B-C. The B portion corresponds to an ActRII polypeptide domain or a GDF trap polypeptide domain. The A and C portions may be independently zero, one, or more than one amino acid, and both the A and C portions when present are heterologous to B. The A and/or C portions may be attached to the B portion via a linker sequence. Exemplary linkers include short polypeptide linkers such as 2-10, 2-5, 2-4, 2-3 glycine residues, such as, for example, a Gly-Gly-Gly linker. Other suitable linkers are described herein above [*e.g.*, a TGGG linker (SEQ ID NO: 53)]. In certain embodiments, an ActRII or GDF trap fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a leader (signal) sequence, B consists of an ActRII or GDF polypeptide domain, and C is a polypeptide portion that enhances one or more of *in vivo* stability, *in vivo* half-life, uptake/administration, tissue localization or distribution, formation of protein complexes, and/or purification. In certain embodiments, an ActRII or GDF trap fusion protein comprises an amino acid sequence as set forth in the formula A-B-C, wherein A is a TPA leader sequence, B consists of an ActRII or GDF polypeptide domain, and C is an immunoglobulin Fc domain. Preferred fusion proteins comprises the amino acid sequence set forth in any one of SEQ ID NOs: 22, 26, 29, 31, 36, 38, 41, 44, and 51.

In certain embodiments, ActRII polypeptides or GDF trap polypeptides of the present disclosure contain one or more modifications that are capable of stabilizing the polypeptides. For example, such modifications enhance the *in vitro* half-life of the polypeptides, enhance circulatory half-life of the polypeptides, and/or reduce proteolytic degradation of the polypeptides. Such stabilizing modifications include, but are not limited to, fusion proteins (including, for example, fusion proteins comprising an ActRII polypeptide domain or a GDF trap polypeptide domain and a stabilizer domain), modifications of a glycosylation site (including, for example, addition of a glycosylation site to a polypeptide of the disclosure), and modifications of carbohydrate moiety (including, for example, removal of carbohydrate moieties from a polypeptide of the disclosure). As used herein, the term "stabilizer domain" not only refers to a fusion domain (*e.g.*, an immunoglobulin Fc domain) as in the case of fusion proteins, but also includes nonproteinaceous modifications such as a carbohydrate moiety, or nonproteinaceous moiety, such as polyethylene glycol.

In preferred embodiments, ActRII polypeptides and GDF traps to be used in accordance with the methods described herein are isolated polypeptides. As used herein, an isolated protein or polypeptide is one which has been separated from a component of its natural environment. In some embodiments, a polypeptide of the disclosure is purified to greater than 95%, 96%, 97%, 98%, or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). Methods for assessment of antibody purity are well known in the art [see, *e.g.*, Flatman *et al.*, (2007) J. Chromatogr. B 848:79-87].

In certain embodiments, ActRII polypeptides and GDF traps of the disclosure can be produced by a variety of art-known techniques. For example, polypeptides of the disclosure can be synthesized using standard protein chemistry techniques such as those described in Bodansky, M. Principles of Peptide Synthesis, Springer Verlag, Berlin (1993) and Grant G. A. (ed.), Synthetic Peptides: A User's Guide, W. H. Freeman and Company, New York (1992). In addition, automated peptide synthesizers are commercially available (see, *e.g.*, Advanced ChemTech Model 396; Milligen/Biosearch 9600). Alternatively, the polypeptides of the disclosure, including fragments or variants thereof, may be recombinantly produced using various expression systems [*e.g.*, E. coli, Chinese Hamster Ovary (CHO) cells, COS cells, baculovirus] as is well known in the art. In a further embodiment, the modified or unmodified polypeptides of the disclosure may be produced by digestion of recombinantly produced full-length ActRII or GDF trap polypeptides by using, for example, a protease, *e.g.*, trypsin, thermolysin, chymotrypsin, pepsin, or paired basic amino acid converting enzyme (PACE). Computer analysis (using a commercially available software, *e.g.*, MacVector, Omega, PCGene, Molecular Simulation, Inc.) can be used to identify proteolytic cleavage sites. Alternatively, such polypeptides may be produced from recombinantly produced full-length ActRII or GDF trap polypeptides using chemical cleavage (*e.g.*, cyanogen bromide, hydroxylamine, *etc.*).

Any of the ActRII polypeptides disclosed herein (*e.g.*, ActRIIA or ActRIIB polypeptides) can be combined with one or more additional ActRII antagonist agents of the disclosure to achieve the desired effect (*e.g.*, increase red blood cell levels and/or hemoglobin in a subject in need thereof, treat or prevent an anemia, treat MDS or sideroblastic anemias, treat or prevent one or more complications of MDS or sideroblastic anemias s). For example, an ActRII polypeptide disclosed herein can be used in combination with i) one or more

additional ActRII polypeptides disclosed herein, ii) one or more GDF traps disclosed herein; iii) one or more ActRII antagonist antibodies disclosed herein (*e.g.,* an anti-activin A antibody, an anti-activin B antibody, an anti-activin C antibody, an anti-activin E antibody, an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-BMP6 antibody, an anti-BMP7 antibody, an anti-ActRIIA antibody, and/or or an anti-ActRIIB antibody); iv) one or more small-molecule ActRII antagonists disclosed herein (*e.g.*, a small-molecule antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); v) one or more of the polynucleotide ActRII antagonists disclosed herein (*e.g.*, a polynucleotide antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); vi) one or more follistatin polypeptides disclosed herein; and/or vii) one or more FLRG polypeptides disclosed herein.

Similarly, any of the GDF traps disclosed herein can be combined with one or more additional ActRII antagonist agents of the disclosure to achieve the desired effect (*e.g.*, increase red blood cell levels and/or hemoglobin in a patient in need thereof, treat or prevent an anemia, treat MDS or sideroblastic anemias, treat or prevent one or more complications of MDS or sideroblastic anemias). For example, a GDF trap disclosed herein can be used in combination with i) one or more additional GDF traps disclosed herein, ii) one or more ActRII polypeptides disclosed herein (*e.g.*, ActRIIA or ActRIIB polypeptides) disclosed herein; iii) one or more ActRII antagonist antibodies disclosed herein (*e.g.,* an anti-activin A antibody, an anti-activin B antibody, an anti-activin C antibody, an anti-activin E antibody, an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-BMP6 antibody, an anti-BMP7 antibody, an anti-ActRIIA antibody, and/or or an anti-ActRIIB antibody); iv) one or more small-molecule ActRII antagonists disclosed herein (*e.g.*, a small-molecule antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); v) one or more of the polynucleotide ActRII antagonists disclosed herein (*e.g.*, a polynucleotide antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); vi) one or more follistatin polypeptides disclosed herein; and/or vii) one or more FLRG polypeptides disclosed herein.

B. Nucleic Acids Encoding ActRII Polypeptides and GDF Traps

In certain embodiments, the present disclosure provides isolated and/or recombinant nucleic acids encoding the ActRII polypeptides and GDF trap polypeptides (including fragments, functional variants, and fusion proteins thereof) disclosed herein. For example, SEQ ID NO:12 encodes the naturally occurring human ActRIIA precursor polypeptide, while SEQ ID NO:13 encodes the processed extracellular domain of ActRIIA. In addition, SEQ ID NO:7 encodes a naturally occurring human ActRIIB precursor polypeptide (the R64 variant described above), while SEQ ID NO:8 encodes the processed extracellular domain of ActRIIB (the R64 variant described above). The subject nucleic acids may be single-stranded or double stranded. Such nucleic acids may be DNA or RNA molecules. These nucleic acids may be used, for example, in methods for making ActRII-based ligand trap polypeptides of the present disclosure.

As used herein, isolated nucleic acid(s) refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

In certain embodiments, nucleic acids encoding ActRII polypeptides and GDF traps of the present disclosure are understood to include nucleic acids that are variants of any one of SEQ ID NOs: 7, 8, 12, 13, 27, 32, 39, 40, 42, 43, 46, 47, and 48. Variant nucleotide sequences include sequences that differ by one or more nucleotide substitutions, additions, or deletions including allelic variants, and therefore, will include coding sequence that differ from the nucleotide sequence designated in any one of SEQ ID NOs: 7, 8, 12, 13, 27, 32, 39, 40, 42, 43, 46, 47, and 48.

In certain embodiments, ActRII polypeptides and GDF traps of the present disclosure are encoded by isolated or recombinant nucleic acid sequences that are at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to SEQ ID NOs: 7, 8, 12, 13, 27, 32, 39, 40, 42, 43, 46, 47, and 48 In some embodiments, GDF traps of the present disclosure are not encoded by nucleic acid sequences that comprise or consist of any one of nucleotide sequences corresponding to any one of SEQ ID NOs: 7, 8, 12, 13, 27, and 32. One of ordinary skill in the art will appreciate that nucleic acid sequences that are at least 80%, 85%, 90%, 95%, 97%, 98%, or 99% identical to the sequences complementary to SEQ ID NOs: 7, 8, 12, 13, 27, 32,

39, 42, 47, and 48, and variants thereof, are also within the scope of the present disclosure. In further embodiments, the nucleic acid sequences of the disclosure can be isolated, recombinant, and/or fused with a heterologous nucleotide sequence, or in a DNA library.

In other embodiments, nucleic acids of the present disclosure also include nucleotide sequences that hybridize under highly stringent conditions to the nucleotide sequence designated in SEQ ID NOs: 7, 8, 12, 13, 27, 32, 39, 40, 42, 43, 46, 47, and 48, complement sequences of SEQ ID NOs: 7, 8, 12, 13, 27, 32, 39, 40, 42, 43, 46, 47, and 48, or fragments thereof. As discussed above, one of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. One of ordinary skill in the art will understand readily that appropriate stringency conditions which promote DNA hybridization can be varied. For example, one could perform the hybridization at 6.0 x sodium chloride/sodium citrate (SSC) at about 45 °C, followed by a wash of 2.0 x SSC at 50 °C. For example, the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50 °C to a high stringency of about 0.2 x SSC at 50 °C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22 °C, to high stringency conditions at about 65 °C. Both temperature and salt may be varied, or temperature or salt concentration may be held constant while the other variable is changed. In one embodiment, the disclosure provides nucleic acids which hybridize under low stringency conditions of 6 x SSC at room temperature followed by a wash at 2 x SSC at room temperature.

Isolated nucleic acids which differ from the nucleic acids as set forth in SEQ ID NOs: 7, 8, 12, 13, 27, 32, 39, 40, 42, 43, 46, 47, and 48 due to degeneracy in the genetic code are also within the scope of the disclosure. For example, a number of amino acids are designated by more than one triplet. Codons that specify the same amino acid, or synonyms (for example, CAU and CAC are synonyms for histidine) may result in "silent" mutations which do not affect the amino acid sequence of the protein. However, it is expected that DNA sequence polymorphisms that do lead to changes in the amino acid sequences of the subject proteins will exist among mammalian cells. One skilled in the art will appreciate that these variations in one or more nucleotides (up to about 3-5% of the nucleotides) of the nucleic acids encoding a particular protein may exist among individuals of a given species due to natural allelic variation. Any and all such nucleotide variations and resulting amino acid polymorphisms are within the scope of this disclosure.

In certain embodiments, the recombinant nucleic acids of the present disclosure may be operably linked to one or more regulatory nucleotide sequences in an expression construct. Regulatory nucleotide sequences will generally be appropriate to the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells. Typically, said one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, and enhancer or activator sequences. Constitutive or inducible promoters as known in the art are contemplated by the disclosure. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter. An expression construct may be present in a cell on an episome, such as a plasmid, or the expression construct may be inserted in a chromosome. In some embodiments, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selectable marker genes are well known in the art and will vary with the host cell used.

In certain aspects of the present disclosure, the subject nucleic acid is provided in an expression vector comprising a nucleotide sequence encoding an ActRII polypeptide or a GDF trap and operably linked to at least one regulatory sequence. Regulatory sequences are art-recognized and are selected to direct expression of the ActRII or GDF trap polypeptide. Accordingly, the term regulatory sequence includes promoters, enhancers, and other expression control elements. Exemplary regulatory sequences are described in Goeddel; *Gene Expression Technology*: *Methods in Enzymology*, Academic Press, San Diego, CA (1990). For instance, any of a wide variety of expression control sequences that control the expression of a DNA sequence when operatively linked to it may be used in these vectors to express DNA sequences encoding an ActRII or GDF trap polypeptide. Such useful expression control sequences, include, for example, the early and late promoters of SV40, *tet* promoter, adenovirus or cytomegalovirus immediate early promoter, RSV promoters, the lac system, the *trp* system, the TAC or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage lambda , the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, *e.g.*, Pho5, the promoters of the yeast α-mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses,

and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed. Moreover, the vector's copy number, the ability to control that copy number and the expression of any other protein encoded by the vector, such as antibiotic markers, should also be considered.

A recombinant nucleic acid of the present disclosure can be produced by ligating the cloned gene, or a portion thereof, into a vector suitable for expression in either prokaryotic cells, eukaryotic cells (yeast, avian, insect or mammalian), or both. Expression vehicles for production of a recombinant ActRII or GDF trap polypeptide include plasmids and other vectors. For instance, suitable vectors include plasmids of the following types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *E. coli*.

Some mammalian expression vectors contain both prokaryotic sequences to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papilloma virus (BPV-1), or Epstein-Barr virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. Examples of other viral (including retroviral) expression systems can be found below in the description of gene therapy delivery systems. The various methods employed in the preparation of the plasmids and in transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see, *e.g.*, Molecular Cloning A Laboratory Manual, 3rd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press, 2001). In some instances, it may be desirable to express the recombinant polypeptides by the use of a baculovirus expression system. Examples of such baculovirus expression systems include pVL-derived vectors (such as pVL1392, pVL1393 and pVL941), pAcUW-derived vectors (such as pAcUW1), and pBlueBac-derived vectors (such as the ß-gal containing pBlueBac III).

In a preferred embodiment, a vector will be designed for production of the subject ActRII or GDF trap polypeptides in CHO cells, such as a Pcmv-Script vector (Stratagene, La Jolla, Calif.), pcDNA4 vectors (Invitrogen, Carlsbad, Calif.) and pCI-neo vectors (Promega, Madison, Wisc.). As will be apparent, the subject gene constructs can be used to cause expression of the subject ActRII polypeptides in cells propagated in culture, *e.g.*, to produce proteins, including fusion proteins or variant proteins, for purification.

This disclosure also pertains to a host cell transfected with a recombinant gene including a coding sequence for one or more of the subject ActRII or GDF trap polypeptides. The host cell may be any prokaryotic or eukaryotic cell. For example, an ActRII or GDF trap polypeptide of the disclosure may be expressed in bacterial cells such as *E. coli*, insect cells (e.g., using a baculovirus expression system), yeast, or mammalian cells [*e.g.* a Chinese hamster ovary (CHO) cell line]. Other suitable host cells are known to those skilled in the art.

Accordingly, the present disclosure further pertains to methods of producing the subject ActRII and GDF trap polypeptides. For example, a host cell transfected with an expression vector encoding an ActRII or GDF trap polypeptide can be cultured under appropriate conditions to allow expression of the ActRII or GDF trap polypeptide to occur. The polypeptide may be secreted and isolated from a mixture of cells and medium containing the polypeptide. Alternatively, the ActRII or GDF trap polypeptide may be retained cytoplasmically or in a membrane fraction and the cells harvested, lysed and the protein isolated. A cell culture includes host cells, media and other byproducts. Suitable media for cell culture are well known in the art. The subject polypeptides can be isolated from cell culture medium, host cells, or both, using techniques known in the art for purifying proteins, including ion-exchange chromatography, gel filtration chromatography, ultrafiltration, electrophoresis, immunoaffinity purification with antibodies specific for particular epitopes of the ActRII or GDF trap polypeptides, and affinity purification with an agent that binds to a domain fused to the ActRII or GDF trap polypeptide (*e.g.*, a protein A column may be used to purify an ActRII-Fc or GDF Trap-Fc fusion protein). In some embodiments, the ActRII or GDF trap polypeptide is a fusion protein containing a domain which facilitates its purification.

In some embodiments, purification is achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange. An ActRII-Fc or GDF trap-Fc protein

may be purified to a purity of >90%, >95%, >96%, >98%, or >99% as determined by size exclusion chromatography and >90%, >95%, >96%, >98%, or >99% as determined by SDS PAGE. The target level of purity should be one that is sufficient to achieve desirable results in mammalian systems, particularly non-human primates, rodents (mice), and humans.

In another embodiment, a fusion gene coding for a purification leader sequence, such as a poly-(His)/enterokinase cleavage site sequence at the N-terminus of the desired portion of the recombinant ActRII or GDF trap polypeptide, can allow purification of the expressed fusion protein by affinity chromatography using a Ni^{2+} metal resin. The purification leader sequence can then be subsequently removed by treatment with enterokinase to provide the purified ActRII or GDF trap polypeptide. See, *e.g.*, Hochuli *et al.* (1987) *J. Chromatography* 411:177; and Janknecht *et al.* (1991) *PNAS USA* 88:8972.

Techniques for making fusion genes are well known. Essentially, the joining of various DNA fragments coding for different polypeptide sequences is performed in accordance with conventional techniques, employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed to generate a chimeric gene sequence. See, *e.g.*, Current Protocols in Molecular Biology, eds. Ausubel *et al.*, John Wiley & Sons: 1992.

C. Antibody Antagonists

In certain aspects, the present disclosure relates to an antibody, or combination of antibodies, that antagonize ActRII activity (*e.g.*, inhibition of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 and/or SMAD 1/5/8 signaling). Such antibodies may bind to and inhibit one or more ligands (e.g., GDF8, GDF11, activin A, activin B, activin C, activin E, BMP6, BMP7 or Nodal) or one or more receptors (e.g., ActRIIA, ActRIIB, ALK4, ALK5). In particular, the disclosure provides methods of using an antibody ActRII antagonist, or combination of antibody ActRII antagonists,alone or in combination with one or more erythropoiesis stimulating agents (*e.g.*, EPO) or other supportive therapies

[*e.g.*, hematopoietic growth factors (*e.g.*, G-CSF or GM-CSF), transfusion of red blood cells or whole blood, iron chelation therapy], to, *e.g.*, increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with *SF3B1, DNMT3A,* and/or *TET2* mutations in a subject in need thereof.

In certain embodiments, a preferred antibody ActRII antagonist of the disclosure is an antibody, or combination of antibodies, that binds to and/or inhibits activity of at least GDF11 (*e.g.*, GDF11-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). Optionally, the antibody, or combination of antibodies, further binds to and/or inhibits activity of GDF8 (*e.g.*, GDF8-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling), particularly in the case of a multispecific antibody that has binding affinity for both GDF11 and GDF8 or in the context of a combination of one or more anti-GDF11 antibodies and one or more anti-GDF8 antibodies. Optionally, an antibody, or combination of antibodies, of the disclosure does not substantially bind to and/or inhibit activity of activin A (*e.g.*, activin A-mediated activation of ActRIIA or ActRIIB signaling transduction, such as SMAD 2/3 signaling). In some embodiments, an antibody, or combination of antibodies, of the disclosure that binds to and/or inhibits the activity of GDF11 and/or GDF8 further binds to and/or inhibits activity of one of more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, and Nodal (*e.g.*, activation of ActRIIA or ActRIIB SMAD 2/3 and/or SMAD 1/5/8 signaling), particularly in the case of a multispecific antibody that has binding affinity for multiple ActRII ligands or in the context of a combination of multiple antibodies – each having binding affinity for a different ActRII ligand.

In certain aspects, an ActRII antagonist of the present disclosure is an antibody, or combination of antibodies, that binds to and/or inhibits activity of at least GDF8 (*e.g.*, GDF8-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). Optionally, the antibody, or combination of antibodies, further binds to and/or inhibits activity of GDF11 (*e.g.*, GDF11-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling), particularly in the case of a

multispecific antibody that has binding affinity for both GDF8 and GDF11 or in the context of a combination of one or more anti-GDF8 antibodies and one or more anti-GDF11 antibodies. Optionally, an antibody, or combination of antibodies, of the disclosure does not substantially bind to and/or inhibit activity of activin A (*e.g.*, activin A-mediated activation of ActRIIA or ActRIIB signaling transduction, such as SMAD 2/3 signaling). In some embodiments, an antibody, or combination of antibodies, of the disclosure that binds to and/or inhibits the activity of GDF8 and/or GDF11 further binds to and/or inhibits activity of one of more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, and Nodal (*e.g.*, activation of ActRIIA or ActRIIB signaling transduction, such as SMAD 2/3 and/or SMAD 1/5/8 signaling), particularly in the case of a multispecific antibody that has binding affinity for multiple ActRII ligands or in the context of a combination multiple antibodies – each having binding affinity for a different ActRII ligand.

In another aspect, an ActRII antagonist of the present disclosure is an antibody, or combination of antibodies, that binds to and/or inhibits activity of an ActRII receptor (*e.g.* an ActRIIA or ActRIIB receptor). In preferred embodiments, an anti-ActRII receptor antibody (*e.g.* an anti-ActRIIA or anti-ActRIIB receptor antibody), or combination of antibodies, of the disclosure binds to an ActRII receptor and prevents binding and/or activation of the ActRII receptor by at least GDF11 (*e.g.*, GDF11-mediated activation of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). Optionally, an anti-ActRII receptor antibody, or combination of antibodies, of the disclosure further prevents binding and/or activation of the ActRII receptor by GDF8. Optionally, an anti-ActRII receptor antibody, or combination of antibodies, of the disclosure does not substantially inhibit activin A from binding to and/or activating an ActRII receptor. In some embodiments, an anti-ActRII receptor antibody, or combination of antibodies, of the disclosure that binds to an ActRII receptor and prevents binding and/or activation of the ActRII receptor by GDF11 and/or GDF8 further prevents binding and/or activation of the ActRII receptor by one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, and Nodal.

The term antibody is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity. An antibody fragment refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments

include but are not limited to Fv, Fab, Fab', Fab'-SH, $F(ab')_2$; diabodies; linear antibodies; single-chain antibody molecules (*e.g.*, scFv); and multispecific antibodies formed from antibody fragments. See, *e.g.*, Hudson *et al.* (2003) Nat. Med. 9:129-134; Plückthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); WO 93/16185; and U.S. Pat. Nos. 5,571,894, 5,587,458, and 5,869,046. Antibodies disclosed herein may be polyclonal antibodies or monoclonal antibodies. In certain embodiments, the antibodies of the present disclosure comprise a label attached thereto and able to be detected (*e.g.*, the label can be a radioisotope, fluorescent compound, enzyme, or enzyme co-factor). In preferred embodiments, the antibodies of the present disclosure are isolated antibodies.

Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, *e.g.*, EP 404,097; WO 1993/01161; Hudson *et al.* (2003) Nat. Med. 9:129-134 (2003); and Hollinger *et al.* (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448. Triabodies and tetrabodies are also described in Hudson *et al.* (2003) Nat. Med. 9:129-134.

Single-domain antibodies are antibody fragments comprising all or a portion of the heavy-chain variable domain or all or a portion of the light-chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody. *See, e.g.*, U.S. Pat. No. 6,248,516.

Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g., E. coli or phage), as described herein.

The antibodies herein may be of any class. The class of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), for example, IgG_1, IgG_2, IgG_3, IgG_4, IgA_1, and IgA_2. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called alpha, delta, epsilon, gamma, and mu.

In general, an antibody for use in the methods disclosed herein specifically binds to its target antigen, preferably with high binding affinity. Affinity may be expressed as a K_D value and reflects the intrinsic binding affinity (*e.g.*, with minimized avidity effects). Typically, binding affinity is measured *in vitro*, whether in a cell-free or cell-associated setting. Any of a number of assays known in the art, including those disclosed herein, can be used to obtain

binding affinity measurements including, for example, surface plasmon resonance (Biacore™ assay), radiolabeled antigen binding assay (RIA), and ELISA. In some embodiments, antibodies of the present disclosure bind to their target antigens (*e.g.* GDF11, GDF8, ActRIIA, ActRIIB, *etc.*) with at least a K_D of 1x 10^{-7} or stronger, $1x10^{-8}$ or stronger, $1x10^{-9}$ or stronger, $1x10^{-10}$ or stronger, $1x10^{-11}$ or stronger, $1x10^{-12}$ or stronger, $1x10^{-13}$ or stronger, or $1x10^{-14}$ or stronger.

In certain embodiments, K_D is measured by RIA performed with the Fab version of an antibody of interest and its target antigen as described by the following assay. Solution binding affinity of Fabs for the antigen is measured by equilibrating Fab with a minimal concentration of radiolabeled antigen (*e.g.*, ^{125}I-labeled) in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate [see, *e.g.*, Chen *et al.* (1999) J. Mol. Biol. 293:865-881]. To establish conditions for the assay, multi-well plates (*e.g.,* MICROTITER® from Thermo Scientific) are coated (*e.g.*, overnight) with a capturing anti-Fab antibody (*e.g.*, from Cappel Labs) and subsequently blocked with bovine serum albumin, preferably at room temperature (approximately 23°C). In a non-adsorbent plate, radiolabeled antigen are mixed with serial dilutions of a Fab of interest [e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta *et al.*, (1997) Cancer Res. 57:4593-4599]. The Fab of interest is then incubated, preferably overnight but the incubation may continue for a longer period (*e.g.*, about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation, preferably at room temperature for about one hour. The solution is then removed and the plate is washed times several times, preferably with polysorbate 20 and PBS mixture. When the plates have dried, scintillant (*e.g.*, MICROSCINT® from Packard) is added, and the plates are counted on a gamma counter (*e.g.*, TOPCOUNT® from Packard).

According to another embodiment, K_D is measured using surface plasmon resonance assays using, for example a BIACORE® 2000 or a BIACORE® 3000 (Biacore, Inc., Piscataway, N.J.) with immobilized antigen CM5 chips at about 10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, Biacore, Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. For example, an antigen can be diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (about 0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block

unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20®) surfactant (PBST) at at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using, for example, a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_D) is calculated as the ratio k_{off} / k_{on} [see, *e.g.*, Chen *et al.*, (1999) J. Mol. Biol. 293:865-881]. If the on-rate exceeds, for example, 10^6 M^{-1} s^{-1} by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (*e.g.*, excitation=295 nm; emission=340 nm, 16 nm band-pass) of a 20 nM anti-antigen antibody (Fab form) in PBS in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO® spectrophotometer (ThermoSpectronic) with a stirred cuvette.

As used herein, anti-GDF11 antibody generally refers to an antibody that is capable of binding to GDF11 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GDF11. In certain embodiments, the extent of binding of an anti-GDF11 antibody to an unrelated, non-GDF11 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than 1% of the binding of the antibody to GDF11 as measured, for example, by a radioimmunoassay (RIA). In certain embodiments, an anti-GDF11 antibody binds to an epitope of GDF11 that is conserved among GDF11 from different species. In certain preferred embodiments, an anti-GDF11 antibody of the present disclosure is an antagonist antibody that can inhibit GDF11 activity. For example, an anti-GDF11 antibody of the disclosure may inhibit GDF11 from binding to a cognate receptor (*e.g.*, ActRIIA or ActRIIB receptor) and/or inhibit GDF11-mediated signal transduction (activation) of a cognate receptor, such as SMAD2/3 signaling by ActRIIA and/or ActRIIB receptors. In some embodiments, anti-GDF11 antibodies of the present disclosure do not substantially bind to and/or inhibit activity of activin A. It should be noted that GDF11 has high sequence homology to GDF8 and therefore antibodies that bind and/or to GDF11, in some cases, may also bind to and/or inhibit GDF8.

An anti-GDF8 antibody refers to an antibody that is capable of binding to GDF8 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting GDF8. In certain embodiments, the extent of binding of an anti-GDF8 antibody to

an unrelated, non-GDF8 protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than 1% of the binding of the antibody to GDF8 as measured, for example, by a radioimmunoassay (RIA). In certain embodiments, an anti-GDF8 antibody binds to an epitope of GDF8 that is conserved among GDF8 from different species. In preferred embodiments, an anti-GDF8 antibody of the present disclosure is an antagonist antibody that can inhibit GDF8 activity. For example, an anti-GDF8 antibody of the disclosure may inhibit GDF8 from binding to a cognate receptor (*e.g.*, ActRIIA or ActRIIB receptor) and/or inhibit GDF8-mediated signal transduction (activation) of a cognate receptor, such as SMAD2/3 signaling by ActRIIA and/or ActRIIB receptors. In some embodiments, anti-GDF8 antibodies of the present disclosure do not substantially bind to and/or inhibit activity of activin A. It should be noted that GDF8 has high sequence homology to GDF11 and therefore antibodies that bind and/or to GDF8, in many cases, may also bind to and/or inhibit GDF11.

An anti-ActRIIA antibody refers to an antibody that is capable of binding to ActRIIA with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ActRIIA. In certain embodiments, the extent of binding of an anti-ActRIIA antibody to an unrelated, non-ActRIIA protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than 1% of the binding of the antibody to ActRIIA as measured, for example, by a radioimmunoassay (RIA). In certain embodiments, an anti-ActRIIA antibody binds to an epitope of ActRIIA that is conserved among ActRIIA from different species. In preferred embodiments, an anti-ActRIIA antibody of the present disclosure is an antagonist antibody that can inhibit ActRIIA activity. For example, an anti-ActRIIA antibody of the present disclosure may inhibit one or more ActRIIA ligands selected from activin A, activin B, activin AB, activin C, activin E, GDF11, GDF8, activin A, BMP6, and BMP7 from binding to the ActRIIA receptor and/or inhibit one of these ligands from activating ActRIIA signaling (*e.g.*, SMAD2/3 and/or SMAD 1/5/8 ActRIIA signaling). In preferred embodiments, anti-ActRIIA antibodies of the present disclosure inhibit GDF11 from binding to the ActRIIA receptor and/or inhibit GDF11 from activating ActRIIA signaling. Optionally, anti-ActRIIA antibodies of the disclosure further inhibit GDF8 from binding to the ActRIIA receptor and/or inhibit GDF8 from activating ActRIIA signaling. Optionally, anti-ActRIIA antibodies of the present disclosure do not substantially inhibit activin A from binding to the ActRIIA receptor and/or do not substantially inhibit activin A-mediated activation of ActRIIA signaling. In some embodiments, an anti-ActRIIA antibody of the

disclosure that inhibits GDF11 and/or GDF8 from binding to and/or activating an ActRIIA receptor further inhibits one or more of activin A, activin B, activin AB, activin C, activin E, activin A, GDF8, BMP6, and BMP7 from binding to and/or activating the ActRIIA receptor.

An anti-ActRIIB antibody refers to an antibody that is capable of binding to ActRIIB with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ActRIIB. In certain embodiments, the extent of binding of an anti-ActRIIB antibody to an unrelated, non-ActRIIB protein is less than about 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or less than 1% of the binding of the antibody to ActRIIB as measured, for example, by a radioimmunoassay (RIA). In certain embodiments, an anti-ActRIIB antibody binds to an epitope of ActRIIB that is conserved among ActRIIB from different species. In preferred embodiments, an anti-ActRIIB antibody of the present disclosure is an antagonist antibody that can inhibit ActRIIB activity. For example, an anti-ActRIIB antibody of the present disclosure may inhibit one or more ActRIIB ligands selected from activin A, activin B, activin AB, activin C, activin E, GDF11, GDF8, activin A, BMP6, and BMP7 from binding to the ActRIIB receptor and/or inhibit one of these ligands from activating ActRIIB signaling (*e.g.*, SMAD2/3 and/or SMAD 1/5/8 ActRIIB signaling). In preferred embodiments, anti-ActRIIB antibodies of the present disclosure inhibit GDF11 from binding to the ActRIIB receptor and/or inhibit GDF11 from activating ActRIIB signaling. Optionally, anti-ActRIIB antibodies of the disclosure further inhibit GDF8 from binding to the ActRIIB receptor and/or inhibit GDF8 from activating ActRIIB signaling. Optionally, anti-ActRIIB antibodies of the present disclosure do not substantially inhibit activin A from binding to the ActRIIB receptor and/or do not substantially inhibit activin A-mediated activation of ActRIIB signaling. In some embodiments, an anti-ActRIIB antibody of the disclosure that inhibits GDF11 and/or GDF8 from binding to and/or activating an ActRIIB receptor further inhibits one or more of activin A, activin B, activin AB, activin C, activin E, activin A, GDF8, BMP6, and BMP7 from binding to and/or activating the ActRIIB receptor.

The nucleic acid and amino acid sequences of human GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, GDF8, BMP6, BMP7, ActRIIB, and ActRIIA are well known in the art and thus antibody antagonists for use in accordance with this disclosure may be routinely made by the skilled artisan based on the knowledge in the art and teachings provided herein.

In certain embodiments, an antibody provided herein (*e.g.,* an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody) is a chimeric

antibody. A chimeric antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species. Certain chimeric antibodies are described, for example, in U.S. Pat. No. 4,816,567; and Morrison *et al.*, (1984) Proc. Natl. Acad. Sci. USA, 81:6851-6855. In some embodiments, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In some embodiments, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. In general, chimeric antibodies include antigen-binding fragments thereof.

In certain embodiments, a chimeric antibody provided herein (*e.g.,* an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody) is a humanized antibody. A humanized antibody refers to a chimeric antibody comprising amino acid residues from non-human hypervariable regions (HVRs) and amino acid residues from human framework regions (FRs). In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, *e.g.*, a non-human antibody, refers to an antibody that has undergone humanization.

Humanized antibodies and methods of making them are reviewed, for example, in Almagro and Fransson (2008) Front. Biosci. 13:1619-1633 and are further described, for example, in Riechmann *et al.*, (1988) Nature 332:323-329; Queen *et al.* (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033; U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, (2005) Methods 36:25-34 [describing SDR (a-CDR) grafting]; Padlan, Mol. Immunol. (1991) 28:489-498 (describing "resurfacing"); Dall'Acqua *et al.* (2005) Methods 36:43-60 (describing "FR shuffling"); Osbourn *et al.* (2005) Methods 36:61-68; and Klimka *et al.* Br. J. Cancer (2000) 83:252-260 (describing the "guided selection" approach to FR shuffling).

Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method [see, *e.g.*, Sims *et al.* (1993) J. Immunol. 151:2296]; framework regions derived from the consensus sequence of

human antibodies of a particular subgroup of light-chain or heavy-chain variable regions [see, *e.g.*, Carter *et al.* (1992) Proc. Natl. Acad. Sci. USA, 89:4285; and Presta *et al.* (1993) J. Immunol., 151:2623]; human mature (somatically mutated) framework regions or human germline framework regions [see, *e.g.*, Almagro and Fransson (2008) Front. Biosci. 13:1619-1633]; and framework regions derived from screening FR libraries [see, *e.g.*, Baca et cd., (1997) J. Biol. Chem. 272:10678-10684; and Rosok et cd., (1996) J. Biol. Chem. 271:22611-22618].

In certain embodiments, an antibody provided herein (*e.g.,* an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody) is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel (2001) Curr. Opin. Pharmacol. 5: 368-74 and Lonberg (2008) Curr. Opin. Immunol. 20:450-459.

Human antibodies may be prepared by administering an immunogen (*e.g.*, a GDF11 polypeptide, GDF8 polypeptide, an ActRIIA polypeptide, or an ActRIIB polypeptide) to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic animals, the endogenous immunoglobulin loci have generally been inactivated. For a review of methods for obtaining human antibodies from transgenic animals, see, for example, Lonberg (2005) Nat. Biotechnol. 23:1117-1125; U.S. Pat. Nos. 6,075,181 and 6,150,584 (describing XENOMOUSE™ technology); U.S. Pat. No. 5,770,429 (describing HuMab® technology); U.S. Pat. No. 7,041,870 (describing K-M MOUSE® technology); and U.S. Patent Application Publication No. 2007/0061900 (describing VelociMouse® technology). Human variable regions from intact antibodies generated by such animals may be further modified, for example, by combining with a different human constant region.

Human antibodies provided herein can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described [see, *e.g.*, Kozbor J. Immunol., (1984) 133: 3001; Brodeur *et al.* (1987) Monoclonal Antibody Production Techniques and Applications, pp. 51-63, Marcel Dekker, Inc., New York; and Boerner *et al.* (1991) J. Immunol., 147: 86]. Human antibodies generated via human B-cell hybridoma technology are also described in Li *et al.*,

(2006) Proc. Natl. Acad. Sci. USA, 103:3557-3562. Additional methods include those described, for example, in U.S. Pat. No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue (2006) 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein (2005) Histol. Histopathol., 20(3):927-937 (2005) and Vollmers and Brandlein (2005) Methods Find Exp. Clin. Pharmacol., 27(3):185-91.

Human antibodies provided herein (*e.g.,* an anti-GDF11 antibody, an anti-activin B antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody) may also be generated by isolating Fv clone variable-domain sequences selected from human-derived phage display libraries. Such variable-domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described herein.

For example, antibodies of the present disclosure may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. A variety of methods are known in the art for generating phage-display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, for example, in Hoogenboom *et al.* (2001) in Methods in Molecular Biology 178:1-37, O'Brien *et al.*, ed., Human Press, Totowa, N.J. and further described, for example, in the McCafferty *et al.* (1991) Nature 348:552-554; Clackson *et al.*, (1991) Nature 352: 624-628; Marks *et al.* (1992) J. Mol. Biol. 222:581-597; Marks and Bradbury (2003) in Methods in Molecular Biology 248:161-175, Lo, ed., Human Press, Totowa, N.J.; Sidhu *et al.* (2004) J. Mol. Biol. 338(2):299-310; Lee *et al.* (2004) J. Mol. Biol. 340(5):1073-1093; Fellouse (2004) Proc. Natl. Acad. Sci. USA 101(34):12467-12472; and Lee *et al.* (2004) J. Immunol. Methods 284(1-2): 119-132.

In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.* (1994) Ann. Rev. Immunol., 12: 433-455. Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen (*e.g.*, GDF11, activin B, ActRIIA, or ActRIIB) without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies directed

against a wide range of non-self and also self-antigens without any immunization as described by Griffiths *et al.* (1993) EMBO J, 12: 725-734. Finally, naive libraries can also be made synthetically by cloning un-rearranged V-gene segments from stem cells and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement in vitro, as described by Hoogenboom and Winter (1992) J. Mol. Biol., 227: 381-388. Patent publications describing human antibody phage libraries include, for example: U.S. Pat. No. 5,750,373, and U.S. Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

In certain embodiments, an antibody provided herein is a multispecific antibody, for example, a bispecific antibody. Multispecific antibodies (typically monoclonal antibodies) have binding specificities for at least two different epitopes (*e.g.*, two, three, four, five, or six or more) on one or more (*e.g.*, two, three, four, five, six or more) antigens.

In certain embodiments, a multispecific antibody of the present disclosure comprises two or more binding specificities, with at least one of the binding specificities being for a GDF11 epitope, and optionally one or more additional binding specificities being for an epitope on a different ActRII ligand (*e.g.*, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6 BMP7 and/or Nodal) and/or an ActRII receptor (*e.g.,* an ActRIIA and/or ActRIIB receptor). In certain embodiments, multispecific antibodies may bind to two or more different epitopes of GDF11. Preferably a multispecific antibody of the disclosure that has binding affinity, in part, for a GDF11 epitope can be used to inhibit a GDF11 activity (*e.g.*, the ability to bind to and/or activate an ActRIIA and/or ActRIIB receptor), and optionally inhibit the activity of one or more different ActRII ligands (*e.g.*, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7 and/or Nodal) and/or an ActRII receptor (*e.g.,* an ActRIIA or ActRIIB receptor). In certain preferred embodiments, multispecific antibodies of the present disclosure that bind to and/or inhibit GDF11 further bind to and/or inhibit at least GDF8. Optionally, multispecific antibodies of the disclosure that bind to and/or inhibit GDF11 do not substantially bind to and/or substantially inhibit activin A. In some embodiments, multispecific antibodies of the disclosure that bind to and/or inhibit GDF11 and GDD8 further bind to and/or inhibit one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7 and/or Nodal.

In certain embodiments, a multispecific antibody of the present disclosure comprises two or more binding specificities, with at least one of the binding specificities being for a

GDF8 epitope, and optionally one or more additional binding specificities being for an epitope on a different ActRII ligand (*e.g.*, GDF11, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7 and/or Nodal) and/or an ActRII receptor (*e.g.,* an ActRIIA and/or ActRIIB receptor). In certain embodiments, multispecific antibodies may bind to two or more different epitopes of GDF8. Preferably a multispecific antibody of the disclosure that has binding affinity, in part, for an GDF8 epitope can be used to inhibit an GDF8 activity (*e.g.*, the ability to bind to and/or activate an ActRIIA and/or ActRIIB receptor), and optionally inhibit the activity of one or more different ActRII ligands (*e.g.*, GDF11, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7 and/or Nodal) and/or an ActRII receptor (*e.g.,* an ActRIIA or ActRIIB receptor). In certain preferred embodiments, multispecific antibodies of the present disclosure that bind to and/or inhibit GDF8 further bind to and/or inhibit at least GDF11. Optionally, multispecific antibodies of the disclosure that bind to and/or inhibit GDF8 do not substantially bind to and/or substantially inhibit activin A. In some embodiments, multispecific antibodies of the disclosure that bind to and/or inhibit GDF8 and GDF11 further bind to and/or inhibit one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7 and/or Nodal.

Engineered antibodies with three or more functional antigen binding sites, including "octopus antibodies," are also included herein (see, *e.g.*, US 2006/0025576A1).

In certain embodiments, the antibodies disclosed herein (*e.g.,* an anti-GDF11 antibody, an anti-activin B antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody) are monoclonal antibodies. Monoclonal antibody refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, *e.g.*, containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different epitopes, each monoclonal antibody of a monoclonal antibody preparation is directed against a single epitope on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present methods may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods,

and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

For example, by using immunogens derived from GDF11 or GDF8, anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols [see, *e.g.*, Antibodies: A Laboratory Manual (1988) ed. by Harlow and Lane, Cold Spring Harbor Press]. A mammal, such as a mouse, hamster, or rabbit can be immunized with an immunogenic form of the GDF11 or GDF8 polypeptide, an antigenic fragment which is capable of eliciting an antibody response, or a fusion protein. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers or other techniques well known in the art. An immunogenic portion of a GDF11 or GDF8 polypeptide can be administered in the presence of adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibody production and/or level of binding affinity.

Following immunization of an animal with an antigenic preparation of GDF11 or GDF8, antisera can be obtained and, if desired, polyclonal antibodies can be isolated from the serum. To produce monoclonal antibodies, antibody-producing cells (lymphocytes) can be harvested from an immunized animal and fused by standard somatic cell fusion procedures with immortalizing cells such as myeloma cells to yield hybridoma cells. Such techniques are well known in the art, and include, for example, the hybridoma technique [see, *e.g.*, Kohler and Milstein (1975) Nature, 256: 495-497], the human B cell hybridoma technique [see, *e.g.*, Kozbar *et al.* (1983) Immunology Today, 4:72], and the EBV-hybridoma technique to produce human monoclonal antibodies [Cole *et al.* (1985) Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. pp. 77-96]. Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with a GDF11 or GDF8 polypeptide, and monoclonal antibodies isolated from a culture comprising such hybridoma cells.

In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein (*e.g.*, an anti-GDF11 antibody, an anti-activin B antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody), thereby generating an Fc-region variant. The Fc-region variant may comprise a human Fc-region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid

modification (*e.g.*, a substitution, deletion, and/or addition) at one or more amino acid positions.

For example, the present disclosure contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half-life of the antibody *in vivo* is important yet for which certain effector functions [*e.g.*, complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC)] are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in, for example, Ravetch and Kinet (1991) Annu. Rev. Immunol. 9:457-492. Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest are described in U.S. Pat. No. 5,500,362; Hellstrom, I. *et al.* (1986) Proc. Nat'l Acad. Sci. USA 83:7059-7063; Hellstrom, I *et al.* (1985) Proc. Nat'l Acad. Sci. USA 82:1499-1502; U.S. Pat. No. 5,821,337; and Bruggemann, M. *et al.* (1987) J. Exp. Med. 166:1351-1361. Alternatively, non-radioactive assay methods may be employed (*e.g.*, ACTI™, non-radioactive cytotoxicity assay for flow cytometry; CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96® non-radioactive cytotoxicity assay, Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, for example, in an animal model such as that disclosed in Clynes *et al.* (1998) Proc. Nat'l Acad. Sci. USA 95:652-656. C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity [see, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402]. To assess complement activation, a CDC assay may be performed [see, *e.g.*, Gazzano-Santoro *et al.* (1996) J. Immunol. Methods 202:163; Cragg, M. S. *et al.* (2003) Blood 101:1045-1052; and Cragg, M. S, and M. J. Glennie (2004) Blood 103:2738-2743]. FcRn binding and *in vivo* clearance/half-life determinations can also be performed using methods known in the art [see, *e.g.*, Petkova, S. B. *et al.* (2006) Int. Immunol. 18(12):1759-1769].

Antibodies of the present disclosure (*e.g.,* an anti-GDF11 antibody, an anti-activin B antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody) with reduced effector

function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

In certain embodiments, it may be desirable to create cysteine-engineered antibodies, *e.g.*, "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy-chain Fc region. Cysteine engineered antibodies may be generated as described, for example., in U.S. Pat. No. 7,521,541.

In addition, the techniques used to screen antibodies in order to identify a desirable antibody may influence the properties of the antibody obtained. For example, if an antibody is to be used for binding an antigen in solution, it may be desirable to test solution binding. A variety of different techniques are available for testing interaction between antibodies and antigens to identify particularly desirable antibodies. Such techniques include ELISAs, surface plasmon resonance binding assays (*e.g.*, the Biacore™ binding assay, Biacore AB, Uppsala, Sweden), sandwich assays (*e.g.*, the paramagnetic bead system of IGEN International, Inc., Gaithersburg, Maryland), western blots, immunoprecipitation assays, and immunohistochemistry.

In certain embodiments, amino acid sequence variants of the antibodies and/or the binding polypeptides provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody and/or binding polypeptide. Amino acid sequence variants of an antibody and/or binding polypeptides may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody and/or binding polypeptide, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into, and/or substitutions of residues within, the amino acid sequences of the antibody and/or binding polypeptide. Any combination of

deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, target-binding (GDF11, GDF8, ActRIIA, and/or ActRIIB binding).

Alterations (*e.g.*, substitutions) may be made in HVRs, for example, to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, *e.g.*, Chowdhury (2008) Methods Mol. Biol. 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described in the art [see, *e.g.*, Hoogenboom *et al.*, in Methods in Molecular Biology 178:1-37, O'Brien *et al.*, ed., Human Press, Totowa, N.J., (2001)]. In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind to the antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two, or three amino acid substitutions.

A useful method for identification of residues or regions of the antibody and/or the binding polypeptide that may be targeted for mutagenesis is called "alanine scanning mutagenesis", as described by Cunningham and Wells (1989) Science, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody or binding polypeptide with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively,

or additionally, a crystal structure of an antigen-antibody complex can be used to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

Amino-acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include fusion of the N- or C-terminus of the antibody to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

In certain embodiments, an antibody and/or binding polypeptide provided herein may be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody and/or binding polypeptide include but are not limited to water-soluble polymers. Non-limiting examples of water-soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody and/or binding polypeptide may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody and/or binding polypeptide to be improved, whether the antibody derivative and/or binding polypeptide derivative will be used in a therapy under defined conditions.

Any of the ActRII antagonist antibodies disclosed herein (*e.g.,* an anti-activin A antibody, an anti-activin B antibody, an anti-activin C antibody, an anti-activin E antibody, an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-BMP6 antibody, an anti-BMP7

antibody, an anti-ActRIIA antibody, and/or or an anti-ActRIIB antibody) can be combined with one or more additional ActRII antagonist agents of the disclosure to achieve the desired effect. For example, an ActRII antagonist antibody disclosed herein (*e.g.*, an anti-GDF11 antibody, an anti-activin B antibody, an anti-activin C antibody, an anti-activin E antibody, an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-BMP6 antibody, an-anti-BMP7 antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody) can be used in combination with i) one or more additional ActRII antagonist antibodies disclosed herein, ii) one or more ActRII polypeptides disclosed herein (*e.g.*, ActRIIA and/or ActRIIB polypeptides), iii) one or more GDF traps disclosed herein; iv) one or more small-molecule ActRII antagonist disclosed herein (*e.g.*, a small molecule antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); v) one or more polynucleotide ActRII antagonists disclosed herein (*e.g.*, a polynucleotide antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); vi) one or more follistatin polypeptides disclosed herein; and/or vii) one or more FLRG polypeptides disclosed herein.

D. Small-Molecule Antagonists

In another aspect, the present disclosure relates to a small molecule, or combination of small molecules, that antagonizes ActRII activity (*e.g.*, inhibition of ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 and/or SMAD 1/5/8 signaling). In particular, the disclosure provides methods of using a small-molecule antagonist, or combination of antibody antagonists, of ActRII , alone or in combination with one or more erythropoiesis stimulating agents (*e.g.*, EPO) or other supportive therapies [*e.g.*, hematopoietic growth factors (*e.g.*, G-CSF or GM-CSF), transfusion of red blood cells or whole blood, iron chelation therapy], to, *e.g.*, increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with *SF3B1, DNMT3A,* and/or *TET2* mutations in a subject in need thereof.

In some embodiments, a preferred ActRII antagonist of the present disclosure is a small-molecule antagonist, or combination of small-molecule antagonists, that direct or indirect inhibits at least GDF11 activity. Optionally, such a small-molecule antagonist, or combination of small-molecule antagonists, may further inhibit, either directly or indirectly, GDF8. Optionally, a small-molecule antagonist, or combination of small-molecule antagonists, of the present disclosure does not substantially inhibit activin A activity. In some embodiments, a small-molecule antagonist, or combination of small-molecule antagonists, of the present disclosure that inhibits, either directly or indirectly, GDF11 and/or GDF8 activity further inhibits, either directly or indirectly, activity of one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB.

In certain embodiments, a small-molecule antagonist, or combination of small-molecule antagonists, of the present disclosure is an indirect inhibitor of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, Nodal, ActRIIA, and ActRIIB. For example, a small-molecule antagonist, or combination of small-molecule antagonists, of the present disclosure may inhibit the expression (*e.g.*, transcription, translation, cellular secretion, or combinations thereof) of at least GDF11. Optionally, such a small-molecule antagonist, or combination of small-molecule antagonists, may further inhibit expression of GDF8. Optionally, a small-molecule antagonist, or combinations of small-molecule antagonists, of the disclosure does not substantially inhibit the expression of activin A. In some embodiments, a small-molecule antagonist, or combination of small-molecule antagonists, of the disclosure that inhibits expression of GDF11 and/or GDF8 may further inhibit the expression of one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB.

In other embodiments, a small-molecule antagonist, or combination of small-molecule antagonists, of the present disclosure is direct inhibitor of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB. For example, a preferred small-molecule antagonist, or combination of small-molecule antagonists, of the present disclosure directly binds to and inhibits at least GDF11 activity (*e.g.* inhibits the ability GDF11 to bind to an ActRIIA and/or ActRIIB receptor; inhibits GDF11-mediated activation of the ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). Optionally, a small-molecule antagonist, or combinations of small-molecule antagonists, of the disclosure may further bind to and inhibit GDF8 activity (*e.g.* inhibits the ability of GDF8 to bind to an ActRIIA and/or ActRIIB receptor; inhibits

GDF8-mediated activation of the ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). Optionally, a small-molecule antagonist, or combinations of small-molecule antagonists, of the disclosure does not substantially bind to or inhibit activin A activity (*e.g.* the ability of activin A to bind to an ActRIIA and/or ActRIIB receptor; activin A-mediated activation of the ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling pathway). In some embodiments, a small-molecule antagonist, or combinations of small-molecule antagonists, of the disclosure that binds to and inhibits the activity of GDF11 and/or GDF8 further binds to and inhibits the activity of one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB.

In some embodiments, a small-molecule antagonist, or combination of small-molecule antagonists, of the present disclosure directly binds to and inhibits at least GDF8 activity (*e.g.* inhibits the ability GDF8 to bind to an ActRIIA and/or ActRIIB receptor; inhibits GDF8-mediated activation of the ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). Optionally, a small-molecule antagonist, or combinations of small-molecule antagonists, of the disclosure may further bind to and inhibit GDF11 activity (*e.g.* inhibit the ability of GDF11 to bind to an ActRIIA and/or ActRIIB receptor; inhibit GDF11-mediated activation of the ActRIIA and/or ActRIIB signaling transduction, such as SMAD 2/3 signaling). Optionally, a small-molecule antagonist, or combinations of small-molecule antagonists, of the disclosure does not substantially bind to or inhibit activin A activity (*e.g.* the ability of activin A to bind to an ActRIIA and/or ActRIIB receptor; activin A-mediated activation of the ActRIIA and/or ActRIIB signaling transduction, SMAD 2/3 signaling). In some embodiments, a small-molecule antagonist, or combinations of small-molecule antagonists, of the disclosure that binds to and inhibits the activity of GDF8 and/or GDF11 further binds to and inhibits the activity of one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB.

In some embodiments, a small-molecule antagonist, or combination of small-molecule antagonists, of the present disclosure directly binds to and inhibits at least ActRIIA activity (*e.g.* ActRII ligand-mediated activation of ActRIIA signaling transduction, such as SMAD 2/3 signaling). For example, a preferred small-molecule antagonist, or combination of small-molecule antagonists, of the disclosure binds to an ActRIIA receptor and inhibits at least GDF11 from binding to and/or activating the ActRIIA receptor. Optionally, such a small-molecule antagonist, or combination of small-molecule antagonists, may further inhibit GDF8 from binding to and/or activating the ActRIIA receptor. Optionally, a small-molecule

antagonist, or combination of small-molecule antagonists, of the disclosure does not substantially inhibit activin A from binding to and/or activating an ActRIIA receptor. In some embodiments, a small-molecule antagonist, or combination of small-molecule antagonists, of the disclosure that inhibits GDF11 and/or GDF8 from binding to and/or activating the ActRIIA receptor further inhibits one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, and Nodal from binding to/and or activating the ActRIIA receptor.

In some embodiments, a small-molecule antagonist, or combination of small-molecule antagonists, of the present disclosure directly binds to and inhibits at least ActRIIB activity (*e.g.* ActRII ligand-mediated activation of ActRIIB signaling transduction, such as SMAD 2/3 signaling). For example, a preferred small-molecule antagonist, or combination of small-molecule antagonists, of the disclosure binds to an ActRIIB receptor and inhibits at least GDF11 from binding to and/or activating the ActRIIB receptor. Optionally, such a small-molecule antagonist, or combination of small-molecule antagonists, may further inhibit GDF8 from binding to and/or activating the ActRIIB receptor. Optionally, a small-molecule antagonist, or combination of small-molecule antagonists, of the disclosure does not substantially inhibit activin A from binding to and/or activating an ActRIIB receptor. In some embodiments, a small-molecule antagonist, or combination of small-molecule antagonists, of the disclosure that inhibits GDF11 and/or GDF8 from binding to and/or activating the ActRIIB receptor further inhibits one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, and Nodal from binding to/and or activating the ActRIIB receptor.

Binding organic small molecule antagonists of the present disclosure may be identified and chemically synthesized using known methodology (*see*, *e.g.*, PCT Publication Nos. WO 00/00823 and WO 00/39585). In general, small-molecule antagonists of the disclosure are usually less than about 2000 daltons in size, alternatively less than about 1500, 750, 500, 250 or 200 daltons in size, wherein such organic small molecules that are capable of binding, preferably specifically, to a polypeptide as described herein (*e.g.*, GDF11, GDF8, ActRIIA, and ActRIIB). Such small-molecule antagonists may be identified without undue experimentation using well-known techniques. In this regard, it is noted that techniques for screening organic small-molecule libraries for molecules that are capable of binding to a polypeptide target are well-known in the art (see, *e.g.*, international patent publication Nos. WO00/00823 and WO00/39585).

Binding organic small molecules of the present disclosure may be, for example, aldehydes, ketones, oximes, hydrazones, semicarbazones, carbazides, primary amines, secondary amines, tertiary amines, N-substituted hydrazines, hydrazides, alcohols, ethers, thiols, thioethers, disulfides, carboxylic acids, esters, amides, ureas, carbamates, carbonates, ketals, thioketals, acetals, thioacetals, aryl halides, aryl sulfonates, alkyl halides, alkyl sulfonates, aromatic compounds, heterocyclic compounds, anilines, alkenes, alkynes, diols, amino alcohols, oxazolidines, oxazolines, thiazolidines, thiazolines, enamines, sulfonamides, epoxides, aziridines, isocyanates, sulfonyl chlorides, diazo compounds, and acid chlorides.

Any of the small-molecule ActRII antagonists disclosed herein (*e.g.*, a small-molecule antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB) can be combined with one or more additional ActRII antagonist agents of the disclosure to achieve the desired effect (*e.g.*, increase red blood cell levels and/or hemoglobin in a subject in need thereof, treat or prevent an anemia, treat MDS or sideroblastic anemias, treat or prevent one or more complications of MDS or sideroblastic anemias). For example, a small-molecule ActRII antagonist disclosed herein (*e.g.*, a small-molecule antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB) can be used in combination with i) one or more additional small molecule ActRII antagonists disclosed herein, ii) one or more ActRII polypeptides disclosed herein (*e.g.*, ActRIIA and/or ActRIIB polypeptides), iii) one or more GDF traps disclosed herein; iv) one or more ActRII antagonist antibodies disclosed herein (*e.g.*, an anti-GDF11 antibody, an anti-activin B antibody, an anti-activin C antibody, an anti-activin E antibody, an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-BMP6 antibody, an-anti-BMP7 antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody); v) one or more polynucleotide ActRII antagonists disclosed herein (*e.g.*, a polynucleotide antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); vi) one or more follistatin polypeptides disclosed herein; and/or vii) one or more FLRG polypeptides disclosed herein.

E. Antagonist Polynucleotides

In another aspect, the present disclosure relates to a polynucleotide, or combination of polynucleotides, that antagonizes ActRII activity (*e.g.*, inhibition of ActRIIA and/or ActRIIB

signaling transduction, such as SMAD 2/3 and/or SMAD 1/5/8 signaling). In particular, the disclosure provides methods of using a polynucleotide ActRII antagonist, or combination of polynucleotide ActRII antagonists, , alone or in combination with one or more erythropoiesis stimulating agents (*e.g.*, EPO) or other supportive therapies [*e.g.*, hematopoietic growth factors (*e.g.*, G-CSF or GM-CSF), transfusion of red blood cells or whole blood, iron chelation therapy], to, *e.g.*, increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with SF3B1, *DNMT3A,* and/or *TET2* mutations in a subject in need thereof.

In some embodiments, a polynucleotide ActRII antagonist, or combination of polynucleotide ActRII antagonists, of the present disclosure can be used to inhibit the activity and/or expression on one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB. In certain preferred embodiments, a polynucleotide ActRII antagonist, or combination of polynucleotide ActRII antagonists, of the disclosure is a GDF-ActRII antagonist.

In some embodiments, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure inhibits the activity and/or expression (*e.g.*, transcription, translation, secretion, or combinations thereof) of at least GDF11. Optionally, such a polynucleotide antagonist, or combination of polynucleotide antagonists, may further inhibit the activity and/or expression of GDF8. Optionally, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure does not substantially inhibit the activity and/or expression of activin A. In some embodiments, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure that inhibits the activity and/or expression of GDF11 and/or GDF8 may further inhibit the activity and or expression of one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB.

In some embodiments, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure inhibits the activity and/or expression (*e.g.*, transcription, translation, secretion, or combinations thereof) of at least GDF8. Optionally, such

polynucleotide antagonist, or combination of polynucleotide antagonists, may further inhibit the activity and/or expression of GDF11. Optionally, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure does not substantially inhibit the activity and/or expression of activin A. In some embodiments, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure that inhibits the activity and/or expression of GDF8 and/or GDF11 may further inhibit the activity and or expression of one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB.

In some embodiments, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure inhibits the activity and/or expression (*e.g.*, transcription, translation, secretion, or combinations thereof) of at least ActRIIA. Optionally, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure does not substantially inhibit the activity and/or expression of activin A. In some embodiments, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure that inhibits the activity and/or expression of ActRIIA may further inhibit the activity and or expression of one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, and/or ActRIIB.

In some embodiments, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure inhibits the activity and/or expression (*e.g.*, transcription, translation, secretion, or combinations thereof) of at least ActRIIB. Optionally, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure does not substantially inhibit the activity and/or expression of activin A. In some embodiments, a polynucleotide antagonist, or combination of polynucleotide antagonists, of the disclosure that inhibits the activity and/or expression of ActRIIB may further inhibit the activity and or expression of one or more of activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, and/or ActRIIA.

The polynucleotide antagonists of the present disclosure may be an antisense nucleic acid, an RNAi molecule [*e.g.*, small interfering RNA (siRNA), small-hairpin RNA (shRNA), microRNA (miRNA)], an aptamer and/or a ribozyme. The nucleic acid and amino acid sequences of human GDF11, GDF8, activin A, activin B, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB are known in the art and thus polynucleotide antagonists for use in accordance with methods of the present disclosure may be routinely made by the skilled artisan based on the knowledge in the art and teachings provided herein.

For example, antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed, for example, in Okano (1991) J. Neurochem. 56:560; Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988). Triple helix formation is discussed in, for instance, Cooney *et al.* (1988) Science 241:456; and Dervan *et al.*, (1991)Science 251:1300. The methods are based on binding of a polynucleotide to a complementary DNA or RNA. In some embodiments, the antisense nucleic acids comprise a single-stranded RNA or DNA sequence that is complementary to at least a portion of an RNA transcript of a gene disclosed herein (*e.g.*, GDF11, GDF8, activin A, activin B, activin C, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB). However, absolute complementarity, although preferred, is not required.

A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double-stranded antisense nucleic acids of a gene disclosed herein (*e.g.*, GDF11, GDF8, activin A, activin B, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB), a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with an RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Polynucleotides that are complementary to the 5' end of the message, for example, the 5'-untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the 3'-untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well [see, *e.g.*, Wagner, R., (1994) Nature 372:333-335]. Thus, oligonucleotides complementary to either the 5'- or 3'-untranslated, noncoding regions of a gene of the disclosure (*e.g.*, GDF11, GDF8, activin A, activin B, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB), could be used in an antisense approach to inhibit translation of an endogenous mRNA. Polynucleotides complementary to the 5'-untranslated region of the mRNA should include the complement of the AUG start codon. Antisense polynucleotides complementary to mRNA coding regions are less efficient inhibitors of

translation but could be used in accordance with the methods of the present disclosure. Whether designed to hybridize to the 5'-untranslated, 3'-untranslated, or coding regions of an mRNA of the disclosure (*e.g.*, an GDF11, GDF8, activin A, activin B, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB mRNA), antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects, the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides, or at least 50 nucleotides.

In one embodiment, the antisense nucleic acid of the present disclosure (*e.g.*, a GDF11, GDF8, activin A, activin B, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, or ActRIIB antisense nucleic acid) is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of a gene of the disclosure. Such a vector would contain a sequence encoding the desired antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding desired genes of the instant disclosure, or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region [see, *e.g.*, Benoist and Chambon (1981) Nature 29:304-310], the promoter contained in the 3' long terminal repeat of Rous sarcoma virus [see, *e.g.*, Yamamoto *et al.* (1980) Cell 22:787-797], the herpes thymidine promoter [see, *e.g.*, Wagner *et al.* (1981) Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445], and the regulatory sequences of the metallothionein gene [see, *e.g.*, Brinster, *et al.* (1982) Nature 296:39-42].

In some embodiments, the polynucleotide antagonists are interfering RNA or RNAi molecules that target the expression of one or more of: GDF11, GDF8, activin A, activin B, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB. RNAi refers to the expression of an RNA which interferes with the expression of the targeted mRNA. Specifically, RNAi silences a targeted gene via interacting with the specific mRNA through a siRNA (small interfering RNA). The ds RNA complex is then targeted for degradation by the cell. An siRNA molecule is a double-stranded RNA duplex of 10 to 50 nucleotides in length, which interferes with the expression of a target gene which is sufficiently complementary (*e.g.*

at least 80% identity to the gene). In some embodiments, the siRNA molecule comprises a nucleotide sequence that is at least 85, 90, 95, 96, 97, 98, 99, or 100% identical to the nucleotide sequence of the target gene.

Additional RNAi molecules include short-hairpin RNA (shRNA); also short-interfering hairpin and microRNA (miRNA). The shRNA molecule contains sense and antisense sequences from a target gene connected by a loop. The shRNA is transported from the nucleus into the cytoplasm, and it is degraded along with the mRNA. Pol III or U6 promoters can be used to express RNAs for RNAi. Paddison *et al.* [Genes & Dev. (2002) 16:948-958, 2002] have used small RNA molecules folded into hairpins as a means to effect RNAi. Accordingly, such short hairpin RNA (shRNA) molecules are also advantageously used in the methods described herein. The length of the stem and loop of functional shRNAs varies; stem lengths can range anywhere from about 25 to about 30 nt, and loop size can range between 4 to about 25 nt without affecting silencing activity. While not wishing to be bound by any particular theory, it is believed that these shRNAs resemble the double-stranded RNA (dsRNA) products of the DICER RNase and, in any event, have the same capacity for inhibiting expression of a specific gene. The shRNA can be expressed from a lentiviral vector. An miRNA is a single-stranded RNA of about 10 to 70 nucleotides in length that are initially transcribed as pre-miRNA characterized by a "stem-loop" structure and which are subsequently processed into mature miRNA after further processing through the RISC.

Molecules that mediate RNAi, including without limitation siRNA, can be produced *in vitro* by chemical synthesis (Hohjoh, FEBS Lett 521:195-199, 2002), hydrolysis of dsRNA (Yang *et al.*, Proc Natl Acad Sci USA 99:9942-9947, 2002), by *in vitro* transcription with T7 RNA polymerase (Donzeet *et al.*, Nucleic Acids Res 30:e46, 2002; Yu *et al.*, Proc Natl Acad Sci USA 99:6047-6052, 2002), and by hydrolysis of double-stranded RNA using a nuclease such as E. coli RNase III (Yang *et al.*, Proc Natl Acad Sci USA 99:9942-9947, 2002).

According to another aspect, the disclosure provides polynucleotide antagonists including but not limited to, a decoy DNA, a double-stranded DNA, a single-stranded DNA, a complexed DNA, an encapsulated DNA, a viral DNA, a plasmid DNA, a naked RNA, an encapsulated RNA, a viral RNA, a double-stranded RNA, a molecule capable of generating RNA interference, or combinations thereof.

In some embodiments, the polynucleotide antagonists of the disclosure are aptamers. Aptamers are nucleic acid molecules, including double-stranded DNA and single-stranded

RNA molecules, which bind to and form tertiary structures that specifically bind to a target molecule, such as a GDF11, GDF8, activin A, activin B, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and ActRIIB polypeptide. The generation and therapeutic use of aptamers are well established in the art. See, *e.g.*, U.S. Pat. No. 5,475,096. Additional information on aptamers can be found in U.S. Patent Application Publication No. 20060148748. Nucleic acid aptamers are selected using methods known in the art, for example via the Systematic Evolution of Ligands by Exponential Enrichment (SELEX) process. SELEX is a method for the in vitro evolution of nucleic acid molecules with highly specific binding to target molecules as described in, *e.g.*, U.S. Pat. Nos. 5,475,096, 5,580,737, 5,567,588, 5,707,796, 5,763,177, 6,011,577, and 6,699,843. Another screening method to identify aptamers is described in U.S. Pat. No. 5,270,163. The SELEX process is based on the capacity of nucleic acids for forming a variety of two- and three-dimensional structures, as well as the chemical versatility available within the nucleotide monomers to act as ligands (form specific binding pairs) with virtually any chemical compound, whether monomeric or polymeric, including other nucleic acid molecules and polypeptides. Molecules of any size or composition can serve as targets. The SELEX method involves selection from a mixture of candidate oligonucleotides and step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve desired binding affinity and selectivity. Starting from a mixture of nucleic acids, which can comprise a segment of randomized sequence, the SELEX method includes steps of contacting the mixture with the target under conditions favorable for binding; partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules; dissociating the nucleic acid-target complexes; amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand enriched mixture of nucleic acids. The steps of binding, partitioning, dissociating and amplifying are repeated through as many cycles as desired to yield highly specific high affinity nucleic acid ligands to the target molecule.

Typically, such binding molecules are separately administered to the animal [see, *e.g.*, O'Connor (1991) J. Neurochem. 56:560], but such binding molecules can also be expressed in vivo from polynucleotides taken up by a host cell and expressed *in vivo* [see, *e.g.*, Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Fla. (1988)].

Any of the polynucleotide ActRII antagonists disclosed herein (*e.g.*, a polynucleotide antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C,

activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB) can be combined with one or more additional ActRII antagonist agents of the disclosure to achieve the desired effect (*e.g.*, increase red blood cell levels and/or hemoglobin in a subject in need thereof, treat or prevent an anemia, treat MDS or sideroblastic anemias, treat or prevent one or more complications of MDS or sideroblastic anemias). For example, an polynucleotide ActRII antagonist disclosed herein (*e.g.*, a polynucleotide antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB) can be used in combination with i) one or more additional polynucleotide ActRII antagonists disclosed herein, ii) one or more ActRII polypeptides disclosed herein (*e.g.*, ActRIIA and/or ActRIIB polypeptides), iii) one or more GDF traps disclosed herein; iv) one or more ActRII antagonist antibodies disclosed herein (*e.g.*, an anti-GDF11 antibody, an anti-activin B antibody, an anti-activin C antibody, an anti-activin E antibody, an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-BMP6 antibody, an-anti-BMP7 antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody); v) one or more small molecule ActRII antagonists disclosed herein (*e.g.*, a small molecule antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); vi) one or more follistatin polypeptides disclosed herein; and/or vii) one or more FLRG polypeptides disclosed herein.

F. Other Antagonists

In other aspects, an agent for use in accordance with the methods disclosed herein is a follistatin polypeptide, which may be used alone or in combination with one or more erythropoiesis stimulating agents (*e.g.*, EPO) or other supportive therapies [*e.g.*, hematopoietic growth factors (*e.g.*, G-CSF or GM-CSF), transfusion of red blood cells or whole blood, iron chelation therapy], to, *e.g.*, increase red blood cell levels in a subject in need thereof, treat or prevent an anemia in a subject in need thereof (including, e.g., reduction of transfusion burden), treat MDS or sideroblastic anemias in a subject in need thereof, and/or treat or prevent one or more complications of MDS or sideroblastic anemias (*e.g.*, anemia, blood transfusion requirement, neutropenia, iron overload, acute myocardial infarction, hepatic failure, hepatomegaly, splenomegaly, progression to acute myeloid lymphoma) and or treat or prevent a disorder associated with *SF3B1, DNMT3A,* and/or *TET2* mutations in a subject in need thereof. The term "follistatin polypeptide" includes polypeptides comprising

any naturally occurring polypeptide of follistatin as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity, and further includes any functional monomer or multimer of follistatin. In certain preferred embodiments, follistatin polypeptides of the disclosure bind to and/or inhibit activin activity, particularly activin A (*e.g.*, activin-mediated activation of ActRIIA and/or ActRIIB SMAD 2/3 signaling). Variants of follistatin polypeptides that retain activin binding properties can be identified based on previous studies involving follistatin and activin interactions. For example, WO2008/030367 discloses specific follistatin domains ("FSDs") that are shown to be important for activin binding. As shown below in SEQ ID NOs: 18-20, the follistatin N-terminal domain ("FSND" SEQ ID NO:18), FSD2 (SEQ ID NO: 20), and to a lesser extent FSD1 (SEQ ID NO: 19) represent exemplary domains within follistatin that are important for activin binding. In addition, methods for making and testing libraries of polypeptides are described above in the context of ActRII polypeptides, and such methods also pertain to making and testing variants of follistatin. Follistatin polypeptides include polypeptides derived from the sequence of any known follistatin having a sequence at least about 80% identical to the sequence of a follistatin polypeptide, and optionally at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or greater identity. Examples of follistatin polypeptides include the mature follistatin polypeptide or shorter isoforms or other variants of the human follistatin precursor polypeptide (SEQ ID NO: 16) as described, for example, in WO2005/025601.

The human follistatin precursor polypeptide isoform FST344 is as follows:

```
  1 mvrarhqpgg lclllllcq fmedrsaqag ncwlrqaknq rcqvlyktel
 51 skeeccstgr lstswteedv ndntlfkwmi fnggapncip cketcenvdc
101 gpgkkcrmnk knkprcvcap dcsnitwkgp vcgldgktyr necallkarc
151 keqpelevqy qgrckktcrd vfcpgsstcv vdqtnnaycv tcnricpepa
201 sseqylcgnd gvtyssachl rkatcllgrs iglayegkci kakscediqc
251 tggkkclwdf kvgrgrcslc delcpdsksd epvcasdnat yasecamkea
301 acssgvllev khsgscnsis edteeeeede dqdysfpiss ilew
```

(SEQ ID NO: 16; NCBI Reference No. NP_037541.1)

The signal peptide is underlined; also underlined above are the last 27 residues which represent the C-terminal extension distinguishing this follistatin isoform from the shorter follistatin isoform FST317 shown below.

The human follistatin precursor polypeptide isoform FST317 is as follows:

```
  1 MVRARHQPGG LCLLLLLLCQ FMEDRSAQAG NCWLRQAKNG RCQVLYKTEL
```

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 51  SKEECCSTGR LSTSWTEEDV NDNTLFKWMI FNGGAPNCIP CKETCENVDC
101  GPGKKCRMNK KNKPRCVCAP DCSNITWKGP VCGLDGKTYR NECALLKARC
151  KEQPELEVQY QGRCKKTCRD VFCPGSSTCV VDQTNNAYCV TCNRICPEPA
201  SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI KAKSCEDIQC
251  TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA
301  ACSSGVLLEV KHSGSCN
```

(SEQ ID NO: 17; NCBI Reference No. NP_006341.1)

The signal peptide is underlined.

The follistatin N-terminal domain (FSND) sequence is as follows:

GNCWLRQAKNGRCQVLYKTELSKEECCSTGRLSTSWTEEDVNDNTLFKWM IFNGGAPNCIPCK (SEQ ID NO: 18; FSND)

The FSD1 and FSD2 sequences are as follows:

ETCENVDCGPGKKCRMNKKNKPRCV (SEQ ID NO: 19; FSD1)

KTCRDVFCPGSSTCVVDQTNNAYCVT (SEQ ID NO: 20; FSD2)

In other aspects, an agent for use in accordance with the methods disclosed herein is a follistatin-like related gene (FLRG), also known as follistatin-related protein 3 (FSTL3). The term "FLRG polypeptide" includes polypeptides comprising any naturally occurring polypeptide of FLRG as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a useful activity. In certain preferred embodiments, FLRG polypeptides of the disclosure bind to and/or inhibit activin activity, particularly activin A (*e.g.*, activin-mediated activation of ActRIIA and/or ActRIIB SMAD 2/3 signaling). Variants of FLRG polypeptides that retain activin binding properties can be identified using routine methods to assay FLRG and activin interactions (see, *e.g.*, US 6,537,966). In addition, methods for making and testing libraries of polypeptides are described above in the context of ActRII polypeptides and such methods also pertain to making and testing variants of FLRG. FLRG polypeptides include polypeptides derived from the sequence of any known FLRG having a sequence at least about 80% identical to the sequence of an FLRG polypeptide, and optionally at least 85%, 90%, 95%, 97%, 99% or greater identity.

The human FLRG precursor (follistatin-related protein 3 precursor) polypeptide is as follows:

```
 1  MRPGAPGPLW PLPWGALAWA VGFVSSMGSG NPAPGGVCWL QQGQEATCSL
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 51  VLQTDVTRAE CCASGNIDTA WSNLTHPGNK INLLGFLGLV HCLPCKDSCD
101  GVECGPGKAC RMLGGRPRCE CAPDCSGLPA RLQVCGSDGA TYRDECELRA
151  ARCRGHPDLS VMYRGRCRKS CEHVVCPRPQ SCVVDQTGSA HCVVCRAAPC
201  PVPSSPGQEL CGNNNVTYIS SCHMRQATCF LGRSIGVRHA GSCAGTPEEP
251  PGGESAEEEE NFV
```

(SEQ ID NO:21; NCBI Reference No. NP_005851.1)

The signal peptide is underlined.

In certain embodiments, functional variants or modified forms of the follistatin polypeptides and FLRG polypeptides include fusion proteins having at least a portion of the follistatin polypeptide or FLRG polypeptide and one or more fusion domains, such as, for example, domains that facilitate isolation, detection, stabilization or multimerization of the polypeptide. Suitable fusion domains are discussed in detail above with reference to the ActRII polypeptides. In some embodiment, an antagonist agent of the disclosure is a fusion protein comprising an activin-binding portion of a follistatin polypeptide fused to an Fc domain. In another embodiment, an antagonist agent of the disclosure is a fusion protein comprising an activin binding portion of an FLRG polypeptide fused to an Fc domain.

Any of the follistatin polypeptides disclosed herein may be combined with one or more additional ActRII antagonist agents of the disclosure to achieve the desired effect (*e.g.*, increase red blood cell levels and/or hemoglobin in a subject in need thereof, treat or prevent an anemia, treat MDS or sideroblastic anemias, treat or prevent one or more complications of MDS or sideroblastic anemias). For example, a follistatin polypeptide disclosed herein can be used in combination with i) one or more additional follistatin polypeptides disclosed herein, ii) one or more ActRII polypeptides disclosed herein (*e.g.*, ActRIIA and/or ActRIIB polypeptides), iii) one or more GDF traps disclosed herein; iv) one or more ActRII antagonist antibodies disclosed herein (*e.g.*, an anti-GDF11 antibody, an anti-activin B antibody, an anti-activin C antibody, an anti-activin E antibody, an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-BMP6 antibody, an-anti-BMP7 antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody); v) one or more small molecule ActRII antagonists disclosed herein (*e.g.*, a small molecule antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); vi) one or more polynucleotide ActRII antagonists disclosed herein (*e.g.*, a polynucleotide antagonist of

one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); and/or one or more FLRG polypeptides disclosed herein.

Similarly, any of the FLRG polypeptides disclosed herein may be combined with one or more additional ActRII antagonist agents of the disclosure to achieve the desired effect. For example, a FLRG polypeptide disclosed herein can be used in combination with i) one or more additional FLRG polypeptides disclosed herein, ii) one or more ActRII polypeptides disclosed herein (*e.g.*, ActRIIA and/or ActRIIB polypeptides), iii) one or more GDF traps disclosed herein; iv) one or more ActRII antagonist antibodies disclosed herein (*e.g.*, an anti-GDF11 antibody, an anti-activin B antibody, an anti-activin C antibody, an anti-activin E antibody, an anti-GDF11 antibody, an anti-GDF8 antibody, an anti-BMP6 antibody, an-anti-BMP7 antibody, an anti-ActRIIA antibody, or an anti-ActRIIB antibody); v) one or more small molecule ActRII antagonists disclosed herein (*e.g.*, a small molecule antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); vi) one or more polynucleotide ActRII antagonists disclosed herein (*e.g.*, a polynucleotide antagonist of one or more of GDF11, GDF8, activin A, activin B, activin AB, activin C, activin E, BMP6, BMP7, Nodal, ActRIIA, and/or ActRIIB); and/or one or more follistatin polypeptides disclosed herein.

3. Screening Assays

In certain aspects, the present disclosure relates to the use of the subject ActRII polypeptides (*e.g.*, ActRIIA and ActRIIB polypeptides) and GDF trap polypeptides to identify compounds (agents) which are agonist or antagonists of ActRIIB polypeptides. Compounds identified through this screening can be tested to assess their ability to modulate red blood cell, hemoglobin, and/or reticulocyte levels *in vivo* or *in vitro*. These compounds can be tested, for example, in animal models.

There are numerous approaches to screening for therapeutic agents for increasing red blood cell or hemoglobin levels by targeting ActRII signaling (*e.g.*, ActRIIA and/or ActRIIB SMAD 2/3 and/or SMAD 1/5/8 signaling). In certain embodiments, high-throughput screening of compounds can be carried out to identify agents that perturb ActRII-mediated effects on a selected cell line. In certain embodiments, the assay is carried out to screen and

identify compounds that specifically inhibit or reduce binding of an ActRII polypeptide or GDF trap polypeptide to its binding partner, such as an ActRII ligand (e.g., activin A, activin B, activin AB, activin C, Nodal, GDF8, GDF11 or BMP7). Alternatively, the assay can be used to identify compounds that enhance binding of an ActRII polypeptide or GDF trap polypeptide to its binding partner such as an ActRII ligand. In a further embodiment, the compounds can be identified by their ability to interact with an ActRII polypeptide or GDF trap polypeptide.

A variety of assay formats will suffice and, in light of the present disclosure, those not expressly described herein will nevertheless be comprehended by one of ordinary skill in the art. As described herein, the test compounds (agents) of the invention may be created by any combinatorial chemical method. Alternatively, the subject compounds may be naturally occurring biomolecules synthesized *in vivo* or *in vitro*. Compounds (agents) to be tested for their ability to act as modulators of tissue growth can be produced, for example, by bacteria, yeast, plants or other organisms (*e.g.*, natural products), produced chemically (*e.g.*, small molecules, including peptidomimetics), or produced recombinantly. Test compounds contemplated by the present invention include non-peptidyl organic molecules, peptides, polypeptides, peptidomimetics, sugars, hormones, and nucleic acid molecules. In certain embodiments, the test agent is a small organic molecule having a molecular weight of less than about 2,000 Daltons.

The test compounds of the disclosure can be provided as single, discrete entities, or provided in libraries of greater complexity, such as made by combinatorial chemistry. These libraries can comprise, for example, alcohols, alkyl halides, amines, amides, esters, aldehydes, ethers and other classes of organic compounds. Presentation of test compounds to the test system can be in either an isolated form or as mixtures of compounds, especially in initial screening steps. Optionally, the compounds may be optionally derivatized with other compounds and have derivatizing groups that facilitate isolation of the compounds. Non-limiting examples of derivatizing groups include biotin, fluorescein, digoxygenin, green fluorescent protein, isotopes, polyhistidine, magnetic beads, glutathione S-transferase (GST), photoactivatible crosslinkers or any combinations thereof.

In many drug-screening programs which test libraries of compounds and natural extracts, high-throughput assays are desirable in order to maximize the number of compounds surveyed in a given period of time. Assays which are performed in cell-free systems, such as may be derived with purified or semi-purified proteins, are often preferred as "primary"

screens in that they can be generated to permit rapid development and relatively easy detection of an alteration in a molecular target which is mediated by a test compound. Moreover, the effects of cellular toxicity or bioavailability of the test compound can be generally ignored in the *in vitro* system, the assay instead being focused primarily on the effect of the drug on the molecular target as may be manifest in an alteration of binding affinity between an ActRII polypeptide or a GDF trap polypeptide and its binding partner (*e.g.*, an ActRII ligand).

Merely to illustrate, in an exemplary screening assay of the present disclosure, the compound of interest is contacted with an isolated and purified ActRIIB polypeptide which is ordinarily capable of binding to an ActRIIB ligand, as appropriate for the intention of the assay. To the mixture of the compound and ActRIIB polypeptide is then added to a composition containing an ActRIIB ligand (*e.g.*, GDF11). Detection and quantification of ActRIIB/ActRIIB ligand complexes provides a means for determining the compound's efficacy at inhibiting (or potentiating) complex formation between the ActRIIB polypeptide and its binding protein. The efficacy of the compound can be assessed by generating dose-response curves from data obtained using various concentrations of the test compound. Moreover, a control assay can also be performed to provide a baseline for comparison. For example, in a control assay, isolated and purified ActRIIB ligand is added to a composition containing the ActRIIB polypeptide, and the formation of ActRIIB/ActRIIB ligand complex is quantitated in the absence of the test compound. It will be understood that, in general, the order in which the reactants may be admixed can be varied, and can be admixed simultaneously. Moreover, in place of purified proteins, cellular extracts and lysates may be used to render a suitable cell-free assay system.

Complex formation between an ActRII polypeptide or GDF trap polypeptide and its binding protein may be detected by a variety of techniques. For instance, modulation of the formation of complexes can be quantitated using, for example, detectably labeled proteins such as radiolabeled (*e.g.*, ^{32}P, ^{35}S, ^{14}C or ^{3}H), fluorescently labeled (*e.g.*, FITC), or enzymatically labeled ActRII polypeptide or GDF trap polypeptide and/or its binding protein, by immunoassay, or by chromatographic detection.

In certain embodiments, the present disclosure contemplates the use of fluorescence polarization assays and fluorescence resonance energy transfer (FRET) assays in measuring, either directly or indirectly, the degree of interaction between an ActRII polypeptide of GDF trap polypeptide and its binding protein. Further, other modes of detection, such as those

based on optical waveguides (see, *e.g.*, PCT Publication WO 96/26432 and U.S. Pat. No. 5,677,196), surface plasmon resonance (SPR), surface charge sensors, and surface force sensors, are compatible with many embodiments of the disclosure.

Moreover, the present disclosure contemplates the use of an interaction trap assay, also known as the "two-hybrid assay," for identifying agents that disrupt or potentiate interaction between an ActRII polypeptide or GDF trap polypeptide and its binding partner. See, *e.g.*, U.S. Pat. No. 5,283,317; Zervos *et al.* (1993) Cell 72:223-232; Madura *et al.* (1993) J Biol Chem 268:12046-12054; Bartel *et al.* (1993) Biotechniques 14:920-924; and Iwabuchi *et al.* (1993) Oncogene 8:1693-1696). In a specific embodiment, the present disclosure contemplates the use of reverse two-hybrid systems to identify compounds (*e.g.*, small molecules or peptides) that dissociate interactions between an ActRII polypeptide or GDF trap and its binding protein [see, *e.g.*, Vidal and Legrain, (1999) Nucleic Acids Res 27:919-29; Vidal and Legrain, (1999) Trends Biotechnol 17:374-81; and U.S. Pat. Nos. 5,525,490; 5,955,280; and 5,965,368].

In certain embodiments, the subject compounds are identified by their ability to interact with an ActRII polypeptide or GDF trap polypeptide. The interaction between the compound and the ActRII polypeptide or GDF trap polypeptide may be covalent or non-covalent. For example, such interaction can be identified at the protein level using *in vitro* biochemical methods, including photo-crosslinking, radiolabeled ligand binding, and affinity chromatography [see, *e.g.*, Jakoby WB *et al.* (1974) Methods in Enzymology 46:1]. In certain cases, the compounds may be screened in a mechanism-based assay, such as an assay to detect compounds which bind to an ActRII polypeptide of GDF trap polypeptide. This may include a solid-phase or fluid-phase binding event. Alternatively, the gene encoding an ActRII polypeptide or GDF trap polypeptide can be transfected with a reporter system (*e.g.*, β-galactosidase, luciferase, or green fluorescent protein) into a cell and screened against the library preferably by high-throughput screening or with individual members of the library. Other mechanism-based binding assays may be used; for example, binding assays which detect changes in free energy. Binding assays can be performed with the target fixed to a well, bead or chip or captured by an immobilized antibody or resolved by capillary electrophoresis. The bound compounds may be detected usually using colorimetric endpoints or fluorescence or surface plasmon resonance.

4. Exemplary Therapeutic Uses

In certain aspects, the disclosure provides methods of treating MDS and sideroblastic anemias, particularly treating or preventing one or more subtypes or complications of MDS, with one or more ActRII antagonists, including the treatment of patients with MDS characterized by the presence of ring sideroblasts and/or one or more mutations in the *SF3B1, DNMT3A,* and/or *TET2* genes. In particular, the disclosure provides methods for using an ActRII antagonist, or combination of ActRII antagonists, to treat or prevent one or more complications of MDS and sideroblastic anemias including, for example, anemia, neutropenia, splenomegaly, blood transfusion requirement, development of acute myeloid leukemia, iron overload, and complications of iron overload, among which are congestive heart failure, cardiac arrhythmia, myocardial infarction, other forms of cardiac disease, diabetes mellitus, dyspnea, hepatic disease, and adverse effects of iron chelation therapy.

In particular, the disclosure provides methods for using an ActRII antagonist, or combination of ActRII antagonists, to treat or prevent anemia or other complications in a subtype of MDS, including MDS patients with elevated numbers of erythroblasts (hypercellularity) in bone marrow; in MDS patients with more than 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% sideroblasts in bone marrow; in MDS patients with refractory anemia with ring sideroblasts (RARS); in MDS patients with refractory anemia with ring sideroblasts and thrombocytosis (RARS-T); in MDS patients with refractory cytopenia with unilineage dysplasia (RCUD); in MDS patients with refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS); in MDS patients with a somatic mutation in *SF3B1*, *SRSF2*, *DNMT3A*, or *TET2*; in MDS patients without a somatic mutation in *ASXL1* or *ZRSR2*; in MDS patients with iron overload; and in MDS patients with neutropenia.

Also in particular, the disclosure provides methods for using an ActRII antagonist, or combination of ActRII antagonists, to treat or prevent anemia or other complications of a sideroblastic anemia, including but not limited to refractory anemia with ring sideroblasts (RARS); refractory anemia with ring sideroblasts and thrombocytosis (RARS-T); refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS); sideroblastic anemia associated with alcoholism; drug-induced sideroblastic anemia; sideroblastic anemia resulting from copper deficiency (zinc toxicity); sideroblastic anemia resulting from

hypothermia; X-linked sideroblastic anemia (XLSA); SLC25A38 deficiency; glutaredoxin 5 deficiency; erythropoietic protoporphyria; X-linked sideroblastic anemia with ataxia (XLSA/A); sideroblastic anemia with B-cell immunodeficiency, fevers, and developmental delay (SIFD); Pearson marrow-pancreas syndrome; myopathy, lactic acidosis, and sideroblastic anemia (MLASA); thiamine-responsive megaloblastic anemia (TRMA); and syndromic/nonsyndromic sideroblastic anemia of unknown cause.

In certain aspects the disclosure provides methods for treating or preventing disorders or complications of a disorder that is associated with germ line or somatic mutations in *SF3B1, DNMT3A,* and/or *TET2*, such as myelodysplastic syndrome, chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML) as well as in breast cancer, pancreatic cancer, gastric cancer, prostate cancer, and uveal melanoma. In certain aspects the disorder may be in a subject that has bone marrow cells that test positive for an *SF3B1, DNMT3A,* and/or *TET2* mutation, particularly myelodysplastic syndrome, CLL and AML. Optionally a mutation in the *SF3B1* gene is in an exon, intron or 5' or 3' untranslated region. Optionally a mutation in *SF3B1, DNMT3A,* and/or *TET2* causes a change in the amino acid sequence or does not cause a change in the amino acid sequence of the protein encoded by the gene. Optionally a mutation in the *SF3B1* gene causes a change in the amino acid of the protein encoded by the gene selected from the following changes: K182E, E491G, R590K, E592K, R625C, R625G, N626D, N626S, H662Y, T663A, K666M, K666Q, K666R, Q670E, G676D, V701I, I704N, I704V, G740R, A744P, D781G, A1188V, N619K, N626H, N626Y, R630S, I704T, G740E, K741N, G742D, D894G, Q903R, R1041H, I1241T, G347V, E622D, Y623C, R625H, R625L, H662D, H662Q, T663I, K666E, K666N, K666T, K700E, and V701F. Optionally a mutation in the *DNMT3A* gene causes a change in the amino acid of the protein encoded by the gene selected from the following changes: R882C, R882H, P904L, and P905P. Optionally a mutation in the *DNMT3A* gene introduces a premature stop codon. For example, in some embodiments, a mutation in the *DNMT3A* gene that introduces a premature stop codon is selected from the following positions: Y436X and W893X. Optionally a mutation in the *TET2* gene causes a change in the amino acid of the protein encoded by the gene selected from the following changes: E47Q, Q1274R, W1291R, G1370R, N1387S, and Y1724H. Optionally a mutation in the *TET2* gene introduces a premature stop codon. For example, in some embodiments, a mutation in the *TET2* gene that introduces a premature stop codon is selected from the following positions: R550X, Q1009X, Y1337X, R1404X, R1516X, and Q1652X.

The terms "subject," an "individual," or a "patient" are interchangeable throughout the specification and generally refer to mammals. Mammals include, but are not limited to, domesticated animals (*e.g.*, cows, sheep, cats, dogs, and horses), primates (*e.g.*, humans and non-human primates such as monkeys), rabbits, and rodents (*e.g.*, mice and rats).

As used herein, a therapeutic that "prevents" a disorder or condition refers to a compound that, in a statistical sample, reduces the occurrence of the disorder or condition in the treated sample relative to an untreated control sample, or delays the onset or reduces the severity of one or more symptoms of the disorder or condition relative to the untreated control sample.

The term "treating" as used herein includes amelioration or elimination of the condition once it has been established. In either case, prevention or treatment may be discerned in the diagnosis provided by a physician or other health care provider and the intended result of administration of the therapeutic agent.

In general, treatment or prevention of a disease or condition as described in the present disclosure is achieved by administering one or more of the ActRII antagonists (*e.g.*, an ActRIIA and/or ActRIIB antagonist) of the present disclosure in an effective amount. An effective amount of an agent refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result. A "therapeutically effective amount" of an agent of the present disclosure may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the agent to elicit a desired response in the individual. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result.

Myelodysplastic syndromes (MDS) are a diverse collection of hematological disorders characterized by ineffective production of myeloid blood cells and risk of transformation to acute myeloid leukemia. In MDS patients, hematopoietic stem cells do not mature into healthy red blood cells, white blood cells, or platelets. MDS disorders include, for example, refractory anemia, refractory cytopenia with unilineage dysplasia (RCUD), refractory anemia with ringed sideroblasts (RARS), refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), refractory anemia with excess blasts (RAEB-1), refractory anemia with excess blasts in transformation (RAEB-2), refractory cytopenia with multilineage dysplasia (RCMD), MDS unclassified (MDS-U), and

myelodysplastic syndrome associated with an isolated 5q chromosome abnormality [MDS with del(5q)].

Allogenic stem-cell transplantation is the only known potentially curative therapy for MDS. However, only a minority of patients undergo this procedure due to advanced age, medical comorbidities, and limited availability of appropriate stem cell donors. Even for those patients who proceed to allogenic stem-cell transplantation, significant treatment-related mortality and morbidity, including acute and chronic graft-versus-host-disease, and high relapse rates compromise long-term disease-free survival [Zeidan et al. (2013) Blood Rev 27:243-259]. For these reasons, the majority of patients with MDS are still managed on a non-curative intent therapeutic paradigm. Treatment is based on prognostic factors that predict survival or progression to acute myeloid leukemia. Survival of lower-risk MDS patients ranges from several months to more than a decade, and most of these patients die from causes directly related to complications of MDS [Dayyani et al. (2010) Cancer 116:2174-2179]. Therefore, therapeutic strategies for lower-risk patients are adapted to the specific patient's situation, including severity and type of cytopenias and expected survival. Lower-risk patients have multiple therapeutic options, including treatment with growth factors, lenalidomide in the case of del(5q) syndrome, thalidomide, pomalidomide, hypomethylating agents such as azacitidine or decitibine, and potentially investigational agents. In contrast, higher-risk MDS patients who are not eligible for allogenic stem-cell transplantation are typically treated with hypomethylating agents, intensive chemotherapy, or investigational agents [Garcia-Manero et al. (2011) 29:516-523].

Since MDS manifest as irreversible defects in both quantity and quality of hematopoietic cells, most MDS patients are afflicted with chronic anemia. Approximately 80% to 90% of MDS patients develop anemia during the course of their disease, of whom at least 40% become RBC transfusion-dependent [Santini (2011) Oncologist 16:35-42; Malcovati et al. (2005) J Clin Oncol 23:7594-7603; Leitch (2011) Blood Rev 25:17-31]. Patients in higher-risk MDS groups (according to IPSS classification) are even more likely to become transfusion-dependent; for example, in one study 79% of high-risk category patients versus 39% of low-risk patients required chronic transfusions to treat or prevent severe anemia [Öscan et al. (2013) Expert Rev Hematol 6:165-189]. Low hemoglobin levels result in poor oxygenation of the brain and peripheral organs; as a result, MDS patients typically suffer from lethargy, decreased mental alertness, physical weakness, and poor concentration. These symptoms are linked to a reduced health-related quality of life. In addition, hemoglobin

thresholds are independently associated with significant morbidity and mortality in MDS. In female and male patients with hemoglobin levels lower than 8 g/dL and 9 g/dL, respectively, the risk of morbidity and mortality increases, mainly due to an increased risk of cardiac complications [Malcovati et al. (2011) Haematologica 96:1433-1440]. Low hemoglobin levels and dependence on red blood cell transfusions have been associated with inferior cardiovascular outcomes and increased mortality in patients with MDS, representing a strong rationale for aggressive management of anemia in MDS [Goldberg et al. (2010) J Clin Oncol 28:2847–2852; Leitch (2011) Blood Rev 25:17-31; Oliva et al. (2011) Am J Blood Res 1:160-166; Özcan et al. (2013) Expert Rev Hematol 6:165-189].

MDS patients eventually require blood transfusions and/or treatment with erythropoietic growth factors (e.g., ESAs such as EPO) alone or in combination with a colony-stimulating factor [e.g., an analog of granulocyte colony-stimulating factor (G-CSF) such as filgrastim or an analog of granulocyte macrophage colony-stimulating factor (GM-GSF) such as sargramostim] to increase red blood cell levels. The frequency of transfusions depends on the extent of the disease and on the presence of comorbidities. Chronic transfusions are known to increase hemoglobin levels, which in turn improve brain and peripheral tissue oxygenation, thereby improving physical activity and mental alertness. However, many MDS patients develop side-effects from the use of such therapies. For example, patients who receive frequent red blood cell transfusions can develop tissue and organ damage from iron accumulation and generation of toxic reactive oxygen species. Accordingly, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator, may be used to treat patients with MDS or sideroblastic anemias. In certain embodiments, patients suffering from MDS or a siderblastic anemia may be treated using one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally in combination with an EPO receptor activator. In other embodiments, patients suffering from MDS or a sideroblastic anemia may be treated using a combination of one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF Trap, *etc.*) and one or more additional therapeutic agents for treating MDS including, for example, ESAs including, e.g., epoetin alfa, epoetin beta (e.g., NeoRecormon), epoetin delta (e.g., Dynepo), epoetin omega, darbepoetin alfa (e.g., Aranesp), methoxy-polyethylene-glycol epoetin beta (e.g., Micera),

and synthetic erythropoiesis protein (SEP); G-CSF analogs, including filgrastim; GM-CSF analogs , including sargramostim; lenalidomide; thalidomide; pomalidomide, hypomethylating agents, including azacitidine and decitabine; iron-chelating agents, including deferoxamine (a.k.a., desferrioxamine B, desferoxamine B, DFO-B, DFOA, DFB, or desferal), deferiprone (a.k.a., Ferriprox), and deferasirox (a.k.a., bis-hydroxyphenyl-triazole, ICL670, or Exjade™).; thrombopoietin mimetics, including romiplostim and eltrombopag; chemotherapeutic agents, including cytarabine (ara-C) alone or in combination with idarubicin, topotecan, or fludarabine; immunosuppressants, including antithymocyte globulin, alemtuzumab, and cyclosporine; histone deacetylase inhibitors (HDAC inhibitors), including vorinostat, valproic acid, phenylbutyrate, entinostat, MGCD0103, and other class I nuclear HDAC inhibitors, class II non-nuclear HDAC inhibitors, pan HDAC inhibitors, and isoform-specific HDAC inhibitors; farnesyltransferase inhibitors, including as tipifarnib and lonafarnib; tumor necrosis factor-alpha (TNF-α) inhibitors, including etanercept or infliximab; inhibitors of glutathione-S-transferase (GST) P1-1, including ezatiostat; and inhitors of CD33, including gemtuzumab ozogamicin.

One or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used to increase red blood cell levels, hemoglobin levels, and/or hematocrit levels in a patient with anemia (MDS or sideroblastic anemia). When monitoring hemoglobin and/or hematocrit levels in humans, a level of less than normal for the appropriate age and gender category may be indicative of anemia, although individual variations are taken into account. For example, a hemoglobin level from 10-12.5 g/dl, and typically about 11.0 g/dl is considered to be within the normal range in healthy adults, although, in terms of therapy, a lower target level may cause fewer cardiovascular side effects. See, *e.g.*, Jacobs et al. (2000) Nephrol Dial Transplant 15, 15-19. Alternatively, hematocrit levels (percentage of the volume of a blood sample occupied by the cells) can be used as a measure of anemia. Hematocrit levels for healthy individuals range from about 41-51% for adult males and from 35-45% for adult females. In certain embodiments, a patient may be treated with a dosing regimen intended to restore the patient to a target level of red blood cells, hemoglobin, and/or hematocrit or allow the reduction or elimination of red blood cell transfusions while maintaining an acceptable level of red blood cells, hemoglobin and/or hematocrit. As

hemoglobin and hematocrit levels vary from person to person, optimally, the target hemoglobin and/or hematocrit level can be individualized for each patient.

Neutropenia denotes a condition of abnormally low levels of circulating granulocytes and is found in approximately 40% of MDS patients [Steensma et al. (2006) Mayo Clin Proc 81:104-130]. Common complaints of patients with neutropenia include fatigue and frequent bacterial infections, especially of the skin. However, neutropenia can also result in serious complications in patients with MDS, and infection is the most common cause of MDS-associated death. Treatment with granulocyte colony-stimulating factor (filgrastim) can help keep neutrophil counts above 1×10^9/L for severely neutropenic patients [Akhtari (2011) Oncology (Williston Park) 25:480-486], but myeloid growth factors do not clearly modify disease history and may only increase production of functionally defective neutrophils lacking bactericidal capacity [Dayyani et al. (2010) Cancer 116:2174-2179; Steensma (2011) Semin Oncol 38:635-647]. Prophylactic antibiotics have no proven role in patients with MDS and are not recommended for patients with neutropenia related to MDS. However, neutropenic fever in MDS patients should be regarded as a medical emergency, requiring immediate administration of empiric broad-spectrum antibiotics and often hospitalization [Barzi et al. (2010) Cleve Clin J Med 77:37-44]. In some embodiments, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies such as a G-CSF or GM-CSF therapy, may be used to treat neutropenia in MDS patients. One or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used to reduce the frequency of granulocyte transfusions in MDS patients.

Patients with MDS or sideroblastic anemia who receive frequent transfusions of red blood cells or whole blood are prone to develop transfusional iron overload, which may partly explain why transfusion dependency in MDS is associated with reduced likelihood of survival. Nevertheless, the use of iron chelation therapy in transfusion-dependent MDS patients remains controversial, because retrospective and registry data suggest chelated patients may live longer than unchelated patients, yet there are no prospective randomized trial data demonstrating a morbidity or mortality benefit from chelation, and currently approved agents are inconvenient (deferroxamine) or costly and poorly tolerated by many

patients (deferasirox) [Steensma et al. (2013) Best Pract Res Clin Haematol 26:431-444; Lyons et al. (2014) Leuk Res 38:149-154].

In some embodiments, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used to prevent or reverse complications of iron overload in patients with MDS or sideroblastic anemia. In certain aspects, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used to prevent or reverse a cardiac complication of iron overload including, *e.g.*, increased cardiac output, cardiomegaly, cardiomyopathy, left ventricular hypertrophy, acute myocardial infarction, arrhythmia, and congestive heart failure. In certain aspects, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used to reduce liver iron content and/or prevent or reverse a hepatic complication of iron overload including, *e.g.*, liver enlargement (hepatomegaly), liver fibrosis (increase in scar tissue), and cirrhosis (extensive scarring). In certain aspects, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used to prevent or reverse an endocrine complication of iron overload including, *e.g.*, diabetes mellitus.

In certain aspects, ActRII antagonist agents of the disclosure may be administered to a subject in need thereof in combination with one or more additional agents [for example, ESAs; G-CSF analogs, including filgrastim; GM-CSF analogs, including sargramostim; lenalidomide; thalidomide; pomalidomide, hypomethylating agents, including azacitidine and decitabine; iron-chelating agents, including deferoxamine and deferasirox; thrombopoietin mimetics, including romiplostim and eltrombopag; chemotherapeutic agents, including cytarabine (ara-C) alone or in combination with idarubicin, topotecan, or fludarabine; immunosuppressants, including antithymocyte globulin, alemtuzumab, and cyclosporine; histone deacetylase inhibitors (HDAC inhibitors), including vorinostat, valproic acid, phenylbutyrate, entinostat, MGCD0103, and other class I nuclear HDAC inhibitors, class II non-nuclear HDAC inhibitors, pan HDAC inhibitors, and isoform-specific HDAC inhibitors;

farnesyltransferase inhibitors, including as tipifarnib and lonafarnib; tumor necrosis factor-alpha (TNF-α) inhibitors, including etanercept or infliximab; inhibitors of glutathione-S-transferase (GST) P1-1, including ezatiostat; and inhitors of CD33, including gemtuzumab ozogamicin.] or supportive therapies [*e.g.*, red blood cell transfusion, granulocyte transfusion, thrombocyte (platelet) transfusion] for treating MDS and sideroblastic anemia or one or more complications of MDS and sideroblastic anemia.

As used herein, "in combination with" or "conjoint administration" refers to any form of administration such that additional therapies (e.g., second, third, fourth, etc.) are still effective in the body (e.g., multiple compounds are simultaneously effective in the patient, which may include synergistic effects of those compounds). Effectiveness may not correlate to measurable concentration of the agent in blood, serum, or plasma. For example, the different therapeutic compounds can be administered either in the same formulation or in separate formulations, either concomitantly or sequentially, and on different schedules. Thus, an individual who receives such treatment can benefit from a combined effect of different therapies. One or more GDF11 and/or activin B antagonist agents (optionally further antagonists of one or more of GDF8, activin A, activin C, activin E, and BMP6) of the disclosure can be administered concurrently with, prior to, or subsequent to, one or more other additional agents or supportive therapies. In general, each therapeutic agent will be administered at a dose and/or on a time schedule determined for that particular agent. The particular combination to employ in a regimen will take into account compatibility of the antagonist of the present disclosure with the therapy and/or the desired therapeutic effect to be achieved.

In certain embodiments, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, antibody *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used in combination with transfusion of either red blood cells or whole blood to treat anemia in patients with MDS or sideroblastic anemias. In patients who receive frequent transfusions of whole blood or red blood cells, normal mechanisms of iron homeostasis can be overwhelmed, eventually leading to toxic and potentially fatal accumulation of iron in vital tissues such as heart, liver, and endocrine glands. Regular red blood cell transfusions require exposure to various donor units of blood and hence a higher risk of alloimmunization. Difficulties with vascular access, availability of and compliance with iron chelation, and high cost are some of the reasons why it can be beneficial to limit the

number of red blood cell transfusions. In some embodiments, the methods of the present disclosure relate to treating MDS or sideroblastic anemia in a subject in need thereof by administering a combination of an ActRII antagonist of the disclosure and one or more blood cell transfusions. In some embodiments, the methods of the present disclosure relate to treating or preventing one or more complications of MDS or sideroblastic anemia in a subject in need thereof by administering a combination of an ActRII antagonist of the disclosure and one or more red blood cell transfusions. In some embodiments, treatment with one or more ActRII antagonists of the disclosure is effective at decreasing the transfusion requirement in a patient with MDS or sideroblastic anemia, *e.g.*, reduces the frequency and/or amount of blood transfusion required to effectively treat MDS or sideroblastic anemia or one or more their complications.

In certain embodiments, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used in combination with one or more iron-chelating molecules to promote iron excretion in the urine and/or stool and thereby prevent or reverse tissue iron overload in patients with MDS or sideroblastic anemias. Effective iron-chelating agents should be able to selectively bind and neutralize ferric iron, the oxidized form of non-transferrin bound iron which likely accounts for most iron toxicity through catalytic production of hydroxyl radicals and oxidation products [see, *e.g.*, Esposito et al. (2003) Blood 102:2670-2677]. These agents are structurally diverse, but all possess oxygen or nitrogen donor atoms able to form neutralizing octahedral coordination complexes with individual iron atoms in stoichiometries of 1:1 (hexadentate agents), 2:1 (tridentate), or 3:1 (bidentate) [Kalinowski *et al.* (2005) Pharmacol Rev 57:547-583]. In general, effective iron-chelating agents also are relatively low molecular weight (*e.g.*, less than 700 daltons), with solubility in both water and lipids to enable access to affected tissues. Specific examples of iron-chelating molecules include deferoxamine, a hexadentate agent of bacterial origin requiring daily parenteral administration, and the orally active synthetic agents deferiprone (bidentate) and deferasirox (tridentate). Combination therapy consisting of same-day administration of two iron-chelating agents shows promise in patients unresponsive to chelation monotherapy and also in overcoming issues of poor patient compliance with dereroxamine alone [Cao *et al.* (2011) Pediatr Rep 3(2):e17; and Galanello *et al.* (2010) Ann NY Acad Sci 1202:79-86].

One or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used to increase red blood cell levels, hemoglobin levels, and/or hematocrit levels in a patient with MDS or sideroblastic anemia. When observing hemoglobin and/or hematocrit levels in humans, a level of less than normal for the appropriate age and gender category may be indicative of anemia, although individual variations are taken into account. For example, a hemoglobin level from 10-12.5 g/dl, and typically about 11.0 g/dl is considered to be within the normal range in healthy adults, although, in terms of therapy, a lower target level may cause fewer cardiovascular side effects. See, *e.g.*, Jacobs et al. (2000) Nephrol Dial Transplant 15, 15-19. Alternatively, hematocrit levels (percentage of the volume of a blood sample occupied by the cells) can be used as a measure for anemia. Hematocrit levels for healthy individuals range from about 41-51% for adult males and from 35-45% for adult females. In certain embodiments, a patient may be treated with a dosing regimen intended to restore the patient to a target level of red blood cells, hemoglobin, and/or hematocrit. As hemoglobin and hematocrit levels vary from person to person, optimally, the target hemoglobin and/or hematocrit level can be individualized for each patient.

One or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) may be used in combination with EPO receptor activators and/or one or more additional therapies to treat anemia. The suboptimal erythropoietin response in some patients with MDS is considered one biologic rationale for treating MDS-related anemia with ESAs [Greenberg et al. (2011) J Natl Compr Canc Netw 9:30–56; Santini (2012) Semin Hematol 49:295–303]. Despite not being approved by the FDA for use in MDS-associated anemia, ESAs are in wide clinical use and are the most commonly used therapy for MDS [Casadevall et al. (2004) Blood 104:321-327; Greenberg et al. (2009) Blood 114:2393-2400]. An analysis of linked SEER-Medicare data between 2001 and 2005 found that 62% of Medicare beneficiaries with MDS received ESAs [Davidoff et al. (2013) Leuk Res 37:675-680]. Greenberg et al. (2009) Blood 114:2393-2400]. Some preclinical and clinical studies suggested that granulocyte colony-stimulating factor (G-CSF) can have synergistic effects with ESAs, and small doses of G-CSF can be tried to improve erythroid responses in some patients, especially those with RARS, either initially or in case of lack of response to sole ESA therapy [Negrin et al. (1996) Blood 87:4076–4081]. Additionally, patients with low-risk MDS and lower levels of serum

EPO (< 200–500 mU/mL) and those who have lower RBC transfusion requirements (< 2 units/month) have higher probabilities of achieving erythroid responses with ESAs [Hellstrom-Lindberg et al. (2003) Br J Haematol 120:1037–1046; Park et al. (2008) 111:574–582]. Therefore, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) may be used in combination with granulocyte colony-stimulating factor (e.g., filgrastim) or granulocyte macrophage colony-stimulating factor (e.g., sargramostim) to treat anemia.

In certain embodiments, the present disclosure provides methods of treating or preventing anemia in an individual in need thereof by administering to the individual a therapeutically effective amount of one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) and a EPO receptor activator. In certain embodiments, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) may be used in combination with EPO receptor activators to reduce the required dose of these activators in patients that are susceptible to adverse effects of ESAs. These methods may be used for therapeutic and prophylactic treatments of a patient.

One or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) may be used in combination with EPO receptor activators to achieve an increase in red blood cells, particularly at lower dose ranges. This may be beneficial in reducing the known off-target effects and risks associated with high doses of EPO receptor activators. The primary adverse effects of ESAs include, for example, an excessive increase in the hematocrit or hemoglobin levels and polycythemia. Elevated hematocrit levels can lead to hypertension (more particularly aggravation of hypertension) and vascular thrombosis. Other adverse effects of ESAs which have been reported, some of which relate to hypertension, are headaches, influenza-like syndrome, obstruction of shunts, myocardial infarctions and cerebral convulsions due to thrombosis, hypertensive encephalopathy, and red cell blood cell aplasia. See, *e.g.*, Singibarti (1994) J. Clin Investig 72(suppl 6), S36-S43; Horl *et al.* (2000) Nephrol Dial Transplant 15(suppl 4), 51-56; Delanty *et al.* (1997) Neurology 49, 686-689; and Bunn (2002) N Engl J Med 346(7), 522-523).

Provided that antagonists of the present disclosure act by a different mechanism that ESAs, these antagonists may be useful for increasing red blood cell and hemoglobin levels in

patients that do not respond well to ESAs or other EPO receptor activators. For example, an ActRII antagonist of the present disclosure may be beneficial for a patient in which administration of a normal to increased (> 300 IU/kg/week) dose of ESA does not result in the increase of hemoglobin level up to the target level. Patients with an inadequate response to ESAs are found in all types of anemia, but higher numbers of non-responders have been observed particularly frequently in patients with cancers and patients with end-stage renal disease. An inadequate response to ESAs can be either constitutive (observed upon the first treatment with ESA) or acquired (observed upon repeated treatment with ESA).

Lenalidomide is a thalidomide derivative approved for patients with lower-risk MDS with del5q and transfusion-dependent anemia. Pomalidomide is another thalidomide derivative. Approval of lenalidomide in the U.S. was based on results of a phase 2 trial in which transfusion independence was achieved in about two thirds of patients studied, and the mean duration of transfusion independence was 2.2 years [List et al. (2006) N Engl J Med 355:1456-1465]. Subsequently approved also in Europe [Giagounidis et al. (2014) Eur J Haematol 93:429-438], lenalidomide is considered the first-line treatment for patients with lower-risk MDS with del5q and anemia. In certain embodiments, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used in combination with lenalidomide for treating patients with MDS.

Aberrant DNA methylation is a poor prognostic feature in MDS [Shen e al. (2010) J Clin Oncol 28:605-613]. Azacitidine and decitabine are two agents with DNA hypomethylating activity currently used to treat patients mainly with high-risk MDS [Kantarjian et al. (2007) Cancer 109:1133-1137; Fenaux et al. (2009) Lancet Oncol 10:223-232]. Since the mechanism underlying their therapeutic effects is uncertain, these agents are sometimes classified according to their chemical structure (azanucleosides) or known activity in vitro (DNA methyltransferase inhibitors). Although there is less experience with azacitidine and decitabine therapeutically in lower-risk MDS, studies indicate that azacytidine and decitabine can produce an erythroid response in 30% to 40% of ESA-resistant patients with lower-risk MDS [Lyons et al. (2009) J Clin Oncol 27:1850-1856]. Platelet responses are also observed in thrombocytopenic patients. On the basis of these results, azacitidine and decitabine are approved in the United States for the treatment of lower-risk MDS with symptomatic cytopenias. In certain embodiments, one or more ActRII

antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used in combination with azacitidine, decitabine, or another DNA methyltransferase inhibitor for treating patients with MDS.

Thrombocytopenia occurs in approximately 35% to 45% of MDS patients, who may complain of easy bruising or frequent minor mucocutaneous bleeding and may display purpura or petechiae [Steensma et al. (2006) Mayo Clin Proc 81:104-130]. In more extreme cases, increased risk of gastrointestinal bleeding or intracranial hemorrhage may occur. For thrombocytopenic patients, platelet transfusions are typically indicated when platelet levels drop to less than 10,000 platelets/μL [Slichter (2007) Hematology Am Soc Hematol Educ Program 2007:172-178]. The supportive use of platelet transfusions is transient and not always effective due to the frequency of sensitization in chronically transfusion-dependent MDS patients. There is considerable interest in using growth factors with thrombopoietic activity in the therapy of MDS. Romiplostim and eltrombopag are thrombomimetic agents approved in the United States for patients with idiopathic thrombocytopenic purpura, and romiplostim is being studied extensively in patients with MDS [Kantarjian et al. (2010) J Clin Oncol 28:437-444; Santini (2012) Semin Hematol 49:295-303]. When used as a single agent, romiplostim can significantly improve platelet counts in approximately 50% of patients with lower-risk MDS with thrombocytopenia. However, a transient increase in marrow blast percentage, sometimes to greater than 20%, can be observed in 15% of patients, consistent with the presence of thrombopoietin receptors on blast cells in MDS. Romiplostim can also significantly reduce thrombocytopenia and/or platelet transfusions in patients with MDS receiving azacitidine, decitabine, or lenalidomide, thalidomide or pomalidomide, and could become an important adjunct to those treatments [Kantarjian et al. (2010) Blood 116:3163-3170]. Eltrombopag is also being developed in MDS. In certain embodiments, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used in combination with thrombomimetic agents such as romiplostim or eltrombopag for treating patients with MDS or sideroblastic anemias.

In certain embodiments, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap,

etc.), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used in combination with hepcidin, a hepcidin analog, or a hepcidin receptor activator for treating patients with MDS or sideroblastic anemias, particularly for complications associated with iron overload. A circulating polypeptide produced mainly in the liver, hepcidin is considered a master regulator of iron metabolism by virtue of its ability to induce the degradation of ferroportin, an iron-export protein localized on absorptive enterocytes, hepatocytes, and macrophages. In broad terms, hepcidin reduces availability of extracellular iron, so hepcidin, hepcidin analogs, or hepcidin receptor activators may be beneficial in the treatment of patients with MDS or sideroblastic anemias, particularly for complications associated with iron overload.

Investigational agents for MDS are in development. These include single-agent inhibitors of histone deacetylase, p38MAPK inhibitors, glutathione S-transferase π inhibitors, and alemtuzumab for patients who meet criteria for immunosuppressive-based therapy [Garcia-Manero et al. (2011) J Clin Oncol 29:516-523;]. For example, high response rates have been reported in MDS patients treated with immunosuppressive therapies incorporating anti-thymocyte globulin or alemtuzumab [Sloand et al. (2010) J Clin Oncol 28:5166-5173]. However, such patients have generally been younger and have had a higher frequency of normal karyotypes than MDS patients overall, which limits the generalizability of those results. Investigational therapies include, for example, histone deacetylase inhibitors (HDAC inhibitors), including vorinostat, valproic acid, phenylbutyrate, entinostat, MGCD0103, and other class I nuclear HDAC inhibitors, class II non-nuclear HDAC inhibitors, pan HDAC inhibitors, and isoform-specific HDAC inhibitors; farnesyltransferase inhibitors, including as tipifarnib and lonafarnib; tumor necrosis factor-alpha (TNF-α) inhibitors, including etanercept or infliximab; inhibitors of glutathione-S-transferase (GST) P1-1, including ezatiostat; and inhitors of CD33, including gemtuzumab ozogamicin. In certain embodiments, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator and/or one or more additional therapies, may be used in combination with one or more of these investigational therapies for MDS.

Increasing evidence suggests that combined use of MDS therapeutic agents with different mechanisms of action offers substantial benefit in the form of diminished side effects, improved overall survival, and delayed progression to acute myeloid leukemia. Multiple studies indicate that when compared with traditional monotherapies, combining

various medications with non-overlapping mechanisms of action and toxicities may result in significant benefit for MDS patients. A variety of combination therapies with growth factors, DNA methytransferase inhibitors, histone deacetylase inhibitors, and immunosuppressant treatments provide encouraging data [Ornstein et al. (2012) Int J Hematol 95:26-33]. Therefore, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), optionally combined with an EPO receptor activator, may be used to increase numbers of red blood cells in MDS patients additionally treated with a combination of two or more agents, including but not limited to growth factors, DNA methyltransferase inhibitors, histone deacetylase inhibitors, or immunosuppressant treatments.

In certain embodiments, the present disclosure provides methods for managing a patient that has been treated with, or is a candidate to be treated with, one or more one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) by measuring one or more hematologic parameters in the patient. The hematologic parameters may be used to evaluate appropriate dosing for a patient who is a candidate to be treated with the antagonist of the present disclosure, to monitor the hematologic parameters during treatment, to evaluate whether to adjust the dosage during treatment with one or more antagonist of the disclosure, and/or to evaluate an appropriate maintenance dose of one or more antagonists of the disclosure. If one or more of the hematologic parameters are outside the normal level, dosing with one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) may be reduced, delayed or terminated.

Hematologic parameters that may be measured in accordance with the methods provided herein include, for example, red blood cell levels, blood pressure, iron stores, and other agents found in bodily fluids that correlate with increased red blood cell levels, using art recognized methods. Such parameters may be determined using a blood sample from a patient. Increases in red blood cell levels, hemoglobin levels, and/or hematocrit levels may cause increases in blood pressure.

In one embodiment, if one or more hematologic parameters are outside the normal range or on the high side of normal in a patient who is a candidate to be treated with one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), then onset of administration of the

one or more antagonists of the disclosure may be delayed until the hematologic parameters have returned to a normal or acceptable level either naturally or via therapeutic intervention. For example, if a candidate patient is hypertensive or pre-hypertensive, then the patient may be treated with a blood pressure lowering agent in order to reduce the patient's blood pressure. Any blood pressure lowering agent appropriate for the individual patient's condition may be used including, for example, diuretics, adrenergic inhibitors (including alpha blockers and beta blockers), vasodilators, calcium channel blockers, angiotensin-converting enzyme (ACE) inhibitors, or angiotensin II receptor blockers. Blood pressure may alternatively be treated using a diet and exercise regimen. Similarly, if a candidate patient has iron stores that are lower than normal, or on the low side of normal, then the patient may be treated with an appropriate regimen of diet and/or iron supplements until the patient's iron stores have returned to a normal or acceptable level. For patients having higher than normal red blood cell levels and/or hemoglobin levels, then administration of the one or more antagonists of the disclosure may be delayed until the levels have returned to a normal or acceptable level.

In certain embodiments, if one or more hematologic parameters are outside the normal range or on the high side of normal in a patient who is a candidate to be treated with one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*), then the onset of administration may not be delayed. However, the dosage amount or frequency of dosing of the one or more antagonists of the disclosure may be set at an amount that would reduce the risk of an unacceptable increase in the hematologic parameters arising upon administration of the one or more antagonists of the disclosure. Alternatively, a therapeutic regimen may be developed for the patient that combines one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) with a therapeutic agent that addresses the undesirable level of the hematologic parameter. For example, if the patient has elevated blood pressure, then a therapeutic regimen may be designed involving administration of one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) and a blood pressure lowering agent. For a patient having lower than desired iron stores, a therapeutic regimen may be developed involving one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) and iron supplementation.

In one embodiment, baseline parameter(s) for one or more hematologic parameters may be established for a patient who is a candidate to be treated with one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) and an appropriate dosing regimen established for that patient based on the baseline value(s). Alternatively, established baseline parameters based on a patient's medical history could be used to inform an appropriate antagonist dosing regimen for a patient. For example, if a healthy patient has an established baseline blood pressure reading that is above the defined normal range it may not be necessary to bring the patient's blood pressure into the range that is considered normal for the general population prior to treatment with the one or more antagonist of the disclosure. A patient's baseline values for one or more hematologic parameters prior to treatment with one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) may also be used as the relevant comparative values for monitoring any changes to the hematologic parameters during treatment with the one or more antagonists of the disclosure.

In certain embodiments, one or more hematologic parameters are measured in patients who are being treated with a one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*). The hematologic parameters may be used to monitor the patient during treatment and permit adjustment or termination of the dosing with the one or more antagonists of the disclosure or additional dosing with another therapeutic agent. For example, if administration of one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) results in an increase in blood pressure, red blood cell level, or hemoglobin level, or a reduction in iron stores, then the dose of the one or more antagonists of the disclosure may be reduced in amount or frequency in order to decrease the effects of the one or more antagonists of the disclosure on the one or more hematologic parameters. If administration of one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) results in a change in one or more hematologic parameters that is adverse to the patient, then the dosing of the one or more antagonists of the disclosure may be terminated either temporarily, until the hematologic parameter(s) return to an acceptable level, or permanently. Similarly, if one or more hematologic parameters are not brought within an acceptable range after reducing the dose or frequency of administration of the one

or more antagonists of the disclosure, then the dosing may be terminated. As an alternative, or in addition to, reducing or terminating the dosing with the one or more antagonists of the disclosure, the patient may be dosed with an additional therapeutic agent that addresses the undesirable level in the hematologic parameter(s), such as, for example, a blood pressure lowering agent or an iron supplement. For example, if a patient being treated with one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) has elevated blood pressure, then dosing with the one or more antagonists of the disclosure may continue at the same level and a blood-pressure-lowering agent is added to the treatment regimen, dosing with the one or more antagonist of the disclosure may be reduced (*e.g.*, in amount and/or frequency) and a blood-pressure-lowering agent is added to the treatment regimen, or dosing with the one or more antagonist of the disclosure may be terminated and the patient may be treated with a blood-pressure-lowering agent.

6. Pharmaceutical Compositions

In certain aspects, one or more ActRII antagonist agents of the disclosure (*e.g.*, a GDF-ActRII antagonist, an ActRIIA polypeptide, an ActRIIB polypeptide, a GDF trap, *etc.*) can be administered alone or as a component of a pharmaceutical formulation (also referred to as a therapeutic composition or pharmaceutical composition). A pharmaceutical formulation refers to a preparation which is in such form as to permit the biological activity of an active ingredient (*e.g.*, an agent of the present disclosure) contained therein to be effective and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. The subject compounds may be formulated for administration in any convenient way for use in human or veterinary medicine. For example, one or more agents of the present disclosure may be formulated with a pharmaceutically acceptable carrier. A pharmaceutically acceptable carrier refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is generally nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, and/or preservative. In general, pharmaceutical formulations for use in the present disclosure are in a pyrogen-free, physiologically-acceptable form when administered to a subject. Therapeutically useful agents other than those described herein, which may optionally be included in the formulation as described above, may be administered in combination with the subject agents in the methods of the present disclosure.

Typically, compounds will be administered parenterally [*e.g.*, by intravenous (I.V.) injection, intraarterial injection, intraosseous injection, intramuscular injection, intrathecal injection, subcutaneous injection, or intradermal injection]. Pharmaceutical compositions suitable for parenteral administration may comprise one or more agents of the disclosure in combination with one or more pharmaceutically acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use. Injectable solutions or dispersions may contain antioxidants, buffers, bacteriostats, suspending agents, thickening agents, or solutes which render the formulation isotonic with the blood of the intended recipient. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical formulations of the present disclosure include water, ethanol, polyols (*e.g.*, glycerol, propylene glycol, polyethylene glycol, *etc.*), vegetable oils (*e.g.*, olive oil), injectable organic esters (*e.g.*, ethyl oleate), and suitable mixtures thereof. Proper fluidity can be maintained, for example, by the use of coating materials (*e.g.*, lecithin), by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

In some embodiments, a therapeutic method of the present disclosure includes administering the pharmaceutical composition systemically, or locally, from an implant or device. Further, the pharmaceutical composition may be encapsulated or injected in a form for delivery to a target tissue site (*e.g.*, bone marrow or muscle). In certain embodiments, compositions of the present disclosure may include a matrix capable of delivering one or more of the agents of the present disclosure to a target tissue site (*e.g.*, bone marrow or muscle), providing a structure for the developing tissue and optimally capable of being resorbed into the body. For example, the matrix may provide slow release of one or more agents of the present disclosure. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material may be based on one or more of: biocompatibility, biodegradability, mechanical properties, cosmetic appearance, and interface properties. The particular application of the subject compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, including, for example, bone or dermal collagen. Further matrices are comprised of pure proteins or

extracellular matrix components. Other potential matrices are non-biodegradable and chemically defined, including, for example, sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above mentioned types of material including, for example, polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics may be altered in composition (*e.g.*, calcium-aluminate-phosphate) and processing to alter one or more of pore size, particle size, particle shape, and biodegradability.

In certain embodiments, pharmaceutical compositions of the present disclosure can be administered orally, for example, in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis such as sucrose and acacia or tragacanth), powders, granules, a solution or a suspension in an aqueous or non-aqueous liquid, an oil-in-water or water-in-oil liquid emulsion, or an elixir or syrup, or pastille (using an inert base, such as gelatin and glycerin, or sucrose and acacia), and/or a mouth wash, each containing a predetermined amount of a compound of the present disclosure and optionally one or more other active ingredients. A compound of the present disclosure and optionally one or more other active ingredients may also be administered as a bolus, electuary, or paste.

In solid dosage forms for oral administration (*e.g.,* capsules, tablets, pills, dragees, powders, and granules), one or more compounds of the present disclosure may be mixed with one or more pharmaceutically acceptable carriers including, for example, sodium citrate, dicalcium phosphate, a filler or extender (*e.g.*, a starch, lactose, sucrose, glucose, mannitol, and silicic acid), a binder (*e.g.* carboxymethylcellulose, an alginate, gelatin, polyvinyl pyrrolidone, sucrose, and acacia), a humectant (*e.g.*, glycerol), a disintegrating agent (*e.g.*, agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, a silicate, and sodium carbonate), a solution retarding agent (*e.g.* paraffin), an absorption accelerator (*e.g.* a quaternary ammonium compound), a wetting agent (*e.g.*, cetyl alcohol and glycerol monostearate), an absorbent (*e.g.*, kaolin and bentonite clay), a lubricant (*e.g.*, a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), a coloring agent, and mixtures thereof. In the case of capsules, tablets, and pills, the pharmaceutical formulation (composition) may also comprise a buffering agent. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using one or more excipients including, e.g., lactose or a milk sugar as well as a high molecular-weight polyethylene glycol.

Liquid dosage forms for oral administration of the pharmaceutical composition may include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active ingredient(s), the liquid dosage form may contain an inert diluent commonly used in the art including, for example, water or other solvent, a solubilizing agent and/or emulsifier [*e.g.*, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, or 1,3-butylene glycol, an oil (*e.g.*, cottonseed, groundnut, corn, germ, olive, castor, and sesame oil), glycerol, tetrahydrofuryl alcohol, a polyethylene glycol, a fatty acid ester of sorbitan, and mixtures thereof]. Besides inert diluents, the oral formulation can also include an adjuvant including, for example, a wetting agent, an emulsifying and suspending agent, a sweetening agent, a flavoring agent, a coloring agent, a perfuming agent, a preservative agent, and combinations thereof.

Suspensions, in addition to the active compounds, may contain suspending agents including, for example, an ethoxylated isostearyl alcohol, polyoxyethylene sorbitol, a sorbitan ester, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar, tragacanth, and combinations thereof.

Prevention of the action and/or growth of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents including, for example, paraben, chlorobutanol, and phenol sorbic acid.

In certain embodiments, it may be desirable to include an isotonic agent including, for example, a sugar or sodium chloride into the compositions. In addition, prolonged absorption of an injectable pharmaceutical form may be brought about by the inclusion of an agent that delays absorption, including, for example, aluminum monostearate and gelatin.

It is understood that the dosage regimen will be determined by the attending physician considering various factors which modify the action of the one or more of the agents of the present disclosure. The various factors include, but are not limited to, the patient's red blood cell count, hemoglobin level, the desired target red blood cell count, the patient's age, the patient's sex, the patient's diet, the severity of any disease that may be contributing to a depressed red blood cell level, the time of administration, and other clinical factors. The addition of other known active agents to the final composition may also affect the dosage. Progress can be monitored by periodic assessment of one or more of red blood cell levels, hemoglobin levels, reticulocyte levels, and other indicators of the hematopoietic process.

In certain embodiments, the present disclosure also provides gene therapy for the *in vivo* production of one or more of the agents of the present disclosure. Such therapy would achieve its therapeutic effect by introduction of the agent sequences into cells or tissues having one or more of the disorders as listed above. Delivery of the agent sequences can be achieved, for example, by using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Preferred therapeutic delivery of one or more of agent sequences of the disclosure is the use of targeted liposomes.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or an RNA virus (*e.g.*, a retrovirus). The retroviral vector may be a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous sarcoma virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. Retroviral vectors can be made target-specific by attaching, for example, a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody. Those of skill in the art will recognize that specific polynucleotide sequences can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the retroviral vector containing one or more of the agents of the present disclosure.

Alternatively, tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes (gag, pol, and env), by conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for one or more of the agents of the present disclosure is a colloidal dispersion system. Colloidal dispersion systems include, for example, macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. In certain embodiments, the preferred colloidal system of this disclosure is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. RNA, DNA, and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form [see, *e.g.*, Fraley, et al. (1981) Trends Biochem. Sci., 6:77].

Methods for efficient gene transfer using a liposome vehicle are known in the art [see, *e.g.*, Mannino, *et al.* (1988) Biotechniques, 6:682, 1988].

The composition of the liposome is usually a combination of phospholipids, which may include a steroid (*e.g.* cholesterol). The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations. Other phospholipids or other lipids may also be used, including, for example a phosphatidyl compound (*e.g.*, phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipid, cerebroside, or a ganglioside), egg phosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoylphosphatidylcholine. The targeting of liposomes is also possible based on, for example, organ specificity, cell specificity, and organelle specificity and is known in the art.

EXEMPLIFICATION

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

Example 1: ActRIIa-Fc Fusion Proteins

Applicants constructed a soluble ActRIIA fusion protein that has the extracellular domain of human ActRIIa fused to a human or mouse Fc domain with a minimal linker in between. The constructs are referred to as ActRIIA-hFc and ActRIIA-mFc, respectively.

ActRIIA-hFc is shown below as purified from CHO cell lines (SEQ ID NO:22):

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEI VKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNP VTPKPPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK

The ActRIIA-hFc and ActRIIA-mFc proteins were expressed in CHO cell lines. Three different leader sequences were considered:

(i) Honey bee mellitin (HBML): MKFLVNVALVFMVVYISYIYA (SEQ ID NO:23)

(ii) Tissue plasminogen activator (TPA): MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO:24)

(iii) Native: MGAAAKLAFAVFLISCSSGA (SEQ ID NO:25).

The selected form employs the TPA leader and has the following unprocessed amino acid sequence:

MDAMKRGLCCVLLLCGAVFVSPGAAILGRSETQECLFFNANWEKDRTNQTG
VEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFC
CCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPPTGGGTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY
RVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREE
MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDK
SRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:26)

This polypeptide is encoded by the following nucleic acid sequence:

ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGC
AGTCTTCGTTTCGCCCGGCGCCGCTATACTTGGTAGATCAGAAACTCAGGAGTGT
CTTTTTTTAATGCTAATTGGGAAAAAGACAGAACCAATCAAACTGGTGTTGAACC
GTGTTATGGTGACAAAGATAAACGGCGGCATTGTTTTGCTACCTGGAAGAATATT
TCTGGTTCCATTGAATAGTGAAACAAGGTTGTTGGCTGGATGATATCAACTGCTA
TGACAGGACTGATTGTGTAGAAAAAAAAGACAGCCCTGAAGTATATTTCTGTTGC
TGTGAGGGCAATATGTGTAATGAAAAGTTTTCTTATTTTCCGGAGATGGAAGTCA
CACAGCCCACTTCAAATCCAGTTACACCTAAGCCACCCACCGGTGGTGGAACTCA
CACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTC
TTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACAT
GCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACG
TGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTAC
AACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGA
ATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGTCCCCATCG
AGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCC
TGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGG
TCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGC
CGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTT
CCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTT

CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC TCCCTGTCTCCGGGTAAATGAGAATTC (SEQ ID NO:27)

Both ActRIIA-hFc and ActRIIA-mFc were remarkably amenable to recombinant expression. As shown in Figure 3, the protein was purified as a single, well-defined peak of protein. N-terminal sequencing revealed a single sequence of –ILGRSETQE (SEQ ID NO:34). Purification could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange. The ActRIIA-hFc protein was purified to a purity of >98% as determined by size exclusion chromatography and >95% as determined by SDS PAGE.

ActRIIA-hFc and ActRIIA-mFc showed a high affinity for ligands, particularly activin A. GDF-11 or activin A were immobilized on a Biacore™ CM5 chip using standard amine-coupling procedure. ActRIIA-hFc and ActRIIA-mFc proteins were loaded onto the system, and binding was measured. ActRIIA-hFc bound to activin with a dissociation constant (K_D) of 5 x 10^{-12} and bound to GDF11 with a K_D of 9.96 x 10^{-9}. See Figure 4. ActRIIA-mFc behaved similarly.

The ActRIIA-hFc was very stable in pharmacokinetic studies. Rats were dosed with 1 mg/kg, 3 mg/kg, or 10 mg/kg of ActRIIA-hFc protein, and plasma levels of the protein were measured at 24, 48, 72, 144 and 168 hours. In a separate study, rats were dosed at 1 mg/kg, 10 mg/kg, or 30 mg/kg. In rats, ActRIIA-hFc had an 11-14 day serum half-life, and circulating levels of the drug were quite high after two weeks (11 μg/ml, 110 μg/ml, or 304 μg/ml for initial administrations of 1 mg/kg, 10 mg/kg, or 30 mg/kg, respectively.) In cynomolgus monkeys, the plasma half-life was substantially greater than 14 days, and circulating levels of the drug were 25 μg/ml, 304 μg/ml, or 1440 μg/ml for initial administrations of 1 mg/kg, 10 mg/kg, or 30 mg/kg, respectively.

Example 2: Characterization of an ActRIIA-hFc Protein

ActRIIA-hFc fusion protein was expressed in stably transfected CHO-DUKX B11 cells from a pAID4 vector (SV40 ori/enhancer, CMV promoter), using a tissue plasminogen leader sequence of SEQ ID NO:9. The protein, purified as described above in Example 1, had a sequence of SEQ ID NO:22. The Fc portion is a human IgG1 Fc sequence, as shown in

SEQ ID NO:22. Protein analysis reveals that the ActRIIA-hFc fusion protein is formed as a homodimer with disulfide bonding.

The CHO-cell-expressed material has a higher affinity for activin B ligand than that reported for an ActRIIa-hFc fusion protein expressed in human 293 cells [see, del Re *et al.* (2004) J Biol Chem. 279(51):53126-53135]. Additionally, the use of the TPA leader sequence provided greater production than other leader sequences and, unlike ActRIIA-Fc expressed with a native leader, provided a highly pure N-terminal sequence. Use of the native leader sequence resulted in two major species of ActRIIA-Fc, each having a different N-terminal sequence.

Example 3. ActRIIA-hFc Increases Red Blood Cell Levels in Non-Human Primates

The study employed four groups of five male and five female cynomolgus monkeys each, with three per sex per group scheduled for termination on Day 29, and two per sex per group scheduled for termination on Day 57. Each animal was administered the vehicle (Group 1) or ActRIIA-Fc at doses of 1, 10, or 30 mg/kg (Groups 2, 3 and 4, respectively) via intravenous (IV) injection on Days 1, 8, 15, and 22. The dose volume was maintained at 3 mL/kg. Various measures of red blood cell levels were assessed two days prior to the first administration and at days 15, 29, and 57 (for the remaining two animals) after the first administration.

The ActRIIA-hFc caused statistically significant increases in mean red blood cell parameters [red blood cell count (RBC), hemoglobin (HGB), and hematocrit (HCT)] for males and females, at all dose levels and time points throughout the study, with accompanying elevations in absolute and relative reticulocyte counts (ARTC; RTC). See Figures 5-8.

Statistical significance was calculated for each treatment group relative to the mean for the treatment group at baseline.

Notably, the increases in red blood cell counts and hemoglobin levels are roughly equivalent in magnitude to effects reported with erythropoietin. The onset of these effects is more rapid with ActRIIA-Fc than with erythropoietin.

Similar results were observed with rats and mice.

Example 4: ActRIIA-hFc Increases Red Blood Cell Levels and Markers of Bone Formation in Human Patients

The ActRIIA-hFc fusion protein described in Example 1 was administered to human subjects in a randomized, double-blind, placebo-controlled study that was conducted to evaluate, primarily, the safety of the protein in healthy, postmenopausal women. Forty-eight subjects were randomized in cohorts of 6 to receive either a single dose of ActRIIA-hFc or placebo (5 active:1 placebo). Dose levels ranged from 0.01 to 3.0 mg/kg intravenously (IV) and 0.03 to 0.1 mg/kg subcutaneously (SC). All subjects were followed for 120 days. In addition to pharmacokinetic (PK) analyses, the biologic activity of ActRIIA-hFc was also assessed by measurement of biochemical markers of bone formation and resorption as well as FSH levels.

To look for potential changes, hemoglobin and RBC numbers were examined in detail for all subjects over the course of the study and compared to the baseline levels. Platelet counts were compared over the same time as the control. There were no clinically significant changes from the baseline values over time for the platelet counts.

Pharmacokinetic (PK) analysis of ActRIIA-hFc revealed a linear profile with dose, and a mean half-life of approximately 25-32 days. The area-under-curve (AUC) for ActRIIA-hFc was linearly related to dose, and the absorption after SC dosing was essentially complete. See Figures 9 and 10. These data indicate that SC is a desirable approach to dosing because it provides equivalent bioavailability and serum-half life for the drug while avoiding the spike in serum concentrations of drug associated with the first few days of IV dosing (see Figure 10). ActRIIA-hFc caused a rapid, sustained dose-dependent increase in serum levels of bone-specific alkaline phosphatase (BAP), which is a marker for anabolic bone growth, and a dose-dependent decrease in C-terminal type 1 collagen telopeptide and tartrate-resistant acid phosphatase 5b levels, which are markers for bone resorption. Other markers such as P1NP showed inconclusive results. BAP levels showed near-saturating effects at the highest dosage of drug, indicating that half-maximal effects on this anabolic bone biomarker could be achieved at a dosage of 0.3 mg/kg, with increases ranging up to 3 mg/kg. Calculated as a relationship of pharmacodynamic effect to AUC for drug, the EC50 was 51,465 (day*ng/ml) (see Figure 11). These bone biomarker changes were sustained for approximately 120 days at the highest dose levels tested. There was also a dose-dependent decrease in serum FSH levels consistent with inhibition of activin.

Overall, there was a very small non-drug related reduction in hemoglobin over the first week of the study probably related to study phlebotomy in the 0.01 and 0.03 mg/kg groups whether given IV or SC. The 0.1 mg/kg SC and IV hemoglobin results were stable or showed modest increases by Day 8-15. At the 0.3 mg/kg IV dose level there was a clear

increase in HGB levels seen as early as Day 2 and often peaking at Day 15-29 that was not seen in the placebo-treated subjects. At the 1.0 mg/kg IV dose and the 3.0 mg/kg IV dose, mean increases in hemoglobin of greater than 1 g/dl were observed in response to the single dose, with corresponding increases in RBC counts and hematocrit. These hematologic parameters peaked at about 60 days after the dose and substantial decrease by day 120. This indicates that dosing for the purpose of increasing red blood cell levels may be more effective if done at intervals less than 120 days (*i.e.*, prior to return to baseline), with dosing intervals of 90 days or less or 60 days or less may be desirable. For a summary of hematological changes, see Figures 12-15.

Overall, ActRIIA-hFc showed a dose-dependent effect on red blood cell counts and reticulocyte counts.

Example 5: Treatment of an Anemic Patient with ActRIIA-hFc

A clinical study was designed to treat patients with multiple doses of ActRIIA-hFc, at three dose levels of 0.1 mg/kg, 0.3 mg/kg, and 1.0 mg/kg, with dosing to occur every 30 days. Normal healthy subjects in the trial exhibited an increase in hemoglobin and hematocrit that is consistent with the increases seen in the Phase I clinical trial reported in Example 4, except that in some instances the hemoglobin (Hg) and hematocrit (Hct) are elevated beyond the normal range. An anemic patient with hemoglobin levels of approximately 7.5 g/dL also received two doses at the 1 mg/kg level, resulting in a hemoglobin level of approximately 10.5 g/dL after two months. The patient's anemia was a microcytic anemia, thought to be caused by chronic iron deficiency.

ActRIIA-Fc fusion proteins have been further demonstrated to be effective in increasing red blood cell levels in various models of anemia including, for example, chemotherapy-induced anemia and anemia associated with chronic kidney disease (see, *e.g.*, U.S. Patent Application Publication No. 2010/0028331).

Example 6: Alternative ActRIIA-Fc Proteins

A variety of ActRIIA variants that may be used according to the methods described herein are described in the International Patent Application published as WO2006/012627 (see e.g., pp. 55-58), incorporated herein by reference in its entirety. An alternative construct may have a deletion of the C-terminal tail (the final 15 amino acids of the extracellular domain of ActRIIA. The sequence for such a construct is presented below (Fc portion underlined) (SEQ ID NO:28):

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQG
CWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMTGGGTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK
TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG
SFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

Example 7: Generation of ActRIIB-Fc fusion proteins

Applicants constructed a soluble ActRIIB fusion protein that has the extracellular domain of human ActRIIB fused to a human or mouse Fc domain with a minimal linker (three glycine amino acids) in between. The constructs are referred to as ActRIIB-hFc and ActRIIB-mFc, respectively.

ActRIIB-hFc is shown below as purified from CHO cell lines (SEQ ID NO:29):

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKK
GCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPT
APTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

The ActRIIB-hFc and ActRIIB-mFc proteins were expressed in CHO cell lines. Three different leader sequences were considered:

(i) Honey bee mellitin (HBML): MKFLVNVALVFMVVYISYIYA (SEQ ID NO:23)

(ii) Tissue plasminogen activator (TPA): MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO:24)

(iii) Native: MGAAAKLAFAVFLISCSSGA (SEQ ID NO:30).

The selected form employs the TPA leader and has the following unprocessed amino acid sequence (SEQ ID NO: 31):

MDAMKRGLCCVLLLCGAVFVSPGASGRGEAETRECIYYNANWELERTNQSGLERCE
GEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCE
GNFCNERFTHLPEAGGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ

VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK

This polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO:32):

A TGGATGCAAT GAAGAGAGGG CTCTGCTGTG TGCTGCTGCT GTGTGGAGCA
GTCTTCGTTT CGCCCGGCGC CTCTGGGCGT GGGGAGGCTG AGACACGGGA
GTGCATCTAC TACAACGCCA ACTGGGAGCT GGAGCGCACC AACCAGAGCG
GCCTGGAGCG CTGCGAAGGC GAGCAGGACA AGCGGCTGCA CTGCTACGCC
TCCTGGCGCA ACAGCTCTGG CACCATCGAG CTCGTGAAGA AGGGCTGCTG
GCTAGATGAC TTCAACTGCT ACGATAGGCA GGAGTGTGTG GCCACTGAGG
AGAACCCCCA GGTGTACTTC TGCTGCTGTG AAGGCAACTT CTGCAACGAG
CGCTTCACTC ATTTGCCAGA GGCTGGGGGC CCGGAAGTCA CGTACGAGCC
ACCCCCGACA GCCCCCACCG GTGGTGGAAC TCACACATGC CCACCGTGCC
CAGCACCTGA ACTCCTGGGG GGACCGTCAG TCTTCCTCTT CCCCCCAAAA
CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT
GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC
AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG
GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCCAG
TCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
CAGGTGTACA CCCTGCCCCC ATCCCGGGAG GAGATGACCA AGAACCAGGT
CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG
AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC
GTGCTGGACT CCGACGGCTC CTTCTTCCTC TATAGCAAGC TCACCGTGGA
CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG
AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT
AAATGA

N-terminal sequencing of the CHO-cell-produced material revealed a major sequence of –GRGEAE (SEQ ID NO:33). Notably, other constructs reported in the literature begin with an –SGR… sequence.

Purification could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange.

ActRIIB-Fc fusion proteins were also expressed in HEK293 cells and COS cells. Although material from all cell lines and reasonable culture conditions provided protein with muscle-building activity *in vivo*, variability in potency was observed perhaps relating to cell line selection and/or culture conditions.

Applicants generated a series of mutations in the extracellular domain of ActRIIB and produced these mutant proteins as soluble fusion proteins between extracellular ActRIIB and an Fc domain. The background ActRIIB-Fc fusion has the sequence of SEQ ID NO:29.

Various mutations, including N- and C-terminal truncations, were introduced into the background ActRIIB-Fc protein. Based on the data presented in Example 1, it is expected that these constructs, if expressed with a TPA leader, will lack the N-terminal serine. Mutations were generated in ActRIIB extracellular domain by PCR mutagenesis. After PCR, fragments were purified through a Qiagen column, digested with SfoI and AgeI and gel purified. These fragments were ligated into expression vector pAID4 (see WO2006/012627) such that upon ligation it created fusion chimera with human IgG1. Upon transformation into E. coli DH5 alpha, colonies were picked and DNAs were isolated. For murine constructs (mFc), a murine IgG2a was substituted for the human IgG1. Sequences of all mutants were verified.

All of the mutants were produced in HEK293T cells by transient transfection. In summary, in a 500ml spinner, HEK293T cells were set up at $6x10^5$ cells/ml in Freestyle (Invitrogen) media in 250ml volume and grown overnight. Next day, these cells were treated with DNA:PEI (1:1) complex at 0.5 ug/ml final DNA concentration. After 4 hrs, 250 ml media was added and cells were grown for 7 days. Conditioned media was harvested by spinning down the cells and concentrated.

Mutants were purified using a variety of techniques, including, for example, a protein A column, and eluted with low pH (3.0) glycine buffer. After neutralization, these were dialyzed against PBS.

Mutants were also produced in CHO cells by similar methodology. Mutants were tested in binding assays and/or bioassays described in WO 2008/097541 and WO 2006/012627 incorporated by reference herein. In some instances, assays were performed with conditioned medium rather than purified proteins. Additional variations of ActRIIB are described in U.S. Patent No. 7,842,663.

Example 8: ActRIIB-Fc Stimulates Erythropoiesis in Non-Human Primates

Cynomolgus monkeys were allocated into seven groups (6/sex/group) and administered ActRIIB(20-134)-hFc as a subcutaneous injection at dosages of 0.6, 3, or 15 mg/kg every 2 weeks or every 4 weeks over a 9-month period. The control group (6/sex/group) received the vehicle at the same dose volume (0.5 ml/kg/dose) as ActRIIB(20-134)-hFc-treated animals. Animals were monitored for changes in general clinical pathology parameters (*e.g.*, hematology, clinical chemistry, coagulation, and urinalysis). Hematology, coagulation, and clinical chemistry parameters (including iron parameters, lipase, and amylase) were evaluated twice prior to initiation of dosing and on Days 59, 143, 199, 227, and on Days 267 (for groups dosed every 4 weeks) or 281 (for groups dosed every 2 weeks). The evaluations on Days 267/281 occurred 2 weeks after the final dose was administered.

Administration of ActRIIB(20-134)-hFc resulted in non-adverse, dose-related changes to hematology parameters in male and female monkeys. These changes included increased red blood cell count, reticulocyte count and red cell distribution width and decreased mean corpuscular volume, mean corpuscular hemoglobin, and platelet count. In males, RBC count was increased at all dose levels, and the magnitude of increase was generally comparable whether ActRIIB(20-134)-hFc was administered every 2 weeks or every 4 weeks. Mean RBC count was increased at all time points between Days 59 and 267/281 (except RBC count was not increased in group 2 males [0.6 mg/kg every 2 weeks] on Day 281). In females, RBC count was increased at $\geq$ 3 mg/kg every 2 weeks and the changes occurred between Days 143 and 281; at 15 mg/kg every 4 weeks mean RBC count was increased between Days 59 and 267.

These effects are consistent with a positive effect of ActRIIB(20-134)-hFc on stimulating erythropoiesis.

Example 9: Generation of a GDF Trap

Applicants constructed a GDF trap as follows. A polypeptide having a modified extracellular domain of ActRIIB (amino acids 20-134 of SEQ ID NO:1 with an L79D substitution) with greatly reduced activin A binding relative to GDF11 and/or myostatin (as a consequence of a leucine-to-aspartate substitution at position 79 in SEQ ID NO:1) was fused to a human or mouse Fc domain with a minimal linker (three glycine amino acids) in between. The constructs are referred to as ActRIIB(L79D 20-134)-hFc and ActRIIB(L79D 20-134)-mFc, respectively. Alternative forms with a glutamate rather than an aspartate at position 79

performed similarly (L79E). Alternative forms with an alanine rather than a valine at position 226 with respect to SEQ ID NO:36, below were also generated and performed equivalently in all respects tested. The aspartate at position 79 (relative to SEQ ID NO: 1, or position 60 relative to SEQ ID NO:36) is indicated with double underlining below. The valine at position 226 relative to SEQ ID NO:36 is also indicated by double underlining below.

The GDF trap ActRIIB(L79D 20-134)-hFc is shown below as purified from CHO cell lines (SEQ ID NO:36).

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKK
GCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPT
APTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK
FNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL
PVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

The ActRIIB-derived portion of the GDF trap has an amino acid sequence set forth below (SEQ ID NO: 37), and that portion could be used as a monomer or as a non-Fc fusion protein as a monomer, dimer, or greater-order complex.

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKK
GCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPT
APT (SEQ ID NO: 37)

The GDF trap protein was expressed in CHO cell lines. Three different leader sequences were considered:

(i) Honey bee melittin (HBML): MKFLVNVALVFMVVYISYIYA (SEQ ID NO:23)

(ii) Tissue plasminogen activator (TPA): MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO:24)

(iii) Native: MTAPWVALALLWGSLCAGS (SEQ ID NO:30).

The selected form employs the TPA leader and has the following unprocessed amino acid sequence:

MDAMKRGLCCVLLLCGAVFVSPGASGRGEAETRECIYYNANWELERTNQSGLERCE
GEQDKRLHCYASWRNSSGTIELVKKGCWDDDFNCYDRQECVATEENPQVYFCCCE
GNFCNERFTHLPEAGGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKD
TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ

VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:38)

This polypeptide is encoded by the following nucleic acid sequence (SEQ ID NO:39):

A TGGATGCAAT GAAGAGAGGG CTCTGCTGTG TGCTGCTGCT GTGTGGAGCA
GTCTTCGTTT CGCCCGGCGC CTCTGGGCGT GGGGAGGCTG AGACACGGGA
GTGCATCTAC TACAACGCCA ACTGGGAGCT GGAGCGCACC AACCAGAGCG
GCCTGGAGCG CTGCGAAGGC GAGCAGGACA AGCGGCTGCA CTGCTACGCC
TCCTGGCGCA ACAGCTCTGG CACCATCGAG CTCGTGAAGA AGGGCTGCTG
GGACGATGAC TTCAACTGCT ACGATAGGCA GGAGTGTGTG GCCACTGAGG
AGAACCCCCA GGTGTACTTC TGCTGCTGTG AAGGCAACTT CTGCAACGAG
CGCTTCACTC ATTTGCCAGA GGCTGGGGGC CCGGAAGTCA CGTACGAGCC
ACCCCCGACA GCCCCCACCG GTGGTGGAAC TCACACATGC CCACCGTGCC
CAGCACCTGA ACTCCTGGGG GGACCGTCAG TCTTCCTCTT CCCCCCAAAA
CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT
GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG
ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC
AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG
GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCCAG
TCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA
CAGGTGTACA CCCTGCCCCC ATCCCGGGAG GAGATGACCA AGAACCAGGT
CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG
AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC
GTGCTGGACT CCGACGGCTC CTTCTTCCTC TATAGCAAGC TCACCGTGGA
CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG
AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT
AAATGA

Purification could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange. In an example of a purification scheme, the cell culture medium is passed over a protein A column, washed in 150 mM Tris/NaCl (pH 8.0), then washed in 50 mM Tris/NaCl (pH 8.0) and eluted with 0.1 M glycine, pH 3.0. The low pH eluate is kept at room temperature for 30 minutes as a viral clearance step. The eluate is

then neutralized and passed over a Q-sepharose ion-exchange column and washed in 50 mM Tris pH 8.0, 50 mM NaCl, and eluted in 50 mM Tris pH 8.0, with an NaCl concentration between 150 mM and 300 mM. The eluate is then changed into 50 mM Tris pH 8.0, 1.1 M ammonium sulfate and passed over a phenyl sepharose column, washed, and eluted in 50 mM Tris pH 8.0 with ammonium sulfate between 150 and 300 mM. The eluate is dialyzed and filtered for use.

Additional GDF traps (ActRIIB-Fc fusion proteins modified so as to reduce the ratio of activin A binding relative to myostatin or GDF11 binding) are described in WO 2008/097541 and WO 2006/012627, incorporated by reference herein.

Example 10: Bioassay for GDF-11- and Activin-Mediated Signaling

An A-204 reporter gene assay was used to evaluate the effects of ActRIIB-Fc proteins and GDF traps on signaling by GDF-11 and activin A. Cell line: human rhabdomyosarcoma (derived from muscle). Reporter vector: pGL3(CAGA)12 (described in Dennler et al, 1998, EMBO 17: 3091-3100). The CAGA12 motif is present in TGF-beta responsive genes (*e.g.*, PAI-1 gene), so this vector is of general use for factors signaling through SMAD2 and 3.

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

Day 2: A-204 cells transfected with 10 ug pGL3(CAGA)12 or pGL3(CAGA)12(10 ug) + pRLCMV (1 μg) and Fugene.

Day 3: Add factors (diluted into medium + 0.1 % BSA). Inhibitors need to be preincubated with factors for 1 hr before adding to cells. Six hrs later, cells were rinsed with PBS and lysed.

This is followed by a luciferase assay. In the absence of any inhibitors, activin A showed 10-fold stimulation of reporter gene expression and an ED50 ~ 2 ng/ml. GDF-11: 16 fold stimulation, ED50: ~ 1.5 ng/ml.

ActRIIB(20-134) is a potent inhibitor of activin A, GDF-8, and GDF-11 activity in this assay. As described below, ActRIIB variants were also tested in this assay.

Example 11: ActRIIB-Fc Variants, Cell-Based Activity

Activity of ActRIIB-Fc proteins and GDF traps was tested in a cell-based assay as described above. Results are summarized in the table below. Some variants were tested in different C-terminal truncation constructs. As discussed above, truncations of five or fifteen

amino acids caused reduction in activity. The GDF traps (L79D and L79E variants) showed substantial loss of activin A inhibition while retaining almost wild-type inhibition of GDF-11.

Soluble ActRIIB-Fc binding to GDF11 and Activin A:

ActRIIB-Fc Variations	Portion of ActRIIB (corresponds to amino acids of SEQ ID NO:1)	GDF11 Inhibition Activity	Activin Inhibition Activity
R64	20-134	+++ (approx. 10^{-8} M K_I)	+++ (approx. 10^{-8} M K_I)
A64	20-134	+ (approx. 10^{-6} M K_I)	+ (approx. 10^{-6} M K_I)
R64	20-129	+++	+++
R64 K74A	20-134	++++	++++
R64 A24N	20-134	+++	+++
R64 A24N	20-119	++	++
R64 A24N K74A	20-119	+	+
R64 L79P	20-134	+	+
R64 L79P K74A	20-134	+	+
R64 L79D	20-134	+++	+
R64 L79E	20-134	+++	+

R64K	20-134	+++	+++
R64K	20-129	+++	+++
R64 P129S P130A	20-134	+++	+++
R64N	20-134	+	+

\+ Poor activity (roughly $1x10^{-6}$ K_I)

++ Moderate activity (roughly $1x10^{-7}$ K_I)

+++ Good (wild-type) activity (roughly $1x10^{-8}$ K_I)

++++ Greater than wild-type activity

Several variants have been assessed for serum half-life in rats. ActRIIB(20-134)-Fc has a serum half-life of approximately 70 hours. ActRIIB(A24N 20-134)-Fc has a serum half-life of approximately 100-150 hours. The A24N variant has activity in the cell-based assay (above) and *in vivo* assays (below) that is equivalent to the wild-type molecule. Coupled with the longer half-life, this means that over time an A24N variant will give greater effect per unit of protein than the wild-type molecule. The A24N variant, and any of the other variants tested above, may be combined with the GDF trap molecules, such as the L79D or L79E variants.

Example 12: GDF-11 and Activin A Binding.

Binding of certain ActRIIB-Fc proteins and GDF traps to ligands was tested in a Biacore™ assay.

The ActRIIB-Fc variants or wild-type protein were captured onto the system using an anti-hFc antibody. Ligands were injected and flowed over the captured receptor proteins. Results are summarized in the tables below.

Ligand-binding specificity IIB variants.

	GDF11		
Protein	**Kon (1/Ms)**	**Koff (1/s)**	**KD (M)**

ActRIIB(20-134)-hFc	1.34e-6	1.13e-4	8.42e-11
ActRIIB(A24N 20-134)-hFc	1.21e-6	6.35e-5	5.19e-11
ActRIIB(L79D 20-134)-hFc	6.7e-5	4.39e-4	6.55e-10
ActRIIB(L79E 20-134)-hFc	3.8e-5	2.74e-4	7.16e-10
ActRIIB(R64K 20-134)-hFc	6.77e-5	2.41e-5	3.56e-11
	GDF8		
Protein	**Kon (1/Ms)**	**Koff (1/s)**	**KD (M)**
ActRIIB(20-134)-hFc	3.69e-5	3.45e-5	9.35e-11
ActRIIB(A24N 20-134)-hFc			
ActRIIB(L79D 20-134)-hFc	3.85e-5	8.3e-4	2.15e-9
ActRIIB(L79E 20-134)-hFc	3.74e-5	9e-4	2.41e-9
ActRIIB(R64K 20-134)-hFc	2.25e-5	4.71e-5	2.1e-10
ActRIIB(R64K 20-129)-hFc	9.74e-4	2.09e-4	2.15e-9
ActRIIB(P129S, P130R 20-134)-hFc	1.08e-5	1.8e-4	1.67e-9
ActRIIB(K74A 20-134)-hFc	2.8e-5	2.03e-5	7.18e-11
	Activin A		
Protein	**Kon (1/Ms)**	**Koff (1/s)**	**KD (M)**
ActRIIB(20-134)-hFc	5.94e6	1.59e-4	2.68e-11
ActRIIB(A24N 20-134)-hFc	3.34e6	3.46e-4	1.04e-10

ActRIIB(L79D 20-134)-hFc			Low binding
ActRIIB(L79E 20-134)-hFc			Low binding
ActRIIB(R64K 20-134)-hFc	6.82e6	3.25e-4	4.76e-11
ActRIIB(R64K 20-129)-hFc	7.46e6	6.28e-4	8.41e-11
ActRIIB(P129S, P130R 20-134)-hFc	5.02e6	4.17e-4	8.31e-11

These data obtained in a cell-free assay confirm the cell-based assay data, demonstrating that the A24N variant retains ligand-binding activity that is similar to that of the ActRIIB(20-134)-hFc molecule and that the L79D or L79E molecule retains myostatin and GDF11 binding but shows markedly decreased (non-quantifiable) binding to activin A.

Other variants have been generated and tested, as reported in WO2006/012627 (incorporated herein by reference in its entirety). See, *e.g.*, pp. 59-60, using ligands coupled to the device and flowing receptor over the coupled ligands. Notably, K74Y, K74F, K74I (and presumably other hydrophobic substitutions at K74, such as K74L), and D80I, cause a decrease in the ratio of activin A (ActA) binding to GDF11 binding, relative to the wild-type K74 molecule. A table of data with respect to these variants is reproduced below:

Soluble ActRIIB-Fc variants binding to GDF11 and Activin A (Biacore™ assay)

ActRIIB	**ActA**	**GDF11**
WT (64A)	KD=1.8e-7M (+)	KD= 2.6e-7M (+)
WT (64R)	na	KD= 8.6e-8M (+++)

+15tail	KD ~2.6 e-8M (+++)	KD= 1.9e-8M (++++)
E37A	*	*
R40A	-	-
D54A	-	*
K55A	++	*
R56A	*	*
K74A	KD=4.35e-9 M +++++	KD=5.3e-9M +++++
K74Y	*	--
K74F	*	--
K74I	*	--
W78A	*	*
L79A	+	*
D80K	*	*
D80R	*	*
D80A	*	*
D80F	*	*
D80G	*	*
D80M	*	*
D80N	*	*
D80I	*	--

F82A	++	-

* No observed binding

-- < 1/5 WT binding

- ~ 1/2 WT binding

\+ WT

++ < 2x increased binding

+++ ~5x increased binding

++++ ~10x increased binding

+++++ ~ 40x increased binding

Example 13: A GDF Trap Increases Red Blood Cell Levels *in vivo*

Twelve-week-old male C57BL/6NTac mice were assigned to one of two treatment groups (N=10). Mice were dosed with either vehicle or with a variant ActRIIB polypeptide ("GDF trap") [ActRIIB(L79D 20-134)-hFc] by subcutaneous injection (SC) at 10 mg/kg twice per week for 4 weeks. At study termination, whole blood was collected by cardiac puncture into EDTA containing tubes and analyzed for cell distribution using an HM2 hematology analyzer (Abaxis, Inc).

Group Designation

Group	**N**	**Mice**	**Injection**	**Dose (mg/kg)**	**Route**	**Frequency**
1	10	C57BL/6	PBS	0	SC	Twice/week
2	10	C57BL/6	GDF trap [ActRIIB(L79D 20-134)-hFc]	10	SC	Twice/week

Treatment with the GDF trap did not have a statistically significant effect on the number of white blood cells (WBC) compared to the vehicle controls. Red blood cell (RBC) numbers were increased in the treated group relative to the controls (see table below). Both the hemoglobin content (HGB) and hematocrit (HCT) were also increased due to the additional red blood cells. The average width of the red blood cells (RDWc) was higher in the treated animals, indicating an increase in the pool of immature red blood cells. Therefore, treatment with the GDF trap leads to increases in red blood cells, with no distinguishable effects on white blood cell populations.

Hematology Results

	RBC 10^{12}/L	**HGB** (g/dL)	**HCT** (%)	**RDWc** (%)
PBS	10.7 ± 0.1	14.8 ± 0.6	44.8 ± 0.4	17.0 ± 0.1
GDF trap	12.4 ± 0.4**	17.0 ± 0.7*	48.8 ± 1.8*	18.4 ± 0.2**

*=p<0.05, **= p<0.01

Example 14: A GDF Trap is Superior to ActRIIB-Fc for Increasing Red Blood Cell Levels *in vivo*.

Nineteen-week-old male C57BL/6NTac mice were randomly assigned to one of three groups. Mice were dosed with vehicle (10 mM Tris-buffered saline, TBS), wild-type ActRIIB(20-134)-mFc, or GDF trap ActRIIB(L79D 20-134)-hFc by subcutaneous injection twice per week for three weeks. Blood was collected by cheek bleed at baseline and after three weeks of dosing and analyzed for cell distribution using a hematology analyzer (HM2, Abaxis, Inc.)

Treatment with ActRIIB-Fc or the GDF trap did not have a significant effect on white blood cell (WBC) numbers compared to vehicle controls. The red blood cell count (RBC), hematocrit (HCT), and hemoglobin levels were all elevated in mice treated with GDF trap compared to either the controls or the wild-type construct (see table below). Therefore, in a

direct comparison, the GDF trap promotes increases in red blood cells to a significantly greater extent than a wild-type ActRIIB-Fc protein. In fact, in this experiment, the wild-type ActRIIB-Fc protein did not cause a statistically significant increase in red blood cells, suggesting that longer or higher dosing would be needed to reveal this effect.

Hematology Results after three weeks of dosing

	RBC (10^{12}/ml)	HCT %	HGB g/dL
TBS	11.06 ± 0.46	46.78 ± 1.9	15.7 ± 0.7
ActRIIB-mFc	11.64 ± 0.09	49.03 ± 0.3	16.5 ± 1.5
GDF trap	13.19 ± 0.2**	53.04 ±0.8**	18.4 ± 0.3**

**=$p<0.01$

Example 15: Generation of a GDF Trap with Truncated ActRIIB Extracellular Domain

As described in Example 9, a GDF trap referred to as ActRIIB(L79D 20-134)-hFc was generated by N-terminal fusion of TPA leader to the ActRIIB extracellular domain (residues 20-134 in SEQ ID NO:1) containing a leucine-to-aspartate substitution (at residue 79 in SEQ ID NO:1) and C-terminal fusion of human Fc domain with minimal linker (three glycine residues) (Figure 16). A nucleotide sequence corresponding to this fusion protein is shown in Figures 17A and 17B.

A GDF trap with truncated ActRIIB extracellular domain, referred to as ActRIIB(L79D 25-131)-hFc, was generated by N-terminal fusion of TPA leader to truncated extracellular domain (residues 25-131 in SEQ ID NO:1) containing a leucine-to-aspartate substitution (at residue 79 in SEQ ID NO:1) and C-terminal fusion of human Fc domain with minimal linker (three glycine residues) (Figure 18). A nucleotide sequence corresponding to this fusion protein is shown in Figures 19A and 19B.

Example 16: Selective Ligand Binding by GDF Trap with Double-Truncated ActRIIB Extracelluar Domain

The affinity of GDF traps and other ActRIIB-hFc proteins for several ligands was evaluated *in vitro* with a Biacore™ instrument. Results are summarized in the table below. Kd values were obtained by steady-state affinity fit due to the very rapid association and dissociation of the complex, which prevented accurate determination of k_{on} and k_{off}.

Ligand Selectivity of ActRIIB-hFc Variants:

Fusion Construct	Activin A (Kd e-11)	Activin B (Kd e-11)	GDF11 (Kd e-11)
ActRIIB(L79 20-134)-hFc	1.6	1.2	3.6
ActRIIB(L79D 20-134)-hFc	1350.0	78.8	12.3
ActRIIB(L79 25-131)-hFc	1.8	1.2	3.1
ActRIIB(L79D 25-131)-hFc	2290.0	62.1	7.4

The GDF trap with a truncated extracellular domain, ActRIIB(L79D 25-131)-hFc, equaled or surpassed the ligand selectivity displayed by the longer variant, ActRIIB(L79D 20-134)-hFc, with pronounced loss of activin A binding, partial loss of activin B binding, and nearly full retention of GDF11 binding compared to ActRIIB-hFc counterparts lacking the L79D substitution. Note that truncation alone (without L79D substitution) did not alter selectivity among the ligands displayed here [compare ActRIIB(L79 25-131)-hFc with ActRIIB(L79 20-134)-hFc].

Example 17: Generation of ActRIIB(L79D 25-131)-hFc with Alternative Nucleotide Sequences

To generate ActRIIB(L79D 25-131)-hFc, the human ActRIIB extracellular domain with an aspartate substitution at native position 79 (SEQ ID NO:1) and with N-terminal and C-terminal truncations (residues 25-131 in SEQ ID NO: 1) was fused N-terminally with a

TPA leader sequence instead of the native ActRIIB leader and C-terminally with a human Fc domain via a minimal linker (three glycine residues) (Figure 18). One nucleotide sequence encoding this fusion protein is shown in Figures 19A and 19B (SEQ ID NO: 42), and an alternative nucleotide sequence encoding exactly the same fusion protein is shown in Figures 22A and 22B (SEQ ID NO: 46). This protein was expressed and purified using the methodology described in Example 9.

Example 18: GDF Trap with a Truncated ActRIIB Extracellular Domain Increases Proliferation of Erythroid Progenitors in Mice

ActRIIB(L79D 25-131)-hFc was evaluated to determine its effect on proliferation of erythroid progenitors. Male C57BL/6 mice (8 weeks old) were treated with ActRIIB(L79D 25-131)-hFc (10 mg/kg, s.c.; n = 6) or vehicle (TBS; n = 6) on Days 1 and 4, then euthanized on Day 8 for collection of spleens, tibias, femurs, and blood. Cells of the spleen and bone marrow were isolated, diluted in Iscove's modified Dulbecco's medium containing 5% fetal bovine serum, suspended in specialized methylcellulose-based medium, and cultured for either 2 or 12 days to assess levels of clonogenic progenitors at the colony-forming unit-erythroid (CFU-E) and burst forming unit-erythroid (BFU-E) stages, respectively. Methylcellulose-based medium for BFU-E determination (MethoCult M3434, Stem Cell Technologies) included recombinant murine stem cell factor, interleukin-3, and interleukin-6, which were not present in methylcellulose medium for CFU-E determination (MethoCult M3334, Stem Cell Technologies), while both media contained erythropoietin, among other constituents. For both BFU-E and CFU-E, the number of colonies were determined in duplicate culture plates derived from each tissue sample, and statistical analysis of the results was based on the number of mice per treatment group.

Spleen-derived cultures from mice treated with ActRIIB(L79D 25-131)-hFc had twice the number of CFU-E colonies as did corresponding cultures from control mice ($P < 0.05$), whereas the number of BFU-E colonies did not differ significantly with treatment *in vivo*. The number of CFU-E or BFU-E colonies from bone marrow cultures also did not differ significantly with treatment. As expected, increased numbers of CFU-E colonies in spleen-derived cultures were accompanied by highly significant ($P < 0.001$) changes in red blood cell level (11.6% increase), hemoglobin concentration (12% increase), and hematocrit level (11.6% increase) at euthanasia in mice treated with ActRIIB(L79D 25-131)-hFc compared to

controls. These results indicate that *in vivo* administration of a GDF trap with truncated ActRIIB extracellular domain can stimulate proliferation of erythroid progenitors as part of its overall effect to increase red blood cell levels.

GDF trap fusion proteins have been further demonstrated to be effective in increasing red blood cell levels in various models of anemia including, for example, chemotherapy-induced anemia, nephrectomy-induced anemia, and in a blood loss anemia (see, *e.g.*, International Patent Application Publication No. WO 2010/019261).

Example 19: GDF Trap with Truncated ActRIIB Extracellular Domain Increases Levels of Red Blood Cells in Non-Human Primates

Two GDF Traps, ActRIIB(L79D 20-134)-hFc and ActRIIB(L79D 25-131)-hFc, were evaluated for their ability to stimulate red blood cell production in cynomolgus monkeys. Monkeys were treated subcutaneously with GDF trap (10 mg/kg; n = 4 males/4 females), or vehicle (n = 2 males/2 females) on Days 1 and 8. Blood samples were collected on Days 1 (pretreatment baseline), 3, 8, 15, 29, and 44, and were analyzed for red blood cell levels (Figure 24), hematocrit (Figure 25), hemoglobin levels (Figure 26), and reticulocyte levels (Figure 27). Vehicle-treated monkeys exhibited decreased levels of red blood cells, hematocrit, and hemoglobin at all post-treatment time points, an expected effect of repeated blood sampling. In contrast, treatment with ActRIIB(L79D 20-134)-hFc or ActRIIB(L79D 25-131)-hFc increased these parameters by the first post-treatment time point (Day 3) and maintained them at substantially elevated levels for the duration of the study (Figures 24-26). Importantly, reticulocyte levels in monkeys treated with ActRIIB(L79D 20-134)-hFc or ActRIIB(L79D 25-131)-hFc were substantially increased at Days 8, 15, and 29 compared to vehicle (Figure 27). This result demonstrates that GDF trap treatment increased production of red blood cell precursors, resulting in elevated red blood cell levels.

Taken together, these data demonstrate that truncated GDF traps, as well as a full-length variants, can be used as selective antagonists of GDF11 and potentially related ligands to increase red blood cell formation *in vivo*.

Example 20: GDF Trap Derived from ActRIIB5

Others have reported an alternate, soluble form of ActRIIB (designated ActRIIB5), in which exon 4, including the ActRIIB transmembrane domain, has been replaced by a different C-terminal sequence (see, *e.g.*, WO 2007/053775).

The sequence of native human ActRIIB5 without its leader is as follows:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVK

KGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEGPWAST

TIPSGGPEATAAAGDQGSGALWLCLEGPAHE (SEQ ID NO:49)

An leucine-to-aspartate substitution, or other acidic substitutions, may be performed at native position 79 (underlined) as described to construct the variant ActRIIB5(L79D), which has the following sequence:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVK

KGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEGPWAST

TIPSGGPEATAAAGDQGSGALWLCLEGPAHE (SEQ ID NO:50)

This variant may be connected to human Fc (double underline) with a TGGG linker (single underline) to generate a human ActRIIB5(L79D)-hFc fusion protein with the following sequence:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVK

KGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEGPWAST

TIPSGGPEATAAAGDQGSGALWLCLEGPAHETGGGTHTCPPCPAPELLGGPSVFL

FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN

STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP

PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY

SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:51).

This construct may be expressed in CHO cells.

Example 21: Effects in Mice of Combined Treatment with EPO and a GDF Trap with a Truncated ActRIIB Extracellular Domain

EPO induces formation of red blood cells by increasing the proliferation of erythroid precursors, whereas GDF traps could potentially affect formation of red blood cells in ways that complement or enhance EPO's effects. Therefore, Applicants investigated the effect of combined treatment with EPO and ActRIIB(L79D 25-131)-hFc on erythropoietic parameters. Male C57BL/6 mice (9 weeks old) were given a single i.p. injection of recombinant human EPO alone (epoetin alfa, 1800 units/kg), ActRIIB(L79D 25-131)-hFc alone (10 mg/kg), both EPO and ActRIIB(L79D 25-131)-hFc, or vehicle (Tris-buffered saline). Mice were euthanized 72 h after dosing for collection of blood, spleens, and femurs.

Spleens and femurs were processed to obtain erythroid precursor cells for flow cytometric analysis. After removal, the spleen was minced in Iscove's modified Dulbecco's medium containing 5% fetal bovine serum and mechanically dissociated by pushing through a 70-μm cell strainer with the plunger from a sterile 1-mL syringe. Femurs were cleaned of any residual muscle or connective tissue and ends were trimmed to permit collection of marrow by flushing the remaining shaft with Iscove's modified Dulbecco's medium containing 5% fetal bovine serum through a 21-gauge needle connected to a 3-mL syringe. Cell suspensions were centrifuged (2000 rpm for 10 min) and the cell pellets resuspended in PBS containing 5% fetal bovine serum. Cells (10^6) from each tissue were incubated with anti-mouse IgG to block nonspecific binding, then incubated with fluorescently labeled antibodies against mouse cell-surface markers CD71 (transferrin receptor) and Ter119 (an antigen associated with cell-surface glycophorin A), washed, and analyzed by flow cytrometry. Dead cells in the samples were excluded from analysis by counterstaining with propidium iodide. Erythroid differentiation in spleen or bone marrow was assessed by the degree of CD71 labeling, which decreases over the course of differentiation, and Ter119 labeling, which is increased during terminal erythroid differentiation beginning with the proerythroblast stage (Socolovsky et al., 2001, Blood 98:3261-3273; Ying et al., 2006, Blood 108:123-133). Thus, flow cytometry was used to determine the number of proerythroblasts ($CD71^{high}Ter119^{low}$), basophilic erythroblasts ($CD71^{high}Ter119^{high}$), polychromatophilic + orthochromatophilic erythroblasts ($CD71^{med}Ter119^{high}$), and late orthochromatophilic erythroblasts + reticulocytes ($CD71^{low}Ter119^{high}$), as described.

Combined treatment with EPO and ActRIIB(L79D 25-131)-hFc led to a surprisingly vigorous increase in red blood cells. In the 72-h time frame of this experiment, neither EPO nor ActRIIB(L79D 25-131)-hFc alone increased hematocrit significantly compared to vehicle, whereas combined treatment with the two agents led to a nearly 25% increase in hematocrit that was unexpectedly synergistic, i.e., greater than the sum of their separate effects (Figure 28). Synergy of this type is generally considered evidence that individual agents are acting through different cellular mechanisms. Similar results were also observed for hemoglobin concentrations (Figure 29) and red blood cell concentrations (Figure 30), each of which was also increased synergistically by combined treatment.

Analysis of erythroid precursor levels revealed a more complex pattern. In the mouse, the spleen is considered the primary organ responsible for inducible ("stress") erythropoiesis. Flow cytometric analysis of splenic tissue at 72 h revealed that EPO markedly altered the erythropoietic precursor profile compared to vehicle, increasing the number of basophilic erythroblasts by more than 170% at the expense of late precursors (late orthochromatophilic erythroblasts + reticulocytes), which decreased by more than one third (Figure 31). Importantly, combined treatment increased basophilic erythroblasts significantly compared to vehicle, but to a lesser extent than EPO alone, while supporting undiminished maturation of late-stage precursors (Figure 31). Thus, combined treatment with EPO and ActRIIB(L79D 25-131)-hFc increased erythropoiesis through a balanced enhancement of precursor proliferation and maturation. In contrast to spleen, the precursor cell profile in bone marrow after combined treatment did not differ appreciably from that after EPO alone. Applicants predict from the splenic precursor profile that combined treatment would lead to increased reticulocyte levels and would be accompanied by sustained elevation of mature red blood cell levels if the experiment were extended beyond 72 h.

Taken together, these findings demonstrate that a GDF trap with a truncated ActRIIB extracellular domain can be administered in combination with EPO to synergistically increase red blood cell formation in vivo. Acting through a complementary but undefined mechanism, a GDF trap can moderate the strong proliferative effect of an EPO receptor activator alone and still permit target levels of red blood cells to be attained with lower doses of an EPO receptor activator, thereby avoiding potential adverse effects or other problems associated with higher levels of EPO receptor activation.

Example 22: Effect of a GDF Trap on Ineffective Erythropoiesis and Anemia in a Mouse Model of MDS

Applicants investigated effects of the GDF trap ActRIIB(L79D 25-131)-mFc (RAP-536) in the NUP98-HOXD13 mouse model of MDS, which is characterized by abortive precursor maturation and ineffective hematopoiesis. In this model, disease severity increases with age, eventually progressing to acute myeloid leukemia in the majority of mice, and they have a mean life span of approximately 14 months. Starting at approximately 4 months of age, these mice exhibit anemia, leukopenia, ineffective erythropoiesis, and bone marrow that is normocellular to hypercellular [Lin et al. (2005) Blood 106:287-295]. To monitor the effects of chronic administration, MDS mice were treated with RAP-536 (10 mg/kg, s.c.) or vehicle twice weekly beginning at 4 months of age and continuing for 7 months, while blood samples (50 μL) were collected at baseline and monthly thereafter for complete blood count analysis. As expected, 6-month-old MDS mice developed severe anemia compared to wild-type mice (Figure 32A), and bone marrow analyses revealed increased numbers of erythroid precursors (Figure 32A) and a lower myeloid/erythroid (M/E) ratio [Suragani et al. (2014) Nat Med 20:408-414] in MDS mice compared to age-matched FVB wild-type mice, indicative of ineffective erythropoiesis. In 6-month-old MDS mice, treatment with RAP-536 significantly increased RBC count (by 16.9%) and hemoglobin concentration (by 12.5%) (Figure 32A), reduced erythroid precursor cell count in bone marrow (Figure 32A), and normalized the M/E ratio to that of wild-type mice [Suragani et al. (2014) Nat Med 20:408-414].

In MDS mice at 12 months of age, RAP-536 treatment significantly increased RBC count (by 18.3%) and hemoglobin level (by 13.0%) (Figure 32B), reduced erythroid precursor cell count (Figure 32B), and improved the M:E ratio [Suragani et al. (2014) Nat Med 20:408-414], as compared to vehicle. RAP-536 treatment did not affect the absolute number of myeloid precursors. Flow cytometry confirmed that RAP-536 treatment reduced erythroid hyperplasia in MDS mice at both ages. A time-course analysis in MDS mice treated with RAP-536 for 7 months showed a sustained elevation in RBC numbers for the duration of the study [Suragani et al. (2014) Nat Med 20:408-414]. Together, these results indicate that treatment with a GDF trap ameliorates anemia, erythroid hyperplasia and ineffective erythropoiesis in MDS mice regardless of disease severity.

Example 23: Cytologic and Genetic Signatures in MDS Patients Therapeutically Responsive to a GDF Trap

A recombinant fusion protein containing modified activin receptor type IIB and IgG Fc [ActRIIB(L79D 25-131)-hFc, also known as luspatercept or ACE-536] is being developed for the treatment of anemias due to ineffective erythropoiesis such as myelodysplastic syndromes (MDS). Patients with MDS often have elevated levels of EPO and may be non-responsive or refractory to erythropoiesis-stimulating agents (ESAs). MDS patients have also been shown to have increased serum levels of GDF11 [Suragani et al. (2014) Nat Med 20:408-414] and increased Smad 2/3 signaling in the bone marrow [Zhou et al. (2008) Blood 112:3434-3443]. ActRIIB(L79D 25-131)-hFc binds to ligands in the TGFβ superfamily, including GDF11, inhibits Smad2/3 signaling, and promotes late-stage erythroid differentiation via a mechanism distinct from ESAs. A murine version, ActRIIB(L79D 25-131)-mFc, reduced Smad2 signaling, increased hemoglobin (Hb) levels, and decreased bone marrow erythroid hyperplasia in a mouse model of MDS [Suragani et al. (2014) Nat Med 20:408-414]. In a healthy-volunteer study, ActRIIB(L79D 25-131)-hFc was well-tolerated and increased Hb levels [Attie et al. (2014) Am J Hematol 89:766-770].

Applicants are conducting an ongoing, phase 2, multicenter, open-label, dose-finding study to evaluate the effects of ActRIIB(L79D 25-131)-hFc on anemia in patients with Low or Int-1 risk MDS who have either high transfusion burden (HTB, defined as ≥ 4 units RBC per 8 weeks prior to baseline) or low transfusion burden (LTB, defined as < 4 units RBC per 8 weeks prior to baseline). Study outcomes include erythroid response (either Hb increase in LTB patients or reduced transfusion burden in HTB patients), safety, tolerability, pharmacokinetics, and pharmacodynamic biomarkers. Inclusion criteria include: Low or Int-1 risk MDS, age ≥ 18 yr, anemia (defined as either being HTB patient or having baseline Hb < 10.0 g/dL in LTB patient), EPO > 500 U/L or nonresponsive/refractory to ESAs, no prior azacitidine or decitabine, and no current treatment with ESA, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), or lenalidomide, thalidomide or pomalidomide. In the dose-escalation phase, ActRIIB(L79D 25-131)-hFc was administered by subcutaneous injection once every 3 weeks in seven sequential cohorts (n = 3-6) at dose levels of 0.125, 0.25, 0.5, 0.75, 1.0, 1.33 and 1.75 mg/kg for up to 5 doses with a 3-month follow-up.

Data were available for 26 patients (seven LTB/19 HTB). Median age was 71 yr (range: 27-88 yr), 50% were female, 54% had prior EPO therapy, and 15% had prior

lenalidomide. Patient classification by WHO subtype was as follows: 15% RARS, 46% RCMD-RS, 15% RCMD, 15% RAEB-1 (including two patients with ≥15% ring sideroblasts) and 8% del (5q). Mean (SD) baseline Hgb for the LTB patients (n = 7) was 9.1 (0.4) g/dL. Mean (SD) units RBC transfused in the 8 weeks prior to treatment was 0.9 (1.1) units for the LTB patients and 6.3 (2.4) units for the HTB patients. Two of the seven LTB patients had an increase in mean Hb ≥ 1.5 g/dL over 8 weeks compared to baseline. Mean maximum Hb increase in the LTB patients was 0.8, 1.0, 2.2, and 3.5 g/dL in the 0.125 (n=1), 0.25 (n = 1), 0.75 (n = 3), and 1.75 (n = 2) mg/kg dose groups, respectively. Six of the seven LTB patients achieved RBC transfusion independence (RBC-TI) for ≥ 8 weeks during the study. The dose levels ranging from 0.75 mg/kg to 1.75 mg/kg were deemed to be active doses. Among the five patients in the active dose groups, four (80%) achieved the pre-specified endpoint of Hgb increase of ≥ 1.5 g/dl for ≥ 2 weeks. Two patients (40%) achieved a HI-E response [International Working Group; Cheson et al. (2000) Blood 96:3671-3674; Cheson et al. (2006) Blood 108:419-425], defined as an Hgb increase of ≥ 1.5 g/dl for ≥ 8 weeks in LTB patients. In HTB patients, the HI-E response is defined as a reduction in transfusion burden of at least four units of red blood cells transfused over an 8 week period as compared to the 8 weeks prior to study start. In the active dose groups, five of 12 (42%) HTB patients met the pre-specified endpoint of a reduction of ≥ 4 RBC units or ≥ 50% reduction in RBC units transfused over an 8-week interval during the treatment period compared to the 8 weeks prior to treatment, and the same patients (five of 12, 42%) achieved a HI-E response; three of 12 (25%) of HTB patients in the active dose groups achieved RBC-TI ≥ 8 weeks during the study. Increases in neutrophil count following study drug administration were observed in some patients. Finally, ActRIIB(L79D 25-131)-hFc was generally well tolerated. No related serious adverse events have been reported to date. The most frequent adverse events regardless of causality were: diarrhea (n = 4, grade 1/2), bone pain, fatigue, muscle spasms, myalgia, and nasopharyngitis (n = 3 each, grade 1/2).

Assessment of bone marrow aspirates demonstrated an association between the presence of ring sideroblasts (considered positive if ≥ 15% of erythroid precursors exhibited ring sideroblast morphology) and responsiveness to ActRIIB(L79D 25-131)-hFc in the active dose groups (0.75 – 1.75 mg/kg). The overall response rate (using HI-E criteria, described above) across both LTB and HTB patients was seven of 17 (41%). Among patients positive for ring sideroblasts at baseline, seven of 13 (54%) patients achieved a HI-E response, and

notably none of the four patients negative for ring sideroblasts at baseline achieved a HI-E response.

Bone marrow aspirates from patients were also evaluated for the presence of mutations in 21 different genes that are known to harbor mutations (primarily somatic mutations) associated with MDS. Genomic DNA was isolated from bone marrow aspirates, selected coding regions of the 21 genes were amplified by polymerase chain reaction, and the DNA sequences of these regions were determined using next-generation sequencing (Myeloid Molecular Profile 21-gene panel, Genoptix, Inc., Carlsbad, CA). This analysis examined activated signaling genes (*KIT*, *JAK2*, *NRAS*, *CBL*, and *MPL*), transcription factors (*RUNX1*, *ETV6*), epigenetic genes (*IDH1*, *IDH2*, *TET2*, *DNMT3A*, *EZH2*, *ASXL1*, and *SETBP1*), RNA splicing genes (*SF3B1*, *U2AF1*, *ZRSR2*, and *SRSF2*), and tumor suppressors/others (*TP53*, *NPM1*, *PHF6*). Analysis of SF3B1 specifically targeted exons 13-16. Of these 21 MDS-associated genes evaluated, mutations in *SF3B1* were more frequently detected in bone marrow cells in the responsive patients than in the nonresponsive patients. Individual *SF3B1* mutations detected in these patients are shown in the following table. The same mutation sometimes occurred in multiple patients.

Nucleotide	Nucleotide Substitution	Amino Acid Substitution	Exon
1873	C → T	R625C	14
1874	G → T	R625L	14
1986	C → G	H662Q	14
2098	A → G	K700E	15
2342	A → G	D781G	16

In patients with *SF3B1* mutations in the active dose groups, six of nine (67%) achieved HI-E responses, including all three patients that achieved transfusion independence for greater than 8 weeks. In patients not having an *SF3B1* mutation, only one of eight (13%) achieved a HI-E reponse. Mutations in *SF3B1* are frequently observed in MDS patients with ring sideroblasts and are associated with ineffective erythropoiesis.

The initial analysis of the clinical trial in process, presented above, was confirmed in a later analysis, for which data were available for 44 patients (15 LTB/29 HTB). Median age was 71 yr (range: 27-88 yr), 43% were female, 61% had prior EPO therapy, and 21% had prior lenalidomide. Mean baseline Hgb for the LTB patients (n = 15) was 9.0 (range: 6.8-10.1) g/dL. Mean units RBC transfused in the 8 weeks prior to treatment was 2 (range 2-2)

units for the 6 LTB patients that received transfusions and 6 (range: 4-14) units for the HTB patients. The dose levels ranging from 0.75 mg/kg to 1.75 mg/kg were deemed to be active doses. Among the 35 LTB and HTB patients in the active dose groups, 22 (63%) achieved the pre-specified endpoint of Hgb increase of ≥ 1.5 g/dl for ≥ 2 weeks for LTB patients and ≥4 unit or 50% reduction in transfusions over 8 weeks for HTB patients . 19 of 35 patients (54%)in the active dose groups achieved a HI-E response [International Working Group; Cheson et al. (2000) Blood 96:3671-3674; Cheson et al. (2006) Blood 108:419-425], defined as an Hgb increase of ≥ 1.5 g/dl for ≥ 8 weeks in LTB patients and defined as a reduction of ≥ 4 RBC units or ≥ 50% reduction in RBC units transfused over an 8-week interval during the treatment period compared to the 8 weeks prior to treatment in HTB patients. 10/28 (36%) patients in the active dose groups that had baseline transfusions achieved transfusion independence for a period of at least 8 weeks. Assessment of bone marrow aspirates demonstrated an association between the presence of ring sideroblasts (considered positive if ≥ 15% of erythroid precursors exhibited ring sideroblast morphology) or a mutation in the SF3B1 gene and responsiveness to ActRIIB(L79D 25-131)- hFc in the active dose groups (0.75 – 1.75 mg/kg). The overall response rate (using HI-E criteria, described above) across both LTB and HTB patients was 19/35 (54%). Among patients positive for ring sideroblasts at baseline, 19/30 (63%) patients achieved a HI-E response, and notably none of the four patients negative for ring sideroblasts at baseline achieved a HI-E response. Among patients positive for SF3B1 mutation at baseline, 16/22 (73%) patients achieved a HI-E response, and notably only 3/13 (23%) patients negative for SF3B1 mutation at baseline achieved a HI-E response. Finally, ActRIIB(L79D 25-131)-hFc was generally well tolerated. The most frequent adverse events regardless of causality were: diarrhea, nasopharyngitis, myalgia, bone pain, bronchitis, headache and and muscle spasms. Two possibly related serious adverse events (SAEs) were reported: grade 3 muscle pain; grade 3 worsening of general condition. One possibly related non-serious grade 3 adverse event of blast cell count increase was reported.

A further data assessment conducted at a later date extended and generally confirmed the above results. Overall, 24 of 49 patients (49%) in the active dose groups achieved an HI-E response, defined in patients with low-transfusion burden (LTB) as an increase in hemoglobin concentration of ≥ 1.5 g/dL for ≥ 8 weeks and defined in patients with high-transfusion burden (HTB) as a reduction of ≥ 4 RBC units, or a reduction of ≥ 50% RBC units, transfused over an 8-week interval during the treatment period compared to the 8

weeks prior to treatment. Fourteen of 40 patients (35%) in the active dose groups that had baseline transfusions achieved transfusion independence for a period of at least 8 weeks.

An assessment of response rate in the presence of certain somatic gene mutations was also conducted for patients in the active dose groups. Mutations in *SF3B1* were detected more frequently in bone marrow cells from the responsive patients than from the nonresponsive patients. Eighteen of 30 patients (60%) in the active dose groups with an *SF3B1* mutation achieved an HI-E response, whereas only 6 of 19 such patients (32%) without a mutation detected in this gene achieved an HI-E response. Individual *SF3B1* mutations detected in these patients are shown in the following table. The same mutation sometimes occurred in multiple patients.

Nucleotide	Nucleotide Change	AA Change	Exon
1868	A → G	Y623C	14
1873	C → T	R625C	14
1874	G → T	R625L	14
1986	C → G	H662Q	14
2098	A → G	K700E	15
2342	A → G	D781G	16
2347	G → A	E783K	16

Similarly, mutations in *DNMT3A* were detected more frequently in bone marrow cells from responsive patients in the active dose groups than from the nonresponsive patients. Seven of 11 patients (64%) in the active dose groups with a *DNMT3A* mutation achieved an HI-E response, whereas 17 of 38 such patients (45%) without a mutation detected in this gene achieved an HI-E response. Individual *DNMT3A* mutations detected in these patients are shown in the following table (IVS refers to intronic mutations and X indicates formation of a premature stop codon).

Nucleotide	Nucleotide Change	AA Change	Exon
1308	C → A	Y436X	10
IVS 2082	+2 T → C	--	--
2193_2195	del CTT	In frame	18
2216	del A	Frame shift	18
IVS 2322	+2 T → C	--	--

2644	C → T	R882C	22
2645	G → A	R882H	22
2678	G → A	W893X	22
2711	C → T	P904L	22
2714	T → C	L905P	22

Similarly, mutations in *TET2* were detected more frequently in bone marrow cells from responsive patients in the active dose groups than from the nonresponsive patients. Eleven of 20 patients (55%) in the active dose groups with a *TET2* mutation achieved an HI-E response, whereas 13 of 29 such patients (45%) without a mutation detected in this gene achieved an HI-E response. Individual *TET2* mutations detected in these patients (excluding known polymorphisms) are shown in the following table.

Nucleotide	Nucleotide Change	AA Change	Exon
73	del T	Frame shift	1
139	G → C	E47Q	1
735	del C	Frame shift	1
1201_1202	ins ACCACCACCAC	Frame shift	1
1337	del T	Frame shift	1
1588_1591	del CAGC	Frame shift	1
1648	C → T	R550X	1
1842_1843	ins G	Frame shift	1
2145	del C	Frame shift	1
2305	del C	Frame shift	1
2784	del T	Frame shift	1
3025	C → T	Q1009X	1
3727_3729	del AAA	In frame	4
3731_3738	del TCTACTCG	Frame shift	4
3821	A → G	Q1274R	5
3854_3856	del TCT	In frame	5
3871	T → A	W1291R	5
IVS 3955	–2 A → G	--	--
4011	T → A	Y1337X	6
4108	G → A	G1370R	7
4109	G → A	G1370E	7
4160	A → G	N1387S	7
4209	del T	Frame shift	8

4210	C → T	R1404X	8
4211_4217	del GAGAATT	Frame shift	8
4546	C → T	R1516X	9
4954	C → T	Q1652X	9
5168	del C	Frame shift	9
5170	T → C	Y1724H	9
5576_5582	del TTGGGGG	Frame shift	9

Mutations in other genes were detected in bone marrow cells from responsive patients with a frequency similar to that in cells from nonresponsive patients. For example, 4 of 8 patients (50%) in the active dose groups with an *ASXL1* mutation achieved an HI-E response, while a similar percentage of such patients (20 of 41 , 49%) without a mutation detected in this gene achieved an HI-E response.

These results indicate that patients with MDS exhibiting ≥ 15% ring sideroblasts (and patients with other forms of sideroblastic anemia) and/or at least one mutation in *SF3B1* are more likely to respond therapeutically to ActRIIB(L79D 25-131)-hFc than MDS patients with < 15% ring sideroblasts and/or no mutation in *SF3B1*. Similarly, patients exhibiting exhibiting ≥ 15% ring sideroblasts (and patients with other forms of sideroblastic anemia) and/or at least one mutation in *DNMT3A or TET2* are more likely to respond therapeutically to ActRIIB(L79D 25-131)-hFc than MDS patients with < 15% ring sideroblasts and/or no mutation in *DNMT3A or TET2*. Based on these data, selective treatment of any of these patient subgroups is expected to greatly increase the benefit/risk ratio of treatment with ActRII inhibitors.

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

While specific embodiments of the subject matter have been discussed, the above specification is illustrative and not restrictive. Many variations will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

WE CLAIM:

1. A method for treating or preventing sideroblastic anemia in a human patient, comprising administering to a patient in need thereof a polypeptide comprising the amino acid sequence of SEQ ID NO: 44, and wherein the patient is on a dosing schedule that comprises administering from 0.75-1.75 mg/kg of the polypeptide to the patient.

2. The method of claim 1, wherein the polypeptide consists of the amino acid sequence of SEQ ID NO: 44.

3. The method of claim 1 or 2, wherein the polypeptide is a dimer.

4. The method of any one of claims 1-3, wherein the polypeptide binds to GDF11.

5. The method of any one of claims 1-3, wherein the polypeptide binds to GDF8.

6. The method of any one of claims 1-3, wherein the polypeptide binds to GDF11 and GDF8.

7. The method of any one of claims 1-6, where the polypeptide comprises one or more amino acid modifications selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, and an amino acid conjugated to a lipid moiety.

8. The method of claim 7, wherein the polypeptide is glycosylated and has a mammalian glycosylation pattern.

9. The method of claim 8, wherein the polypeptide has a glycosylation pattern obtainable from a Chinese hamster ovary cell line.

10. The method of any one of claims 1-9, wherein the polypeptide is subcutaneously administered to the patient.

11. The method of any one of claims 1-10, wherein the dosing schedule further comprises administering the polypeptide to the patient once every three weeks.

12. The method of any one of claims 1-11, wherein the patient has undesirably high levels of endogenous EPO.

13. The method of any one of claims 1-12, wherein the patient has previously been treated with one or more EPO receptor agonists.

14. The method of claim 13, wherein the patient has an inadequate response to the EPO receptor agonist.

15. The method of claim 13, wherein the patient is no longer responsive to the EPO receptor agonist.

16. The method of any one of claims 13-15, wherein the EPO receptor agonist is EPO.

17. The method of any one of claims 1-16, wherein the treatment increases red blood cell levels.

18. The method of any one of claims 1-17, wherein the treatment increases hemoglobin levels.

19. The method of claim 18, wherein the treatment results in an increase in hemoglobin of ≥ 1.5 g/dL for ≥ two weeks.

20. The method of claims 18, wherein the treatment results in an increase in hemoglobin of ≥ 1.5 g/dL for ≥ eight weeks.

21. The method of any one of claims 1-20, wherein the patient has been administered one or more blood cell transfusions prior to the start of treatment.

22. The method of any one of claims 1-21, wherein the patient is a low transfusion burden patient.

23. The method of any one of claims 1-21, wherein the patient is a high transfusion burden patient.

24. The method any one of claims 21-23, wherein the treatment decreases blood cell transfusion burden.

25. The method of claim 24, wherein the treatment decreases blood cell transfusion by ≥ 50% for at least four weeks relative to the equal time prior to start of treatment.

26. The method of claim 24, wherein the treatment decreases blood cell transfusion by ≥ 50% for at least eight weeks relative to the equal time prior to start of treatment.

27. The method of any one of claims 1-26, wherein the patient has myelodysplastic syndrome.

28. The method of claim 27, wherein the patient has an International Prognostic Scoring System (IPSS) or IPSS-R score of low or intermediate.

29. The method of any one of claims 1-28, wherein the sideroblastic anemia patient has at least 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% ring blasts as a percentage of bone marrow erythroid precursors in his or her bone marrow.

30. The method of any one of claims 1-29, wherein the treatment increases neutrophil levels.

31. The method of any one of claims 1-30, wherein the patient has bone marrow cells that test positive for one or more mutations in SF3B1.

32. The method of any one of claims 1-31, wherein the patient has bone marrow cells that test positive for one or more mutations in DNMT3A.

33. The method of any one of claims 1-32, wherein the patient has bone marrow cells that test positive for one or more mutations in TET2.

34. The method of any one of claims 1-33, wherein the treatment decreases iron overload.

35. A method for treating or preventing a bone marrow disorder in a subject in need thereof, the method comprising administering to the subject an ActRII antagonist, wherein the subject has bone marrow cells that test positive for an *SF3B1* mutation, optionally a mutation in the SF3B1 gene is in an exon, intron or 5' or 3' untranslated region, optionally a mutation in SF3B1 causes a change in the amino acid sequence or does not cause a change in the amino acid sequence of the protein encoded by the gene, and optionally a mutation in the SF3B1 gene causes a change in the amino acid of the protein encoded by the gene selected from the following changes: K182E, E491G, R590K, E592K, R625C, R625G, N626D, N626S, H662Y, T663A, K666M, K666Q, K666R, Q670E, G676D, V701I, I704N, I704V, G740R, A744P, D781G, A1188V, N619K, N626H, N626Y, R630S, I704T, G740E, K741N, G742D, D894G, Q903R, R1041H, I1241T, G347V, E622D, Y623C, R625H, R625L, H662D, H662Q, T663I, K666E, K666N, K666T, K700E, and V701F.

36. The method of claim 35, wherein the subject has a disorder selected from: sideroblastic anemia, chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML).

37. The method of claim 35 or 36, wherein administration of the ActRII antagonist treats or prevents one or more complications of sideroblastic anemia.

38. The method of claim 35, wherein the subject has MDS.

39. The method of claim 38, wherein the subject has ring blasts.

40. The method of any one of claims 35-39, wherein the subject has a somatic mutation in one or more of *SF3B1*, *SRSF2*, *DNMT3A*, and *TET2*.

41. The method of any one of claims 38-40, wherein the subject has an International Prognostic Scoring System (IPSS) or IPSS-R score of low or intermediate.

42. The method of any one of claims 35-41, wherein the subject has at least 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% ring blasts as a percentage of bone marrow erythroid precursors in his or her bone marrow.

43. The method of any one of claims 35-42, wherein the subject has previously been treated with one or more EPO receptor agonists.

44. The method of claim 43, wherein the subject has an inadequate response to the EPO receptor agonist.

45. The method of claim 43, wherein the subject is no longer responsive to the EPO receptor agonist.

46. The method of any one of claims 43-45, wherein the EPO receptor agonist is EPO.

47. A method for treating or preventing MDS in a subject in need thereof, the method comprising administering to the subject an ActRII antagonist, wherein the subject has previously been treated with an EPO receptor agonist.

48. The method of claim 47, wherein the subject has a subtype of MDS selected from: MDS with refractory cytopenia with unilineage dysplasia (RCUD); MDS with refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS); MDS with a somatic mutation in one or more of *SF3B1*, *SRSF2*, *DNMT3A*, and *TET2*; MDS without a somatic mutation in *ASXL1* or *ZRSR2*; MDS with iron overload; and MDS with neutropenia.

49. The method of claim 47 or 48, wherein the subject has an International Prognostic Scoring System (IPSS) or IPSS-R score of low or intermediate.

50. The method of any one of claims 47-49, wherein the subject has ring blasts.

51. The method of claim 50, wherein the subject has at least 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% ring blasts as a percentage of bone marrow erythroid precursors in his or her bone marrow.

52. The method of any one of claims 47-51, wherein the subject has bone marrow cells that test positive for a mutation in the SF3B1 gene.

53. The method of any one of claims 47-52, wherein the subject has previously been treated with one or more EPO receptor agonists.

54. The method of claim 53, wherein the subject has an inadequate response to the EPO receptor agonist.

55. The method of claim 53, wherein the subject is no longer responsive to the EPO receptor agonist.

56. The method of any one of claims 53-55, wherein the EPO receptor agonist is EPO.

57. The method of any one of claims 47-56, wherein the treatment increases red blood cell levels.

58. The method of any one of claims 47-57, wherein the subject has been administered one or more blood cell transfusion prior to start of the ActRII antagonist treatment.

59. The method of any one of claims 47-58, wherein the treatment decreases blood cell transfusion burden.

60. The method of claim 59, wherein the treatment decreases blood cell transfusion by greater than 50% for 4 to 8 weeks relative to the equal time prior to start of the ActRII antagonist treatment.

61. The method of any one of claims 47-60, wherein the treatment decreases iron overload.

62. The method of any one of claims 47-61, wherein the treatment decreases liver iron content.

63. The method of any one of claims 47-62, wherein the treatment increases neutrophil levels.

64. The method of any one of claims 47-63, wherein the treatment delays conversion to acute myeloid leukemia (AML).

65. A method for treating or preventing a bone marrow disorder in a subject, comprising administering to a subject in need thereof an effective amount of an ActRII antagonist, wherein the subject has bone marrow cells that test positive for one or more mutations in a gene selected from the group consisting of: SF3B1, DNMT3A, and TET2.

66. The method of claim 65, wherein the subject tests positive for one or more SF3B1 mutations.

67. The method of claim 66, wherein one or more of the SF3B1 mutations are in a SF3B1 exon.

68. The method of claim 66 or 67, wherein one or more of the SF3B1 mutations are in a SF3B1 intron.

69. The method of any one of claims 66-68, wherein one or more of the SF3B1 mutations are in a SF3B1 5' and/or 3' region.

70. The method of any one of claims 66-69, wherein one or more of the SF3B1 mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated SF3B1 gene.

71. The method of claim 70, wherein the one or more SF3B1 mutations causes a substitution of one or more amino acid selected from the group consisting of: K182E, E491G, R590K, E592K, R625C, R625G, N626D, N626S, H662Y, T663A, K666M, K666Q, K666R, Q670E, G676D, V701I, I704N, I704V, G740R, A744P, D781G, A1188V, N619K, N626H, N626Y, R630S, I704T, G740E, K741N, G742D, D894G, Q903R, R1041H, I1241T, G347V, E622D, Y623C, R625H, R625L, H662D, H662Q, T663I, K666E, K666N, K666T, K700E, V701F, and E783K.

72. The method of claim 67, wherein the one or more SF3B1 mutations are in a SF3B1 exon selected from the group consisting of: exon 14, exon 15 and exon 16.

73. The method of any one of claims 65-72, wherein the subject tests positive for one or more DNMT3A mutations.

74. The method of claim 73, wherein one or more of the DNMT3A mutations are in a DNMT3A exon.

75. The method of claim 73 or 74, wherein one or more of the DNMT3A mutations are in a DNMT3A intron.

76. The method of any one of claims 73-75, wherein one or more of the DNMT3A mutations are in a DNMT3A 5' and/or 3' region.

77. The method of any one of claims 73-76, wherein one or more of the DNMT3A mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated DNMT3A gene.

78. The method of claim 77, wherein the one or more DNMT3A mutations causes a substitution of one or more amino acid selected from the group consisting of: R882C, R882H, P904L, and P905P.

79. The method of claim 74, wherein the one or more DNMT3A mutations are in a DNMT3A exon selected from the group consisting of: exon 10, exon 18 and exon 22.

80. The method of claim 73, wherein the one or more DNMT3A mutations introduces a premature stop codon.

81. The method of claim 80, wherein the one or more DNMT3A mutations is selected from the group consisting of Y436X and W893X.

82. The method of any one of claims 65-81, wherein the subject tests positive for one or more TET2 mutations.

83. The method of claim 82, wherein one or more of the TET2 mutations are in a TET2 exon.

84. The method of claim 82 or 83, wherein one or more of the TET2 mutations are in a TET2 intron.

85. The method of any one of claims 82-84, wherein one or more of the TET2 mutations are in a TET2 5' and/or 3' region.

86. The method of any one of claims 82-85, wherein one or more of the TET2 mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated TET2 gene.

87. The method of claim 86, wherein the one or more TET2 mutations causes a substitution of one or more amino acid selected from the group consisting of: E47Q, Q1274R, W1291R, G1370R, G1370E, N1387S, and Y1724H.

88. The method of claim 83, wherein the one or more TET2 mutations are in a TET2 exon selected from the group consisting of: exon 1, exon 4, exon 5, exon 6, exon 7, exon 8, and exon 9.

89. The method of claim 82, wherein the one or more TET2 mutations introduces a premature stop codon.

90. The method of claim 89, wherein the one or more TET2 mutations is selected from the group consisting of: R550X, Q1009X, Y1337X, R1404X, R1516X, and Q1652X.

91. The method of any one of claims 65-90, wherein the subject has anemia.

92. The method of any one of claims 65-91, wherein the subject has undesirably high levels of endogenous EPO.

93. The method of any one of claims 65-92, wherein the subject has previously been treated with one or more EPO receptor agonists.

94. The method of claim 93, wherein the subject has an inadequate response to the EPO receptor agonist.

95. The method of any one of claims 93, wherein the subject is no longer responsive to the EPO receptor agonist.

96. The method of any one of claims 93-95, wherein the EPO receptor agonist is EPO.

97. The method of any one of claims 65-96, wherein treatment delays conversion to leukemia.

98. The method of claim 96, wherein the treatment delays conversion to acute myeloid leukemia.

99. The method of any one of claims 65-98, wherein the subject is a pre-leukemia patient.

100. The method of any one of claims 65-99, wherein the treatment increases red blood cell levels and/or hemoglobin levels in the subject.

101. The method of any one of claims 65-100, wherein the subject has been administered one or more blood cell transfusions prior to the start of the ActRII antagonist treatment.

102. The method of any one of claims 65-101, wherein the treatment decreases blood cell transfusion burden.

103. The method of claim 102, wherein the treatment decreases blood cell transfusion by greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% for 4 to 8 weeks relative to the equal time prior to the start of the ActRII antagonist treatment.

104. The method of claim 102, wherein the treatment decreases blood cell transfusion by greater than about 50% for 4 to 8 weeks relative to the equal time prior to the start of the ActRII antagonist treatment.

105. The method of any one of claims 65-104, wherein the treatment decreases iron overload.

106. The method of any one of claims 65-105, wherein the treatment decrease iron content in the liver and/or spleen.

107. A method for treating or preventing myelodysplastic syndrome (MDS) and/or one or more complications of MDS, comprising administering to a subject in need thereof an effective amount of an ActRII antagonist, wherein the subject has bone marrow cells that test positive for one or more mutations in a gene selected from the group consisting of: SF3B1, DNMT3A, and TET2.

108. The method of claim 107, wherein the subject tests positive for one or more SF3B1 mutations.

109. The method of claim 108, wherein one or more of the mutations are in a SF3B1 exon.

110. The method of claim 107 or 108, wherein one or more of the SF3B1 mutations are in a SF3B1 intron.

111. The method of any one of claims 107-110, wherein one or more of the SF3B1 mutations are in a SF3B1 5' and/or 3' region.

112. The method of any one of claims 107-111, wherein one or more of the SF3B1 mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated SF3B1 gene.

113. The method of claim 112, wherein the one or more SF3B1 mutations causes a substitution of one or more amino acid selected from the group consisting of: K182E, E491G, R590K, E592K, R625C, R625G, N626D, N626S, H662Y, T663A, K666M, K666Q, K666R, Q670E, G676D, V701I, I704N, I704V, G740R, A744P, D781G, A1188V, N619K, N626H, N626Y, R630S, I704T, G740E, K741N, G742D, D894G, Q903R, R1041H, I1241T, G347V, E622D, Y623C, R625H, R625L, H662D, H662Q, T663I, K666E, K666N, K666T, K700E, V701F, and E783K.

114. The method of claim 109, wherein the one or more SF3B1 mutations are in a SF3B1 exon selected from the group consisting of: exon 14, exon 14 and exon 16.

115. The method of any one of claims 107-114, wherein the subject tests positive for one or more DNMT3A mutations.

116. The method of claim 115, wherein one or more of the DNMT3A mutations are in a DNMT3A exon.

117. The method of claim 115 or 116, wherein one or more of the DNMT3A mutations are in a DNMT3A intron.

118. The method of any one of claims 115-117, wherein one or more of the DNMT3A mutations are in a DNMT3A 5' and/or 3' region.

119. The method of any one of claims 115-118, wherein one or more of the DNMT3A mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated DNMT3A gene.

120. The method of claim 119, wherein the one or more DNMT3A mutations causes a substitution of one or more amino acid selected from the group consisting of: R882C, R882H, P904L, and P905P.

121. The method of claim 116, wherein the one or more DNMT3A mutations are in a DNMT3A exon selected from the group consisting of: exon 10, exon 18, and exon 22.

122. The method of claim 115, wherein the one or more DNMT3A mutations introduces a premature stop codon.

123. The method of claim 122, wherein the one or more DNMT3A mutations is selected from the group consisting of Y436X and W893X.

124. The method of any one of claims 107-123, wherein the subject tests positive for one or more TET2 mutations.

125. The method of claim 124, wherein one or more of the TET2 mutations are in a TET2 exon.

126. The method of claim 124 or 125, wherein one or more of the TET2 mutations are in a TET2 intron.

127. The method of any one of claims 124-126, wherein one or more of the TET2 mutations are in a TET2 5' and/or 3' region.

128. The method of any one of claims 124-127, wherein one or more of the TET2 mutations causes a deletion, addition, and/or substitution of an amino acid in the protein encoded by the mutated TET2 gene.

129. The method of claim 128, wherein the one or more TET2 mutations causes a substitution of one or more amino acid selected from the group consisting of: E47Q, Q1274R, W1291R, G1370R, G1370E, N1387S, and Y1724H.

130. The method of claim 125, wherein the one or more TET2 mutations are in a TET2exon selected from the group consisting of: exon 1, exon 4, exon 5, exon 6, exon 7, exon 8, and exon 9.

131. The method of claim 124, wherein the one or more TET2 mutations introduces a premature stop codon.

132. The method of claim 131, wherein the one or more TET2 mutations is selected from the group consisting of: R550X, Q1009X, Y1337X, R1404X, R1516X, and Q1652X.

133. The method of any one of claims 107-132, wherein the subject has anemia.

134. The method of any one of claims 107-133, wherein the subject has undesirably high levels of endogenous EPO.

135. The method of any one of claims 107-134, wherein the subject has previously been treated with one or more EPO receptor agonists.

136. The method of claim 135, wherein the subject has an inadequate response to the EPO receptor agonist.

137. The method of any one of claims 136, wherein the subject is no longer responsive to the EPO receptor agonist.

138. The method of any one of claims 135-137, wherein the EPO receptor agonist is EPO.

139. The method of any one of claims 107-138, wherein treatment delays conversion to leukemia.

140. The method of claim 139, wherein the treatment delays conversion to acute myeloid leukemia.

141. The method of any one of claims 107-140, wherein the treatment increases red blood cell levels and/or hemoglobin levels in the subject.

142. The method of any one of claims 107-141, wherein the subject has been administered one or more blood cell transfusions prior to the start of the ActRII antagonist treatment.

143. The method of any one of claims 107-142, wherein the treatment decreases blood cell transfusion burden.

144. The method of claim 143, wherein the treatment decreases blood cell transfusion by greater than about 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100% for 4 to 8 weeks relative to the equal time prior to the start of the ActRII antagonist treatment.

145. The method of claim 143, wherein the treatment decreases blood cell transfusion by greater than about 50% for 4 to 8 weeks relative to the equal time prior to the start of the ActRII antagonist treatment.

146. The method of any one of claims 107-145, wherein the treatment decreases iron overload.

147. The method of any one of claims 107-145, wherein the treatment decrease iron content in the liver and/or spleen.

148. The method of any one of claims 107-147, wherein the subject has a subtype of MDS selected from: MDS with refractory cytopenia with unilineage dysplasia (RCUD); MDS with refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS); MDS with a somatic mutation in one or more of *SF3B1*, *SRSF2*, *DNMT3A*, and *TET2*; MDS without a somatic mutation in *ASXL1* or *ZRSR2*; MDS with iron overload; and MDS with neutropenia.

149. The method of any one of claims 107-148, wherein the subject has an International Prognostic Scoring System (IPSS) score selected from: low, intermediate 1, or intermediate 2.

150. The method of any one of claims 107-149, wherein the subject has ring blasts.

151. The method of claim 150, wherein the subject has at least 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95% ring blasts as a percentage of bone marrow erythroid precursors in his or her bone marrow.

152. The method of any one of claims 1-151, wherein the ActRII antagonist is an ActRIIA polypeptide.

153. The method of claim 152, wherein the ActRIIA polypeptide is selected from:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:10 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:10;

b) a polypeptide comprising the amino acid sequence of SEQ ID NO:11 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:11;

c) a polypeptide comprising the amino acid sequence of SEQ ID NO:22 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:22;

d) a polypeptide comprising the amino acid sequence of SEQ ID NO:26 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:26;

e) a polypeptide comprising the amino acid sequence of SEQ ID NO:28 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:28; and

f) a polypeptide comprising an amino acid sequence that is identical to amino acids 30-110 of SEQ ID NO:9 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of amino acid 30-110 of SEQ ID NO:9.

154. The method of any one of claims 1-151, wherein the ActRII antagonist is an ActRIIB polypeptide.

155. The method of claim 154, wherein the ActRIIB polypeptide selected from:

a) a polypeptide comprising an amino acid sequence that is identical to amino acids 29-109 of SEQ ID NO:1 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of amino acids 29-109 of SEQ ID NO:1;

b) a polypeptide comprising an amino acid sequence that is identical to amino acids 25-131 of SEQ ID NO:1 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the sequence of amino acids 25-131 of SEQ ID NO:1;

c) a polypeptide comprising the amino acid sequence of SEQ ID NO:2 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:2;

d) a polypeptide comprising the amino acid sequence of SEQ ID NO:3 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:3;

e) a polypeptide comprising the amino acid sequence of SEQ ID NO:4 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:4;

f) a polypeptide comprising the amino acid sequence of SEQ ID NO:5 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:5;

g) a polypeptide comprising the amino acid sequence of SEQ ID NO:6 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:6;

h) a polypeptide comprising the amino acid sequence of SEQ ID NO:36 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:36;

i) a polypeptide comprising the amino acid sequence of SEQ ID NO:37 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:37;

j) a polypeptide comprising the amino acid sequence of SEQ ID NO:38 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:38;

k) a polypeptide comprising the amino acid sequence of SEQ ID NO:49 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:49;

l) a polypeptide comprising the amino acid sequence of SEQ ID NO:50 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:50;

m) a polypeptide comprising the amino acid sequence of SEQ ID NO:51 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:51;

n) a polypeptide comprising the amino acid sequence of SEQ ID NO:41 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:41;

o) a polypeptide comprising the amino acid sequence of SEQ ID NO:44 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:44;

p) a polypeptide comprising the amino acid sequence of SEQ ID NO:45 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:45; and

q) a polypeptide comprising the amino acid sequence of SEQ ID NO:29 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:29.

156. The method of claim 155, wherein the ActRIIB polypeptide comprises an acidic amino acid at position 79 with respect to SEQ ID NO:1.

157. The method of any one of claims 1-156, wherein the ActRII antagonist is a GDF trap polypeptide.

158. The method of any one of claims 152-157, wherein the polypeptide is a fusion protein comprising, in addition to an ActRII polypeptide domain, one or more heterologous polypeptide domains that enhance one or more of: *in vivo* half-life, *in vitro* half-life, administration, tissue localization or distribution, formation of protein complexes, and purification.

159. The method claim 158, wherein the fusion protein comprises a heterologous polypeptide domain selected from: an immunoglobulin Fc domain and a serum albumin.

160. The method of claim 159, wherein the immunoglobulin Fc domain is an IgG1 Fc domain.

161. The method of claim 159, wherein the immunoglobulin Fc domain comprises an amino acid sequence selected from SEQ ID NO: 15 or 16.

162. The method of any one of claims 158-161, wherein the fusion protein further comprises a linker domain positioned between the ActRII polypeptide domain and the immunoglobulin Fc domain.

163. The method of claim 162, wherein the linker domain is a TGGG or GGG linker.

164. The method of claim 158, wherein the polypeptide is an ActRIIA-Fc fusion protein comprising a polypeptide selected from:

a) a polypeptide comprising the amino acid sequence of SEQ ID NO:22 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:22; and

b) a polypeptide comprising the amino acid sequence of SEQ ID NO:28 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:26.

165. The method of claim 158, wherein the polypeptide is an ActRIIB-Fc fusion protein comprising a polypeptide selected from:

a) a polypeptide comprising that comprises the amino acid sequence of SEQ ID NO:29 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:29;

b) a polypeptide comprising the amino acid sequence of SEQ ID NO:36 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:36;

c) a polypeptide comprising the amino acid sequence of SEQ ID NO:29 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:29;

d) a polypeptide comprising the amino acid sequence of SEQ ID NO:38 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:38;

e) a polypeptide comprising the amino acid sequence of SEQ ID NO:41 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:41;

f) a polypeptide comprising the amino acid sequence of SEQ ID NO:44 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:44;

g) a polypeptide comprising the amino acid sequence of SEQ ID NO:45 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:45; and

h) a polypeptide comprising the amino acid sequence of SEQ ID NO:51 or comprising an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:51.

166. The method of claim 165, wherein the ActRIIB-Fc fusion protein comprises an acidic amino acid at position 79 with respect to SEQ ID NO:1.

167. The method of any one of claims 152-166, wherein the polypeptide comprises one or more amino acid modifications selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and amino acid conjugated to an organic derivatizing agent.

168. The method of claim 167, wherein the polypeptide is glycosylated and has a mammalian glycosylation pattern.

169. The method of claim 168, wherein the polypeptide is glycosylated and has a glycosylation pattern obtainable from a Chinese hamster ovary cell line.

170. The method of any one of claims 152-169, wherein the polypeptide binds to GDF11.

171. The method of any one of claims 152-170, wherein the polypeptide binds to GDF8.

172. The method of any one of claims 152-171, wherein the polypeptide binds to activin.

173. The method of claim 172, wherein the polypeptide binds to activin A.

174. The method of claim 172 or 173, wherein the polypeptide binds to activin B.

175. The method of any one of claims 1-151, wherein the ActRII antagonist is an anti-GDF11 antibody.

176. The method of any one of claims 1-151, wherein the ActRII antagonist is an anti-GDF8 antibody.

177. The method of any one of claims 1-151, wherein the ActRII antagonist is an anti-activin antibody.

178. The method of claim 177, wherein the ActRII antagonist binds to activin A.

179. The method of claim 177, wherein the ActRII antagonist binds to activin B.

180. The method of claims 177, wherein the ActRII antagonist binds to activin A and activin B.

181. The method of any one of claims 175-180, wherein the ActRII antagonist is a multispecific antibody that further binds to one or more of: GDF11, GDF8, activin A, activin AB, activin C, activin E, BMP7, GDF3, and BMP6.

182. The method of any one of claims 1-151, wherein the ActRII antagonist is an anti-ActRII antibody.

183. The method claim 182, wherein the anti-ActRII antibody binds to ActRIIA.

184. The method of claim 182, wherein the anti-ActRII antibody binds to ActRIIB.

185. The method of claim 182, wherein the anti-ActRII antibody binds to ActRIIA and ActRIIB.

186. The method of any one of claims 175-185, wherein the antibody is a chimeric antibody, a humanized antibody, or a human antibody.

187. The method of any one of claims 175-186, wherein the antibody is a single-chain antibody, an $F(ab')_2$ fragment, a single-chain diabody, a tandem single-chain Fv fragment, a tandem single-chain diabody, a or a fusion protein comprising a single-chain diabody and at least a portion of an immunoglobulin heavy-chain constant region.

188. The method of any one of claims 1-187, wherein the method further comprises administering one or more supportive therapy for sideroblastic anemia.

189. The method of any one of claims 1-187, wherein the method further comprises administering one or more supportive therapy for MDS.

190. The method of claim 188 or 189, wherein the supportive therapy is transfusion of one or more of: red blood cells, granulocytes, and thrombocytes.

191. The method of any one of claims 188-190, wherein the supportive therapy comprises administration of an iron-chelating agent or multiple iron-chelating agents.

192. The method of claim 191, wherein the iron-chelating agent or multiple iron-chelating agents are selected from:

a) deferoxamine;

b) deferiprone; and

c) deferasirox.

193. The method of any one of claims 188-192, wherein the supportive therapy comprises administering an EPO receptor activator.

194. The method of claim 193, wherein the EPO receptor activator is selected from: EPO, epoetin alfa, epoetin beta, epoetin delta, epoetin omega, darbepoetin alfa, methoxy-polyethylene-glycol epoetin beta, and synthetic erythropoiesis protein (SEP).

195. The method of any one of claims 188-194, wherein the supportive therapy comprises administration of one or more agents selected from the group consisting of: a G-CSF analog, a GM-CSF analog, an iron-chelating agent, hepcidin or a hepcidin receptor activator, lenalidomide, thalidomide, pomalidomide, azacitidine, decitabine, antithymocyte globulin, and thrombomimetic agent, a histone deacetylase inhibitor, a p38MAPK inhibitor, a glutathione S-transferase π inhibitor, alemtuzumab, a DNA methyltransferase inhibitor, and a histone deacetylase inhibitor.

ActRIIa
ActRIIb
ILGRSETQEC
GRGEAETREC
LFFNANWEKD
IYYNANWELE
RTNQTGVEPC
RTNQSGLERC
YGDKDKRRHC
EGEQDKRLHC
FAMWKNISGS
YASWRNSSGT
IEIVRQGCWL
IEILVKKGCWL
DDINCYDRTD
DDFNCYDRQE
CVEKKDSPEV
CVATEENPQV
YFCCCEGNMC
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EVTQPTSNPV
GGPEVTYEPP
TPKPPT
PTAPT

FIG. 1

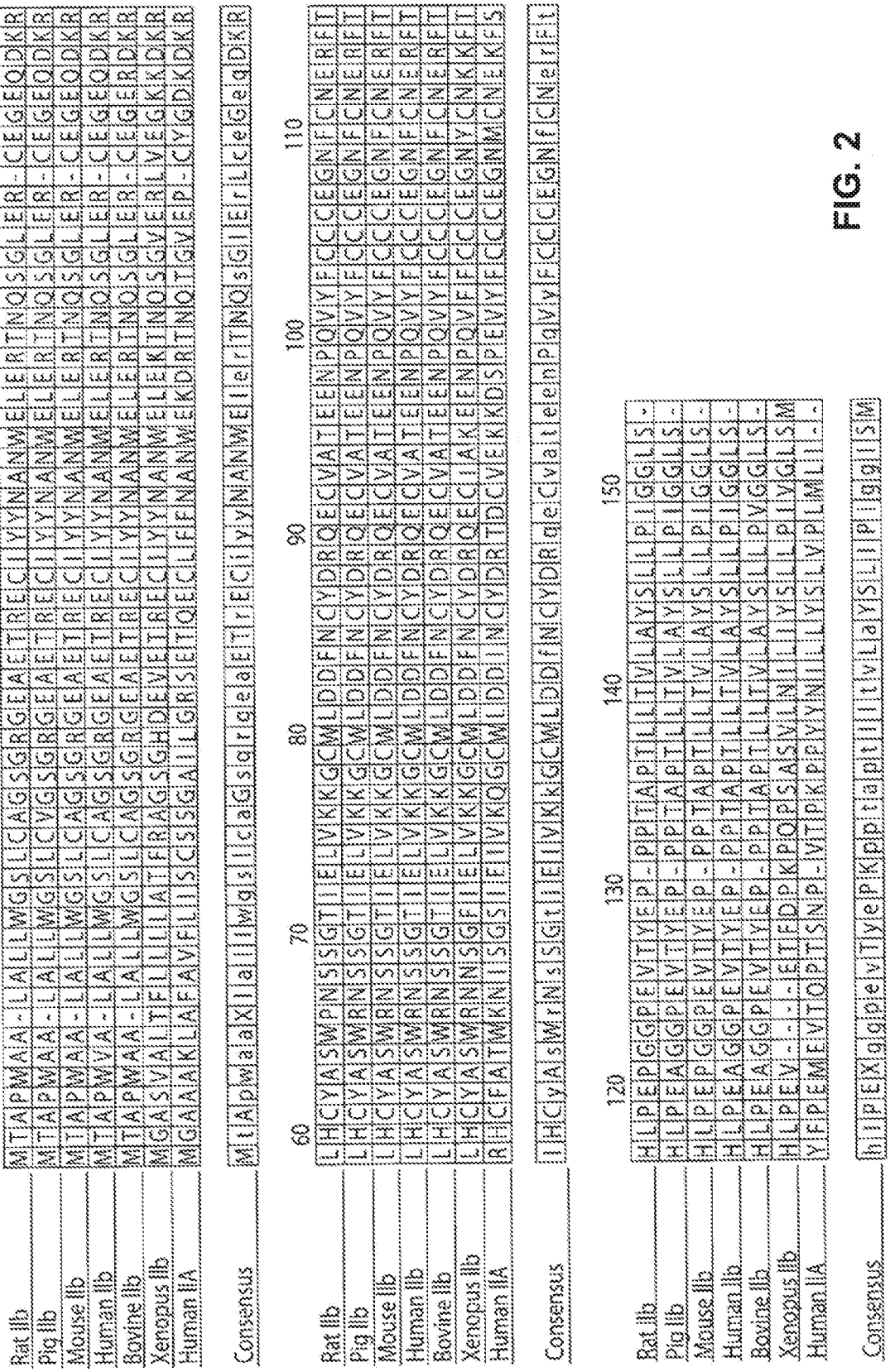
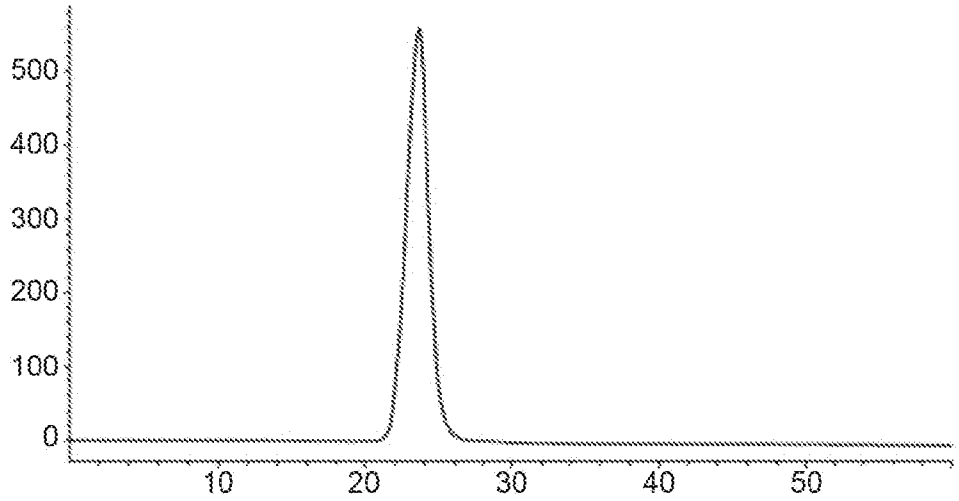


FIG. 2



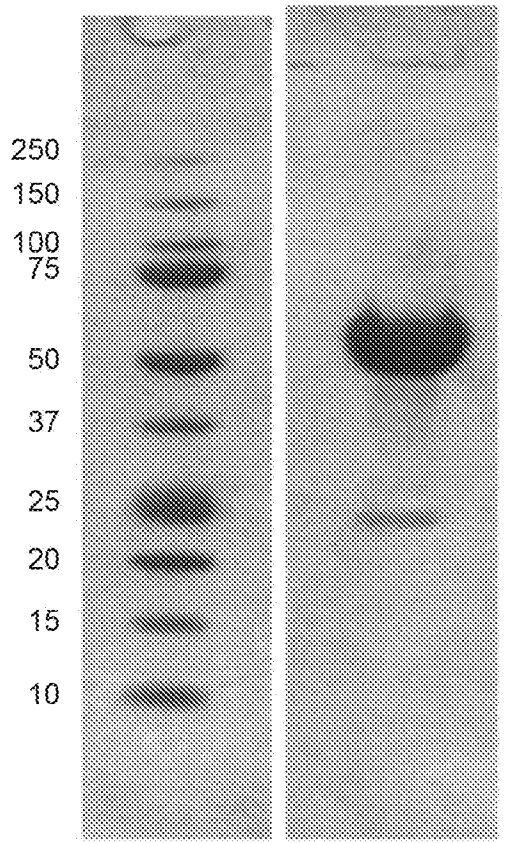
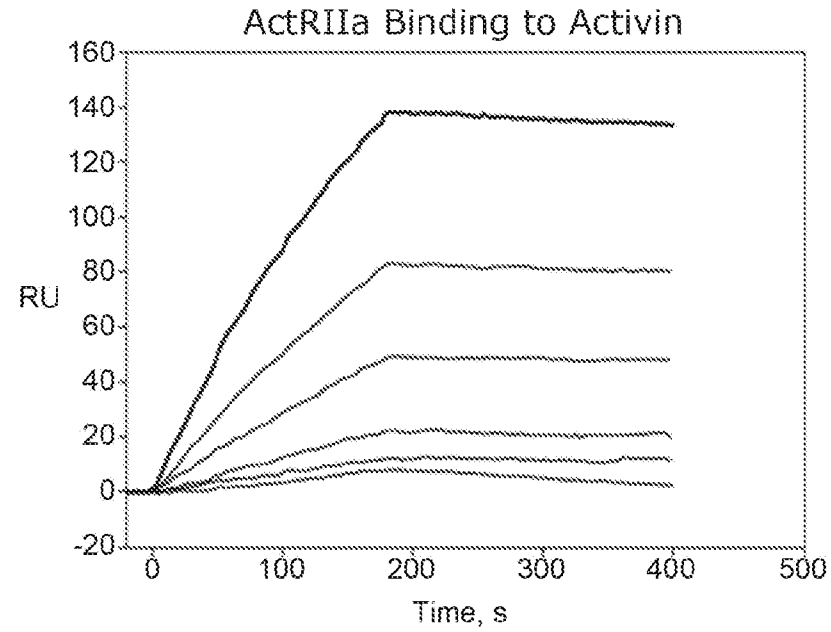


FIG. 3


ActRIIa Binding to Activin
160
140
120
100
80
60
40
20
0
-20
RU
0
100
200
300
400
500
Time, s
Kd 5 e-12 M

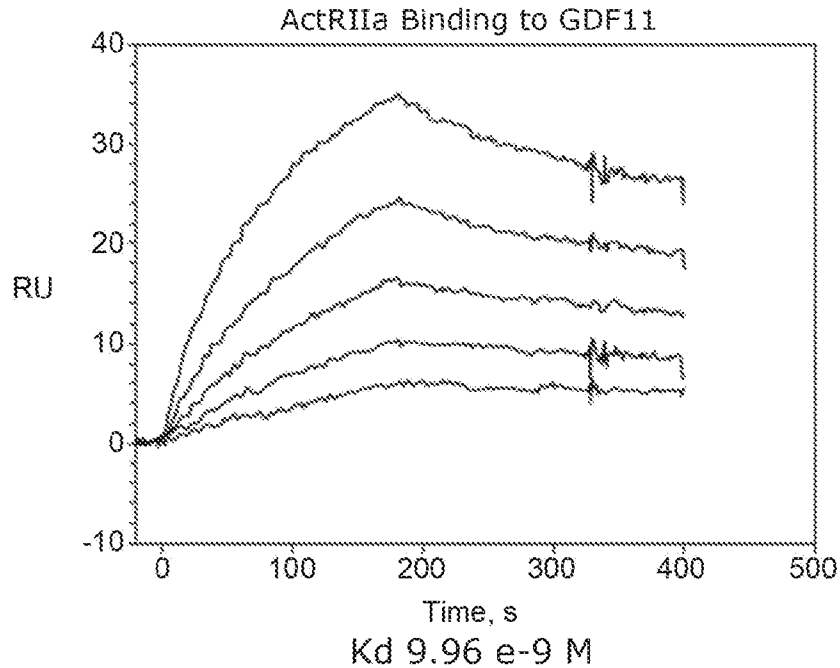

ActRIIa Binding to GDF11
40
30
20
10
0
-10
RU
0
100
200
300
400
500
Time, s
Kd 9.96 e-9 M

FIG. 4

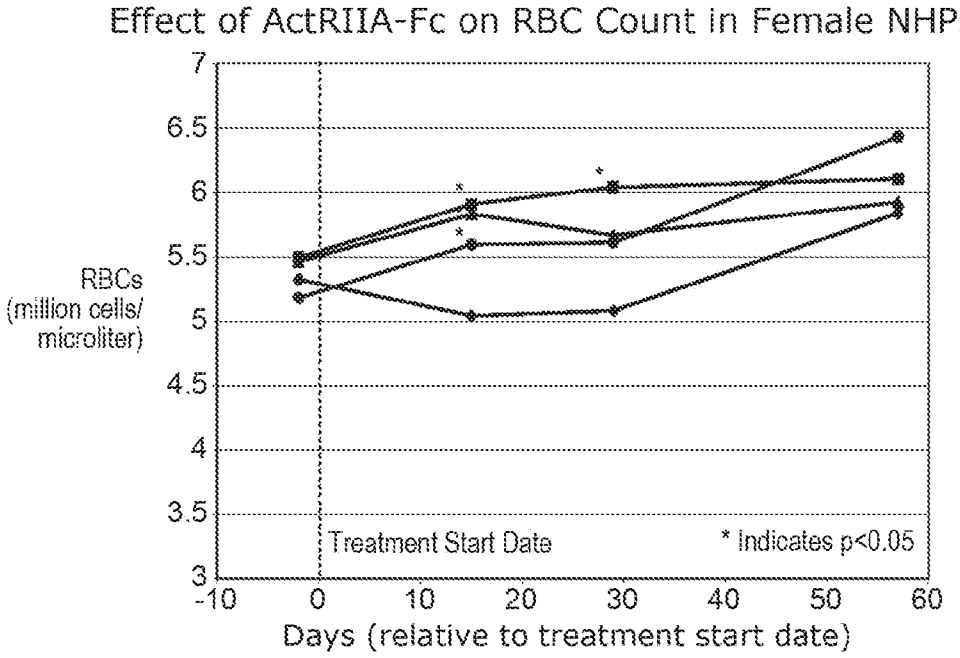
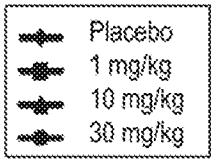

Effect of ActRIIA-Fc on RBC Count in Female NHPs
RBCs (million cells/ microliter)
7
6.5
6
5.5
5
4.5
4
3.5
3
Treatment Start Date
* Indicates p<0.05
-10
0
10
20
30
40
50
60
Days (relative to treatment start date)

FIG. 5A


Placebo
1 mg/kg
10 mg/kg
30 mg/kg

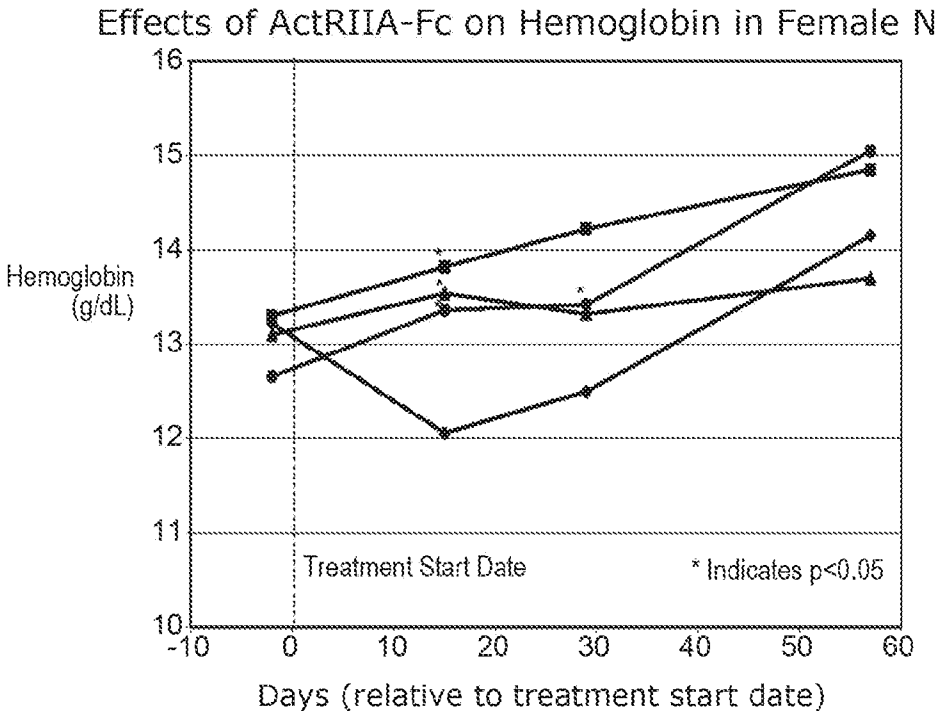

Effects of ActRIIA-Fc on Hemoglobin in Female NHPs
Hemoglobin (g/dL)
16
15
14
13
12
11
10
Treatment Start Date
* Indicates p<0.05
-10
0
10
20
30
40
50
60
Days (relative to treatment start date)

FIG. 5B

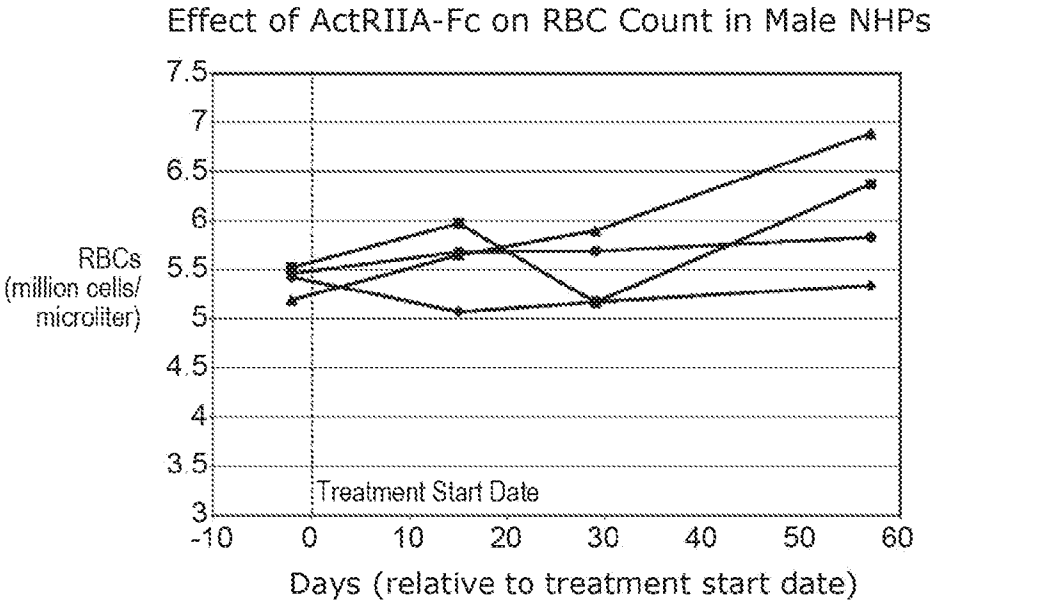
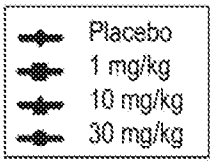

Effect of ActRIIA-Fc on RBC Count in Male NHPs
RBCs (million cells/ microliter)
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
Treatment Start Date
-10
0
10
20
30
40
50
60
Days (relative to treatment start date)

FIG. 6A


Placebo
1 mg/kg
10 mg/kg
30 mg/kg

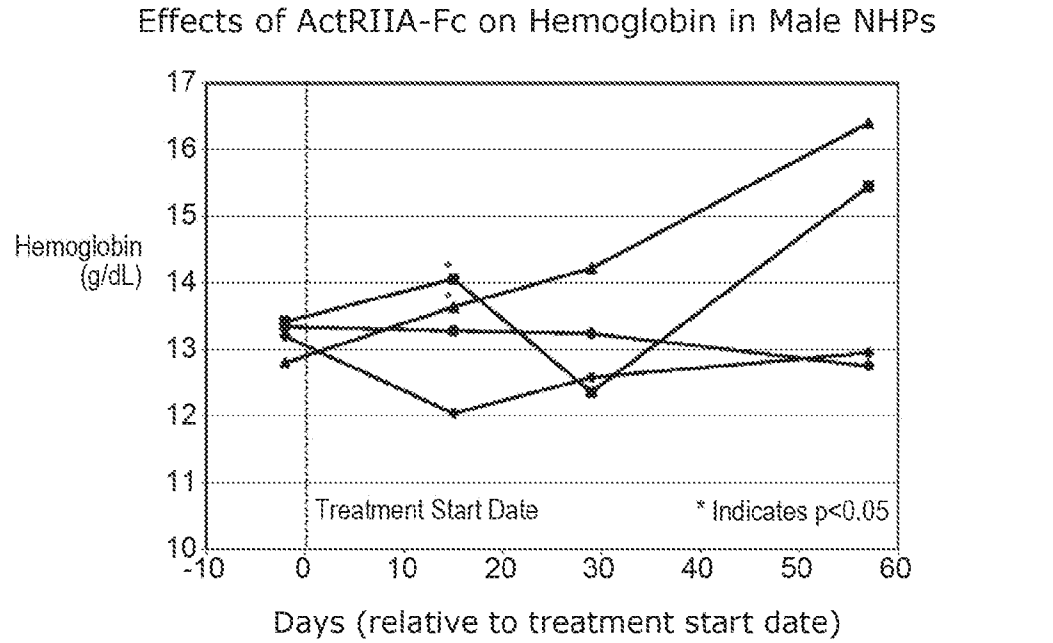

Effects of ActRIIA-Fc on Hemoglobin in Male NHPs
Hemoglobin (g/dL)
17
16
15
14
13
12
11
10
Treatment Start Date
* Indicates p<0.05
-10
0
10
20
30
40
50
60
Days (relative to treatment start date)

FIG. 6B

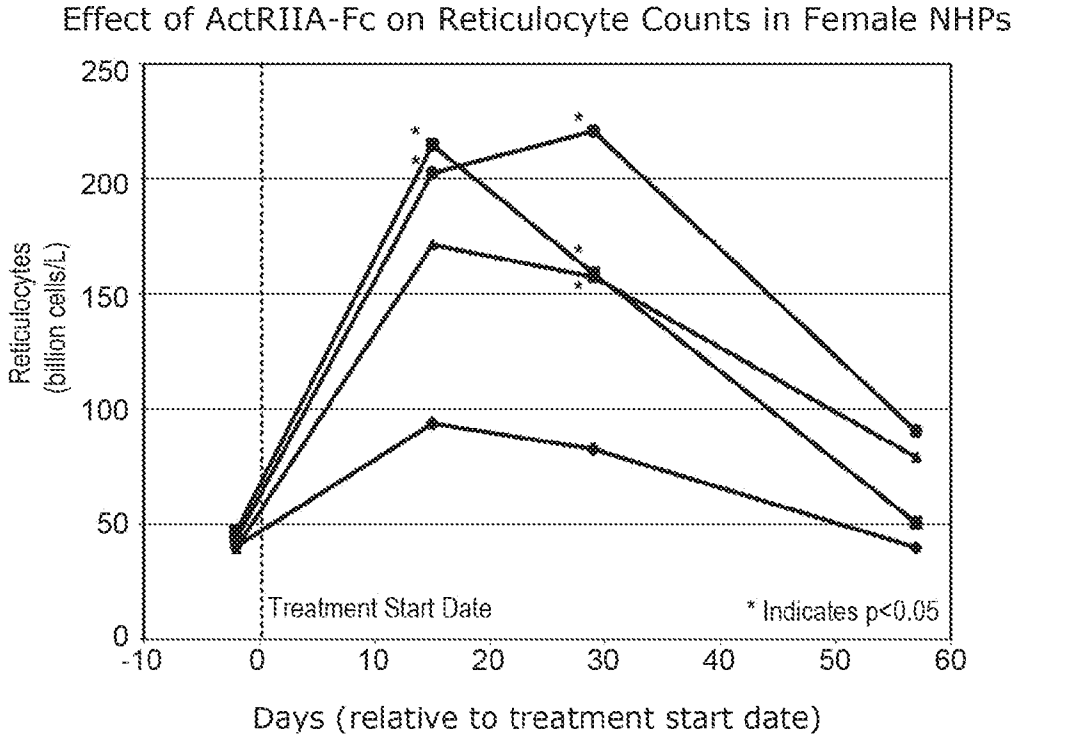

Effect of ActRIIA-Fc on Reticulocyte Counts in Female NHPs
Reticulocytes (billion cells/L)
250
200
150
100
50
0
-10
0
10
20
30
40
50
60
Treatment Start Date
* Indicates p<0.05
Days (relative to treatment start date)
Placebo
1 mg/kg
10 mg/kg
30 mg/kg

FIG. 7A

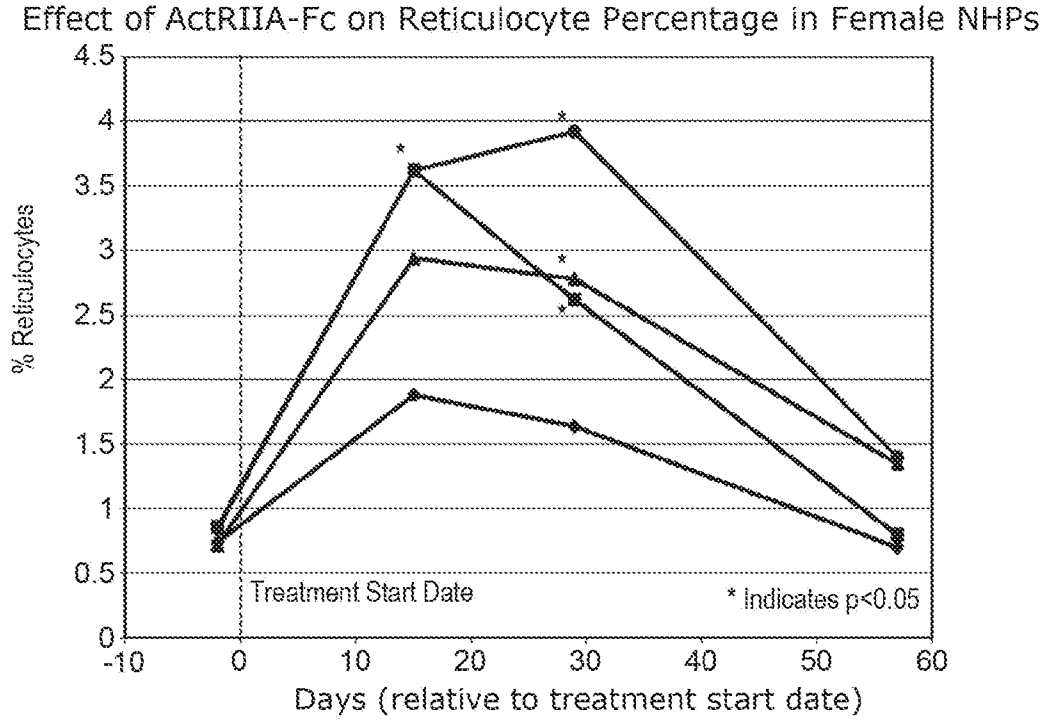

Effect of ActRIIA-Fc on Reticulocyte Percentage in Female NHPs
% Reticulocytes
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-10
0
10
20
30
40
50
60
Treatment Start Date
* Indicates p<0.05
Days (relative to treatment start date)

FIG. 7B

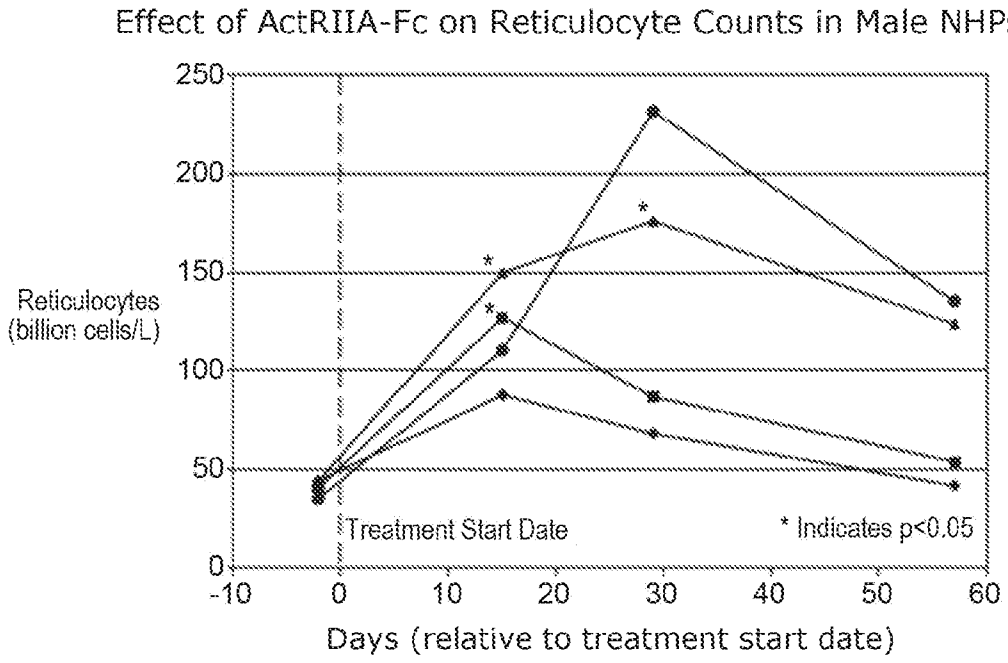
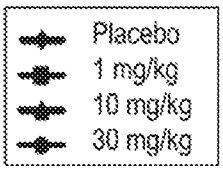

Effect of ActRIIA-Fc on Reticulocyte Counts in Male NHPs
Reticulocytes (billion cells/L)
250
200
150
100
50
0
-10
0
10
20
30
40
50
60
Treatment Start Date
* Indicates p<0.05
Days (relative to treatment start date)

FIG. 8A


Placebo
1 mg/kg
10 mg/kg
30 mg/kg

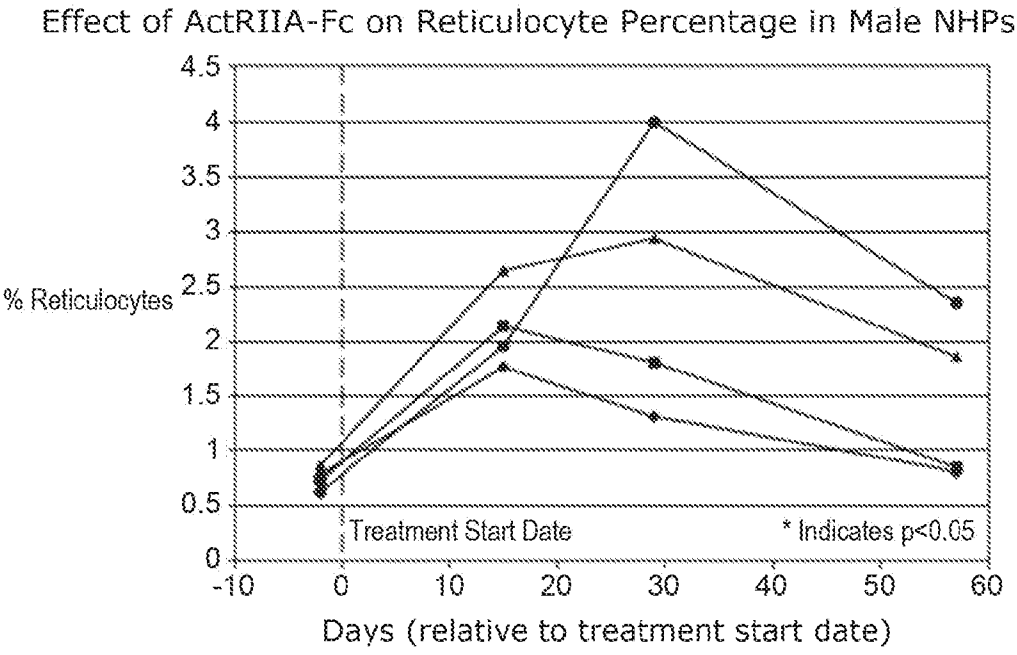

Effect of ActRIIA-Fc on Reticulocyte Percentage in Male NHPs
% Reticulocytes
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
-10
0
10
20
30
40
50
60
Treatment Start Date
* Indicates p<0.05
Days (relative to treatment start date)

FIG. 8B

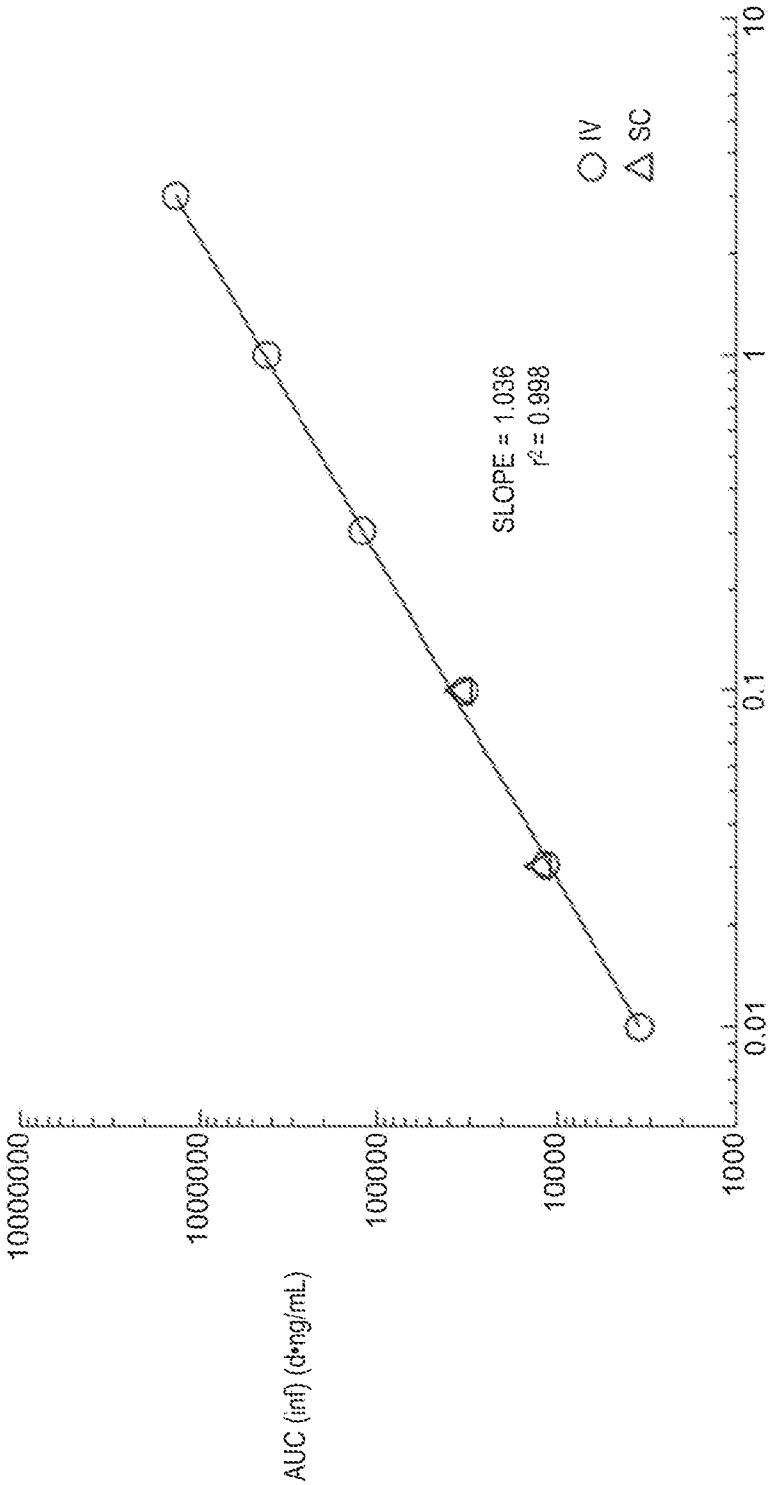

AUC (inf) (d*ng/mL)
10000000
1000000
100000
10000
1000
0.01
0.1
1
10
SLOPE = 1.036
r² = 0.998
IV
SC

FIG. 9

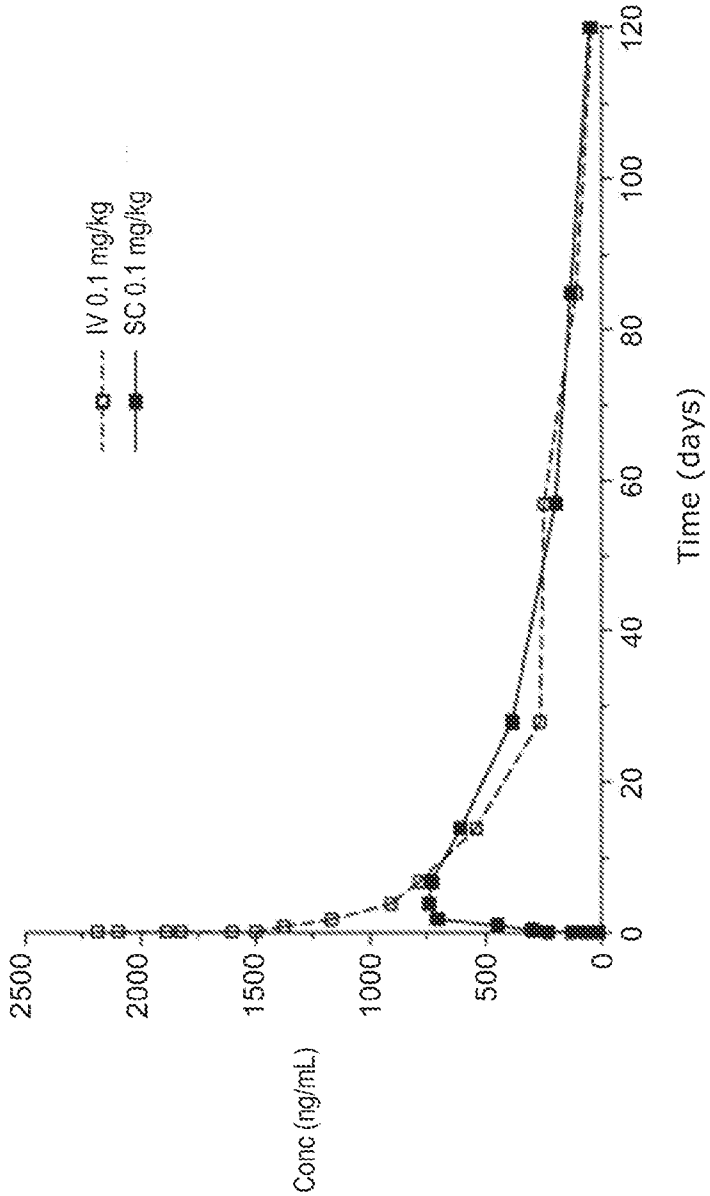

IV 0.1 mg/kg
SC 0.1 mg/kg
Conc (ng/mL)
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
Time (days)

FIG. 10

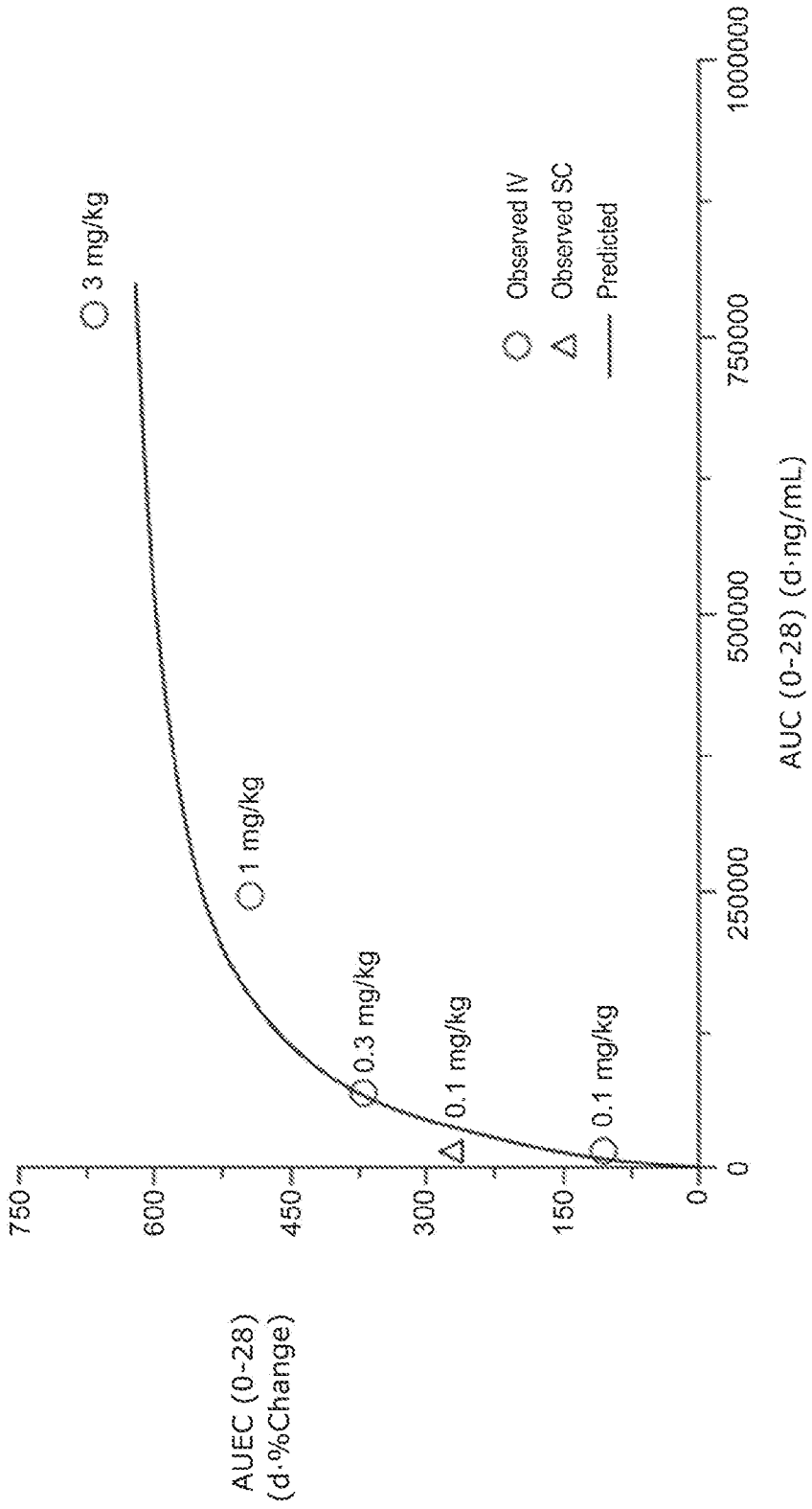

AUEC (0-28)
(d·%Change)
750
600
450
300
150
0
AUC (0-28) (d·ng/mL)
0
250000
500000
750000
1000000
3 mg/kg
1 mg/kg
0.3 mg/kg
0.1 mg/kg
0.1 mg/kg
Observed IV
Observed SC
Predicted

FIG. 11

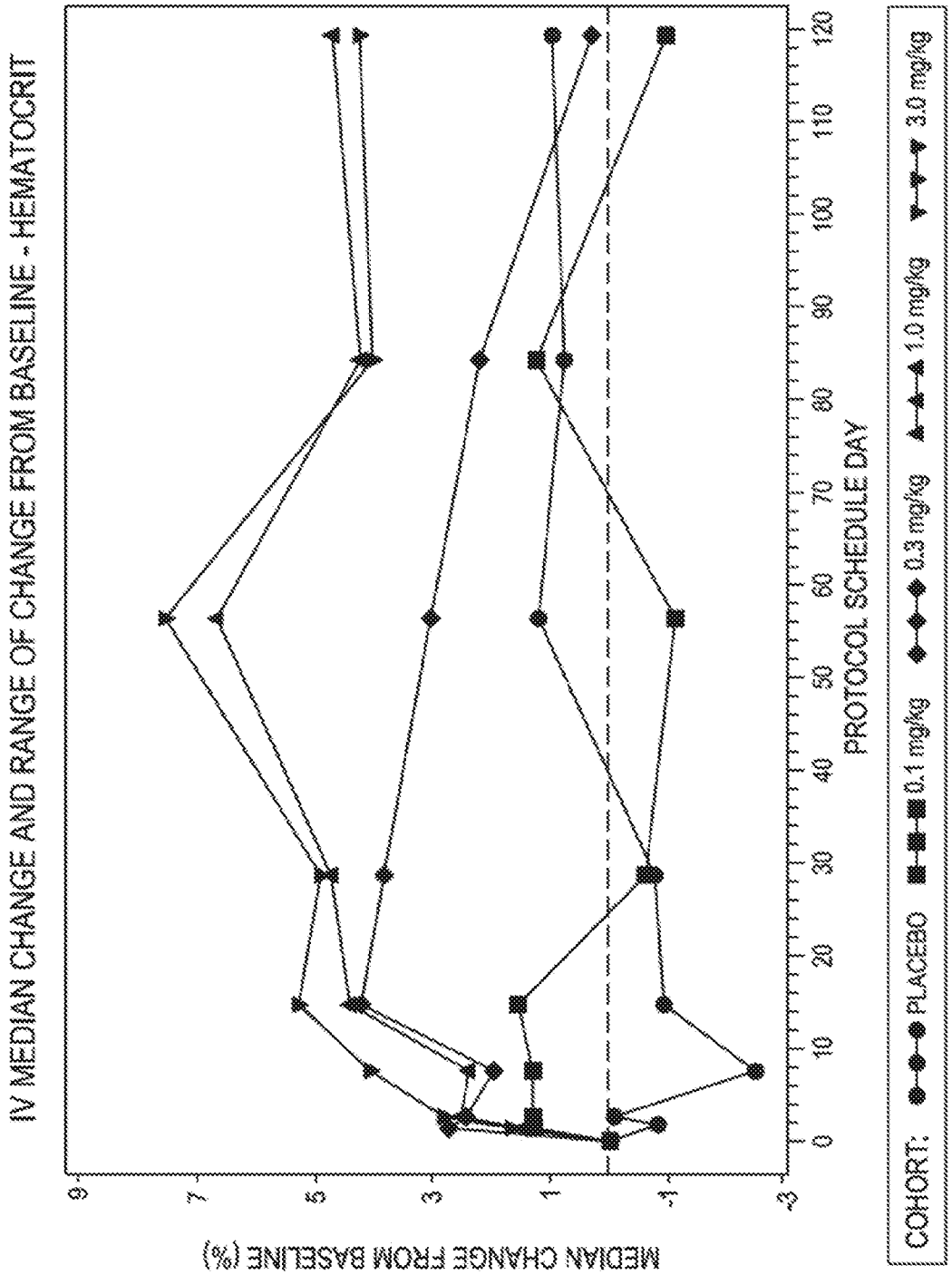

IV MEDIAN CHANGE AND RANGE OF CHANGE FROM BASELINE - HEMATOCRIT
MEDIAN CHANGE FROM BASELINE (%)
9
7
5
3
1
-1
-3
0
10
20
30
40
50
60
70
80
90
100
110
120
PROTOCOL SCHEDULE DAY
COHORT:
PLACEBO
0.1 mg/kg
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg

FIG. 12

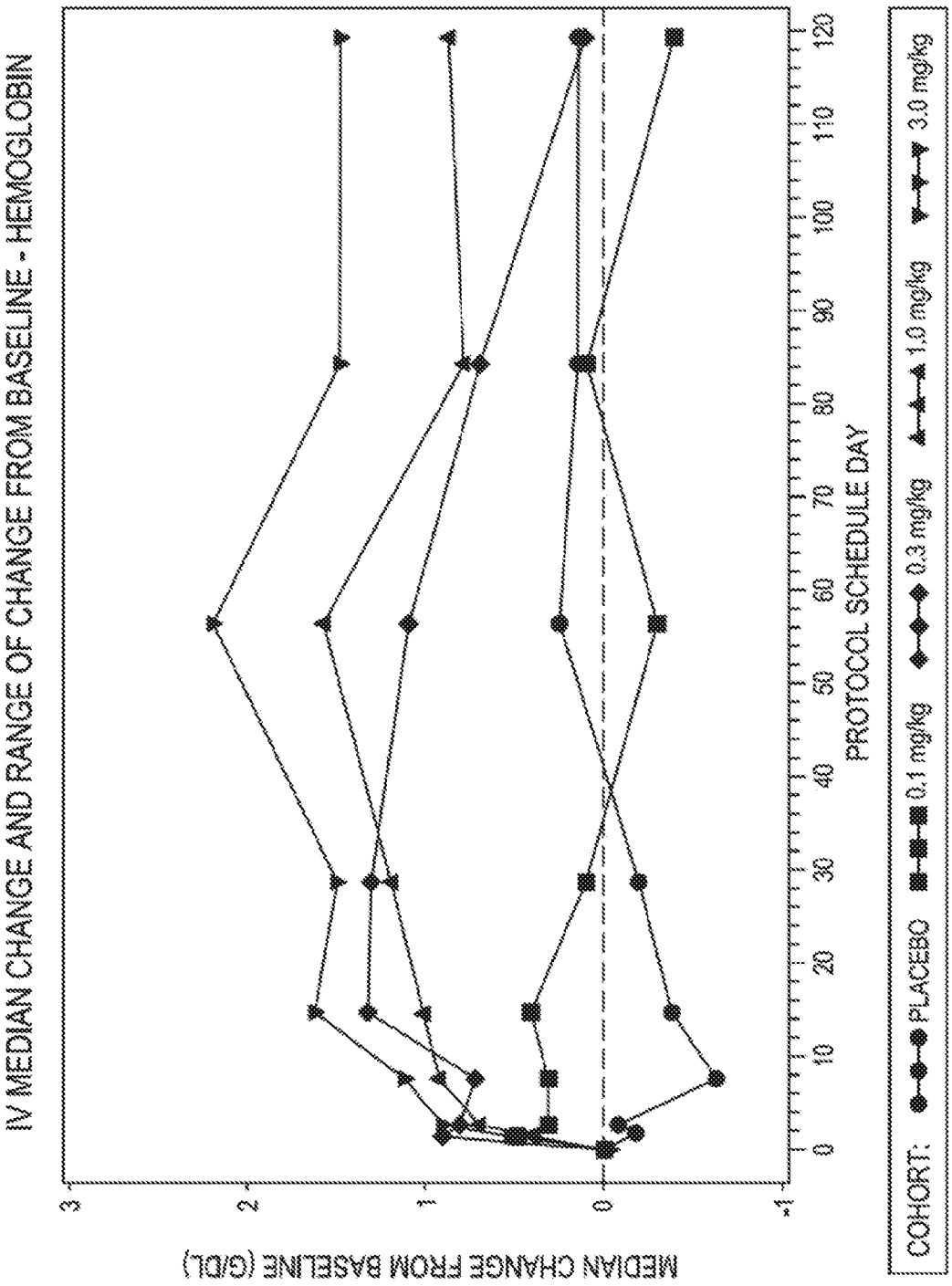

IV MEDIAN CHANGE AND RANGE OF CHANGE FROM BASELINE - HEMOGLOBIN
MEDIAN CHANGE FROM BASELINE (G/DL)
3
2
1
0
-1
0
10
20
30
40
50
60
70
80
90
100
110
120
PROTOCOL SCHEDULE DAY
COHORT:
PLACEBO
0.1 mg/kg
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg

FIG. 13

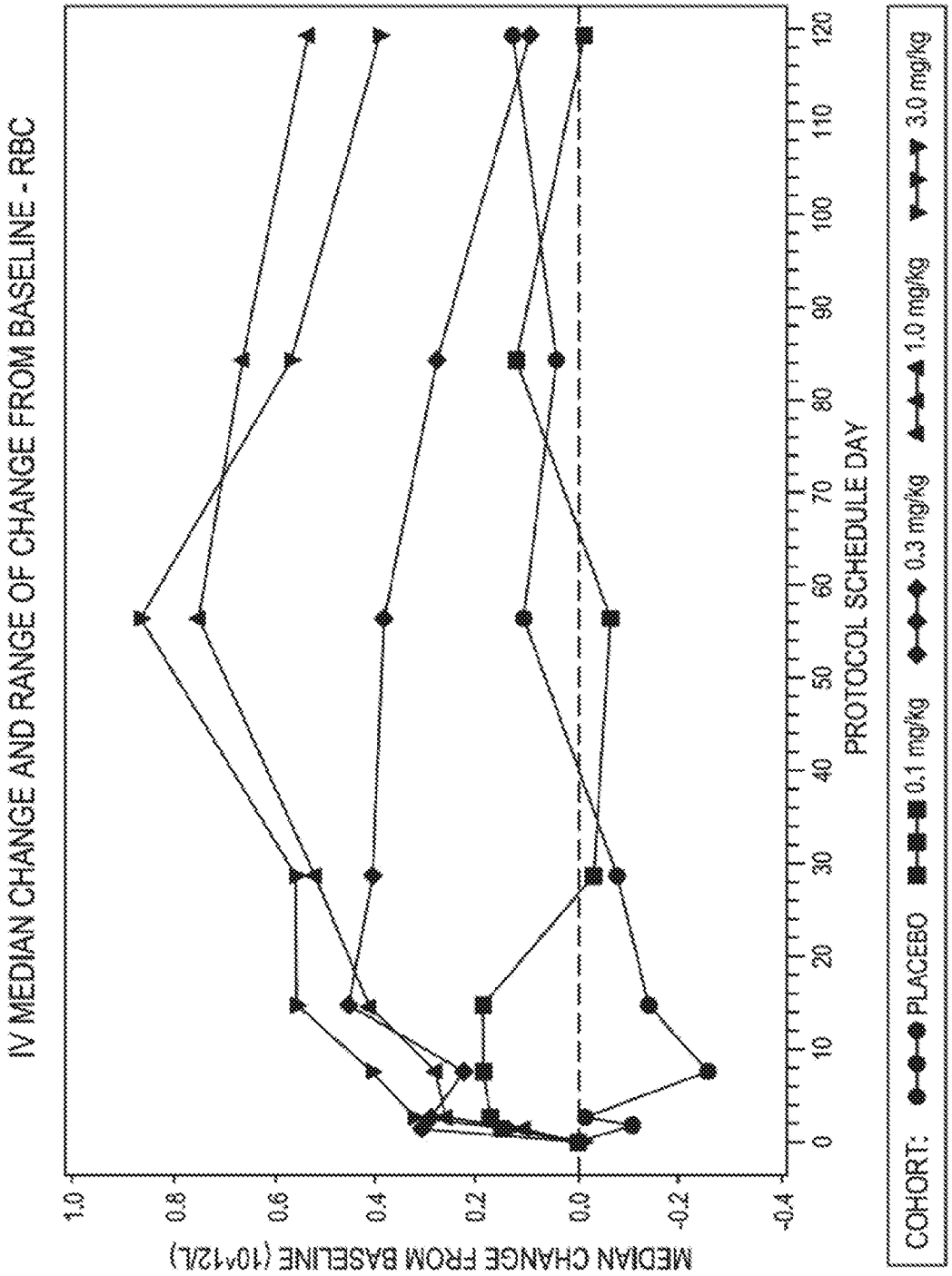

IV MEDIAN CHANGE AND RANGE OF CHANGE FROM BASELINE - RBC
MEDIAN CHANGE FROM BASELINE (10^12/L)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
0
10
20
30
40
50
60
70
80
90
100
110
120
PROTOCOL SCHEDULE DAY
COHORT: PLACEBO 0.1 mg/kg 0.3 mg/kg 1.0 mg/kg 3.0 mg/kg

FIG. 14

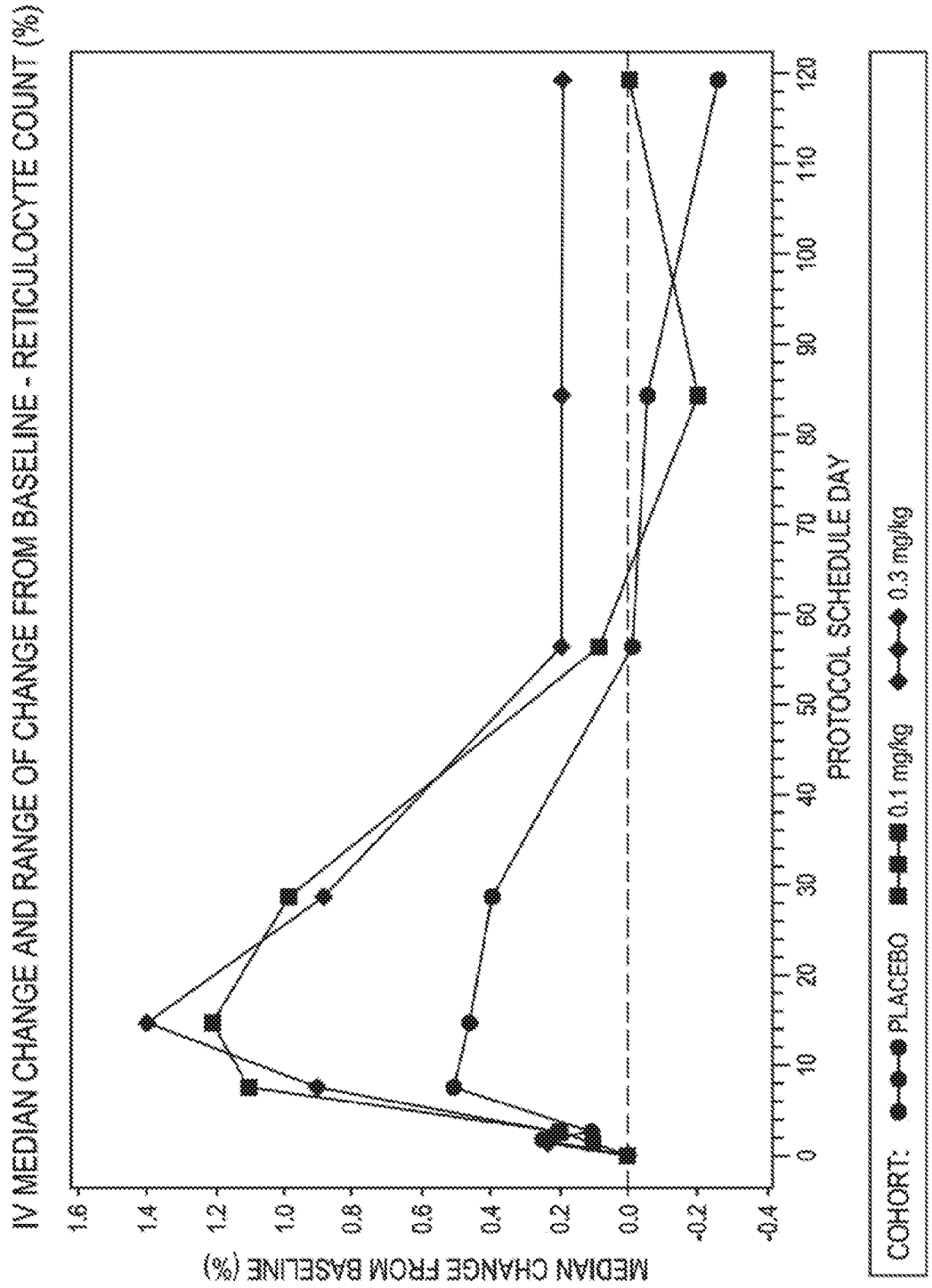

IV MEDIAN CHANGE AND RANGE OF CHANGE FROM BASELINE - RETICULOCYTE COUNT (%)
MEDIAN CHANGE FROM BASELINE (%)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
0
10
20
30
40
50
60
70
80
90
100
110
120
PROTOCOL SCHEDULE DAY
COHORT: PLACEBO 0.1 mg/kg 0.3 mg/kg

FIG. 15

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWDDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251  APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
301  EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK (SEQ ID NO:30)
```

FIG. 16

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
      TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

  51  AGTCTTCGTT TCGCCCGGCG CCTCTGGGCG TGGGGAGGCT GAGACACGGG
      TCAGAAGCAA AGCGGGCCGC GGAGACCCGC ACCCCTCCGA CTCTGTGCCC

 101  AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
      TCACGTAGAT GATGTTGCGG TTGACCCTCG ACCTCGCGTG GTTGGTCTCG

 151  GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
      CCGGACCTCG CGACGCTTCC GCTCGTCCTG TTCGCCGACG TGACGATGCG

 201  CTCCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
      GAGGACCGCG TTGTCGAGAC CGTGGTAGCT CGAGCACTTC TTCCCGACGA

 251  GGGATGATGA CTTCAACTGC TACGATAGGC AGGAGTGTGT GGCCACTGAG
      CCCTACTACT GAAGTTGACG ATGCTATCCG TCCTCACACA CCGGTGACTC

 301  GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
      CTCTTGGGGG TCCACATGAA GACGACGACA CTTCCGTTGA AGACGTTGCT

 351  GCGCTTCACT CATTTGCCAG AGGCTGGGGG CCCGGAAGTC ACGTACGAGC
      CGCGAAGTGA GTAAACGGTC TCCGACCCCC GGGCCTTCAG TGCATGCTCG

 401  CACCCCCGAC AGCCCCCACC GGTGGTGGAA CTCACACATG CCCACCGTGC
      GTGGGGGCTG TCGGGGGTGG CCACCACCTT GAGTGTGTAC GGGTGGCACG

 451  CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
      GGTCGTGGAC TTGAGGACCC CCCTGGCAGT CAGAAGGAGA AGGGGGGTTT

 501  ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
      TGGGTTCCTG TGGGAGTACT AGAGGGCCTG GGGACTCCAG TGTACGCACC

 551  TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
      ACCACCTGCA CTCGGTGCTT CTGGGACTCC AGTTCAAGTT GACCATGCAC

 601  GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
      CTGCCGCACC TCCACGTATT ACGGTTCTGT TTCGGCGCCC TCCTCGTCAT

 651  CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
      GTTGTCGTGC ATGGCACACC AGTCGCAGGA GTGGCAGGAC GTGGTCCTGA

 701  GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
      CCGACTTACC GTTCCTCATG TTCACGTTCC AGAGGTTGTT TCGGGAGGGT

 751  GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC
      CGGGGGGTAGC TCTTTTGGTA GAGGTTTCGG TTTCCCGTCG GGGCTCTTGG
```

FIG. 17A

```
 801   ACAGGTGTAC ACCCTGCCCC CATCCCGGGA GGAGATGACC AAGAACCAGG
       TGTCCACATG TGGGACGGGG GTAGGGCCCT CCTCTACTGG TTCTTGGTCC

 851   TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
       AGTCGGACTG GACGGACCAG TTTCCGAAGA TAGGGTCGCT GTAGCGGCAC

 901   GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
       CTCACCCTCT CGTTACCCGT CGGCCTCTTG TTGATGTTCT GGTGCGGAGG

 951   CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
       GCACGACCTG AGGCTGCCGA GGAAGAAGGA GATATCGTTC GAGTGGCACC

1001   ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
       TGTTCTCGTC CACCGTCGTC CCCTTGCAGA AGAGTACGAG GCACTACGTA

1051   GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCCCCGGG
       CTCCGAGACG TGTTGGTGAT GTGCGTCTTC TCGGAGAGGG ACAGGGGCCC

1101   TAAATGA (SEQ ID NO:39)
       ATTTACT (SEQ ID NO:40)
```

FIG. 17B

```
  1 MDAMKRGLCC VLLLCGAVFV SPGAAETREC IYYNANWELE RTNQSGLERC
 51 EGEQDKRLHC YASWRNSSGT IELVKKGCWD DDFNCYDRQE CVATEENPQV
101 YFCCCEGNFC NERFTHLPEA GGPEVTYEPP PTGGGTHTCP PCPAPELLGG
151 PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251 KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP
301 ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351 QKSLSLSPGK (SEQ ID NO: 41)
```

FIG. 18

```
   1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
     TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

                                     E  T  R  E  C  I  Y  Y
  51 AGTCTTCGTT TCGCCCGGCG CCGCTGAGAC ACGGGAGTGC ATCTACTACA
     TCAGAAGCAA AGCGGGCCGC GGCGACTCTG TGCCCTCACG TAGATGATGT

     N  A  N  W  E  L  E  R  T  N  Q  S  G  L  E  R  C
 101 ACGCCAACTG GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC
     TGCGGTTGAC CCTCGACCTC GCGTGGTTGG TCTCGCCGGA CCTCGCGACG

      E  G  E  Q  D  K  R  L  H  C  Y  A  S  W  R  N  S
 151 GAAGGCGAGC AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG
     CTTCCGCTCG TCCTGTTCGC CGACGTGACG ATGCGGAGGA CCGCGTTGTC

       S  G  T  I  E  L  V  K  K  G  C  W  D  D  D  F
 201 CTCTGGCACC ATCGAGCTCG TGAAGAAGGG CTGCTGGGAC GATGACTTCA
     GAGACCGTGG TAGCTCGAGC ACTTCTTCCC GACGACCCTG CTACTGAAGT

     N  C  Y  D  R  Q  E  C  V  A  T  E  E  N  P  Q  V
 251 ACTGCTACGA TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG
     TGACGATGCT ATCCGTCCTC ACACACCGGT GACTCCTCTT GGGGGTCCAC

      Y  F  C  C  C  E  G  N  F  C  N  E  R  F  T  H  L
 301 TACTTCTGCT GCTGTGAAGG CAACTTCTGC AACGAGCGCT TCACTCATTT
     ATGAAGACGA CGACACTTCC GTTGAAGACG TTGCTCGCGA AGTGAGTAAA

       P  E  A  G  G  P  E  V  T  Y  E  P  P  P  T
 351 GCCAGAGGCT GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGGTG
     CGGTCTCCGA CCCCCGGGCC TTCAGTGCAT GCTCGGTGGG GGCTGTCCAC

 401 GTGGAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA
     CACCTTGAGT GTGTACGGGT GGCACGGGTC GTGGACTTGA GGACCCCCCT

 451 CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
     GGCAGTCAGA AGGAGAAGGG GGGTTTTGGG TTCCTGTGGG AGTACTAGAG

 501 CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC
     GGCCTGGGGA CTCCAGTGTA CGCACCACCA CCTGCACTCG GTGCTTCTGG

 551 CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC
     GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG
```

FIG. 19A

```
 601  AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG
      TTCTGTTTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAGTC

 651  CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
      GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA

 701  GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
      CGTTCCAGAG GTTGTTTCGG GAGGGTCGGG GGTAGCTCTT TTGGTAGAGG

 751  AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC
      TTTCGGTTTC CCGTCGGGGC TCTTGGTGTC CACATGTGGG ACGGGGGTAG


 801  CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
      GGCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

 851  GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
      CGAAGATAGG GTCGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

 901  GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT
      CTCTTGTTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

 951  CTTCCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
      GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTCGTCCCCT

1001  ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
      TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051  CAGAAGAGCC TCTCCCTGTC CCCGGGTAAA TGA   (SEQ ID NO: 42)
      GTCTTCTCGG AGAGGGACAG GGGCCCATTT ACT   (SEQ ID NO: 43)
```

FIG. 19B

```
  1  ETPECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK
 51  KGCWQDDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV
101  TYEPPPTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV
151  VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD
201  WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ
251  VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV
301  DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 44)
```

FIG. 20

```
  1  ETRECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK
 51  KGCWLDDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV
101  TYEPPPT  (SEQ ID NO: 45)
```

FIG. 21

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
      TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

                                    E  T     R  E  C     I  Y  Y
  51  AGTCTTCGTT TCGCCCGGCG CCGCCGAAAC CCGCGAATGT ATTTATTACA
      TCAGAAGCAA AGCGGGCCGC GGCGGCTTTG GGCGCTTACA TAAATAATGT

      N  A  N  W     E  L  E     R  T  N     Q  S  G  L     E  R  C
 101  ATGCTAATTG GGAACTCGAA CGGACGAACC AATCCGGGCT CGAACGTGT
      TACGATTAAC CCTTGAGCTT GCCTGCTTGG TTAGGCCCGA GCTTGCCACA

        E  G  E     Q  D  K  R     L  H  C     Y  A  S     W  R  N  S
 151  GAGGGGGAAC AGGATAAACG CCTCCATTGC TATGCGTCGT GGAGGAACTC
      CTCCCCCTTG TCCTATTTGC GGAGGTAACG ATACGCAGCA CCTCCTTGAG

           S  G  T     I  E  L     V  K  K  G     C  W  D     D  D  F
 201  CTCCGGCACG ATTGAACTGG TCAAGAAAGG GTGCTGGGAC GACGATTTCA
      GAGGCCCTGC TAACTTGACC AGTTCTTTCC CACGACCCTG CTGCTAAAGT

      N  C  Y  D     R  Q  E     C  V  A     T  E  E  N     P  Q  V
 251  ATTGTTATGA CCGCCAGGAA TGTGTCGCGA CCGAAGAGAA TCCGCAGGTC
      TAACAATACT GGCGGTCCTT ACACAGCGCT GGCTTCTCTT AGGCGTCCAG

        Y  F  C     C  C  E  G     N  F  C     N  E  R     F  T  H  L
 301  TATTTCTGTT GTTGCGAGGG GAATTTCTGT AATGAACGGT TTACCCACCT
      ATAAAGACAA CAACGCTCCC CTTAAAGACA TTACTTGCCA AATGGGTGGA

           P  E  A     G  G  P     E  V  T  Y     E  P  P     P  T
 351  CCCCGAAGCC GCCGGGCCCG AGGTGACCTA TGAACCCCCG CCGACCGGTG
      GGGGCTTCGG CGGCCCGGGC TCCACTGGAT ACTTGGGGGC GGGTGGCCAC

 401  GTGGAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA
      CACCTTGAGT GTGTACGGGT GGCACGGGTC GTGGACTTGA GGACCCCCCT

 451  CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
      GGCAGTCAGA AGGAGAAGGG GGGTTTTGGG TTCCTGTGGG AGTACTAGAG

 501  CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC
      GGCCTGGGGA CTCCAGTGTA CGCACCACCA CCTGCACTCG GTGCTTCTGG

 551  CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC
      GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG
```

FIG. 22A

```
601 AACACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG
    TTCTGTTTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAGTC

651 CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
    GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA

701 GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
    CGTTCCAGAG GTTGTTTCGG GAGGGTCGGG GGTAGCTCTT TTGGTAGAGG

751 AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC
    TTTCGGTTTC CCGTCGGGGC TCTTGGTGTC CACATGTGGG ACGGGGGTAG

801 CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
    GGCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

851 GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
    CGAAGATAGG GTCGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

901 GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT
    CTCTTGTTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

951 CTTCCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
    GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTCGTCCCCT

1001 ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
     TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051 CACAAGAGCC TCTCCCTGTC CCCGGGTAAA TGA (SEQ ID NO: 46)
     GTCTTCTCGG AGAGGGACAG GGGCCCATTT ACT (SEQ ID NO: 47)
```

FIG. 22B

GAAAC CCGCGAATGT ATTTATTACA ATGCTAATTG GGAACTCGAA
CGGACGAACC AATCCGGGCT CGAACGGTGT GAGGGGGAAC AGGATAAACG
CCTCCATTGC TATGCGTCGT GGAGGAACTC CTCCGGGACG ATTGAACTGG
TCAAGAAAGG GTGCTGGGAC GACGATTTCA ATTGTTATGA CCGCCAGGAA
TGTGTCGCGA CCGAAGAGAA TCCGCAGGTC TATTTCTGTT GTTGCGAGGG
GAATTTCTGT AATGAACGGT TTACCCACCT CCCCGAAGCC GGCGGGCCCG
AGGTGACCTA TGAACCCCCG CCCACC (SEQ ID NO: 48)

FIG. 23

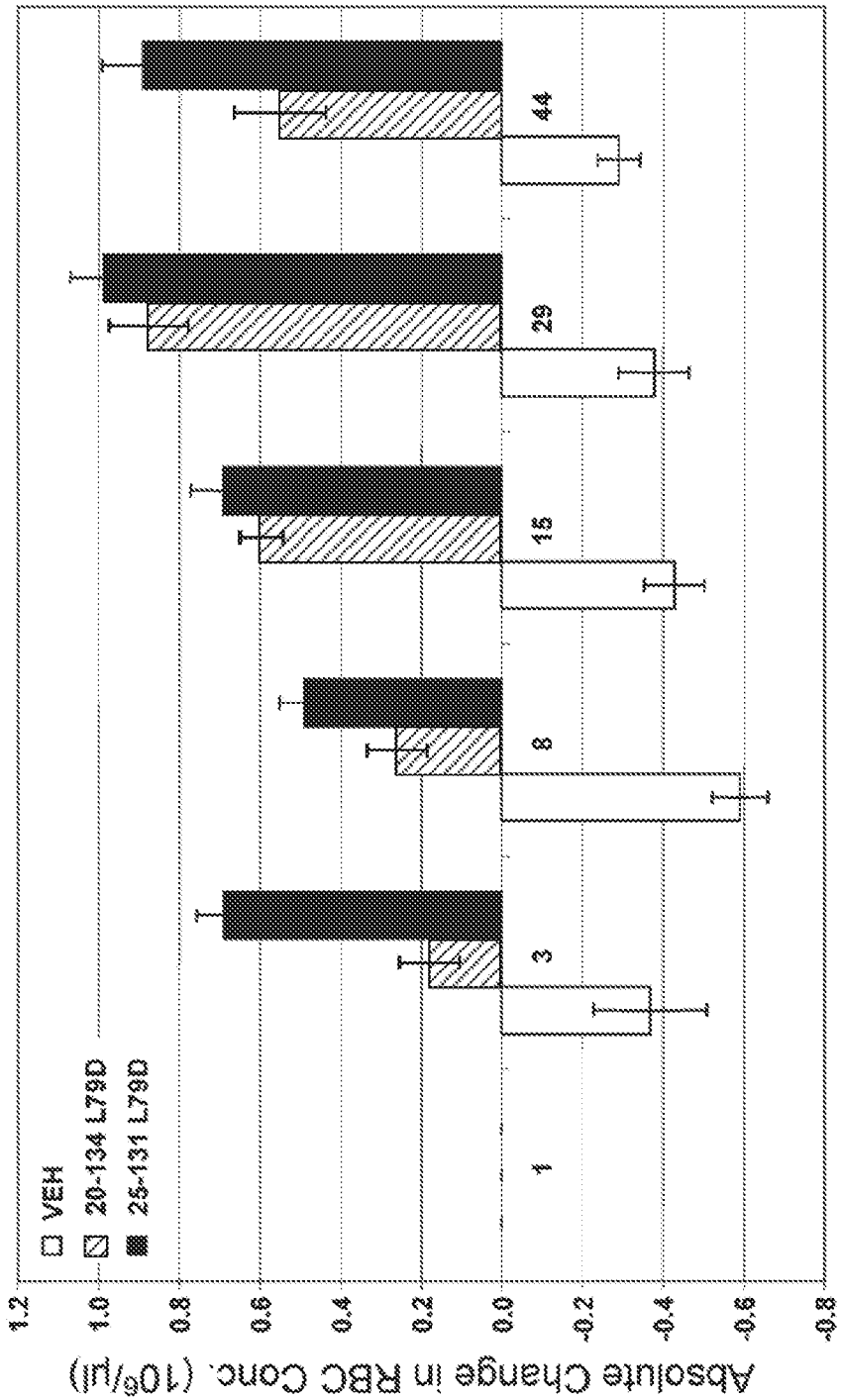

Absolute Change in RBC Conc. (10⁶/µl)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
VEH
20-134 L79D
25-131 L79D
1
3
8
15
29
44
Study Day

FIG. 24

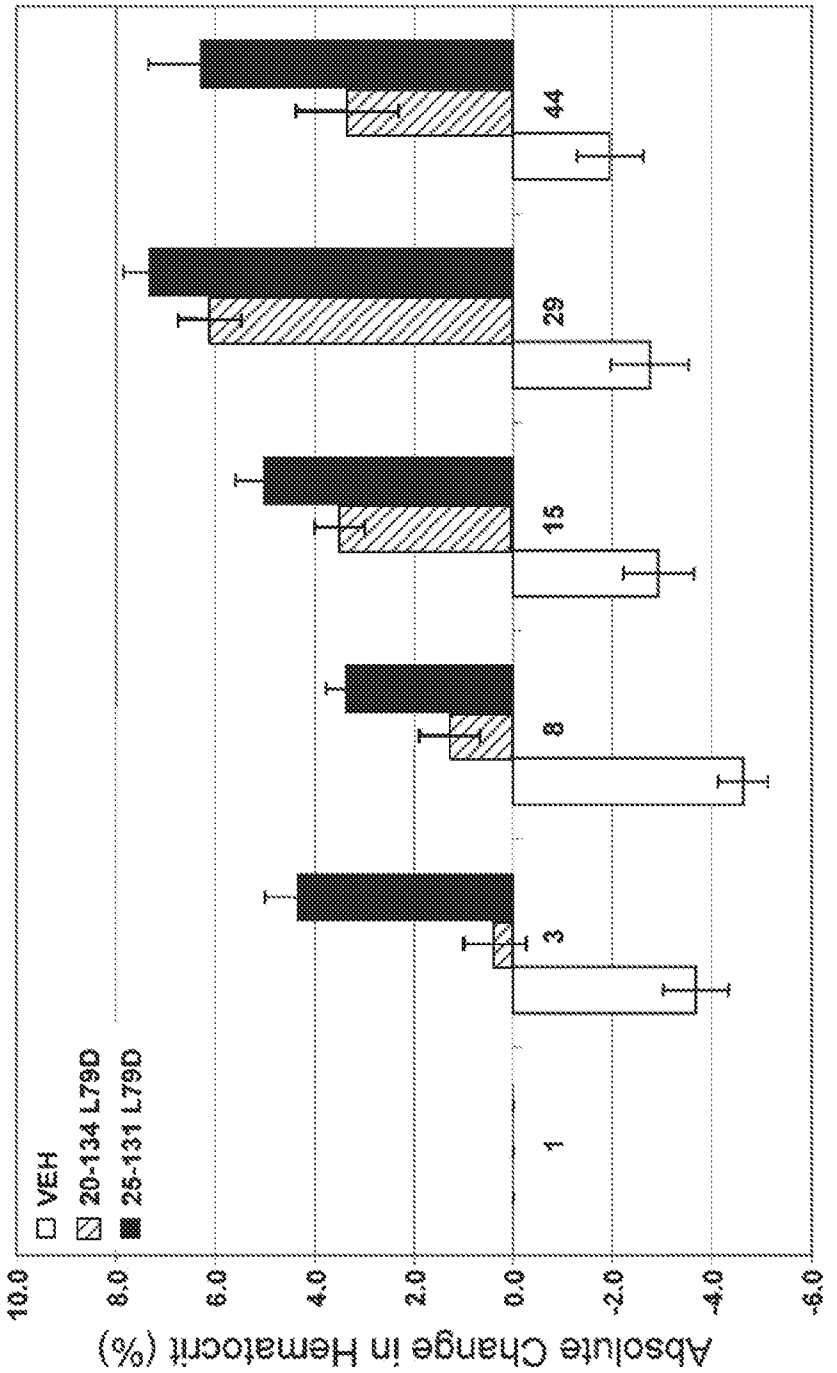

Absolute Change in Hematocrit (%)
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
VEH
20-134 L79D
25-131 L79D
1
3
8
15
29
44
Study Day

FIG. 25

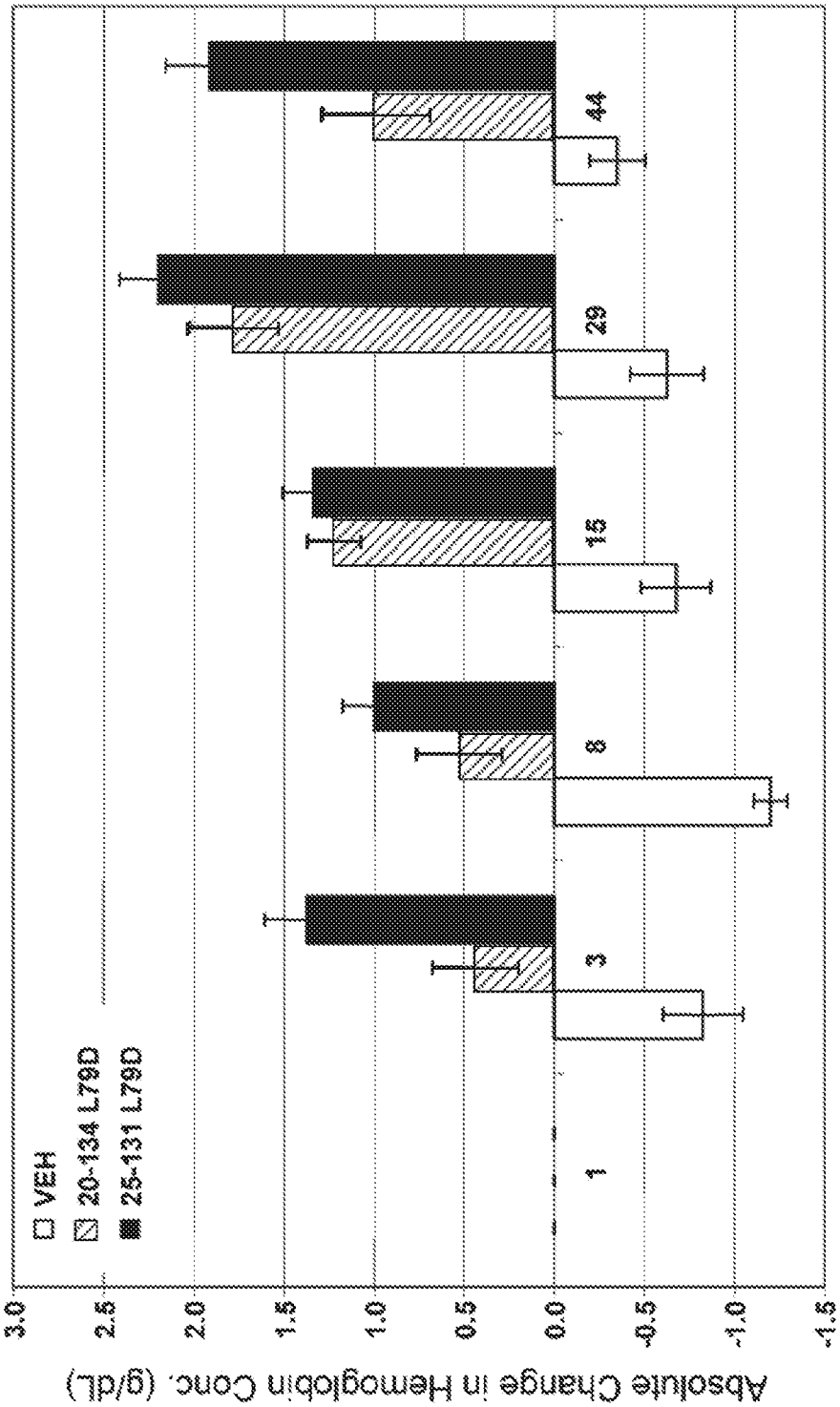

Absolute Change in Hemoglobin Conc. (g/dL)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
VEH
20-134 L79D
25-131 L79D
1
3
8
15
29
44
Study Day

FIG. 26

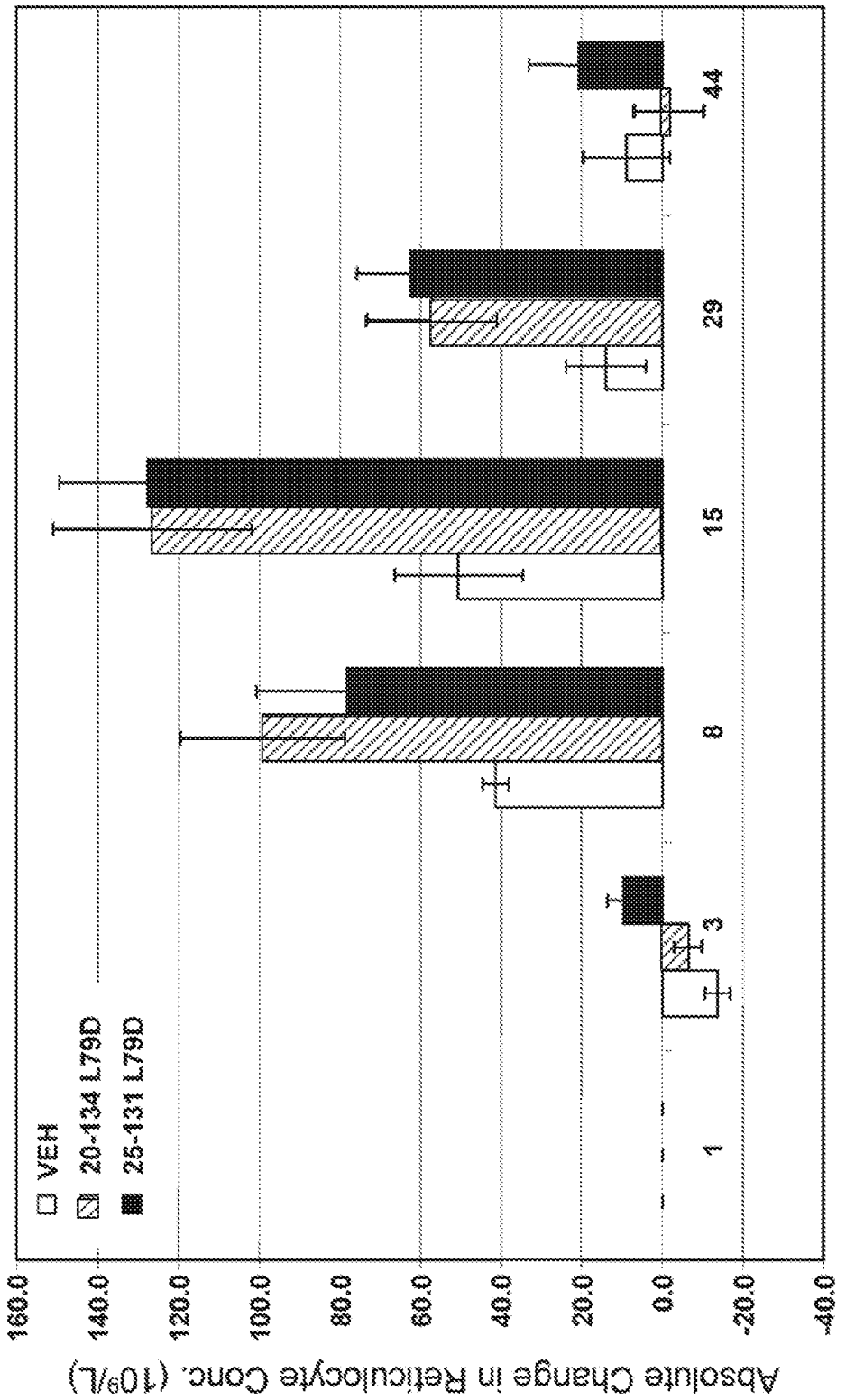

Absolute Change in Reticulocyte Conc. (10^9/L)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-20.0
-40.0
VEH
20-134 L79D
25-131 L79D
1
3
8
15
29
44
Study Day

FIG. 27

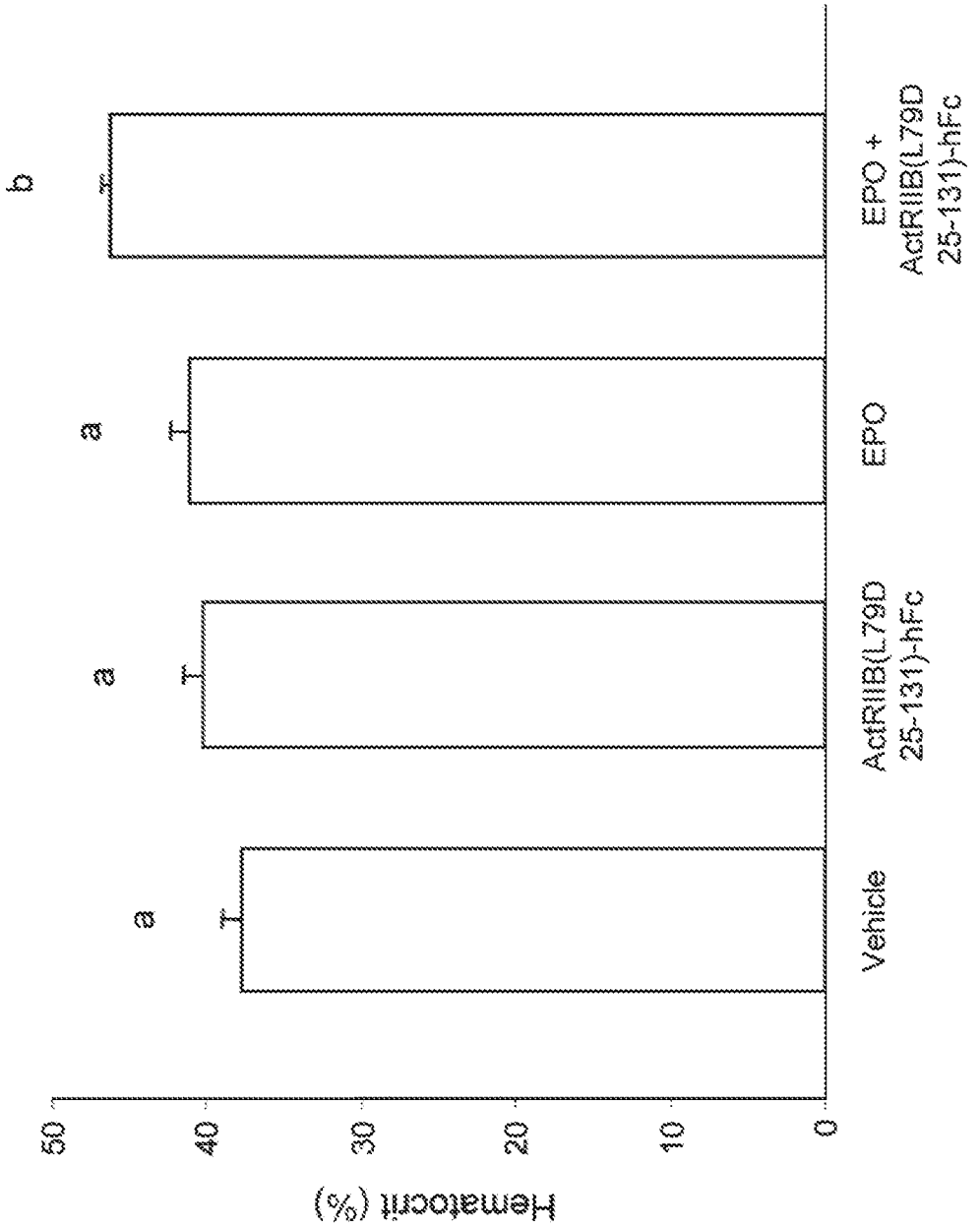

Hematocrit (%)
0
10
20
30
40
50
Vehicle
ActRIIB(L79D 25-131)-hFc
EPO
EPO + ActRIIB(L79D 25-131)-hFc
a
a
a
b

FIG. 28

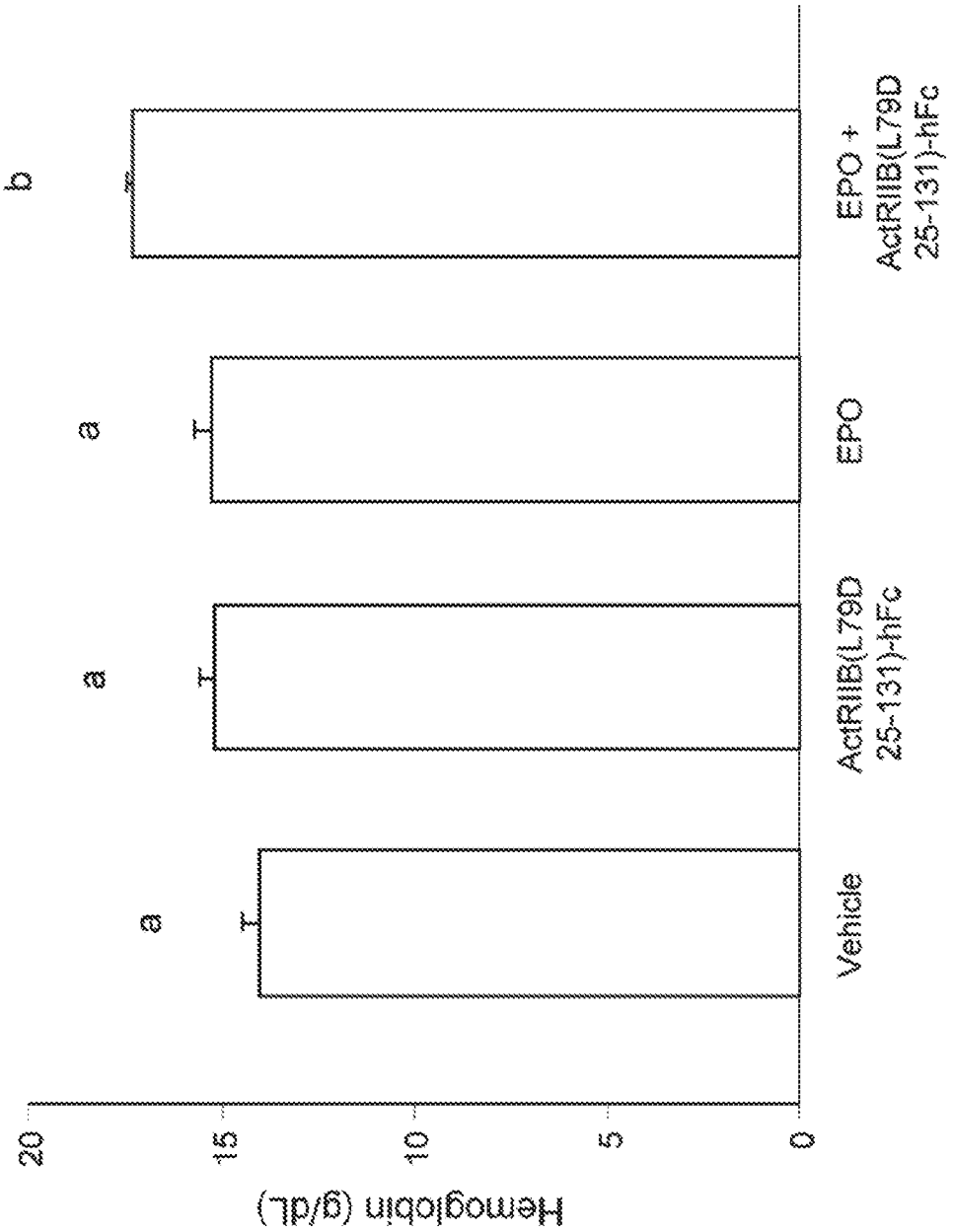

Hemoglobin (g/dL)
0
5
10
15
20
a
a
a
b
Vehicle
ActRIIB(L79D 25-131)-hFc
EPO
EPO + ActRIIB(L79D 25-131)-hFc

FIG. 29

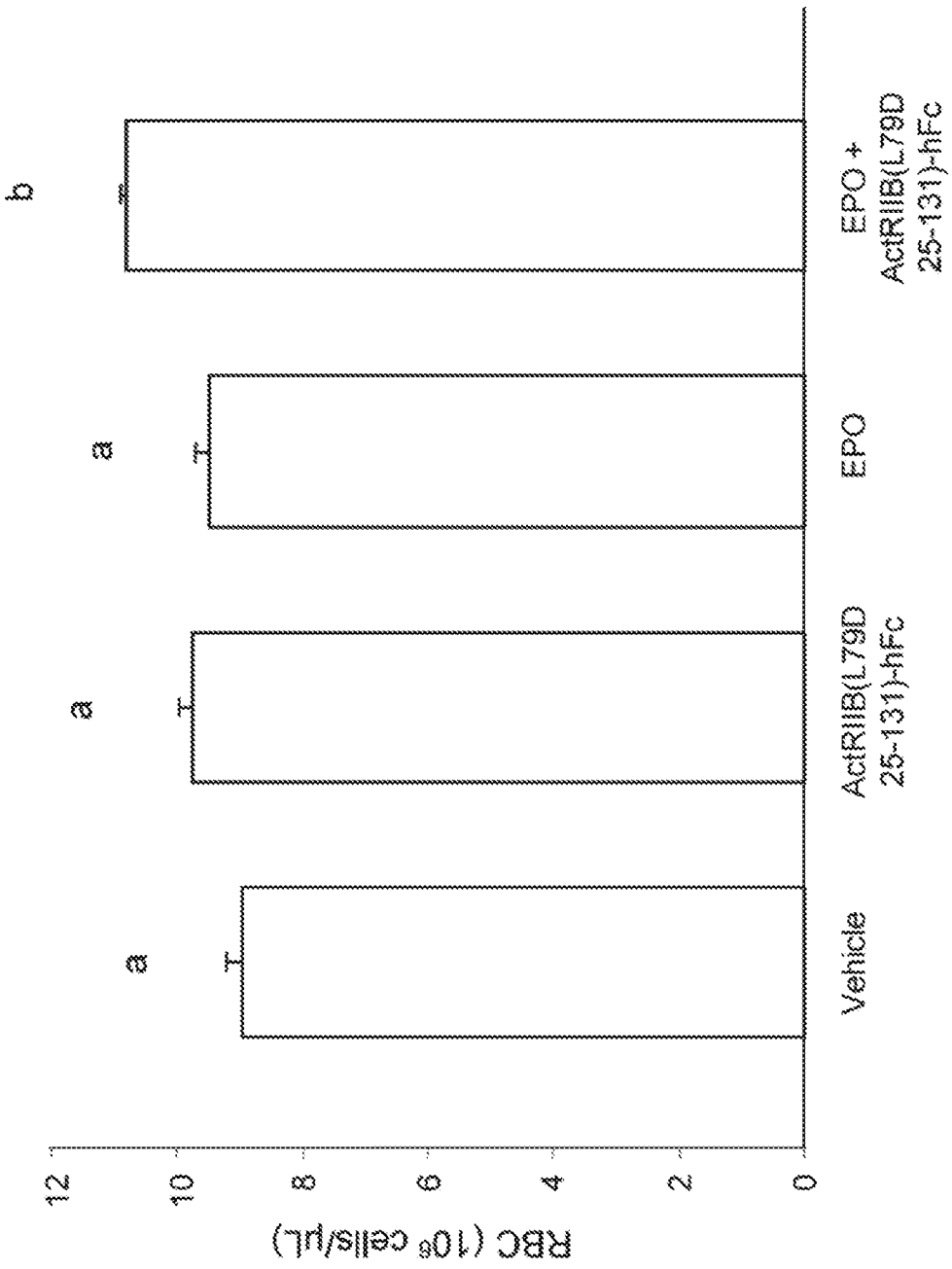

RBC (10^6 cells/μL)
0
2
4
6
8
10
12
a
a
a
b
Vehicle
ActRIIB(L79D 25-131)-hFc
EPO
EPO + ActRIIB(L79D 25-131)-hFc

FIG. 30

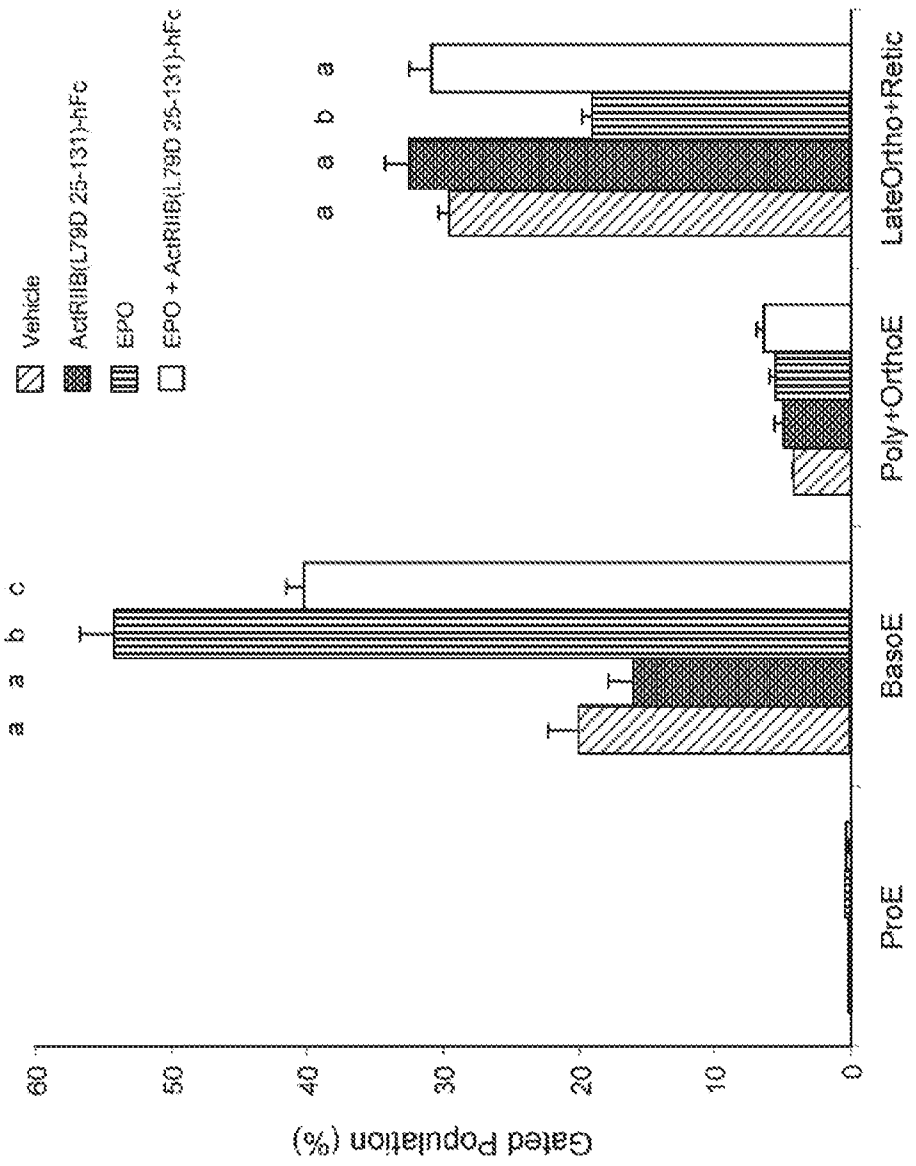

Vehicle
ActRIIB(L79D 25-131)-hFc
EPO
EPO + ActRIIB(L79D 25-131)-hFc
a a b c
a a b a
a a b a
Gated Population (%)
0
10
20
30
40
50
60
ProE
BasoE
Poly+OrthoE
LateOrtho+Retic
Erythropoietic Stage

FIG. 31

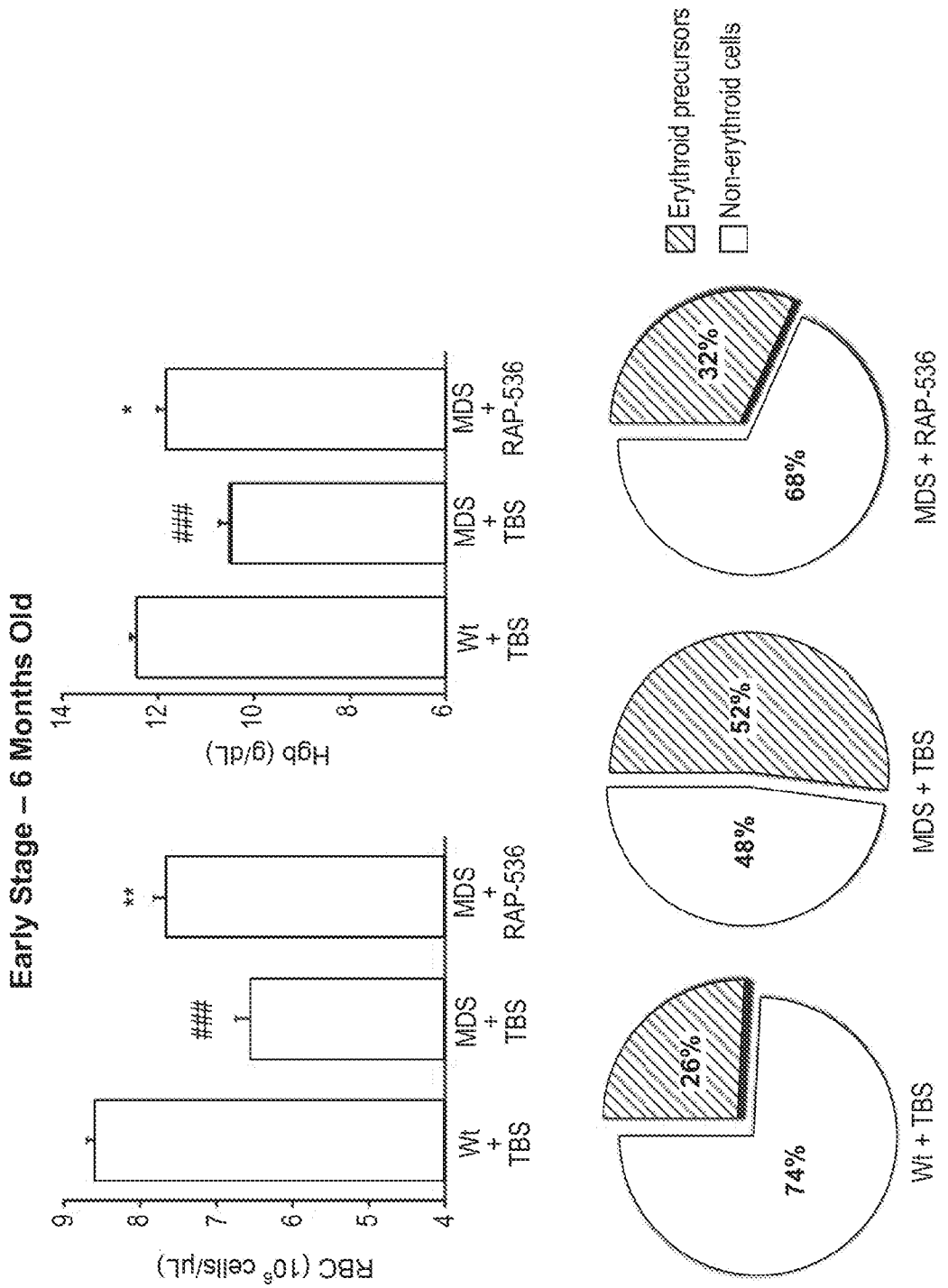

Early Stage – 6 Months Old
RBC (10^6 cells/μL)
9
8
7
6
5
4
Wt + TBS
MDS + TBS
MDS + RAP-536
###
**
Hgb (g/dL)
14
12
10
8
6
Wt + TBS
MDS + TBS
MDS + RAP-536
###
*
Erythroid precursors
Non-erythroid cells
26%
74%
Wt + TBS
52%
48%
MDS + TBS
32%
68%
MDS + RAP-536

FIG. 32A

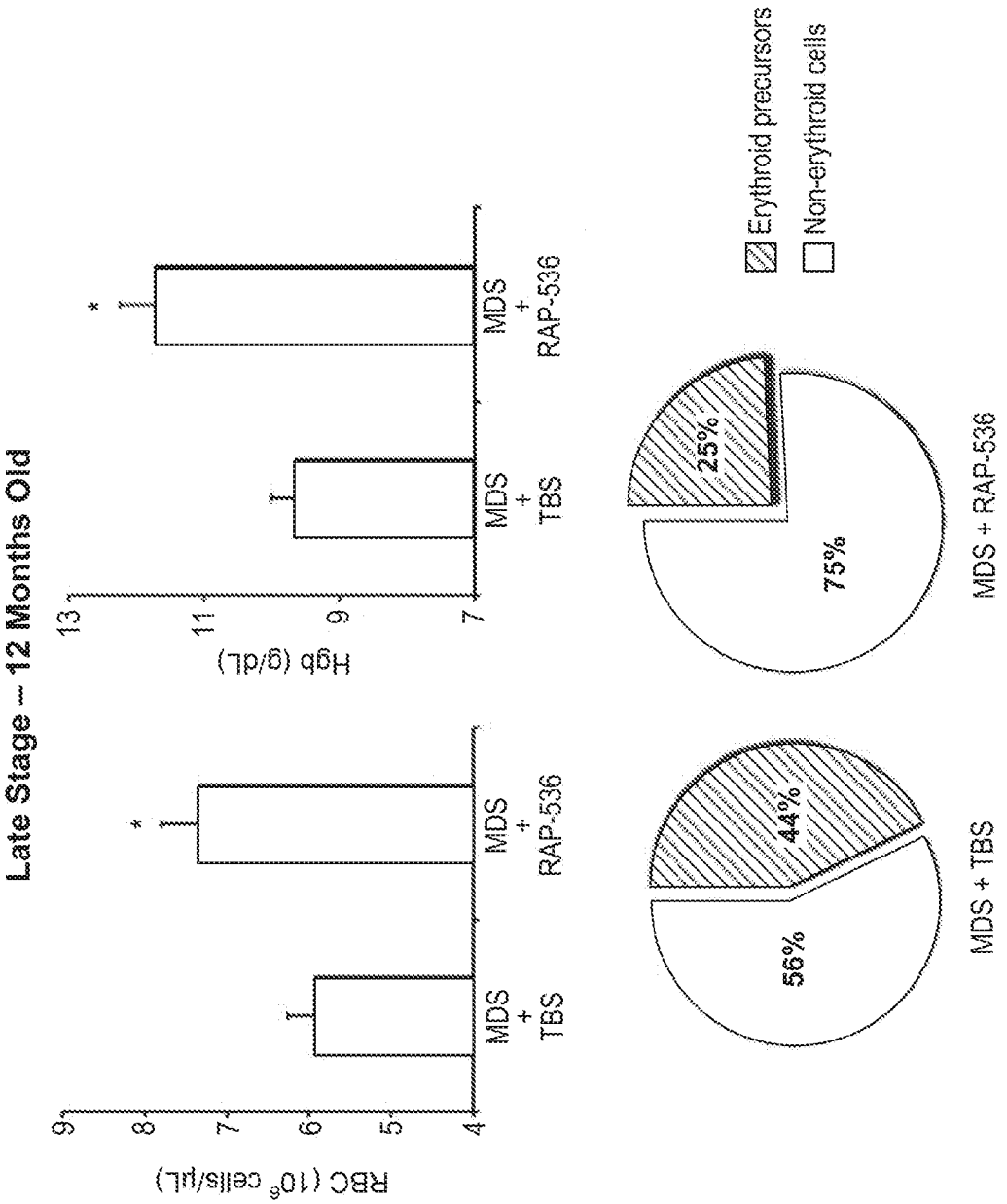

Late Stage – 12 Months Old
RBC (10^6 cells/μL)
9
8
7
6
5
4
MDS + TBS
MDS + RAP-536
*
Hgb (g/dL)
13
11
9
7
MDS + TBS
MDS + RAP-536
*
Erythroid precursors
Non-erythroid cells
56%
44%
MDS + TBS
25%
75%
MDS + RAP-536

FIG. 32B

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

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

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

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

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/063835

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

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

1. ☐ Claims Nos.:

because they relate to subject matter not required to be searched by this Authority, namely:

the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including

2. ☐ Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

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

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

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2015/063835

A. CLASSIFICATION OF SUBJECT MATTER

A61K 38/18 (2006.01) A61K 39/395 (2006.01) A61P 7/06 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPODOC, WPIAD, MEDLINE, WPIDS, BIOSIS & Keywords: anaemia, sideroblastic, bone marrow disorder, CLL, AML, MDS, SF3B1, DNMT3A, TET2, mutation, ActRII antagonist, Activin (A, B, AB, C, E), GDF (11, 8), BMP (6, 7), NODAL, SMAD, MYOSTATIN, FOLLISTATIN, GDF TRAP and similar term

GENOMEQUEST SEQ ID NO:44 and SEQ ID NO: 10, 11, 22, 26, 28, 9, 1, 2, 3, 4, 5, 6, 36, 37, 38, 49, 50, 51, 41, 45

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 February 2016	22 February 2016
Name and mailing address of the ISA/AU	**Authorised officer**
AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustralia.gov.au	Nathalie Tochon-Danguy AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. 0262833101

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	**PCT/US2015/063835**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/076437 A2 (ACCELERON PHARMA INC.) 26 June 2008 Abstract, pages 42-44, 47, SEQ ID NOs:2, 3, 12, 7, 13, 46, 20, 38, 24, 34, 16, 21, 15 at least, Figures	47, 53-59, 64, 152-195
X	WO 2011/020045 A1 (ACCELERON PHARMA INC.) 17 February 2011 Abstract, pages 2-4, 25, 50, 62, 82-84, Figures 5, 7, 19-22, claims and SEQ ID NOs:7, 11, 15, 2, 3 at least	47, 57, 64, 152-190, 193 and 194
X	WO 2013/059347 A1 (ACCELERON PHARMA INC.) 25 April 2013 pages 30, 52-58, 93, 94, Examples 13, 19, 20, 22, claims, SEQ ID NOs 2, 3, 19 at least	1-30, 34, 47, 48, 50, 53-59, 61, 62, 64, 152-195
A	WO 2014/066487 A2 (CELGENE CORPORATION) 01 May 2014 Whole document	1-195
A	SURAGANI R.N.V.S. et al., "Transforming growth factor-β superfamily ligand trap ACE-536 corrects anemia by promoting late-stage erythropoiesis", NATURE MEDICINE, April 2014, vol. 20, no. 4, pages 408-414. Published online 23 March 2014 Abstract, Introduction, Figures 1 and 2	1-195
P,X	WO 2015/161220 A1 (ACCELERON PHARMA, INC) 22 October 2015 abstract, pages 130, 140, 143-147, SEQ ID NOs 44, 1, 10, 22, 28, 3, 29, 37, 41, 45 at least, Figures, Examples 19, 21, claims	47,50, 53-59, 61, 62, 64 and 152-195

Supplemental Box

Continuation of: **Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-34 (fully) and 152-195 (partially) are directed to method of treating sideroblastic anaemia with polypeptide of SEQ ID NO: 44 (truncated ActRIIB). The feature of treating sideroblastic anaemia is specific to this group of claims.

- Claims 35-46, 65-106, 107-151 (fully) and 152-195 (partially) are directed to method of treating bone marrow disorders, myelodysplastic syndrome (MSD) and/or 1 or more complication of MDS in patient with at least 1 mutation selected from SF3B1, DNMT3A or TET2 with an ActRII antagonist. The feature of treating patient with at least 1 mutation in gene selected from SF3B1, DNMT3A or TET2 is specific to this group of claims.

- Claims 47-64 (fully) and 152-195 (partially) are directed to method of treating MDS in patient previously treated with EPO receptor agonist with an ActRII antagonist. The feature of treating MDS in patient previously treated with EPO receptor agonist is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions and which provides a technical relationship among them is the use of ActRII antagonists for the treatment of dysregulated production of blood cellular components

However this feature does not make a contribution over the prior art because it is disclosed in:

D1: WO 2008/076437 A2

D2: WO 2011/020045 A1

Therefore in the light of this document this common feature cannot be a special technical feature. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a posteriori*.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2015/063835**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2008/076437 A2	26 June 2008	WO 2008076437 A2	26 Jun 2008
		AU 2007334333 A1	26 Jun 2008
		AU 2007334333 A2	12 May 2011
		AU 2007334333 B2	30 May 2013
		AU 2009262968 A1	30 Dec 2009
		BR PI0720476 A2	14 Jan 2014
		CA 2672758 A1	26 Jun 2008
		CA 2729054 A1	30 Dec 2009
		CA 2729098 A1	30 Dec 2009
		CN 101687015 A	31 Mar 2010
		CN 102131515 A	20 Jul 2011
		CN 104524548 A	22 Apr 2015
		CO 6230995 A2	20 Dec 2010
		CR 10889 A	01 Sep 2009
		EA 200970603 A1	30 Dec 2009
		EA 018037 B1	30 May 2013
		EA 201201199 A1	30 Oct 2013
		EC SP099514 A	28 Aug 2009
		EP 2124999 A2	02 Dec 2009
		EP 2124999 B1	03 Oct 2012
		EP 2303917 A2	06 Apr 2011
		EP 2318028 A2	11 May 2011
		EP 2446896 A1	02 May 2012
		EP 2468290 A1	27 Jun 2012
		EP 2468290 B1	01 Apr 2015
		EP 2468291 A1	27 Jun 2012
		HK 1137368 A1	01 Mar 2013
		IL 199451 A	31 Aug 2015
		JP 2010513506 A	30 Apr 2010
		JP 5415279 B2	12 Feb 2014
		JP 2011526626 A	13 Oct 2011
		JP 5773868 B2	02 Sep 2015
		JP 2013127002 A	27 Jun 2013
		JP 2014040483 A	06 Mar 2014
		JP 2014088438 A	15 May 2014
		JP 2015187180 A	29 Oct 2015

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2015/063835**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		JP 2016020391 A	04 Feb 2016
		KR 20090096517 A	10 Sep 2009
		KR 20110031951 A	29 Mar 2011
		KR 20150064239 A	10 Jun 2015
		MX 2009006651 A	26 Aug 2009
		NZ 577847 A	29 Jun 2012
		NZ 590326 A	31 May 2013
		NZ 602471 A	31 Oct 2014
		NZ 617196 A	26 Jun 2015
		RS 52537 B	30 Apr 2013
		US 2009047281 A1	19 Feb 2009
		US 7988973 B2	02 Aug 2011
		US 2009163417 A1	25 Jun 2009
		US 8007809 B2	30 Aug 2011
		US 2010028331 A1	04 Feb 2010
		US 8895016 B2	25 Nov 2014
		US 2010028332 A1	04 Feb 2010
		US 2010204092 A1	12 Aug 2010
		US 2012003218 A1	05 Jan 2012
		US 2012052067 A1	01 Mar 2012
		US 2015183845 A1	02 Jul 2015
		WO 2009158015 A2	30 Dec 2009
		WO 2009158033 A2	30 Dec 2009

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2015/063835**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
WO 2011/020045 A1	17 February 2011	WO 2011020045 A1	17 Feb 2011
		AU 2009282441 A1	18 Feb 2010
		AU 2009282441 B2	22 Jan 2015
		AU 2010282361 A1	15 Mar 2012
		AU 2010282361 B2	19 Mar 2015
		CA 2733911 A1	18 Feb 2010
		CA 2770822 A1	17 Feb 2011
		CN 102655872 A	05 Sep 2012
		CN 102655872 B	20 Jan 2016
		EP 2340031 A1	06 Jul 2011
		EP 2464369 A1	20 Jun 2012
		JP 2011530599 A	22 Dec 2011
		JP 2013501816 A	17 Jan 2013
		JP 2014224154 A	04 Dec 2014
		KR 20120054024 A	29 May 2012
		MX 2012001916 A	22 May 2012
		NZ 598348 A	30 May 2014
		NZ 623113 A	30 Oct 2015
		RU 2012109393 A	20 Sep 2013
		TW 201006487 A	16 Feb 2010
		TW I472336 B	11 Feb 2015
		TW 201513879 A	16 Apr 2015
		US 2010068215 A1	18 Mar 2010
		US 8058229 B2	15 Nov 2011
		US 2011038831 A1	17 Feb 2011
		US 8216997 B2	10 Jul 2012
		US 2012015877 A1	19 Jan 2012
		US 8361957 B2	29 Jan 2013
		US 2013071393 A1	21 Mar 2013
		US 8703927 B2	22 Apr 2014
		US 2013244324 A1	19 Sep 2013
		US 2014314759 A1	23 Oct 2014
		WO 2010019261 A1	18 Feb 2010
WO 2013/059347 A1	25 April 2013	WO 2013059347 A1	25 Apr 2013
		AU 2012326207 A1	15 May 2014

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/US2015/063835**

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
		CA 2852683 A1	25 Apr 2013
		CN 103987403 A	13 Aug 2014
		EA 201490809 A1	30 Sep 2014
		EP 2797620 A1	05 Nov 2014
		HK 1203413 A1	30 Oct 2015
		JP 2014530253 A	17 Nov 2014
		KR 20140084211 A	04 Jul 2014
		US 2013243743 A1	19 Sep 2013
WO 2014/066487 A2	01 May 2014	WO 2014066487 A2	01 May 2014
		AU 2013334660 A1	14 May 2015
		CA 2889286 A1	01 May 2014
		CN 104981250 A	14 Oct 2015
		EP 2911682 A2	02 Sep 2015
		JP 2016503398 A	04 Feb 2016
		US 2015266950 A1	24 Sep 2015
WO 2015/161220 A1	22 October 2015	WO 2015161220 A1	22 Oct 2015
		US 2015361163 A1	17 Dec 2015

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

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(54)发明名称

用于治疗骨髓增生异常综合征和铁粒幼红细胞性贫血的方法

(57)摘要

在某些方面,本公开提供用于在脊椎动物(包括啮齿动物和灵长类动物,并且特别是人)中增加红细胞和/或血红蛋白水平的组合物和方法。在一些实施方案中,本公开的组合物可用于治疗或预防铁粒幼红细胞性贫血和骨髓增生异常综合征或者铁粒幼红细胞性贫血和骨髓增生异常综合征有关的一种或多种并发症。

CN 107405383 A

1. 一种用于在人患者中治疗或预防铁粒幼红细胞性贫血的方法,所述方法包括给予有需要的患者包含SEQ ID NO: 44的氨基酸序列的多肽,并且其中患者给药方案包括给予患者0.75-1.75 mg/kg的多肽。

2. 权利要求1的方法,其中所述多肽由SEQ ID NO: 44的氨基酸序列组成。

3.权利要求1或2的方法,其中所述多肽为二聚体。

4.权利要求1-3中任何一项的方法,其中所述多肽结合于GDF11。

5.权利要求1-3中任何一项的方法,其中所述多肽结合于GDF8。

6.权利要求1-3中任何一项的方法,其中所述多肽结合于GDF11和GDF8。

7.权利要求1-6中任何一项的方法,其中所述多肽包含一种或多种选自以下的氨基酸修饰:糖基化氨基酸、聚乙二醇化氨基酸、法尼基化氨基酸、乙酰化氨基酸、生物素化的氨基酸及缀合于脂质部分的氨基酸。

8.权利要求7的方法,其中所述多肽为糖基化的并具有哺乳动物的糖基化模式。

9.权利要求8的方法,其中所述多肽具有可得自中国仓鼠卵巢细胞系的糖基化模式。

10.权利要求1-9中任何一项的方法,其中所述多肽皮下给予患者。

11.权利要求1-10中任何一项的方法,其中所述给药方案进一步包括每3周1次给予患者多肽。

12.权利要求1-11中任何一项的方法,其中所述患者具有不期望的高水平内源性EPO。

13.权利要求1-12中任何一项的方法,其中所述患者先前已用一种或多种EPO受体激动剂治疗。

14.权利要求13的方法,其中所述患者对EPO受体激动剂反应不充分。

15.权利要求13的方法,其中所述患者对EPO受体激动剂不再反应。

16.权利要求13-15中任何一项的方法,其中所述EPO受体激动剂为EPO。

17.权利要求1-16中任何一项的方法,其中所述治疗增加红细胞水平。

18.权利要求1-17中任何一项的方法,其中所述治疗增加血红蛋白水平。

19. 权利要求18的方法,其中所述治疗导致血红蛋白增加≥1.5 g/dL ≥2周。

20. 权利要求18的方法,其中所述治疗导致血红蛋白增加≥1.5 g/dL ≥8周。

21.权利要求1-20中任何一项的方法,其中所述患者已在治疗开始前被给予一次或多次血细胞输注。

22.权利要求1-21中任何一项的方法,其中所述患者为输注负担低的患者。

23.权利要求1-21中任何一项的方法,其中所述患者为输注负担高的患者。

24.权利要求21-23中任何一项的方法,其中所述治疗降低血细胞输注负担。

25.权利要求24的方法,其中相对于治疗开始前的相同时间,所述治疗降低血细胞输注≥50%至少4周。

26.权利要求24的方法,其中相对于治疗开始前的相同时间,所述治疗降低血细胞输注≥50%至少8周。

27.权利要求1-26中任何一项的方法,其中所述患者患有骨髓增生异常综合征。

28. 权利要求27的方法,其中所述患者具有低或中等的国际预后评分系统(International Prognostic Scoring System) (IPSS)或IPSS-R分数。

29.权利要求1-28中任何一项的方法,其中所述铁粒幼红细胞性贫血患者具有作为骨

髓红细胞前体在他或她骨髓中的百分数，至少5%、6%、7%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%或95%的环形未成熟细胞。

30.权利要求1-29中任何一项的方法，其中所述治疗增加嗜中性粒细胞水平。

31.权利要求1-30中任何一项的方法，其中所述患者具有对一种或多种SF3B1突变检测呈阳性的骨髓细胞。

32.权利要求1-31中任何一项的方法，其中所述患者具有对一种或多种DNMT3A突变检测呈阳性的骨髓细胞。

33.权利要求1-32中任何一项的方法，其中所述患者具有对一种或多种TET2突变检测呈阳性的骨髓细胞。

34.权利要求1-33中任何一项的方法，其中所述治疗降低铁超负荷。

35.一种用于在有需要的受试者中治疗或预防骨髓障碍的方法，所述方法包括给予受试者ActRII拮抗剂，其中所述受试者具有对*SF3B1*突变检测呈阳性的骨髓细胞，任选地*SF3B1*基因的突变在外显子、内含子或者5'或3'非翻译区，任选地SF3B1的突变造成基因编码蛋白质的氨基酸序列发生变化，或者不造成氨基酸序列发生改变，和任选地*SF3B1*基因的突变造成基因编码的蛋白质的氨基酸发生选自以下的变化：K182E、E491G、R590K、E592K、R625C、R625G、N626D、N626S、H662Y、T663A、K666M、K666Q、K666R、Q670E、G676D、V701I、I704N、I704V、G740R、A744P、D781G、A1188V、N619K、N626H、N626Y、R630S、I704T、G740E、K741N、G742D、D894G、Q903R、R1041H、I1241T、G347V、E622D、Y623C、R625H、R625L、H662D、H662Q、T663I、K666E、K666N、K666T、K700E和V701F。

36.权利要求35的方法，其中所述受试者患有选自以下的障碍：铁粒幼红细胞性贫血、慢性淋巴细胞白血病(CLL)和急性髓性白血病(AML)。

37.权利要求35或36的方法，其中给予所述ActRII拮抗剂治疗或预防铁粒幼红细胞性贫血的一种或多种并发症。

38.权利要求35的方法，其中所述受试者患有MDS。

39.权利要求38的方法，其中所述受试者具有环形未成熟细胞。

40.权利要求35-39中任何一项的方法，其中所述受试者具有*SF3B1*、*SRSF2*、*DNMT3A*和*TET2*的一种或多种的体细胞突变。

41. 权利要求38-40中任何一项的方法，其中所述受试者具有低或中等的国际预后评分系统(International Prognostic Scoring System) (IPSS)或IPSS-R分数。

42.权利要求35-41中任何一项的方法，其中所述受试者具有作为骨髓红细胞前体在他或她骨髓中的百分数，至少5%、6%、7%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%或95%的环形未成熟细胞。

43.权利要求35-42中任何一项的方法，其中所述受试者先前已用一种或多种EPO受体激动剂治疗。

44.权利要求43的方法，其中所述受试者对EPO受体激动剂反应不充分。

45.权利要求43的方法，其中所述受试者对EPO受体激动剂不再反应。

46.权利要求43-45中任何一项的方法，其中所述EPO受体激动剂为EPO。

47.一种用于在有需要的受试者中治疗或预防MDS的方法，所述方法包括给予受试者ActRII拮抗剂，其中所述受试者先前已用EPO受体激动剂治疗。

48.权利要求47的方法，其中所述受试者具有选自以下的MDS亚型：难治性血细胞减少伴单系发育不良(RCUD)的MDS、难治性血细胞减少伴多系发育不良和环形铁粒幼红细胞增多(RCMD-RS)的MDS、伴*SF3B1*、*SRSF2*、*DNMT3A*和*TET2*的一种或多种的体细胞突变的MDS、没有*ASXL1*或*ZRSR2*的体细胞突变的MDS、伴铁超负荷的MDS及伴嗜中性白血球减少症的MDS。

49. 权利要求47或48的方法，其中所述受试者具有低或中等的国际预后评分系统(International Prognostic Scoring System) (IPSS)或IPSS-R分数。

50.权利要求47-49中任何一项的方法，其中所述受试者具有环形未成熟细胞。

51.权利要求50的方法，其中所述受试者具有作为骨髓红细胞前体在他或她骨髓中的百分数，至少5%、6%、7%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%或95%的环形未成熟细胞。

52.权利要求47-51中任何一项的方法，其中所述受试者具有对SF3B1基因突变检测呈阳性的骨髓细胞。

53.权利要求47-52中任何一项的方法，其中所述受试者先前已用一种或多种EPO受体激动剂治疗。

54.权利要求53的方法，其中所述受试者对EPO受体激动剂反应不充分。

55.权利要求53的方法，其中所述受试者对EPO受体激动剂不再反应。

56.权利要求53-55中任何一项的方法，其中所述EPO受体激动剂为EPO。

57.权利要求47-56中任何一项的方法，其中所述治疗增加红细胞水平。

58.权利要求47-57中任何一项的方法，其中所述受试者已在ActRII拮抗剂治疗开始前被给予一次或多次血细胞输注。

59.权利要求47-58中任何一项的方法，其中所述治疗降低血细胞输注负担。

60. 权利要求59的方法，其中相对于ActRII拮抗剂治疗开始前的相同时间，所述治疗降低血细胞输注大于50% 4-8周。

61.权利要求47-60中任何一项的方法，其中所述治疗降低铁超负荷。

62.权利要求47-61中任何一项的方法，其中所述治疗降低肝脏铁含量。

63.权利要求47-62中任何一项的方法，其中所述治疗增加嗜中性粒细胞水平。

64.权利要求47-63中任何一项的方法，其中所述治疗延迟转化为急性髓性白血病(AML)。

65.一种用于在受试者中治疗或预防骨髓障碍的方法，所述方法包括给予有需要的受试者有效量的ActRII拮抗剂，其中所述受试者具有对选自以下的基因的一种或多种突变检测呈阳性的骨髓细胞：SF3B1、DNMT3A和TET2。

66. 权利要求65 的方法，其中所述受试者对一种或多种SF3B1突变检测呈阳性。

67. 权利要求66 的方法，其中所述SF3B1突变中的一种或多种处于SF3B1外显子。

68.权利要求66或67的方法，其中所述SF3B1突变中的一种或多种处于SF3B1内含子。

69. 权利要求66-68中任何一项的方法，其中所述SF3B1突变中的一种或多种处于SF3B1 5’和/或3’区。

70.权利要求66-69中任何一项的方法，其中所述SF3B1突变中的一种或多种造成由突

变SF3B1基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。

71.权利要求70的方法，其中所述一种或多种SF3B1突变造成选自以下的一种或多种氨基酸替换：K182E、E491G、R590K、E592K、R625C、R625G、N626D、N626S、H662Y、T663A、K666M、K666Q、K666R、Q670E、G676D、V701I、I704N、I704V、G740R、A744P、D781G、A1188V、N619K、N626H、N626Y、R630S、I704T、G740E、K741N、G742D、D894G、Q903R、R1041H、I1241T、G347V、E622D、Y623C、R625H、R625L、H662D、H662Q、T663I、K666E、K666N、K666T、K700E、V701F和E783K。

72.权利要求67的方法，其中所述一种或多种SF3B1突变处于选自以下的SF3B1外显子：外显子14、外显子15和外显子16。

73.权利要求65-72中任何一项的方法，其中所述受试者对一种或多种DNMT3A突变检测呈阳性。

74.权利要求73的方法，其中所述DNMT3A突变中的一种或多种处于DNMT3A外显子。

75.权利要求73或74的方法，其中所述DNMT3A突变中的一种或多种处于DNMT3A内含子。

76. 权利要求73-75中任何一项的方法，其中所述DNMT3A突变中的一种或多种处于DNMT3A 5’和/或3’区。

77.权利要求73-76中任何一项的方法，其中所述DNMT3A突变中的一种或多种造成由突变DNMT3A基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。

78.权利要求77的方法，其中所述一种或多种DNMT3A突变造成选自以下的一种或多种氨基酸替换：R882C、R882H、P904L和P905P。

79.权利要求74的方法，其中所述一种或多种DNMT3A突变处于选自以下的DNMT3A外显子：外显子10、外显子18和外显子22。

80.权利要求73的方法，其中所述一种或多种DNMT3A突变引入提前终止密码子。

81.权利要求80的方法，其中所述一种或多种DNMT3A突变选自Y436X和W893X。

82.权利要求65-81中任何一项的方法，其中所述受试者对一种或多种TET2突变检测呈阳性。

83.权利要求82的方法，其中所述TET2突变中的一种或多种处于TET2外显子。

84.权利要求82或83的方法，其中所述TET2突变中的一种或多种处于TET2内含子。

85. 权利要求82-84中任何一项的方法，其中所述TET2突变中的一种或多种处于TET2 5’和/或3’区。

86.权利要求82-85中任何一项的方法，其中所述TET2突变中的一种或多种造成由突变TET2基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。

87.权利要求86的方法，其中所述一种或多种TET2突变造成选自以下的一种或多种氨基酸替换：E47Q、Q1274R、W1291R、G1370R、G1370E、N1387S和Y1724H。

88.权利要求83的方法，其中所述一种或多种TET2突变处于选自以下的TET2外显子：外显子1、外显子4、外显子5、外显子6、外显子7、外显子8和外显子9。

89.权利要求82的方法，其中所述一种或多种TET2突变引入提前终止密码子。

90.权利要求89的方法，其中所述一种或多种TET2突变选自：R550X、Q1009X、Y1337X、R1404X、R1516X和Q1652X。

91.权利要求65-90中任何一项的方法，其中所述受试者患有贫血。

92.权利要求65-91中任何一项的方法，其中所述受试者具有不期望的高水平内源性EPO。

93.权利要求65-92中任何一项的方法，其中所述受试者先前已用一种或多种EPO受体激动剂治疗。

94.权利要求93的方法，其中所述受试者对EPO受体激动剂反应不充分。

95.权利要求93中任何一项的方法，其中所述受试者对EPO受体激动剂不再反应。

96.权利要求93-95中任何一项的方法，其中所述EPO受体激动剂为EPO。

97.权利要求65-96中任何一项的方法，其中所述治疗延迟转化为白血病。

98.权利要求96的方法，其中所述治疗延迟转化为急性髓性白血病。

99.权利要求65-98中任何一项的方法，其中所述受试者为前-白血病患者。

100.权利要求65-99中任何一项的方法，其中所述治疗增加受试者的红细胞水平和/或血红蛋白水平。

101.权利要求65-100中任何一项的方法，其中所述受试者已在ActRII拮抗剂治疗开始前被给予一次或多次血细胞输注。

102.权利要求65-101中任何一项的方法，其中所述治疗降低血细胞输注负担。

103. 权利要求102的方法，其中相对于ActRII拮抗剂治疗开始前的相同时间，所述治疗降低血细胞输注大于约30%、40%、50%、60%、70%、80%、90%或100% 4-8周。

104. 权利要求102的方法，其中相对于ActRII拮抗剂治疗开始前的相同时间，所述治疗降低血细胞输注大于约50% 4-8周。

105.权利要求65-104中任何一项的方法，其中所述治疗降低铁超负荷。

106.权利要求65-105中任何一项的方法，其中所述治疗降低肝和/或脾脏的铁含量。

107.一种用于治疗或预防骨髓增生异常综合征(MDS)和/或MDS的一种或多种并发症的方法，所述方法包括给予有需要的受试者有效量的ActRII拮抗剂，其中所述受试者具有对选自以下的基因的一种或多种突变检测呈阳性的骨髓细胞：SF3B1、DNMT3A和TET2。

108.权利要求107的方法，其中所述受试者对一种或多种SF3B1突变检测呈阳性。

109.权利要求108的方法，其中所述突变中的一种或多种处于SF3B1外显子。

110.权利要求107或108的方法，其中所述SF3B1突变中的一种或多种处于SF3B1内含子。

111. 权利要求107-110中任何一项的方法，其中所述SF3B1突变中的一种或多种处于SF3B1 5’和/或3’区。

112.权利要求107-111中任何一项的方法，其中所述SF3B1突变中的一种或多种造成由突变SF3B1基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。

113.权利要求112的方法，其中所述一种或多种SF3B1突变造成选自以下的一种或多种氨基酸替换：K182E、E491G、R590K、E592K、R625C、R625G、N626D、N626S、H662Y、T663A、K666M、K666Q、K666R、Q670E、G676D、V701I、I704N、I704V、G740R、A744P、D781G、A1188V、N619K、N626H、N626Y、R630S、I704T、G740E、K741N、G742D、D894G、Q903R、R1041H、I1241T、G347V、E622D、Y623C、R625H、R625L、H662D、H662Q、T663I、K666E、K666N、K666T、K700E、V701F和E783K。

114.权利要求109的方法，其中所述一种或多种SF3B1突变处于选自以下的SF3B1外显

子:外显子14、外显子14和外显子16。

115.权利要求107-114中任何一项的方法,其中所述受试者对一种或多种DNMT3A突变检测呈阳性。

116.权利要求115的方法,其中所述DNMT3A突变中的一种或多种处于DNMT3A外显子。

117.权利要求115或116的方法,其中所述DNMT3A突变中的一种或多种处于DNMT3A内含子。

118. 权利要求115-117中任何一项的方法,其中所述DNMT3A突变中的一种或多种处于DNMT3A 5'和/或3'区。

119.权利要求115-118中任何一项的方法,其中所述DNMT3A突变中的一种或多种造成由突变DNMT3A基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。

120.权利要求119的方法,其中所述一种或多种DNMT3A突变造成选自以下的一种或多种氨基酸替换:R882C、R882H、P904L和P905P。

121.权利要求116的方法,其中所述一种或多种DNMT3A突变处于选自以下的DNMT3A外显子:外显子10、外显子18和外显子22。

122.权利要求115的方法,其中所述一种或多种DNMT3A突变引入提前终止密码子。

123.权利要求112的方法,其中所述一种或多种DNMT3A突变选自Y436X和W893X。

124.权利要求107-123中任何一项的方法,其中所述受试者对一种或多种TET2突变检测呈阳性。

125.权利要求124的方法,其中所述TET2突变中的一种或多种处于TET2外显子。

126.权利要求124或125的方法,其中所述TET2突变中的一种或多种处于TET2内含子。

127. 权利要求124-126中任何一项的方法,其中所述TET2突变中的一种或多种处于TET2 5'和/或3'区。

128.权利要求124-127中任何一项的方法,其中所述TET2突变中的一种或多种造成由突变TET2基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。

129.权利要求128的方法,其中所述一种或多种TET2突变造成选自以下的一种或多种氨基酸替换:E47Q、Q1274R、W1291R、G1370R、G1370E、N1387S和Y1724H。

130.权利要求125的方法,其中所述一种或多种TET2突变处于选自以下的TET2外显子:外显子1、外显子4、外显子5、外显子6、外显子7、外显子8和外显子9。

131.权利要求124的方法,其中所述一种或多种TET2突变引入提前终止密码子。

132.权利要求131的方法,其中所述一种或多种TET2突变选自:R550X、Q1009X、Y1337X、R1404X、R1516X和Q1652X。

133.权利要求107-132中任何一项的方法,其中所述受试者患有贫血。

134.权利要求107-133中任何一项的方法,其中所述受试者具有不期望的高水平内源性EPO。

135.权利要求107-134中任何一项的方法,其中所述受试者先前已用一种或多种EPO受体激动剂治疗。

136.权利要求135的方法,其中所述受试者对EPO受体激动剂反应不充分。

137.权利要求136中任何一项的方法,其中所述受试者对EPO受体激动剂不再反应。

138.权利要求135-137中任何一项的方法,其中所述EPO受体激动剂为EPO。

139.权利要求107-138中任何一项的方法，其中所述治疗延迟转化为白血病。

140.权利要求139的方法，其中所述治疗延迟转化为急性髓性白血病。

141.权利要求107-140中任何一项的方法，其中所述治疗增加受试者的红细胞水平和/或血红蛋白水平。

142.权利要求107-141中任何一项的方法，其中所述受试者已在ActRII拮抗剂治疗开始前被给予一次或多次血细胞输注。

143.权利要求107-142中任何一项的方法，其中所述治疗降低血细胞输注负担。

144. 权利要求143的方法，其中相对于ActRII拮抗剂治疗开始前的相同时间，所述治疗降低血细胞输注大于约30%、40%、50%、60%、70%、80%、90%或100% 4-8周。

145. 权利要求143的方法，其中相对于ActRII拮抗剂治疗开始前的相同时间，所述治疗降低血细胞输注大于约50% 4-8周。

146.权利要求107-145中任何一项的方法，其中所述治疗降低铁超负荷。

147.权利要求107-145中任何一项的方法，其中所述治疗降低肝和/或脾脏的铁含量。

148.权利要求107-147中任何一项的方法，其中所述受试者具有选自以下的MDS亚型：难治性血细胞减少伴单系发育不良(RCUD)的MDS、难治性血细胞减少伴多系发育不良和环形铁粒幼红细胞增多(RCMD-RS)的MDS、伴*SF3B1*、*SRSF2*、*DNMT3A*和*TET2*中的一种或多种的体细胞突变MDS、没有*ASXL1*或*ZRSR2*体细胞突变的MDS、伴铁超负荷的MDS及伴嗜中性白血球减少症的MDS。

149. 权利要求107-148中任何一项的方法，其中所述受试者具有选自以下的国际预后评分系统(International Prognostic Scoring System) (IPSS)分数：低、中等1或中等2。

150.权利要求107-149中任何一项的方法，其中所述受试者具有环形未成熟细胞。

151.权利要求150的方法，其中所述受试者具有作为骨髓红细胞前体在他或她骨髓中的百分数，至少5%、6%、7%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%或95%的环形未成熟细胞。

152.权利要求1-151中任何一项的方法，其中所述ActRII拮抗剂为ActRIIA多肽。

153.权利要求152的方法，其中所述ActRIIA多肽选自：

a) 一种包含SEQ ID NO:10的氨基酸序列或包含与SEQ ID NO:10的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

b) 一种包含SEQ ID NO:11的氨基酸序列或包含与SEQ ID NO:11的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

c) 一种包含SEQ ID NO:22的氨基酸序列或包含与SEQ ID NO:22的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

d) 一种包含SEQ ID NO:26的氨基酸序列或包含与SEQ ID NO:26的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

e) 一种包含SEQ ID NO:28的氨基酸序列或包含与SEQ ID NO:28的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；和

f) 一种包含与SEQ ID NO:9的氨基酸30-110相同的氨基酸序列或包含与SEQ ID NO:9的氨基酸30-110序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽。

154.权利要求1-151中任何一项的方法，其中所述ActRII拮抗剂为ActRIIB多肽。

155.权利要求154的方法，其中所述ActRIIB多肽选自：

a) 一种包含与SEQ ID NO:1的氨基酸29-109相同的氨基酸序列或包含与SEQ ID NO:1的氨基酸29-109序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

b) 一种包含与SEQ ID NO:1的氨基酸25-131相同的氨基酸序列或包含与SEQ ID NO:1的氨基酸25-131序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

c) 一种包含SEQ ID NO:2的氨基酸序列或包含与SEQ ID NO:2的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

d) 一种包含SEQ ID NO:3的氨基酸序列或包含与SEQ ID NO:3的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

e) 一种包含SEQ ID NO:4的氨基酸序列或包含与SEQ ID NO:4的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

f) 一种包含SEQ ID NO:5的氨基酸序列或包含与SEQ ID NO:5的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

g) 一种包含SEQ ID NO:6的氨基酸序列或包含与SEQ ID NO:6的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

h) 一种包含SEQ ID NO:36的氨基酸序列或包含与SEQ ID NO:36的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

i) 一种包含SEQ ID NO:37的氨基酸序列或包含与SEQ ID NO:37的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

j) 一种包含SEQ ID NO:38的氨基酸序列或包含与SEQ ID NO:38的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

k) 一种包含SEQ ID NO:49的氨基酸序列或包含与SEQ ID NO:49的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

l) 一种包含SEQ ID NO:50的氨基酸序列或包含与SEQ ID NO:50的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

m) 一种包含SEQ ID NO:51的氨基酸序列或包含与SEQ ID NO:51的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

n) 一种包含SEQ ID NO:41的氨基酸序列或包含与SEQ ID NO:41的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

o) 一种包含SEQ ID NO:44的氨基酸序列或包含与SEQ ID NO:44的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；

p) 一种包含SEQ ID NO:45的氨基酸序列或包含与SEQ ID NO:45的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽；和

q) 一种包含SEQ ID NO:29的氨基酸序列或包含与SEQ ID NO:29的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽。

156. 权利要求155的方法，其中所述ActRIIB多肽在相对于SEQ ID NO:1的79位包含酸性氨基酸。

157.权利要求1-156中任何一项的方法，其中所述ActRII拮抗剂为GDF捕获多肽。

158.权利要求152-157中任何一项的方法，其中所述多肽为一种融合蛋白，其除ActRII

多肽结构域外还包含一个或多个增强一种或多种以下性能的异源多肽结构域:体内半衰期、体外半衰期、给药、组织定位或分布、蛋白复合物形成和纯化。

159.权利要求158的方法,其中所述融合蛋白包含选自以下的异源多肽结构域:免疫球蛋白Fc结构域和血清白蛋白。

160. 权利要求159的方法,其中所述免疫球蛋白Fc结构域为IgG1 Fc结构域。

161. 权利要求159的方法,其中所述免疫球蛋白Fc结构域包含选自SEQ ID NO: 15或16的氨基酸序列。

162.权利要求158-161中任何一项的方法,其中所述融合蛋白进一步包含位于ActRII多肽结构域和免疫球蛋白Fc结构域之间的连接区域。

163.权利要求162的方法,其中所述连接区域为TGGG或GGG连接子。

164. 权利要求158的方法,其中所述多肽为包含选自以下的多肽的ActRIIA-Fc融合蛋白:

a) 一种包含SEQ ID NO:22的氨基酸序列或包含与SEQ ID NO:22的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽;和

b) 一种包含SEQ ID NO:28的氨基酸序列或包含与SEQ ID NO:28的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽。

165.权利要求158的方法,其中所述多肽为包含选自以下的多肽的ActRIIB-Fc融合蛋白:

a) 一种包含SEQ ID NO:29的氨基酸序列或包含与SEQ ID NO:29的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽;

b) 一种包含SEQ ID NO:36的氨基酸序列或包含与SEQ ID NO:36的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽;

c) 一种包含SEQ ID NO:29的氨基酸序列或包含与SEQ ID NO:29的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽;

d) 一种包含SEQ ID NO:38的氨基酸序列或包含与SEQ ID NO:38的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽;

e) 一种包含SEQ ID NO:41的氨基酸序列或包含与SEQ ID NO:41的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽;

f) 一种包含SEQ ID NO:44的氨基酸序列或包含与SEQ ID NO:44的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽;

g) 一种包含SEQ ID NO:45的氨基酸序列或包含与SEQ ID NO:45的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽;和

h) 一种包含SEQ ID NO:51的氨基酸序列或包含与SEQ ID NO:51的氨基酸序列至少80%、85%、90%、95%、96%、97%、98%或99%相同的氨基酸序列的多肽。

166. 权利要求165的方法,其中所述ActRIIB-Fc融合蛋白在相对于SEQ ID NO:1的79位包含酸性氨基酸。

167.权利要求152-166中任何一项的方法,其中所述多肽包含一种或多种选自以下的氨基酸修饰:糖基化氨基酸、聚乙二醇化氨基酸、法尼基化氨基酸、乙酰化氨基酸、生物素化的氨基酸、缀合于脂质部分的氨基酸及缀合于有机衍生化试剂的氨基酸。

168.权利要求167的方法，其中所述多肽为糖基化的并具有哺乳动物的糖基化模式。

169.权利要求168的方法，其中所述多肽为糖基化的并具有可得自中国仓鼠卵巢细胞系的糖基化模式。

170.权利要求152-169中任何一项的方法，其中所述多肽结合于GDF11。

171.权利要求152-170中任何一项的方法，其中所述多肽结合于GDF8。

172.权利要求152-171中任何一项的方法，其中所述多肽结合于激活素。

173.权利要求172的方法，其中所述多肽结合于激活素A。

174.权利要求172或173的方法，其中所述多肽结合于激活素B。

175.权利要求1-151中任何一项的方法，其中所述ActRII拮抗剂为抗-GDF11抗体。

176.权利要求1-151中任何一项的方法，其中所述ActRII拮抗剂为抗-GDF8抗体。

177.权利要求1-151中任何一项的方法，其中所述ActRII拮抗剂为抗-激活素抗体。

178.权利要求177的方法，其中所述ActRII拮抗剂结合于激活素A。

179.权利要求177的方法，其中所述ActRII拮抗剂结合于激活素B。

180.权利要求177的方法，其中所述ActRII拮抗剂结合于激活素A和激活素B。

181.权利要求175-180中任何一项的方法，其中所述ActRII拮抗剂为进一步结合于以下一种或多种的多特异性抗体：GDF11、GDF8、激活素A、激活素AB、激活素C、激活素E、BMP7、GDF3和BMP6。

182.权利要求1-151中任何一项的方法，其中所述ActRII拮抗剂为抗-ActRII抗体。

183.权利要求182的方法，其中所述抗-ActRII抗体结合于ActRIIA。

184.权利要求182的方法，其中所述抗-ActRII抗体结合于ActRIIB。

185.权利要求182的方法，其中所述抗-ActRII抗体结合于ActRIIA和ActRIIB。

186.权利要求175-185中任何一项的方法，其中所述抗体为嵌合抗体、人源化抗体或人抗体。

187.权利要求175-186中任何一项的方法，其中所述抗体为单链抗体、$F(ab')_2$片段、单链双价抗体、串联单链Fv片段、串联单链双价抗体或者包含单链双价抗体和至少一部分免疫球蛋白重链恒定区的融合蛋白。

188.权利要求1-187中任何一项的方法，其中所述方法进一步包含给予铁粒幼红细胞性贫血的一种或多种支持疗法。

189.权利要求1-187中任何一项的方法，其中所述方法进一步包含给予MDS的一种或多种支持疗法。

190.权利要求188或189的方法，其中所述支持疗法为输注以下一种或多种：红细胞、粒细胞和凝血细胞。

191.权利要求188-190中任何一项的方法，其中所述支持疗法包括给予铁螯合剂或多种铁螯合剂。

192.权利要求191的方法，其中所述铁螯合剂或多种铁螯合剂选自：

a) 去铁胺；

b) 去铁酮；和

c) 去铁斯若。

193.权利要求188-192中任何一项的方法，其中所述支持疗法包括给予EPO受体激活

剂。

194.权利要求193的方法，其中所述EPO受体激活剂选自：EPO、依泊汀α、依泊汀β、依泊汀δ、依泊汀ω、达贝泊汀α、甲氧基聚乙二醇依泊汀β及合成的红细胞生成蛋白(SEP)。

195.权利要求188-194中任何一项的方法，其中所述支持疗法包括给予选自以下的一种或多种试剂：G-CSF类似物、GM-CSF类似物、铁螯合剂、铁调素或铁调素受体激活剂、来那度胺、沙利度胺、泊马度胺、阿扎胞苷、地西他滨、抗胸腺细胞球蛋白和促血小板生成药物、组蛋白去乙酰化酶抑制剂、p38MAPK抑制剂、谷胱甘肽S-转移酶π抑制剂、阿仑单抗、DNA甲基转移酶抑制剂和组蛋白去乙酰化酶抑制剂。

用于治疗骨髓增生异常综合征和铁粒幼红细胞性贫血的方法

[0001] 相关申请的交叉参考

本申请权利要求2014年12月3号递交的美国临时申请序列号62/086977、2014年12月5号递交的美国临时申请序列号62/088087和2015年4月30号递交的美国临时申请序列号62/155395的优先权的利益。前述申请中每一个的公开通过参照以其全部结合到本文中。

[0002] 发明背景

本公开涉及血细胞成分(包括红细胞、嗜中性粒细胞和血小板)产生失调的治疗。造血作用为在产后生活期间自主要位于骨髓、脾脏或淋巴结的自我更新造血干细胞形成血液的细胞成分。血细胞可分类为属于淋巴细胞系、髓细胞系或红细胞系。通过称为淋巴细胞增殖的过程,普通的淋巴系祖细胞产生T细胞、B细胞、自然杀伤细胞和树突细胞。通过称为骨髓细胞生成的过程,普通的髓系祖细胞产生巨噬细胞、粒细胞(嗜碱性粒细胞、嗜中性粒细胞、嗜酸性粒细胞和肥大细胞)及凝血细胞(血小板)。最后,通过称为红细胞生成的过程,红系祖细胞产生红细胞(RBC,红细胞)。

[0003] 产后红细胞生成主要发生于骨髓和脾脏的红髓。各种信号通路的协同作用调控细胞增殖、分化、存活和死亡的平衡。在正常情况下,红细胞以保持体内红细胞总量不变的速率产生,并且这种产生可应答于各种刺激而增加或减少,包括氧张力或组织需求的增加或减少。红细胞生成过程开始于谱系限制性的前体细胞(lineage-committed precursor cell)形成,并通过一系列不同的前体细胞类型进行。当网织红细胞释放入血并失去其线粒体和核糖体,同时呈现成熟红细胞的形态时,发生红细胞生成的最后阶段。血液中的网织红细胞水平升高或者网织红细胞:红细胞比率升高表明红细胞的产生速率增加。成熟的红细胞(RBC)负责脊椎动物循环系统中的氧输送。红细胞含有高浓度的血红蛋白,蛋白在肺中相对高的氧分压(pO_2)下结合氧,并把氧气传递至相对低pO_2的身体部位。

[0004] 促红细胞生成素(EPO)广泛认为是脊椎动物产后红细胞生成的显著正调控因子。EPO调控对组织氧张力减少(缺氧)和红细胞水平低或血红蛋白水平低的代偿性红细胞生成反应。在人类, EPO水平升高通过刺激骨髓和脾脏中产生红系祖细胞促进红细胞的形成。在小鼠,EPO主要在脾脏增强红细胞生成。

[0005] EPO的作用通过属于细胞因子受体超家族的细胞表面受体介导。人EPO受体基因编码483个氨基酸的跨膜蛋白,然而,认为活性EPO受体即使在没有配体的情况下作为多聚复合物存在(参见例如美国专利第6319499号)。在哺乳动物细胞表达的克隆全长EPO受体,具有与红系祖细胞上的天然受体相似的亲和力结合EPO。EPO结合其受体引起构象变化,导致受体激活和生物效应,包括未成熟成红细胞的增殖增多、未成熟成红细胞的分化增多和红系祖细胞的凋亡减少[参见例如Liboi等. (1993) Proc Natl Acad Sci USA 90:11351-11355;Koury等. (1990) Science 248:378-381]。

[0006] 各种形式的重组EPO用于医生在各种临床环境下,特别是在贫血治疗中增加红细胞水平。贫血为一种广泛定义的病症,特征为血液中的血红蛋白或红细胞低于正常水平。在一些情况下,贫血由红细胞产生或存活中的原发性障碍(例如骨髓增生异常综合症)引起。更常见地,贫血继发于其他系统的疾病[参见例如Weatherall & Provan (2000) Lancet

355, 1169-1175]。贫血可能由于红细胞的产生速率减小或破坏速率增大或者由于红细胞因出血而损失造成。贫血可能由各种障碍造成，包括例如急性或慢性肾衰竭或终末期肾病、化疗治疗、骨髓增生异常综合征、类风湿性关节炎和骨髓移植。

[0007] 用EPO治疗一般造成健康人经数周时间血红蛋白升高约1-3 g/dL。当给予贫血的个体时，这种治疗方案通常提供血红蛋白和红细胞水平显著增加，并导致改善生活质量和延长生存期。然而，EPO不是一律有效的，并且许多个体即使大剂量也是难治的[参见例如Horl等. (2000) Nephrol Dial Transplant 15, 43-50]。例如，超过50%的癌症患者对EPO反应不充分，并且约10%的终末期肾病患者对EPO反应低[参见例如Glaspy等. (1997) J Clin Oncol 15, 1218-1234; Demetri等. (1998) J Clin Oncol 16, 3412-3425]。尽管抗EPO的分子机制目前还不清楚，但是几种因素(包括炎症、铁和维生素缺乏、透析不充分、铝中毒和甲状旁腺功能亢进)可预测治疗反应不佳。另外，最近的证据表明，较高剂量的EPO可能与心血管疾病的发病率、肿瘤生长的风险增加以及在某些患者人群的死亡率有关[参见例如Krapf等. (2009) Clin J Am Soc Nephrol 4:470-480; Glaspy (2009) Annu Rev Med 60:181-192]。因此，有人推荐以最低剂量给予基于EPO的治疗化合物(例如促红细胞生成素刺激剂, ESAs)，使得患者能够避免红细胞输注[参见例如Jelkmann等. (2008) Crit Rev Oncol. Hematol 67:39-61]。

[0008] 以遗传性和获得性两种形式发生的铁粒幼红细胞性贫血，特征为在骨髓中存在“环形铁粒幼红细胞”。这些独特的红细胞前体(成红细胞)可根据核周含铁颗粒的存在来确认，后者通过用普鲁士蓝进行组织学染色显示，并表明线粒体中有病理性铁沉积[参见例如Mufti等. (2008) Haematologica 93:1712-1717;Bottomley等. (2014) Hematol Oncol Clin N Am 28:653-670]。获得性铁粒幼红细胞性贫血最常发生在骨髓增生异常综合征(MDS)的情况下，MDS为一组异质性造血干细胞障碍，估计在美国每年影响30000-40000名患者[Bejar等. (2014) Blood 124:2793-2803]。这些障碍特征为造血作用无效、异常“发育不良的”细胞形态学及克隆进化到急性髓性白血病的可能性。如以下讨论的那样，MDS遗传基础的最新进展有潜力极大地改善其诊断和治疗。

[0009] 高度需要对MDS、铁粒幼红细胞性贫血以及这些障碍的并发症的有效治疗，但这种需要尚未得到满足。内源性EPO水平在MDS患者亚群通常升高，因此表明EPO在这些患者身上的疗效有所下降。据估计，不到10%的MDS患者对EPO反应良好[Estey (2003) Curr Opin Hematol 10, 60-67]，而最近的一项元分析发现，EPO响应率依研究而定在30%-60%范围内[Moyo等. (2008) Ann Hematol 87:527-536]。与其他MDS患者相比较，具有环形铁粒幼红细胞的患者往往处于明显更低的出现急性髓性白血病的风险下，并因此长期受益于不造成全身性的铁负荷并相反有助于减少经常出现在这种患者身上的铁超负荷的抗贫血治疗剂[参见例如Temraz等, 2014, Crit Rev Oncol Hematol 91:64-73]。

[0010] 因此，本公开的一个目的是提供用本文公开的ActRII拮抗剂治疗患有MDS和铁粒幼红细胞性贫血的患者的方法，并且具体地讲，指导选择最有可能由于治疗而显示红细胞、嗜中性粒细胞及其他血细胞治疗性有益增加的MDS患者。

[0011] 发明概述

在某种程度上，本公开提供用一种或多种ActRII拮抗剂治疗MDS和铁粒幼红细胞性贫血，特别是治疗或预防MDS的一种或多种并发症或亚型的方法，方法包括特征为骨髓中存在

铁粒幼红细胞的MDS患者。

[0012] 在某种程度上，本公开提供用一种或多种ActRII拮抗剂治疗或预防与生殖系或体细胞*SF3B1*突变有关的障碍或障碍的并发症，比如骨髓增生异常综合征(MDS)、慢性淋巴细胞白血病(CLL)和急性髓性白血病(AML)以及乳腺癌、胰腺癌、胃癌、前列腺癌和葡萄膜黑色素瘤的方法。在某些方面，这种障碍可存在于具有对*SF3B1*突变检测呈阳性的骨髓细胞，特别是骨髓增生异常综合征、CLL和AML的受试者。任选地，*SF3B1*基因突变在外显子、内含子或者5'和/或3'非翻译区。例如，在一些实施方案中，*SF3B1*基因突变在*SF31B*的外显子14、15和/或16。任选地，*SF3B1*的突变造成基因编码蛋白质的氨基酸序列发生变化，或者不造成氨基酸序列发生改变。任选地，*SF3B1*基因的突变造成基因编码的蛋白质氨基酸发生选自以下的变化：K182E、E491G、R590K、E592K、R625C、R625G、N626D、N626S、H662Y、T663A、K666M、K666Q、K666R、Q670E、G676D、V701I、I704N、I704V、G740R、A744P、D781G、A1188V、N619K、N626H、N626Y、R630S、I704T、G740E、K741N、G742D、D894G、Q903R、R1041H、I1241T、G347V、E622D、Y623C、R625H、R625L、H662D、H662Q、T663I、K666E、K666N、K666T、K700E、E783K和V701F。

[0013] 在某些方面，本公开提供用于在人受试者治疗或预防铁粒幼红细胞性贫血的方法，方法包括给予有需要的受试者包含与SEQ ID NO:1的氨基酸29-109至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%相同的氨基酸序列的多肽，其中多肽在相对于SEQ ID NO:1的79位包含酸性氨基酸[天然氨基酸(例如D或E)或人工氨基酸]，其中受试者给药方案包括给予受试者0.125-1.75 mg/kg (例如0.75-1.75 mg/kg)的多肽。在其他方面，本公开提供用于在人受试者治疗或预防铁粒幼红细胞性贫血的方法，方法包括给予有需要的受试者包含与SEQ ID NO:1的氨基酸25-125至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%相同的氨基酸序列的多肽，其中多肽在相对于SEQ ID NO:1的79位包含酸性氨基酸[天然氨基酸(例如D或E)或人工氨基酸]，其中受试者给药方案包括给予受试者0.125-1.75 mg/kg (例如0.75-1.75 mg/kg)的多肽。在甚至其他方面，本公开提供用于在人受试者治疗或预防铁粒幼红细胞性贫血的方法，方法包括给予有需要的受试者包含、基本由以下组成或由与SEQ ID NO:44的氨基酸序列至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%相同的氨基酸序列组成的多肽，其中多肽在相对于SEQ ID NO:1的79位包含酸性氨基酸[天然氨基酸(例如D或E)或人工氨基酸]，其中受试者给药方案包括给予受试者0.125-1.75 mg/kg (例如0.75-1.75 mg/kg)的多肽。在某些方面，本公开提供用于在人受试者治疗或预防铁粒幼红细胞性贫血的一种或多种并发症的方法，方法包括给予有需要的受试者包含与SEQ ID NO:1的氨基酸29-109至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%相同的氨基酸序列的多肽，其中多肽在相对于SEQ ID NO:1的79位包含酸性氨基酸[天然氨基酸(例如D或E)或人工氨基酸]，其中受试者给药方案包括给予受试者0.125-1.75 mg/kg (例如0.75-1.75 mg/kg)的多肽。在其他方面，本公开提供用于在人受试者治疗或预防铁粒幼红细胞性贫血的一种或多种并发症的方法，方法包括给予有需要的受试者包含与SEQ ID NO:1的氨基酸25-125至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%相同的氨基酸序列的多肽，其中多肽在相对于SEQ ID NO:1的79位包含酸性氨基酸[天然氨基酸(例如D或E)或人工氨基酸]，其中受试者给药方案包括给予受试者0.125-1.75

mg/kg (例如0.75-1.75 mg/kg)的多肽。在甚至其他方面,本公开提供用于在人受试者治疗或预防铁粒幼红细胞性贫血的一种或多种并发症的方法,方法包括给予有需要的受试者包含、基本由以下组成或由与SEQ ID NO:44的氨基酸序列至少70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%相同的氨基酸序列组成的多肽,其中多肽在相对于SEQ ID NO:1的79位包含酸性氨基酸[天然氨基酸(例如D或E)或人工氨基酸],其中受试者给药方案包括给予受试者0.125-1.75 mg/kg (例如0.75-1.75 mg/kg)的多肽。在某些优选的实施方案中,要按本文描述的方法使用的多肽为二聚体(例如包含两个通过共价或非共价相互作用连接起来的相应于SEQ ID NO:44的氨基酸序列的多肽的同源二聚体)。任选地,多肽可结合于TGFβ超家族的一个或多个配体。例如,在一些实施方案中,本文描述的多肽(例如ActRIIA 和ActRIIB多肽及其变体比如GDF捕获物)可结合于GDF11。在其他的实施方案中,本文描述的多肽(例如ActRIIA 和ActRIIB多肽及其变体比如GDF捕获物)可结合于GDF8。在仍然其他的实施方案中,本文描述的多肽(例如ActRIIA 和ActRIIB多肽及其变体比如GDF捕获物)可结合于GDF11和GDF8。任选地,多肽可包含一种或多种选自以下的氨基酸修饰:糖基化氨基酸、聚乙二醇化氨基酸、法尼基化氨基酸、乙酰化氨基酸、生物素化的氨基酸及缀合于脂质部分的氨基酸。在某些优选的实施方案中,多肽为糖基化的并具有哺乳动物的糖基化模式。任选地,多肽具有可得自中国仓鼠卵巢细胞系的糖基化模式。任选地,方法包括皮下给予受试者多肽。任选地,给药方案进一步包括每周2次、每周1次、每3周1次、每4周1次、每5周1次、每6周1次、每7周1次、每8周1次、每9周1次、每10周1次、每12周1次、每14周1次、每16周1次、每18周1次、每20周1次、每24周1次、每26周1次、每28周1次、每30周1次、每32周1次、每34周1次或每36周1次给予患者多肽。在某些优选的实施方案中,给药方案进一步包括每3周1次给予患者多肽。任选地,受试者具有不期望的高水平内源性EPO。任选地,受试者先前已用一种或多种EPO受体激动剂治疗。任选地,受试者对EPO受体激动剂反应不充分。任选地,受试者对EPO受体激动剂不再反应。任选地,EPO受体激动剂为EPO。任选地,治疗增加红细胞水平。任选地,治疗增加血红蛋白水平。任选地,其中治疗导致血红蛋白增加≥1.5 g/dL ≥2周。任选地,治疗导致血红蛋白增加≥1.5 g/dL ≥8周。任选地,受试者已在治疗开始前被给予一次或多次血细胞输注。任选地,其中受试者为输注负担低的受试者。任选地,受试者为输注负担高的受试者。任选地,治疗降低血细胞输注负担。任选地,相对于治疗开始前的相同时间,治疗降低血细胞输注≥50%至少4周。任选地,相对于治疗开始前的相同时间,治疗降低血细胞输注≥50%至少8周。任选地,患者患有骨髓增生异常综合征。任选地,患者具有低或中等的国际预后评分系统(International Prognostic Scoring System) (IPSS)或IPSS-R分数。任选地,铁粒幼红细胞性贫血受试者具有作为骨髓红细胞前体在他或她骨髓中的百分数,至少5%、6%、7%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%或95%的环形未成熟细胞。任选地,治疗增加嗜中性粒细胞水平。任选地,受试者具有对一种或多种SF3B1突变检测呈阳性的骨髓细胞。任选地,受试者具有对一种或多种DNMT3A突变检测呈阳性的骨髓细胞。任选地,受试者具有对一种或多种TET2突变检测呈阳性的骨髓细胞。任选地,治疗降低铁超负荷。在一些实施方案中,治疗降低组织铁超负荷(例如肾、肝和/或脾脏的铁超负荷)。在一些实施方案中,治疗降低血清铁超负荷。

[0014] 在某种程度上,本公开提供用一种或多种ActRII拮抗剂治疗或预防与生殖系或体

细胞*DNMT3A*突变有关的障碍或障碍的并发症，比如骨髓增生异常综合征(MDS)、慢性淋巴细胞白血病(CLL)和急性髓性白血病(AML)的方法。在某些方面，这种障碍可存在于具有对*DNMT3A*突变检测呈阳性的骨髓细胞，特别是骨髓增生异常综合征、CLL和AML的受试者。任选地，*DNMT3A*基因突变在外显子、内含子和/或5’或3’ 非翻译区。例如，在一些实施方案中，*DNMT3A*基因突变在*DNMT3A*的外显子10、18和/或22。任选地，*DNMT3A*的突变造成基因编码蛋白质的氨基酸序列发生变化，或者不造成氨基酸序列发生改变。任选地，*DNMT3A*基因的突变造成基因编码的蛋白质氨基酸发生选自以下的变化：R882C、R882H、P904L和P905P。任选地，*DNMT3A*基因的突变引入提前终止密码子。例如，在一些实施方案中，引入提前终止密码子的*DNMT3A*基因的突变选自以下位置：Y436X和W893X。

[0015] 在某种程度上，本公开提供用一种或多种ActRII拮抗剂治疗或预防与生殖系或体细胞*TET2*突变有关的障碍或障碍的并发症，比如骨髓增生异常综合征(MDS)、慢性淋巴细胞白血病(CLL)和急性髓性白血病(AML)的方法。在某些方面，这种障碍可存在于具有对*TET2*突变检测呈阳性的骨髓细胞，特别是骨髓增生异常综合征、CLL和AML的受试者。任选地，*TET2*基因突变在外显子、内含子和/或5’或3’非翻译区。例如，在一些实施方案中，*TET2*基因突变在*TET2*的外显子1、外显子4、外显子5、外显子6、外显子7、外显子8和/或外显子9。任选地，*TET2*的突变造成基因编码蛋白质的氨基酸序列发生变化，或者不造成氨基酸序列发生改变。任选地，*TET2*基因的突变造成基因编码蛋白质的氨基酸发生选自以下的变化：E47Q、Q1274R、W1291R、G1370R、N1387S和Y1724H。任选地，*TET2*基因的突变引入提前终止密码子。例如，在一些实施方案中，引入提前终止密码子的*TET2*基因的突变选自以下位置：R550X、Q1009X、Y1337X、R1404X、R1516X和Q1652X。

[0016] 在某些方面，本公开提供用于在受试者治疗或预防骨髓障碍的方法，方法包括给予有需要的受试者有效量的ActRII拮抗剂，其中受试者具有对选自以下的基因的一种或多种突变检测呈阳性的骨髓细胞：SF3B1、DNMT3A和TET2。在一些实施方案中，受试者对一种或多种SF3B1突变检测呈阳性。任选地，SF3B1突变中的一种或多种处于SF3B1外显子。任选地，SF3B1突变中的一种或多种处于SF3B1内含子。任选地，SF3B1突变中的一种或多种处于SF3B1 5’和/或3’区。任选地，SF3B1突变中的一种或多种造成由突变SF3B1基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。任选地，一种或多种SF3B1突变造成选自以下的一种或多种氨基酸替换：K182E、E491G、R590K、E592K、R625C、R625G、N626D、N626S、H662Y、T663A、K666M、K666Q、K666R、Q670E、G676D、V701I、I704N、I704V、G740R、A744P、D781G、A1188V、N619K、N626H、N626Y、R630S、I704T、G740E、K741N、G742D、D894G、Q903R、R1041H、I1241T、G347V、E622D、Y623C、R625H、R625L、H662D、H662Q、T663I、K666E、K666N、K666T、K700E、V701F和E783K。任选地，一种或多种SF3B1突变处于选自以下的SF3B1外显子：外显子14、外显子15和外显子16。在一些实施方案中，受试者对一种或多种DNMT3A突变检测呈阳性。任选地，DNMT3A突变中的一种或多种处于DNMT3A外显子。任选地，DNMT3A突变中的一种或多种处于DNMT3A内含子。任选地，DNMT3A突变中的一种或多种处于DNMT3A 5’和/或3’区。任选地，DNMT3A突变中的一种或多种造成由突变DNMT3A基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。任选地，一种或多种DNMT3A突变造成选自以下的一种或多种氨基酸替换：R882C、R882H、P904L和P905P。任选地，一种或多种DNMT3A突变处于选自以下的DNMT3A外显子：外显子10、外显子18和外显子22。任选地，一种或多种DNMT3A突变引入提前终止密码子。

任选地，一种或多种DNMT3A突变选自Y436X和W893X。在一些实施方案中，受试者对一种或多种TET2突变检测呈阳性。任选地，TET2突变中的一种或多种处于TET2外显子。任选地，TET2突变中的一种或多种处于TET2内含子。任选地，TET2突变中的一种或多种处于TET2 5'和/或3'区。任选地，TET2突变中的一种或多种造成由突变TET2基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。任选地，一种或多种TET2突变造成选自以下的一种或多种氨基酸替换：E47Q、Q1274R、W1291R、G1370R、G1370E、N1387S和Y1724H。任选地，一种或多种TET2突变处于选自以下的TET2外显子：外显子1、外显子4、外显子5、外显子6、外显子7、外显子8和外显子9。任选地，一种或多种TET2突变引入提前终止密码子。任选地，一种或多种TET2突变选自R550X、Q1009X、Y1337X、R1404X、R1516X和Q1652X。任选地，受试者患有贫血。任选地，受试者具有不期望的高水平内源性EPO。任选地，受试者先前已用一种或多种EPO受体激动剂治疗。任选地，受试者对EPO受体激动剂反应不充分。任选地，受试者对EPO受体激动剂不再反应。任选地，EPO受体激动剂为EPO。任选地，治疗延迟转化为白血病。任选地，治疗延迟转化为急性髓性白血病。任选地，受试者为前-白血病患者。任选地，治疗增加受试者的红细胞水平和/或血红蛋白水平。任选地，受试者已在ActRII拮抗剂治疗开始前被给予一次或多次血细胞输注。任选地，治疗降低血细胞输注负担。任选地，相对于ActRII拮抗剂治疗开始前的相同时间，治疗降低血细胞输注大于约30%、40%、50%、60%、70%、80%、90%或100% 4-8周。任选地，相对于ActRII拮抗剂治疗开始前的相同时间，治疗降低血细胞输注大于约50% 4-8周。任选地，治疗降低铁超负荷。任选地，治疗降低肝和/或脾脏的铁含量。

[0017] 在某些方面，本公开提供用于治疗或预防骨髓增生异常综合征(MDS)和/或MDS的一种或多种并发症的方法，方法包括给予有需要的受试者有效量的ActRII拮抗剂，其中受试者具有对选自以下的基因的一种或多种突变检测呈阳性的骨髓细胞：SF3B1、DNMT3A和TET2。在一些实施方案中，受试者对一种或多种SF3B1突变检测呈阳性。任选地，突变中的一种或多种处于SF3B1外显子。任选地，SF3B1突变中的一种或多种处于SF3B1内含子。任选地，SF3B1突变中的一种或多种处于SF3B1 5'和/或3'区。任选地，SF3B1突变中的一种或多种造成由突变SF3B1基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。任选地，一种或多种SF3B1突变造成选自以下的一种或多种氨基酸替换：K182E、E491G、R590K、E592K、R625C、R625G、N626D、N626S、H662Y、T663A、K666M、K666Q、K666R、Q670E、G676D、V701I、I704N、I704V、G740R、A744P、D781G、A1188V、N619K、N626H、N626Y、R630S、I704T、G740E、K741N、G742D、D894G、Q903R、R1041H、I1241T、G347V、E622D、Y623C、R625H、R625L、H662D、H662Q、T663I、K666E、K666N、K666T、K700E、V701F和E783K。任选地，一种或多种SF3B1突变处于选自以下的SF3B1外显子：外显子14、外显子14和外显子16。在一些实施方案中，受试者对一种或多种DNMT3A突变检测呈阳性。任选地，DNMT3A突变中的一种或多种处于DNMT3A外显子。任选地，DNMT3A突变中的一种或多种处于DNMT3A内含子。任选地，DNMT3A突变中的一种或多种处于DNMT3A 5'和/或3'区。任选地，DNMT3A突变中的一种或多种造成由突变DNMT3A基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。任选地，一种或多种DNMT3A突变造成选自以下的一种或多种氨基酸替换：R882C、R882H、P904L和P905P。任选地，一种或多种DNMT3A突变处于选自以下的DNMT3A外显子：外显子10、外显子18和外显子22。任选地，一种或多种DNMT3A突变引入提前终止密码子。任选地，一种或多种DNMT3A突变选自Y436X和W893X。在一些实施方案中，受试者对一种或多种TET2突变检测呈阳性。任选地，TET2突变中的一种或多

种处于TET2外显子。任选地，TET2突变中的一种或多种处于TET2内含子。任选地，TET2突变中的一种或多种处于TET2 5’和/或3’区。任选地，TET2突变中的一种或多种造成由突变TET2基因编码的蛋白质的氨基酸发生缺失、添加和/或替换。任选地，一种或多种TET2突变造成选自以下的一种或多种氨基酸替换：E47Q、Q1274R、W1291R、G1370R、G1370E、N1387S和Y1724H。任选地，一种或多种TET2突变处于选自以下的TET2外显子：外显子1、外显子4、外显子5、外显子6、外显子7、外显子8和外显子9。任选地，一种或多种TET2突变引入提前终止密码子。任选地，一种或多种TET2突变选自R550X、Q1009X、Y1337X、R1404X、R1516X和Q1652X。任选地，受试者患有贫血。任选地，受试者具有不期望的高水平内源性EPO。任选地，受试者具有不期望的高水平内源性EPO。任选地，受试者先前已用一种或多种EPO受体激动剂治疗。任选地，受试者对EPO受体激动剂反应不充分。任选地，受试者对EPO受体激动剂不再反应。任选地，EPO受体激动剂为EPO。任选地，治疗延迟转化为白血病。任选地，治疗延迟转化为急性髓性白血病。任选地，治疗增加受试者的红细胞水平和/或血红蛋白水平。任选地，受试者已在ActRII拮抗剂治疗开始前被给予一次或多次血细胞输注。任选地，治疗降低血细胞输注负担。任选地，相对于ActRII拮抗剂治疗开始前的相同时间，治疗降低血细胞输注大于约30%、40%、50%、60%、70%、80%、90%或100% 4-8周。任选地，相对于ActRII拮抗剂治疗开始前的相同时间，治疗降低血细胞输注大于约50% 4-8周。任选地，治疗降低铁超负荷。任选地，治疗降低肝和/或脾脏的铁含量。任选地，受试者具有选自以下的MDS亚型：难治性贫血伴环形铁粒幼红细胞增多(RARS)的MDS、难治性贫血伴环形铁粒幼红细胞和血小板增多(RARS-T)的MDS、难治性血细胞减少伴单系发育不良(RCUD)的MDS、难治性血细胞减少伴多系发育不良和环形铁粒幼红细胞增多(RCMD-RS)的MDS、伴*SF3B1*、*SRSF2*、*DNMT3A*和*TET2*中的一种或多种体细胞突变的MDS、没有*ASXL1*或*ZRSR2*体细胞突变的MDS、伴铁超负荷的MDS及伴嗜中性白血球减少症的MDS。任选地，受试者具有选自以下的国际预后评分系统(International Prognostic Scoring System) (IPSS)分数：低、中等1或中等2。任选地，受试者具有铁粒幼红细胞。任选地，受试者具有作为骨髓红细胞前体在他或她骨髓中的百分数，至少5%、6%、7%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%或95%的铁粒幼红细胞。

[0018] 本公开的ActRII拮抗剂包括例如可抑制信号转导通路的ActRII受体(例如ActRIIA和/或ActRIIB受体)介导的激活(例如经细胞内介质比如SMAD 1、2、3、5和/或8的信号转导激活)的试剂、可抑制一种或多种ActRII配体(例如激活素A、激活素B、激活素AB、激活素C、激活素E、GDF11、GDF8、BMP6、BMP7、Nodal等)例如结合和/或激活ActRII受体的试剂、抑制ActRII配体和/或ActRII受体表达(例如转录、翻译、细胞分泌或其组合)的试剂以及可抑制ActRII信号通路的一种或多种细胞内介质(例如SMADs 1、2、3、5和/或8)的试剂。

[0019] 具体地讲，本公开提供使用ActRII拮抗剂或ActRII拮抗剂的组合，治疗或预防MDS和铁粒幼红细胞性贫血的一种或多种并发症(包括例如贫血、嗜中性白血球减少症、脾肿大、输血需求、急性髓性白血病的发展、铁超负荷及铁超负荷的并发症，其中有充血性心力衰竭、心律失常、心肌梗死、其他形式的心脏病、糖尿病、呼吸困难、肝脏疾病，以及铁螯合疗法的不良反应及其他实例)的方法，以在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血

需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和/或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍。

[0020] 具体地讲,本公开提供使用ActRII拮抗剂或ActRII拮抗剂的组合,在MDS的亚型治疗或预防并发症的方法,包括骨髓中成红细胞数目升高(细胞过多)的MDS患者;在具有作为红细胞前体在骨髓中的百分数多于1%、2%、3%、4%、5%、6%、7%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%或95%的铁粒幼红细胞的MDS患者;在难治性贫血伴环形铁粒幼红细胞增多(RARS)的MDS患者;在难治性贫血伴环形铁粒幼红细胞和血小板增多(RARS-T)的MDS患者;在难治性血细胞减少伴单系发育不良(RCUD)的MDS患者;在难治性血细胞减少伴多系发育不良和环形铁粒幼红细胞增多(RCMD-RS)的MDS患者;在伴*SF3B1*、*SRSF2*、*DNMT3A*、*TET2*或*SETBP1*体细胞突变的MDS患者;在没有*ASXL1*或*ZRSR2*体细胞突变的MDS患者;在伴铁超负荷的MDS患者及在伴嗜中性白血球减少症的MDS患者。

[0021] 还具体地讲,本公开提供使用ActRII拮抗剂或ActRII拮抗剂的组合,治疗或预防铁粒幼红细胞性贫血的并发症的方法,包括难治性贫血伴环形铁粒幼红细胞增多(RARS)、难治性贫血伴环形铁粒幼红细胞和血小板增多(RARS-T)、难治性血细胞减少伴多系发育不良和环形铁粒幼红细胞增多(RCMD-RS)、与酗酒有关的铁粒幼细胞性贫血、药物诱发的铁粒幼细胞性贫血、铜缺乏引起的铁粒幼细胞性贫血(锌毒性)、低温引起的铁粒幼细胞性贫血、遗传性铁粒幼细胞性贫血(X-linked sideroblastic anemia) (XLSA)、SLC25A38缺乏、谷氧还蛋白5缺乏、红细胞生成性原卟啉病、遗传性铁粒幼细胞性贫血伴共济失调(X-linked sideroblastic anemia with ataxia) (XLSA/A)、铁粒幼细胞性贫血伴B细胞免疫缺陷、发热及发育迟缓(SIFD);皮尔森骨髓胰腺综合征、肌病、乳酸性酸中毒和铁粒幼红细胞性贫血(MLASA);硫胺素反应性巨幼细胞性贫血(TRMA)以及不明原因的综合征性/非综合征性铁粒幼细胞性贫血。

[0022] 在某些实施方案中,要按本文公开的方法使用的优选的ActRII拮抗剂为结合和/或抑制GDF11和/或GDF8的试剂(例如抑制GDF11-和/或GDF8-介导的ActRIIA和/或ActRIIB信号转导,比如SMAD 2/3信号传递的激活的试剂)。这种拮抗剂统称为GDF-ActRII拮抗剂。任选地,这种GDF-ActRII拮抗剂可进一步抑制激活素A、激活素B、激活素AB、激活素C、激活素E、GDF11、GDF8、BMP6、BMP7和Nodal中的一种或多种。因此,在一些实施方案中,本公开提供使用一种或多种ActRII拮抗剂(包括例如可溶性ActRIIA多肽、可溶性ActRIIB多肽、GDF捕获多肽、抗-ActRIIA抗体、抗-ActRIIB抗体、抗-ActRII配体抗体(例如抗-GDF11抗体、抗-GDF8抗体、抗-激活素A抗体、抗-激活素B抗体、抗-激活素AB抗体、抗-激活素C抗体、抗-激活素E抗体、抗-BMP6抗体、抗-BMP7抗体和抗-Nodal抗体)、ActRIIA的小分子抑制剂、ActRIIB的小分子抑制剂、一种或多种ActRII配体的小分子抑制剂(例如激活素A、激活素B、激活素AB、激活素C、激活素E、GDF11、GDF8、BMP6、BMP7、Nodal等)、ActRIIA的抑制剂核苷酸(基于核苷酸的抑制剂)、ActRIIB的抑制剂核苷酸、一种或多种ActRII配体的抑制剂核苷酸(例如激活素A、激活素B、激活素AB、激活素C、激活素E、GDF11、GDF8、BMP6、BMP7、Nodal等)或其组合)的方法,以在有需要的受试者增加红细胞水平和/或血红蛋白水平、在有需要的受试者治疗或预防贫血、在有需要的受试者治疗铁粒幼红细胞性贫血或MDS,在有需要的受试者治疗铁

粒幼红细胞性贫血或MDS的一种或多种并发症，以及作为其他实例，在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍。在某些实施方案中，要按本文公开的方法使用的ActRII拮抗剂基本上不结合和/或抑制激活素A (例如激活素A介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。

[0023] 在某种程度上，本公开证实ActRII拮抗剂，包括变体，细胞外(可溶性) ActRIIB结构域，其结合并抑制GDF11活性(例如GDF11-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递)，可用于增加体内红细胞的水平、治疗各种病症/障碍引起的贫血和治疗铁粒幼红细胞性贫血或MDS。因此，在某些实施方案中，要按本文公开的方法(例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*突变有关的障碍等的方法)使用的优选的ActRII拮抗剂为结合和/或抑制GDF11 (例如GDF11-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)的可溶性ActRII多肽(例如可溶性ActRIIA或ActRIIB多肽)。尽管可溶性ActRIIA和可溶性ActRIIB ActRII拮抗剂可通过非GDF11拮抗的机制影响红细胞形成和/或形态学，但是本公开证实，关于本文公开的方法合乎需要的治疗剂，可基于GDF11拮抗或ActRII拮抗或两者进行选择。任选地，这种可溶性ActRII多肽拮抗剂可进一步结合和/或抑制GDF8 (例如抑制GDF8-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。在一些实施方案中，结合和/或抑制GDF11和/或GDF8的本公开可溶性ActRIIA和ActRIIB多肽，可进一步结合和/或抑制一种或多种另外的选自以下的ActRII配体:激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和Nodal。

[0024] 在某些方面，本公开提供为变体ActRII多肽(例如ActRIIA和ActRIIB多肽)，包括具有氨基-和羧基-末端截短和/或其他序列改变(一种或多种氨基酸替换、添加、缺失或其组合)的ActRII多肽的GDF捕获物。任选地，本发明的GDF捕获物可被设计为优先拮抗ActRII受体的一种或多种配体，比如GDF8 (也称为肌肉生长抑制素)、GDF11、Nodal、BMP6和BMP7(也称为OP-1)。如本文公开的那样，GDF捕获物的实例包括一组源自ActRIIB，对激活素特别是激活素A的亲和力大大减弱的变体。这些变体对红细胞呈现合乎需要的效果，同时减少对其他组织的影响。这种变体的实例包括在相应于SEQ ID NO:1的79位的位置具有酸性氨基酸[例如天冬氨酸(D)或谷氨酸(E)]的那些变体。在某些实施方案中，要按本文公开的方法(例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍等的方法)使用的优选的

GDF捕获物结合和/或抑制GDF11。任选地，这种GDF捕获物可进一步结合和/或抑制GDF8。在一些实施方案中，结合和/或抑制GDF11和/或GDF8的GDF捕获物可进一步结合和/或抑制一种或多种另外的ActRII配体(例如激活素B、激活素E、BMP6、BMP7和Nodal)。在一些实施方案中，要按本文公开的方法使用的GDF捕获物基本上不结合和/或抑制激活素A (例如激活素A-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。在某些实施方案中，GDF捕获多肽包含其包含、由以下组成或基本由以下组成的氨基酸序列：SEQ ID NOs: 36、37、41、44、45、50或51的氨基酸序列，及与上述中任何一种至少80%、85%、90%、95%、96%、97%、98%或99%相同的多肽。在其他的实施方案中，GDF捕获多肽包含其包含、由以下组成或基本上由以下组成的氨基酸序列：SEQ ID NOs: 2、3、4、5、6、10、11、22、26、28、29、31或49的氨基酸序列，及与上述中任何一种至少80%、85%、90%、95%、96%、97%、98%或99%相同的多肽。在仍然其他的实施方案中，GDF捕获多肽包含其包含以下的氨基酸序列：SEQ ID NOs: 2、3、4、5、6、29、31或49的氨基酸序列，及与上述中任何一种至少80%、85%、90%、95%、96%、97%、98%或99%相同的多肽，其中在相应于SEQ ID NO: 1、4或50的79的位置为酸性氨基酸。GDF捕获物可包括天然ActRII多肽的功能片段，比如包含选自SEQ ID NOs: 1、2、3、4、5、6、9、10、11或49的序列或SEQ ID NO: 2、5、10、11或49的序列，缺乏C-末端1、2、3、4、5或10-15个氨基酸和缺乏N-末端1、2、3、4或5个氨基酸的至少10、20或30个氨基酸的片段。相对于SEQ ID NO: 2或5，优选的多肽包含N-末端的2-5个氨基酸和C-末端的不多于3个氨基酸的截短。用于这种制备的优选的GDF捕获物由以下组成，或基本上由SEQ ID NO: 36的氨基酸序列组成。

[0025] 任选地，包含改变的ActRII配体结合域的GDF捕获物的激活素A结合K_d与GDF11和/或GDF8结合K_d的比率，相对于野生型配体结合域的比率至少大2-、5-、10-、20、50-、100-或者甚至1000-倍。任选地，包含改变的配体结合域的GDF捕获物的抑制激活素A的IC_{50}与抑制GDF11和/或GDF8的IC_{50}的比率，相对于野生型ActRII配体结合域至少大2-、5-、10-、20-、25-、50-、100-或者甚至1000-倍。任选地，包含改变的配体结合域的GDF捕获物，抑制GDF11和/或GDF8的IC_{50}比抑制激活素A的IC_{50}小至少2、5、10、20、50或者甚至100倍。这些GDF捕获物可为包含免疫球蛋白Fc结构域(野生型或突变型)的融合蛋白。在某些情况下，研究对象可溶性GDF捕获物为GDF8和/或GDF11的拮抗剂(抑制剂)。

[0026] 在某些方面，本公开提供包含改变的配体结合(例如GDF11-结合)域为可溶性ActRIIB多肽的GDF捕获物。具有改变的配体结合域的GDF捕获物，可在氨基酸残基比如人ActRIIB的E37、E39、R40、K55、R56、Y60、A64、K74、W78、L79、D80、F82和F101(编号为相对于SEQ ID NO: 1)包含例如一个或多个突变。任选地，相对于ActRIIB受体的野生型配体结合域，改变的配体结合域可增加对配体比如GDF8/GDF11的选择性。为了说明起见，本文证实以下这些突变增加改变的配体结合域对GDF11 (并因此可假定对GDF8)超过以下激活素的选择性：K74Y、K74F、K74I、L79D、L79E和D80I。以下突变具有相反的效果，增加激活素结合的比率超过GDF11: D54A、K55A、L79A和F82A。通过包含“尾”区，或者可推测为非结构化连接区，以及通过利用K74A突变，可增加整体(GDF11和激活素)结合活性。造成配体亲和力整体下降的其他突变包括：R40A、E37A、R56A、W78A、D80K、D80R、D80A、D80G、D80F、D80M和D80N。突变可结合起来达到预期效果。例如，影响GDF11: 激活素结合比率的许多这些突变对配体结合具有整体的负面影响，并因此可把这些突变与通常增加配体结合的突变结合起来，以产生具有配体选择性的改良结合蛋白。在示例性的实施方案中，GDF捕获物为包含任选地与另外的

氨基酸替换、添加或缺失组合的L79D或L79E突变的ActRIIB多肽。

[0027] 在某些实施方案中，要按本文公开的方法使用的ActRII拮抗剂为ActRIIB多肽或基于ActRIIB的GDF捕获多肽。通常这种ActRIIB多肽和基于ActRIIB的GDF捕获多肽为可溶性多肽，其包含源自SEQ ID NO:1、4或49的ActRIIB序列的一部分/域，特别是细胞外，源自SEQ ID NO:1、4或49的ActRIIB序列的配体结合部分/域。在一些实施方案中，源自ActRIIB的部分对应于一种序列，这种序列在SEQ ID NO:1或4的氨基酸21-29中的任何一个(例如21、22、23、24、25、26、27、28或29)开始[任选地在SEQ ID NO:1或4的22-25 (例如22、23、24或25)开始]，和在SEQ ID NO:1或4的氨基酸109-134中的任何一个(例如109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134)结尾。在一些实施方案中，源自ActRIIB的部分对应于一种序列，这种序列在SEQ ID NO:1或4的氨基酸20-29中的任何一个(例如20、21、22、23、24、25、26、27、28或29)开始[任选地在SEQ ID NO:1或4的22-25 (例如22、23、24或25)开始]，和在SEQ ID NO:1或4的氨基酸109-133中的任何一个(例如109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129、130、131、132或133)结尾。在一些实施方案中，源自ActRIIB的部分对应于一种序列，这种序列在SEQ ID NO:1或4的氨基酸20-24中的任何一个(例如20、21、22、23或24)开始[任选地在SEQ ID NO:1或4的22-25 (例如22、23、24或25)开始]，和在SEQ ID NO:1或4的氨基酸109-133中的任何一个(例如109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129、130、131、132或133)结尾。在一些实施方案中，源自ActRIIB的部分对应于一种序列，这种序列在SEQ ID NO:1或4的氨基酸21-24中的任何一个(例如21、22、23或24)开始，和在SEQ ID NO:1或4的氨基酸109-134中的任何一个(例如109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134)结尾。在一些实施方案中，源自ActRIIB的部分对应于一种序列，这种序列在SEQ ID NO:1或4的氨基酸20-24中的任何一个(例如20、21、22、23或24)开始，和在SEQ ID NO:1或4的氨基酸118-133中的任何一个(例如118、119、120、121、122、123、124、125、126、127、128、129、130、131、132或133)结尾。在一些实施方案中，源自ActRIIB的部分对应于一种序列，这种序列在SEQ ID NO:1或4的氨基酸21-24中的任何一个(例如21、22、23或24)开始，和在SEQ ID NO:1或4的氨基酸118-134中的任何一个(例如118、119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134)结尾。在一些实施方案中，源自ActRIIB的部分对应于一种序列，这种序列在SEQ ID NO:1或4的氨基酸20-24中的任何一个(例如20、21、22、23或24)开始，和在SEQ ID NO:1或4的氨基酸128-133中的任何一个(例如128、129、130、131、132或133)结尾。在一些实施方案中，源自ActRIIB的部分对应于一种序列，这种序列在SEQ ID NO:1或39的氨基酸20-24中的任何一个(例如20、21、22、23或24)开始，和在SEQ ID NO:1或39的氨基酸128-133中的任何一个(例如128、129、130、131、132或133)结尾。在一些实施方案中，源自ActRIIB的部分对应于一种序列，这种序列在SEQ ID NO:1或4的氨基酸21-29中的任何一个(例如21、22、23、24、25、26、27、28或29)开始，和在SEQ ID NO:1或4的氨基酸118-134中的任何一个(例如118、119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134)结尾。在一些实施方案中，源自ActRIIB的部分对应于一种序列，这种序列在SEQ ID NO:1或4的氨基酸20-29中的任何一个(例如20、21、22、23、24、25、26、27、28或29)开始，和在

SEQ ID NO:1或4的氨基酸118-133中的任何一个(例如118、119、120、121、122、123、124、125、126、127、128、129、130、131、132或133)结尾。在一些实施方案中,源自ActRIIB的部分对应于一种序列,这种序列在SEQ ID NO:1或4的氨基酸21-29中的任何一个(例如21、22、23、24、25、26、27、28或29)开始,和在SEQ ID NO:1或4的氨基酸128-134中的任何一个(例如128、129、130、131、132、133或134)结尾。在一些实施方案中,源自ActRIIB的部分对应于一种序列,这种序列在SEQ ID NO:1或4的氨基酸20-29中的任何一个(例如20、21、22、23、24、25、26、27、28或29)开始,和在SEQ ID NO:1或4的氨基酸128-133中的任何一个(例如128、129、130、131、132或133)结尾。出人意外地,在SEQ ID NO:1或4的22-25 (例如22、23、24或25)开始的ActRIIB和基于ActRIIB的GDF捕获物构建体具有比包含人ActRIIB的完整细胞外结构域的蛋白更高的活性水平。在一个优选的实施方案中,ActRIIB多肽和基于ActRIIB的GDF捕获多肽包含、基本上由以下组成、或由在SEQ ID NO: 1或4的25位氨基酸开始和在SEQ ID NO: 1或4的131位氨基酸结尾的氨基酸序列组成。上述ActRIIB多肽或基于ActRIIB的GDF捕获多肽中的任何一种可作为同源二聚体产生。上述ActRIIB多肽或基于ActRIIB的GDF捕获多肽中的任何一种可进一步包含其含有IgG重链恒定区比如Fc结构域的异源性部分。任何一种以上基于ActRIIB的GDF捕获多肽,可任选地与相对于SEQ ID NO: 1的一种或多种另外的氨基酸替换、缺失或插入组合,在相应于SEQ ID NO: 1的79位的位置包含酸性氨基酸。以上ActRIIB多肽或基于ActRIIB的GDF捕获多肽中的任何一种,包括其同源二聚体和/或融合蛋白,可在基于细胞的试验中结合和/或抑制经激活素的信号传递(例如激活素A、激活素B、激活素C或激活素AB)。以上ActRIIB多肽或基于ActRIIB的GDF捕获多肽中的任何一种,包括其同源二聚体和/或融合蛋白,可在基于细胞的试验中结合和/或抑制经GDF11和/或GDF8的信号传递。任选地,以上ActRIIB多肽或基于ActRIIB的GDF捕获多肽中的任何一种,包括其同源二聚体和/或融合蛋白,可在基于细胞的试验中结合和/或抑制激活素B、激活素C、激活素E、BMP6、BMP7和Nodal中一种或多种的信号传递。

[0028] 考虑其他ActRIIB多肽和基于ActRIIB的GDF捕获多肽,比如以下多肽。ActRIIB多肽或GDF捕获多肽,包含与SEQ ID NO: 1或4的氨基酸29-109序列至少80% (例如85%、90%、95%、96%、97%、98%、99%或100%)相同的氨基酸序列,其中相应于SEQ ID NO: 1的64的位置为R或K,和其中ActRIIB多肽或基于ActRIIB的GDF捕获多肽在基于细胞的试验中抑制经激活素、GDF8和/或GDF11的信号传递。如上ActRIIB多肽或基于ActRIIB的GDF捕获多肽,其中对于SEQ ID NO: 1或4的序列的至少一个改变位于配体结合口袋的外面。如上ActRIIB多肽或基于ActRIIB的GDF捕获多肽,其中对于SEQ ID NO: 1或4的序列的至少一个改变为位于配体结合口袋中的保守性改变。如上ActRIIB多肽或基于ActRIIB的GDF捕获多肽,其中对于SEQ ID NO: 1或4的序列的至少一个改变为选自K74、R40、Q53、K55、F82和L79的一个或多个位置的改变。

[0029] 考虑其他ActRIIB多肽和基于ActRIIB的GDF捕获多肽,比如以下多肽。ActRIIB多肽或基于ActRIIB的GDF捕获多肽,包含与SEQ ID NO: 1或4的氨基酸29-109序列至少80%(例如85%、90%、95%、96%、97%、98%、99%或100%)相同的氨基酸序列,和其中蛋白在非内源性ActRIIB的N-X-S/T序列的位置和在配体结合口袋外面的位置,包含至少一个N-X-S/T序列。如上ActRIIB多肽或基于ActRIIB的GDF捕获多肽,其中ActRIIB多肽或基于ActRIIB的GDF捕获多肽,在相应于SEQ ID NO: 1或4的24位的位置包含N和在相应于SEQ ID NO: 1或4的26

位的位置包含S或T，和其中ActRIIB多肽或基于ActRIIB的GDF捕获多肽，在基于细胞的试验中抑制经激活素、GDF8和/或GDF11的信号传递。如上ActRIIB多肽或基于ActRIIB的GDF捕获多肽，其中ActRIIB多肽或基于ActRIIB的GDF捕获多肽在相应于SEQ ID NO: 1或4的64位的位置包含R或K。如上ActRIIB多肽或基于ActRIIB的GDF捕获多肽，其中ActRIIB多肽或基于ActRIIB的GDF捕获多肽在相应于SEQ ID NO: 1或4的79位的位置包含D或E，和其中ActRIIB多肽或基于ActRIIB的GDF捕获多肽，在基于细胞的试验中抑制经激活素、GDF8和/或GDF11的信号传递。如上ActRIIB多肽或基于ActRIIB的GDF捕获多肽，其中对于SEQ ID NO: 1或4的序列的至少一个改变为位于配体结合口袋中的保守性改变。如上ActRIIB多肽或基于ActRIIB的GDF捕获多肽，其中对于SEQ ID NO: 1或4的序列的至少一个改变为选自K74、R40、Q53、K55、F82和L79的一个或多个位置的改变。以上ActRIIB多肽或基于ActRIIB的GDF捕获多肽，其中ActRIIB多肽或基于ActRIIB的GDF捕获多肽为进一步包含一个或多个异源性部分的融合蛋白。以上ActRIIB多肽或基于ActRIIB的GDF捕获多肽或其融合蛋白中的任何一种可作为同源二聚体产生。以上ActRIIB融合蛋白或基于ActRIIB的GDF捕获物融合蛋白中的任何一种可具有其包含IgG重链恒定区比如Fc结构域的异源性部分。

[0030] 在某些实施方案中，用于本文公开的方法用途的优选的ActRIIB多肽，包含氨基酸序列(其包含、由以下组成或基本上由SEQ ID NOs: 2、3、5、6、29、31或49的氨基酸序列组成)及与上述任何一种至少80%、85%、90%、95%、96%、97%、98%或99%相同的多肽。ActRIIB多肽可包括天然ActRIIB多肽的功能片段，比如包含选自SEQ ID NOs: 2、3、5、6、29、31或49的序列或SEQ ID NO: 2或5的序列，缺乏C-末端1、2、3、4、5或10-15个氨基酸和缺乏N-末端1、2、3、4或5个氨基酸的至少10、20或30个氨基酸的片段。相对于SEQ ID NO: 2或5，优选的多肽包含N-末端的2-5个氨基酸和C-末端的不多于3个氨基酸的截短。用于本文描述的方法用途的优选的GDF捕获物由以下组成，或基本上由SEQ ID NO: 29的氨基酸序列组成。

[0031] 活性(例如配体结合) ActRII多肽的通式为包括在SEQ ID NO:9的氨基酸30开始和在氨基酸110结尾的多肽的通式。因此，本公开的ActRIIA多肽和基于ActRIIA的GDF捕获物可包含、由以下组成或基本上由与SEQ ID NO:9的氨基酸30-110至少80%、85%、90%、95%、96%、97%、98%、99%或100%相同的多肽组成。任选地，本公开的ActRIIA多肽和基于ActRIIA的GDF捕获多肽包含、由以下组成或基本上由与SEQ ID NO:9的氨基酸12-82至少80%、85%、90%、95%、96%、97%、98%、99%或100%相同，任选地分别在SEQ ID NO:9的1-5 (例如1、2、3、4或5)或3-5 (例如3、4或5)范围内的位置开始和在110-116 (例如110、111、112、113、114、115或116)或110-115 (例如110、111、112、113、114或115)范围内的位置结尾的多肽组成，并且相对于SEQ ID NO:9在配体结合口袋中包含不多于1、2、5、10或15个保守性氨基酸改变，和在配体结合口袋的40、53、55、74、79和/或82位包含0、1或多个非保守性改变。上述ActRIIA多肽或基于ActRIIA的GDF捕获多肽中的任何一种可作为同源二聚体产生。上述ActRIIA多肽或基于ActRIIA的GDF捕获多肽中的任何一种可进一步包含其含有IgG重链恒定区比如Fc结构域的异源性部分。以上ActRIIA多肽或基于ActRIIA的GDF捕获多肽中的任何一种，包括其同源二聚体和/或融合蛋白，可在基于细胞的试验中结合和/或抑制经激活素(例如激活素A、激活素B、激活素C或激活素AB)的信号传递。以上ActRIIA多肽或基于ActRIIA的GDF捕获多肽中的任何一种，包括其同源二聚体和/或融合蛋白，可在基于细胞的试验中结合和/或抑制经GDF11和/或GDF8的信号传递。任选地，以上ActRIIA多肽或基于ActRIIB的GDF捕获

多肽中的任何一种，包括其同源二聚体和/或融合蛋白，可在基于细胞的试验中结合和/或抑制激活素B、激活素C、激活素E、GDF7和Nodal中一种或多种的信号传递。

[0032] 在某些实施方案中，用于本文公开的方法用途的优选的ActRIIA多肽和基于ActRIIA的GDF捕获多肽，包含氨基酸序列(其含有、由以下组成或基本上由SEQ ID NOs: 9、10、22、26或28的氨基酸序列组成)和与上述中任何一种至少80%、85%、90%、95%、96%、97%、98%或99%相同的多肽。ActRIIA多肽或基于ActRIIA的GDF捕获多肽可包括天然ActRIIA多肽的功能片段，比如包含选自SEQ ID NOs: 9、10、22、26或28的序列或SEQ ID NO: 10的序列，缺乏C-末端1、2、3、4、5或10-15个氨基酸和缺乏N-末端1、2、3、4或5个氨基酸的至少10、20或30个氨基酸的片段。相对于SEQ ID NO: 10，优选的多肽包含N-末端的2-5个氨基酸和C-末端的不多于3个氨基酸的截短。用于本文描述的方法用途的优选的ActRIIA多肽由以下组成，或基本上由SEQ ID NO: 26或28的氨基酸序列组成。

[0033] 本公开的ActRII多肽(例如ActRIIA或ActRIIB多肽)或GDF捕获多肽，相对于天然ActRII多肽，可在ActRII多肽的氨基酸序列(例如在配体结合域)包括一种或多种改变(例如氨基酸添加、缺失、替换或其组合)。相对于天然ActRII多肽，当在哺乳动物、昆虫或其他真核细胞产生时，氨基酸序列的改变可例如改变多肽的糖基化，或者改变多肽的溶蛋白性裂解。

[0034] 任选地，本公开的ActRII多肽(例如ActRIIA或ActRIIB多肽)或GDF捕获多肽包含一个或多个选自以下的修饰的氨基酸残基：糖化氨基酸、聚乙二醇化氨基酸、法尼基化氨基酸、乙酰化氨基酸、生物素化的氨基酸、缀合于脂质部分的氨基酸及缀合于有机衍生化试剂的氨基酸。

[0035] 在一些实施方案中，本公开的ActRII多肽(例如ActRIIA或ActRIIB多肽)或GDF捕获多肽可为一种融合蛋白，其作为一个结构域，具有ActRII多肽或GDF捕获多肽(例如ActRII受体的配体结合域，任选地具有一种或多种序列变异)和一种或多种另外的提供合乎需要的性能比如改善的药物代谢动力学、更易于纯化、靶向特定组织等的异源结构域。例如，融合蛋白结构域可增强体内稳定性、体内半衰期、摄取/给药、组织定位或分布、蛋白复合物形成、融合蛋白多聚化和/或纯化中的一种或多种。ActRII多肽和GDF捕获物融合蛋白可包括免疫球蛋白Fc结构域(野生型或突变型)或血清白蛋白。在某些实施方案中，ActRII多肽和GDF捕获物融合蛋白包含位于Fc结构域与ActRII或GDF捕获物结构域之间的相对非结构化连接子。这种非结构化连接子可能对应于ActRII或GDF捕获物的细胞外结构域C-末端(“尾部”)的大约15个氨基酸的非结构化区域，或者其可为3-5、15、20、30、50或更多个氨基酸之间的相对无二级结构的人工序列。连接子可富含甘氨酸和脯氨酸残基，并可例如含有苏氨酸/丝氨酸和甘氨酸的重复序列[例如TG_4 (SEQ ID NO:52)、TG_3 (SEQ ID NO:53)或SG_4 (SEQ ID NO:54)单重或重复]或者一系列的3个甘氨酸。融合蛋白可包括纯化子序列，比如表位标签、FLAG标签、多组氨酸序列和GST融合物。在某些实施方案中，ActRII融合蛋白或GDF捕获融合物包含前导序列。前导序列可为天然的ActRII前导序列(例如天然ActRIIA或ActRIIB前导序列)或异源前导序列。在某些实施方案中，前导序列为组织纤溶酶原激活物(TPA)前导序列。在一些实施方案中，ActRII融合蛋白或GDF捕获物融合蛋白包含如在式A-B-C中阐述的氨基酸序列。B部分为如本文描述的N-和C-末端截短的ActRII或GDF捕获多肽。A和C部分可独立地为0、1或多于1个氨基酸，并且A和C两个部分为与B异源。A和/或C部分

可经连接序列附着于B部分。

[0036] 任选地，用于本文公开的方法用途的ActRII多肽(例如ActRIIA和ActRIIB多肽)或GDF捕获多肽，包括其变体和融合蛋白，结合一种或多种ActRIIB配体(例如激活素A、激活素B、激活素AB、激活素C、激活素E、GDF11、GDF8、BMP6、BMP7和/或Nodal)，Kd小于10微摩尔、小于1微摩尔、小于100纳摩尔、小于10纳摩尔或小于1纳摩尔。任选地，这种ActRII多肽或GDF捕获多肽抑制ActRII信号传递，比如ActRII配体触发的ActRIIA和/或ActRIIB细胞内信号转导事件(例如SMAD 2/3和/或SMAD 1/5/8信号传递)。

[0037] 在某些方面，本公开提供包含本公开的ActRII拮抗剂(例如ActRIIA多肽、ActRIIB多肽、GDF捕获多肽)和药学上可接受的载体的药用制剂。药用制剂还可包含一种或多种另外的化合物，比如用于治疗本文描述的障碍或病症的化合物(例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*突变有关的障碍的另外的化合物)。优选地，本公开的药用制剂为基本上无热原的。通常，优选的是ActRIIA多肽、ActRIIB多肽或GDF捕获多肽在哺乳动物细胞系表达，其适当地介导多肽的天然糖基化，以减少患者不利免疫反应的可能性。人类和CHO细胞系已被成功应用，并且预计其他常见的哺乳动物表达载体将是有用的。在一些实施方案中，优选的ActRIIA多肽、ActRIIB多肽和GDF捕获多肽被糖基化，并具有可得自哺乳动物细胞，优选地得自CHO细胞的糖基化模式。在某些实施方案中，本公开提供包装的药品，这种药品包含本文描述的药用制剂，并被标注用于以下中的一种或多种：在哺乳动物(优选地为人)增加红细胞水平和/或血红蛋白、在哺乳动物(优选地为人)治疗或预防贫血、在哺乳动物(优选地为人)治疗铁粒幼红细胞性贫血或MDS和/或在哺乳动物(优选地为人)治疗或预防铁粒幼红细胞性贫血或MDS的一种或多种并发症(例如贫血、血管阻塞危象等)或者在哺乳动物(优选地为人)治疗或预防与*SF3B1*突变有关的障碍。

[0038] 在某些方面，本公开提供编码ActRIIA多肽(例如ActRIIA或ActRIIB多肽)或GDF捕获多肽的核酸。分离的多核苷酸可包含可溶性ActRII多肽或GDF捕获多肽的编码序列，比如本文描述的。例如，分离的核酸可包括编码ActRII多肽或GDF捕获物(包含ActRII多肽的细胞外结构域(例如配体结合域)，具有一种或多种序列变异))的序列和编码ActRII多肽的部分或全部跨膜域和/或胞浆域的序列，但是终止密码子位于跨膜域或胞浆域内，或者位于细胞外结构域与跨膜域或胞浆域之间。例如，编码GDF捕获物的分离的多核苷酸可包含全长ActRII多核苷酸序列比如SEQ ID NO: 1、4或9，或具有一种或多种变异，或为部分截短的版本，所述分离的多核苷酸在3'-末端之前进一步包含至少600个核苷酸的转录终止密码子，或者在其他位置，以使多核苷酸的翻译产生任选地融合于全长ActRII的截短部分的细胞外结构域。本文公开的核酸可切实可行地连接于表达启动子，并且本公开提供用这种重组多核苷酸转化的细胞。优选地，细胞为哺乳动物细胞，比如CHO细胞。

[0039] 在某些方面，本公开提供用于制备ActRII多肽或GDF捕获物的方法。这种方法可包括在合适的细胞，比如中国仓鼠卵巢(CHO)细胞表达本文公开的任何一种核酸(例如SEQ ID NO: 8、13、27、32、39、42、46或48)。这种方法可包括：a) 在适合于表达GDF捕获多肽的条件

下培养细胞，其中所述细胞用GDF捕获物表达构建体转化；和b) 回收如此表达的GDF捕获多肽。GDF捕获多肽可作为粗品、采用自细胞培养物获得蛋白的任何一种熟知技术部分纯化或高度纯化的部分进行回收。

[0040] 在某些方面，本公开涉及拮抗ActRII活性(例如抑制ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3和/或SMAD 1/5/8信号传递)的抗体或抗体组合。具体地讲，本公开提供采用抗体ActRII拮抗剂或抗体ActRII拮抗剂的组合，以例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍的方法。

[0041] 在某些实施方案中，优选的本公开抗体ActRII拮抗剂为结合和/或抑制至少GDF11的活性(例如GDF11-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)的抗体或抗体组合。任选地，抗体或抗体组合进一步结合和/或抑制GDF8的活性(例如GDF8-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)，特别是在对GDF11和GDF8两者具有亲和力的多特异性抗体的情况下，或者在一种或多种抗-GDF11抗体和一种或多种抗-GDF8抗体组合的情况下。任选地，本公开的抗体或抗体组合基本上不结合和/或抑制激活素A的活性(例如激活素A-介导的ActRIIA或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。在一些实施方案中，结合和/或抑制GDF11和/或GDF8的活性的本公开抗体或抗体组合进一步结合和/或抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和Nodal中一种或多种的活性(例如ActRIIA或ActRIIB 信号转导，比如SMAD 2/3和/或SMAD 1/5/8信号传递的激活)，特别是在对多种ActRII配体具有亲和力的多特异性抗体的情况下，或者在组合多种抗体-每一种对不同的ActRII配体具有亲和力的情况下。

[0042] 在某种程度上，本公开证实ActRII拮抗剂可与EPO受体激活剂组合使用(例如同时或不同时，但是通常以获得叠加的药理作用这样的方式给予)，以增加红细胞水平(红细胞生成)，或者作为实例，在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍。在某种程度上，本公开证实GDF捕获物可与EPO受体激活剂组合给予，以协同增加患者，特别是铁粒幼红细胞性贫血或MDS患者的红细胞形成。因此，这种联合治疗的效果可以明显大于ActRII拮抗剂与EPO受体激活剂以其各自剂量分别给予时的效果总和。在某些实施方案中，这种协同作用可能是有利的，因为这使得能够用较低剂量的EPO受体激活剂达到目标红细胞水平，从而避免潜在的不良反应或与较高水平的EPO受体激活有关的其他问题。因此，在某些实施方案中，本公开的方法(例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治

疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍的方法)，包括给予有需要的患者与一种或多种EPO受体激活剂组合的一种或多种ActRII拮抗剂(例如ActRIIA多肽、ActRIIB多肽和/或GDF捕获多肽)。

[0043] EPO受体激活剂可通过直接接触和激活EPO受体而刺激红细胞生成。在某些实施方案中，EPO受体激活剂为一类基于165个氨基酸的天然EPO序列并通常称为红细胞生成刺激剂(ESAs)的化合物之一，其实例为依泊汀α、依泊汀β、依泊汀δ和依泊汀ω。在其他的实施方案中，ESAs包括合成的EPO蛋白(SEPs)和具有非肽类修饰，赋予合乎需要的药代动力学性质(延长的循环半衰期)的EPO衍生物，其实例为达贝泊汀α和甲氧基聚乙二醇依泊汀β。在某些实施方案中，EPO受体激活剂可为不包含EPO多肽骨架或通常不归类为ESA的EPO受体激动剂。这种EPO受体激动剂可非限制性地包括EPO的肽类和非肽类拟似物、靶向EPO受体的激动性抗体、包含EPO模拟结构域的融合蛋白及促红细胞生成素受体延长期限制激动剂(EREDLA)。

[0044] 在某些实施方案中，EPO受体激活剂可通过增强内源性EPO的产生而不接触EPO受体本身间接刺激红细胞生成。例如，缺氧诱导转录因子(HIFs)为EPO基因表达的内源性刺激剂，其在常氧条件下通过细胞调控机制受到抑制(失去稳定)。在某种程度上，通过与GDF捕获物和具有HIF稳定性能的间接EPO受体激活剂比如脯氨酰羟化酶抑制剂联合治疗，本公开增加患者的红细胞生成。

[0045] 在某些情况下，当给予GDF捕获多肽用于这些其他的治疗适应症时，可能合乎需要的是监测给予ActRII拮抗剂期间对红细胞的影响，或者确定或调整ActRII拮抗剂的剂量，以减少对红细胞不期望的影响。例如，增加红细胞水平、血红蛋白水平或血细胞比容水平可能造成血压升高。

[0046] 附图简述

所递交的该专利或专利申请包含至少一张着色绘图。带有彩图的该专利或专利申请出版物的副本根据要求和支付必要的费用由办公室提供。

[0047] 图1显示人ActRIIA (SEQ ID NO: 56)和人ActRIIB (SEQ ID NO: 2)的细胞外结构域的比对，其中本文基于多种ActRIIB和ActRIIA晶体结构的复合分析推导直接接触配体的残基用方框显示。

[0048] 图2显示各种脊椎动物ActRIIB蛋白和人ActRIIA (SEQ ID NOs: 57-64)的多序列比对。

[0049] 图3显示在CHO细胞表达的ActRIIA-hFc的纯化。蛋白被纯化为单一的、轮廓分明的峰态，如通过分级柱(顶部)和考马斯亮蓝染色SDS-PAGE (底部) (左侧: 分子量标准; 右侧: ActRIIA-hFc)可见的那样。

[0050] 图4显示ActRIIA-hFc与激活素和GDF-11的结合，如经Biacore™试验测量的那样。

[0051] 图5A和5B显示ActRIIA-hFc对雌性非人灵长类动物(NHPs)红细胞计数的影响。在第0天、第7天、第14天和第21天用安慰剂或1 mg/kg、10 mg/kg或30 mg/kg的ActRIIA-hFc治疗雌性食蟹猴(4组，每组5只猴子)。图5A显示红细胞(RBC)计数。图5B显示血红蛋白水平。每个治疗组的统计显著性为相对于基线。在第57天，每组剩下两只猴子。

[0052] 图6A和6B显示ActRIIA-hFc对雄性非人灵长类动物红细胞计数的影响。在第0天、第7天、第14天和第21天用安慰剂或1 mg/kg、10 mg/kg或30 mg/kg的ActRIIA-hFc治疗雄性

食蟹猴(4组,每组5只猴子)。图6A显示红细胞(RBC)计数。图6B显示血红蛋白水平。每个治疗组的统计显著性为相对于基线。在第57天,每组剩下两只猴子。

[0053] 图7A和7B显示ActRIIA-hFc对雌性非人灵长类动物网织红细胞计数的影响。在第0天、第7天、第14天和第21天用安慰剂或1 mg/kg、10 mg/kg或30 mg/kg的ActRIIA-hFc治疗食蟹猴(4组,每组5只猴子)。图7A显示绝对的网织红细胞计数。图7B显示网织红细胞相对于RBCs的百分数。每组的统计显著性为相对于基线。在第57天,每组剩下两只猴子。

[0054] 图8A和8B显示ActRIIA-hFc对雄性非人灵长类动物网织红细胞计数的影响。在第0天、第7天、第14天和第21天用安慰剂或1 mg/kg、10 mg/kg或30 mg/kg的ActRIIA-hFc治疗食蟹猴(4组,每组5只猴子)。图8A显示绝对的网织红细胞计数。图8B显示网织红细胞相对于RBCs的百分数。每组的统计显著性为相对于基线。在第57天,每组剩下两只猴子。

[0055] 图9显示实施例5中描述的人临床试验的结果,其中曲线下面积(AUC)和ActRIIA-hFc的给予剂量线性相关,而不管ActRIIA-hFc是静脉(IV)还是皮下(SC)给予。

[0056] 图10显示IV或SC给予患者的ActRIIA-hFc血清水平的比较。

[0057] 图11显示响应不同剂量水平ActRIIA-hFc的骨碱性磷酸酶(BAP)水平。BAP为合成骨生长的标记物。

[0058] 图12描绘实施例5中描述的人临床试验的血细胞比容水平自基线的中值变化。ActRIIA-hFc以指明的剂量静脉注射(IV)给予。

[0059] 图13描绘实施例5中描述的人临床试验的血红蛋白水平自基线的中值变化。ActRIIA-hFc以指明的剂量静脉注射(IV)给予。

[0060] 图14描绘实施例5中描述的人临床试验的RBC (红细胞)计数自基线的中值变化。ActRIIA-hFc以指明的剂量静脉注射(IV)给予。

[0061] 图15描绘实施例5中描述的人临床试验的网织红细胞计数自基线的中值变化。ActRIIA-hFc以指明的剂量静脉注射(IV)给予。

[0062] 图16显示GDF捕获物ActRIIB (L79D 20-134)-hFc (SEQ ID NO:38)的完整氨基酸序列,包括TPA前导序列(双下划线的)、ActRIIB细胞外结构域(SEQ ID NO: 1的残基20-134;下划线的)和hFc结构域。在天然序列79位替换的天冬氨酸被双下划线和突出显示,如通过对成熟融合蛋白的N-末端残基测序显示为甘氨酸。

[0063] 图17A和17B显示编码ActRIIB (L79D 20-134)-hFc的核苷酸序列。SEQ ID NO:39对应有义链,和SEQ ID NO:40对应反义链。TPA前导(核苷酸1-66)为双下划线的,和ActRIIB细胞外结构域(核苷酸76-420)为下划线的。

[0064] 图18显示截短的GDF捕获物ActRIIB (L79D 25-131)-hFc (SEQ ID NO:41)的完整氨基酸序列,包括TPA前导(双下划线的)、截短的ActRIIB细胞外结构域(SEQ ID NO: 1的残基25-131;下划线的)和hFc结构域。在天然序列79位替换的天冬氨酸被双下划线和突出显示,如通过对成熟融合蛋白的N-末端残基测序显示为谷氨酸。

[0065] 图19A和19B显示编码ActRIIB (L79D 25-131)-hFc的核苷酸序列。SEQ ID NO:42对应有义链,和SEQ ID NO:43对应反义链。TPA前导(核苷酸1-66)为双下划线的,和截短的ActRIIB细胞外结构域(核苷酸76-396)为下划线的。ActRIIB细胞外结构域的氨基酸序列(SEQ ID NO: 1的残基25-131)也得到显示。

[0066] 图20显示没有前导(SEQ ID NO:44)的截短的GDF捕获物ActRIIB (L79D 25-131)-

hFc的氨基酸序列。截短的ActRIIB细胞外结构域(SEQ ID NO: 1的残基25-131)为下划线的。在天然序列79位替换的天冬氨酸被双下划线和突出显示,如通过对成熟融合蛋白的N-末端残基测序显示为谷氨酸。

[0067] 图21显示没有前导、hFc结构域和连接子(SEQ ID NO:45)的截短的GDF捕获物ActRIIB (L79D 25-131)的氨基酸序列。在天然序列79位替换的天冬氨酸被双下划线和突出显示,如通过对成熟融合蛋白的N-末端残基测序显示为谷氨酸。

[0068] 图22A和22B显示编码ActRIIB (L79D 25-131)-hFc的备选核苷酸序列。SEQ ID NO:46对应有义链,和SEQ ID NO:47对应反义链。TPA前导(核苷酸1-66)为双下划线的,截短的ActRIIB细胞外结构域(核苷酸76-396)为下划线的,和细胞外结构域的野生型核苷酸序列的替换被双下划线和突出显示(与SEQ ID NO:42, 图19A和19B相比较)。ActRIIB细胞外结构域的氨基酸序列(SEQ ID NO: 1的残基25-131)也得到显示。

[0069] 图23显示在图22A和22B显示的备选核苷酸序列(SEQ ID NO:46)的核苷酸76-396(SEQ ID NO:48)。在图22A和22B中显示的相同核苷酸替换在此也被下划线和突出显示。SEQ ID NO:48仅编码具有L79D替换的截短的ActRIIB细胞外结构域(对应SEQ ID NO:1的残基25-131),例如ActRIIB (L79D 25-131)。

[0070] 图24显示用ActRIIB (L79D 20-134)-hFc (灰色)或ActRIIB (L79D 25-131)-hFc(黑色)治疗对食蟹猴红细胞浓度自基线的绝对变化的影响。VEH=媒介物。数据为平均值±SEM。n=4-8每组。

[0071] 图25显示用ActRIIB (L79D 20-134)-hFc (灰色)或ActRIIB (L79D 25-131)-hFc(黑色)治疗对食蟹猴血细胞比容自基线的绝对变化的影响。VEH=媒介物。数据为平均值±SEM。n=4-8每组。

[0072] 图26显示用ActRIIB (L79D 20-134)-hFc (灰色)或ActRIIB (L79D 25-131)-hFc(黑色)治疗对食蟹猴血红蛋白浓度自基线的绝对变化的影响。VEH=媒介物。数据为平均值±SEM。n=4-8每组。

[0073] 图27显示用ActRIIB (L79D 20-134)-hFc (灰色)或ActRIIB (L79D 25-131)-hFc(黑色)治疗对食蟹猴循环网织红细胞浓度自基线的绝对变化的影响。VEH=媒介物。数据为平均值±SEM。n=4-8每组。

[0074] 图28显示用促红细胞生成素(EPO)和ActRIIB (L79D 25-131)-hFc联合治疗72小时对小鼠血细胞比容的影响。数据为平均值±SEM (n=4每组),和明显彼此不同($p<0.05$,非配对t检验)的平均值用不同字母指明。与媒介物相比较,联合治疗增加血细胞比容23%,协同增加大于EPO和ActRIIB (L79D 25-131)-hFc单独效果的总和。

[0075] 图29显示用EPO和ActRIIB (L79D 25-131)-hFc联合治疗72小时对小鼠血红蛋白浓度的影响。数据为平均值±SEM (n=4每组),和明显彼此不同($p<0.05$)的平均值用不同字母指明。与媒介物相比较,联合治疗增加血红蛋白浓度23%,其也为协同效应。

[0076] 图30显示用EPO和ActRIIB (L79D 25-131)-hFc联合治疗72小时对小鼠红细胞浓度的影响。数据为平均值±SEM (n=4每组),和明显彼此不同($p<0.05$)的平均值用不同字母指明。与媒介物相比较,联合治疗增加红细胞浓度20%,其也为协同效应。

[0077] 图31显示用EPO和ActRIIB (L79D 25-131)-hFc联合治疗72小时对小鼠脾脏红细胞生成前体细胞数目的影响。数据为平均值±SEM (n=4每组),和明显彼此不同($p<0.01$)的

平均值用不同字母指明。而单独EPO以晚期前体成熟为代价显著增加嗜碱性成红细胞(BasoE)的数目，联合治疗在较小但仍然显著的程度上增加BasoE数目，同时支持不减少晚期前体的成熟。

[0078] 图32显示GDF捕获物可在MDS小鼠模型的疾病严重程度的多个阶段减少无效红细胞生成并改善贫血。(A) 在用媒介物(Tris缓冲盐水，TBS，n=5)处理的野生型(Wt)小鼠、用TBS (n=5)处理的MDS小鼠和用ActRIIB (L79D 25-131)-mFc (RAP-536，10 mg/kg，n=6)处理的MDS小鼠，每周两次，持续8周，在约6个月龄时结束(早期)，RBC数目和血红蛋白浓度(顶部)及骨髓中造血前体的形态列举(底部)。*P<0.05，**P<0.01，对比TBS处理的MDS小鼠；###P<0.001对比野生型小鼠。(B) 在用RAP-536 (10 mg/kg，每周两次，n=5)或TBS (n=4)处理的MDS小鼠，持续7周，在约12个月龄时结束(晚期)，与在子图A中相同的终点。*P<0.05对比TBS处理的MDS小鼠。数据为平均值±SEM。

[0079] 发明详述

1．综述

转化生长因子-β (TGF-β)超家族含有拥有共同序列元件和结构基序的多种生长因子。已知这些蛋白对脊椎动物和无脊椎动物两者的各种各样细胞类型发挥生物效应。该超家族成员在胚胎发育过程中的模式形成和组织特化方面执行重要的功能，并可影响各种分化过程，包括脂肪生成、肌细胞生成、软骨形成、心脏发育、造血作用、神经发生和上皮细胞分化。通过操控TGF-β家族成员的活性，通常可引起生物体内重要的生理变化。例如，皮埃蒙特和比利时蓝牛品种在GDF8 (也称为肌肉生长抑制素)基因携带着功能丧失突变，造成肌肉质量明显增加[参见例如Grobet等. (1997) Nat Genet. 17(1):71-4]。此外，在人类，GDF8的无效等位基因与肌肉质量增加、并且据报道与力量异常有关[参见例如Schuelke等. (2004) N Engl J Med, 350:2682-8]。

[0080] TGF-β信号由I型和II型丝氨酸/苏氨酸激酶受体的异聚复合体介导，其在配体刺激时磷酸化并激活下游SMAD蛋白(例如SMAD蛋白1、2、3、5和8) [参见例如Massagué (2000) Nat. Rev. Mol. Cell Biol. 1:169-178]。这些I型和II型受体为跨膜蛋白，由含有半胱氨酸富集区的细胞外配体结合域、跨膜域及具有预期的丝氨酸/苏氨酸特异性的胞浆域组成。I型受体是信号传递必要的。II型受体是配体结合和I型受体激活需要的。I和II型激活素受体在配体结合后形成稳定的复合物，导致I型受体通过II型受体磷酸化。

[0081] 两种相关的II型受体(ActRII) (ActRIIA和ActRIIB)已被确认为激活素的II型受体[参见例如Mathews and Vale (1991) Cell 65:973-982；和Attisano等*1*. (1992) Cell 68: 97-108]。除了激活素以外，ActRIIA和ActRIIB可与几种其他TGF-β家族蛋白(包括例如BMP6、BMP7、Nodal、GDF8和GDF11)发生生物化学相互作用[参见例如Yamashita等. (1995) J. Cell Biol. 130:217-226；Lee and McPherron (2001) Proc. Natl. Acad. Sci. USA 98:9306-9311；Yeo and Whitman (2001) Mol. Cell 7: 949-957；和Oh等. (2002) Genes Dev. 16:2749-54]。ALK4为激活素，特别是激活素A的主要I型受体，和ALK-7也可用作其他激活素，特别是激活素B的受体。在某些实施方案中，本公开涉及用本文公开的一种或多种抑制剂，特别是可拮抗激活素A、激活素B、激活素C、激活素E、GDF11和/或GDF8中一种或多种的抑制剂拮抗ActRII受体的配体(也称为ActRII配体)。

[0082] 激活素为属于TGF-β超家族的二聚体多肽生长因子。有三种主要的激活素形式(A、

B和AB)，其为两种密切相关的β亚基的同源/异质二聚体(分别为$\beta_A\beta_A$、$\beta_B\beta_B$和$\beta_A\beta_B$)。人类基因组也编码激活素C和激活素E，其主要在肝脏表达，并且含有β_C或β_E的异质二聚体形式也是已知的。

[0083] 在TGF-β超家族中，激活素是独特的和多功能因子，可刺激卵巢和胎盘细胞产生激素，支持神经元细胞存活，依细胞类型而定影响细胞周期的积极或负向进展，并且至少在两栖类胚胎诱导中胚层分化[DePaolo等. (1991) Proc Soc Ep Biol Med. 198:500-512；Dyson等. (1997) Curr Biol. 7:81-84;和Woodruff (1998) Biochem Pharmacol. 55:953-963]。此外，据发现分离自被刺激的人单核细胞白血病细胞的红细胞分化因子(EDF)与激活素A相同[Murata等. (1988) PNAS, 85:2434]。表明激活素A促进骨髓红细胞生成。在一些组织中，激活素信号传递受到其相关的异质二聚体抑制素的拮抗。例如，在促卵泡激素(FSH)自垂体的释放过程中，激活素促进FSH分泌和合成，而抑制素阻止FSH分泌和合成。可调节激活素生物活性和/或与激活素结合的其他蛋白包括卵泡抑素(FS)、卵泡抑素相关蛋白(FSRP，也称为FLRG或FSTL3)和α_2-巨球蛋白。

[0084] 如本文描述的那样，结合“激活素A”的试剂为特异性地结合β_A亚基的试剂，无论是在孤立的β_A亚基还是作为二聚体复合物(例如$\beta_A\beta_A$同源二聚体或$\beta_A\beta_B$异质二聚体)的情况下。在异质二聚体复合物(例如$\beta_A\beta_B$异质二聚体)的情况下，结合“激活素A”的试剂对存在于β_A亚基内的表位是特异性的，但是不结合存在于复合物非β_A亚基(例如复合物的β_B亚基)内的表位。类似地，本文公开的拮抗(抑制)“激活素A”的试剂为抑制由β_A亚基介导的一种或多种活性的试剂，无论是在孤立的β_A亚基还是作为二聚体复合物(例如$\beta_A\beta_A$同源二聚体或$\beta_A\beta_B$异质二聚体)的情况下。在$\beta_A\beta_B$异质二聚体的情况下，抑制“激活素A”的试剂为特异性地抑制β_A亚基的一种或多种活性，但是不抑制复合物的非β_A亚基基(例如复合物的β_B亚基)活性的试剂。该原则也适用于结合和/或抑制“激活素B”、“激活素C”和“激活素E”的试剂。本文公开的拮抗“激活素AB”的试剂为抑制β_A亚基介导的一种或多种活性和β_B亚基介导的一种或多种活性的试剂。

[0085] Nodal蛋白在早期胚胎发生中，在中胚层和内胚层诱导和形成以及随后轴向结构比如心脏和胃的组织化方面具有功能。已经证实发育中脊椎动物胚胎的背部组织主要有助于脊索和脊索前板的轴向结构，同时其募集周围细胞形成非轴向胚胎结构。Nodal似乎通过I型和II型受体两者及称为SMAD蛋白的胞内效应器传递信号。研究支持ActRIIA和ActRIIB用作Nodal的II型受体这种观点[参见例如Sakuma等. (2002) Genes Cells. 2002, 7:401-12]。提示Nodal配体与其辅助因子(例如cripto)相互作用，激活激活素I型和II型受体，使SMAD2磷酸化。Nodal蛋白参与对早期脊椎动物胚胎至关重要的许多事件，包括中胚层形成、前部模式化和左右轴特化。实验证据已经表明Nodal信号激活pAR3-Lux (一种先前显示对激活素和TGF-β特异性反应的萤光素酶报告基因)。然而，Nodal不能诱导pTlx2-Lux(一种对骨形态生成蛋白特异性反应的报告基因)。最近的研究结果提供了直接的生物化学证据，即Nodal信号通过两种激活素-TGF-β途径SMADs即SMAD2和SMAD3介导。进一步的证据已经显示，胞外cripto蛋白为Nodal信号传递需要的，使其不同于激活素或TGF-β信号传递。

[0086] 生长和分化因子-8 (GDF8)也称为肌肉生长抑制素。GDF8 为骨骼肌质量的负调控因子。GDF8在发育中和成人骨骼肌中高度表达。转基因小鼠的GDF8无效突变特征为骨骼肌的明显肥大和增生[McPherron等, Nature (1997) 387:83-90]。骨骼肌质量的类似增加在

牛天然发生的GDF8突变是明显的[参见例如Ashmore等. (1974) Growth, 38:501-507; Swatland and Kieffer (1994) J. Anim. Sci. 38:752-757;McPherron and Lee (1997) Proc. Natl. Acad. Sci. USA 94:12457-12461;和Kambadur等. (1997) Genome Res. 7: 910-915],并且在人是显著的[参见例如Schuelke等. (2004) N Engl J Med 350:2682-8]。研究还显示与人HIV-感染有关的肌肉萎缩伴随GDF8蛋白表达增加[参见例如Gonzalez-Cadavid等. (1998) PNAS 95:14938-43]。另外,GDF8可调节肌肉特异性酶(例如肌酸激酶)的产生和调节成肌细胞的细胞增殖[参见例如国际专利申请出版物第WO 00/43781号]。GDF8前肽可非共价结合成熟的GDF8结构域二聚体,灭活其生物学活性[参见例如Miyazono等. (1988) J. Biol. Chem., 263: 6407-6415;Wakefield等. (1988) J. Biol. Chem., 263: 7646-7654;和Brown等. (1990) Growth Factors, 3: 35-43]。结合GDF8或结构相关蛋白并抑制其生物学活性的其它蛋白包括卵泡抑素,并且潜在地抑制卵泡抑素相关蛋白[参见例如Gamer等. (1999) Dev. Biol., 208: 222-232]。

[0087] 生长和分化因子-11 (GDF11)也称为BMP11,为一种分泌性蛋白[McPherron等. (1999) Nat. Genet. 22: 260-264]。GDF11在小鼠发育期间,在尾芽、肢芽、上颌弓和下颌弓及背根神经节表达[参见例如Nakashima等. (1999) Mech. Dev. 80: 185-189]。GDF11于中胚层和神经组织两者,在模式化(patterning)方面起独特作用[参见例如Gamer等. (1999) Dev Biol., 208:222-32]。GDF11被显示为发育中小鸡腿的软骨形成和肌细胞生成的负调控因子[参见例如Gamer 等. (2001) Dev Biol. 229:407-20]。GDF11在肌肉中的表达也表明其在以类似于GDF8的方式调节肌肉生长方面的作用。另外,GDF11在脑中的表达表明GDF11还可具有涉及神经系统功能的活性。有趣的是,发现GDF11抑制嗅觉上皮的神经发生[参见例如Wu等. (2003) Neuron. 37:197-207]。

[0088] 骨形态生成蛋白(BMP7)也称为成骨蛋白-1 (OP-1),众所周知其诱导软骨和骨形成。另外,BMP7调节广泛的生理过程。例如,BMP7可为负责上皮骨生成现象的骨生成诱导因子。还发现BMP7在钙调节和骨稳态方面起作用。类似于激活素,BMP7结合II型受体ActRIIA和ActRIIB。然而,BMP7和激活素募集不同的I型受体到异聚受体复合物中。观察到的主要BMP7 I型受体是ALK2,而激活素仅结合于ALK4 (ActRIIB)。BMP7和激活素引发不同的生物反应和激活不同的SMAD途径[参见例如Macias-Silva等. (1998) J Biol Chem. 273: 25628-36]。

[0089] 如本文证实的那样,ActRIIB多肽(例如ActRIIA和ActRIIB多肽)可用于增加体内红细胞水平。在某些实例中,已经表明GDF捕获多肽(具体地讲变体ActRIIB多肽)特征为与野生型(未修饰的) ActRII多肽的相应样品相比较独特的生物学特性。这种GDF捕获物在某种程度上特征为显著丧失对激活素A的亲和力,并且因此显著降低拮抗激活素A活性的能力,但是保持接近野生型的GDF11结合和抑制水平。在体内,GDF捕获物与野生型ActRIIB多肽相比较更有效增加红细胞水平,并在多种贫血模型具有有益效果。应该注意的是,造血作用是个复杂过程,受多种因素调控,包括促红细胞生成素、G-CSF和铁稳态。术语“增加红细胞水平”和“促进红细胞形成”指临床上可观察的量度指标,如血细胞比容、红细胞计数和血红蛋白测量,并且意欲这种变化发生的机制是中立的。

[0090] 因此本公开的数据表明,所观察到的ActRII多肽关于红细胞水平的生物活性不依赖于激活素A抑制作用。然而应该注意的是,未修饰的ActRIIB多肽其保持激活素A结合,但

仍然显示出增加体内红细胞的能力。此外，与对激活素A亲和力减弱的GDF捕获物相比较，保持激活素A抑制作用的ActRIIB或ActRIIA多肽在一些应用中可能更合乎需要，此时红细胞水平的较为温和增长是合乎需要的和/或此时某种程度的偏离目标的活性是可接受的(或者甚至合乎需要的)。

[0091] 因此，本公开的方法通常涉及任选地与一种或多种支持疗法组合的一种或多种本文描述的ActRII拮抗剂，在有需要的受试者增加红细胞形成、在有需要的受试者治疗或预防贫血、治疗骨髓增生异常综合征；在有需要的受试者治疗铁粒幼红细胞性贫血和治疗或预防铁粒幼红细胞性贫血或骨髓增生异常综合征的一种或多种并发症(例如贫血、输血需求、铁超负荷、嗜中性白血球减少症、脾肿大、进展为急性髓细胞性淋巴瘤)，和任选地在具有环形铁粒幼红细胞和/或骨髓细胞*SF3B1*、*DNMT3A*和/或*TET2*基因的一种或多种突变的患者亚群的用途。被确认为特别有可能对ActRII拮抗剂作出反应的另一个患者亚群为在用EPO疗法或其他EPO受体激活剂疗法治疗前已经失败的患者。

[0092] 如本文证明的那样，所描述的ActRII拮抗剂可与EPO受体激活剂组合使用或用于用EPO受体激活剂治疗已经失败的患者。EPO为参与红系祖细胞生长和成熟为红细胞的糖蛋白激素。EPO在胎儿期由肝脏和在成人由肾脏产生。通常由于肾衰竭在成人发生EPO产生减少导致贫血。EPO已经通过基因工程技术(基于自用EPO基因转染的宿主细胞表达与分泌蛋白)产生。给予这种重组EPO已有效治疗贫血。例如，Eschbach等(1987, N Engl J Med 316:73)描述了使用EPO克服慢性肾衰竭引起的贫血。

[0093] EPO的作用通过其结合和激活属于细胞因子受体超家族并指定EPO受体的细胞表面受体来介导。人和鼠的EPO受体已被克隆和表达[参见例如D'Andrea等. (1989) Cell 57:277;Jones等. (1990) Blood 76:31;Winkelman等. (1990) Blood 76:24;和美国专利第5278065号]。人EPO受体基因编码包含约224个氨基酸的细胞外结构域的483个氨基酸的跨膜蛋白，并与鼠的EPO受体呈现约82%的氨基酸序列同一性(参见例如美国专利第6319499号)。在哺乳动物细胞表达的克隆的全长EPO受体(66-72 kDa)结合EPO，亲和力(K_D=100-300 nM)与红系祖细胞上的天然受体的类似。因此，认为这种形式含有主要EPO结合决定因素，并称为EPO受体。通过从其他密切相关的细胞因子受体类推，认为EPO受体在激动剂结合时成为二聚物。然而，其可能为多聚复合物的EPO受体的详细结构及其具体激活机制还不完全理解(参见例如美国专利第6319499号)。

[0094] EPO受体的激活导致几种生物效应。这些效应包括未成熟成红细胞的增殖增多、未成熟成红细胞的分化增多和红系祖细胞的凋亡减少[参见例如Liboi等. (1993) Proc Natl Acad Sci USA 90:11351-11355;Koury等. (1990) Science 248:378-381]。介导增殖和分化的EPO受体信号转导途径似乎不同[参见例如Noguchi等. (1988) Mol Cell Biol 8:2604;Patel等. (1992) J Biol Chem, 267:21300;和Liboi et al. (1993) Proc Natl Acad Sci USA 90:11351-11355]。一些结果表明，可能需要一种辅助蛋白用于介导分化信号[参见例如Chiba等. (1993) Nature 362:646;和Chiba等. (1993) Proc Natl Acad Sci USA 90:11593]。然而，关于辅助蛋白在分化方面的作用存在争议，因为受体的组成性激活形式可刺激增殖和分化两方面[参见例如Pharr等. (1993) Proc Natl Acad Sci USA 90:938]。

[0095] EPO受体激活剂包括小分子红细胞生成刺激剂(ESAs)及基于EPO的化合物。前者的

实例为共价连接于聚乙二醇的基于二聚肽的激动剂(专有名称Hematide™和Omontys®),其在健康志愿者和具有慢性肾病与内源性抗-EPO抗体两者的患者显示具有红细胞生成刺激性[参见例如Stead等. (2006) Blood 108:1830-1834;和Macdougall等. (2009) N Engl J Med 361:1848-1855]。其他实例包括基于非肽的ESAs [参见例如Qureshi等. (1999) Proc Natl Acad Sci USA 96:12156-12161]。

[0096] EPO受体激活剂还包括通过增强内源性EPO的产生而不接触EPO受体本身间接刺激红细胞生成的化合物。例如,缺氧诱导转录因子(HIFs)为EPO基因表达的内源性刺激剂,EPO基因表达在常氧条件下,通过细胞调控机制受到抑制(失去稳定)。因此,HIF脯氨酰羟化酶的抑制剂正被研究体内EPO诱导活性。EPO受体的其他间接激活剂包括紧张性(tonically)抑制EPO基因表达的GATA-2转录因子的抑制剂[参见例如Nakano等. (2004) Blood 104:4300-4307]和起EPO受体信号转导负调控因子作用的造血细胞磷酸酶(HCP或SHP-1)的抑制剂[参见例如Klingmuller *et al*. (1995) Cell 80:729-738]。

[0097] 如本文描述的那样,呈现环形铁粒幼红细胞的患者可能特别适合用ActRII拮抗剂治疗。铁粒幼红细胞性贫血可大致分为先天性(遗传性)和获得性形式的,其可进一步如表1中显示的那样细分。

表 1. 铁粒幼红细胞性贫血的分类*

分类	基因	贫血严重程度	铁稳态
先天性的			
非综合征性的			
X 连锁	*ALAS2*	轻度到重度	铁超负荷
SLC25A38 缺乏	*SLC25A38*	重度	铁超负荷
谷氧还蛋白 5 缺乏	*GLRX5*	轻度到重度	铁超负荷
红细胞生成性原卟啉病	*FECH*	轻度	---
综合征性的			
X 连锁与共济失调	*ABCB7*	轻度至中度	---
SIFD	未知	重度	铁超负荷
皮尔森骨髓-胰腺综合征	mtDNA	重度	---
肌病、乳酸性酸中毒和铁粒幼红细胞性贫血(MLASA)	*PUS1/YARS2*	轻度至重度	---
硫胺素反应性巨幼细胞性贫血(TRMA)	*SLC19A2*	重度	---
不明原因的综合征/非综合征性	未知	可变的	---
获得性的			
克隆的/肿瘤的			
MDS**	可变的	轻度至重度	铁超负荷
代谢的			
酗酒	---	可变的	---
药物引起的	---	可变的	---
铜缺乏(锌毒性)	---	可变的	---
体温过低	---	可变的	---

* 参见 Bottomley 等, 2014, Hematol Oncol Clin N Am 28:653-670。

** 参见下表根据世界卫生组织(World Health Organization)的 MDS 子分类。

[0098] MDS代表成人最常见的一类获得性骨髓衰竭综合征。尽管MDS从生物学的角度越来越清楚地被理解,但是对于患有这些障碍的大多数患者改善的病理学认识尚未转化为高效或治愈性的疗法。MDS细胞分化的失败增加与演化为继发性急性髓性白血病(AML)有关,AML目前由WHO定义为在血液或骨髓中具有至少20%成髓细胞,或者存在几种AML定义的核型异常之一而不管未成熟细胞的比例[参见例如Vardiman等. (2009) Blood 114:937-951]。

AML最终在多达30%的MDS患者得到诊断。因为构成MDS基础的生物异质性转化为临床结果的广泛差异，已开发出预后分类方案，以预测MDS的自然进程并建议患者[Zeidan等 (2013) Curr Hematol Malig Rep 8:351-360]。国际预后评分系统(IPSS)为一种这样分类的实例，其根据风险状况对患者进行分类，范围从低度(生存中值5.7年)到中度1 (生存中值3.5年)到中度2 (生存中值1.2年)到高度(生存中值0.4年)。一种称为IPSS-R的备选系统也可用于患者分层。从患者的角度来看，预后有助于确定疾病的严重程度并对如何可能施加影响设定期望。从医生的角度来看，预后为以可用于帮助直接治疗的方式把疾病分期提供了一种手段。值得注意的是，这种方案通常不考虑患者并发症，并且不打算预测与特定治疗相关的临床受益。

[0099] 已提出了另外的MDS分类系统，以促进MDS患者的适当治疗和管理。分类已经演变了几十年，以在理解这些复杂综合征方面取得进展。MDS的法国-美国-英国(FAB)分类方案于1982年提出[Bennett等. (1982) Br J Haematol 51:189-199]，并用作由WHO在2001年建立的一种改进分类系统的基础。如在以下表2中概述的那样，WHO分类的当前版本(2008年修订)基于(1) 骨髓和外周血中成髓细胞的百分比；(2) 发育不良的类型和程度，(3) 存在环形铁粒幼红细胞，和(4) 存在细胞遗传学异常。因此，环形铁粒幼红细胞为RARS的特征，但是也可存在于MDS的其他亚型[参见例如Juneja等. (1983) J Clin Pathol 36:566-569;Malcovati等. (2013) Best Pract Res Clin Haematol 26:377-385]。依MDS亚型而定，贫血可在例如环形铁粒幼红细胞存在或不存在下发生，单独或与嗜中性粒细胞数目异常低(嗜中性白血球减少症)、血小板数低(血小板减少症)或血小板水平升高(血小板增多症)组合发生。与血小板显著增多有关的伴环形铁粒幼红细胞增多的难治性贫血(RARS-T)目前作为不能分类的MDS肿瘤(MDS-U)组内WHO分类的临时条目(表2)列入。RARS-T定义为伴发育不良的无效红细胞生成和环形铁粒幼红细胞≥红细胞前体的15%，外周血中无未成熟细胞和骨髓中<5%，和血小板计数≥450 x 10^9/L的血小板增多症的贫血[Malcovati等. (2013) Best Pract Res Clin Haematol 26:377-385]。由于免疫系统受损，嗜中性白血球减少症患者可能处于严重的感染甚至脓毒症的风险下，并且因此重要的是治疗这种病症。血小板减少症患者处于增大的内出血风险下，并且依严重程度而定治疗这种病症也可能是有益的。

表 2. 2008 年 WHO 的 MDS 分类系统*

MDS 亚型	血液检查	骨髓检查
难治性血细胞减少伴单系发育不良(RCUD)：(a) 难治性贫血，(b) 难治性嗜中性白血球减少症，或(c) 难治性血小板减少症	主要是单系血细胞减少(unicytopenia)	单系发育不良：≥ 一个髓系中细胞的 10% < 5%未成熟细胞 < 红细胞前体的 15%为环形铁粒幼红细胞
难治性贫血伴环形铁粒幼红细胞增多(RARS)	贫血 无未成熟细胞	≥ 红细胞前体的 15%为环形铁粒幼红细胞 红细胞发育不良仅< 5%未成熟细胞
难治性血细胞减少伴多系发育不良(RCMD)	血细胞减少 无或很少未成熟细胞(< 1%) 无奥氏小体 < 1 x 10^9 每 L 单核细胞	发育不良在≥2 髓系发育不良的≥ 10%的细胞 (嗜中性粒细胞和/或红细胞前体和/或巨核细胞) < 5%未成熟细胞 无奥氏小体 ± 15%环形铁粒幼红细胞
难治性贫血伴未成熟细胞过多-1 (RAEB-1)	血细胞减少 < 5%未成熟细胞 无奥氏小体 < 1 x 10^9 每 L 单核细胞	多系或单系发育不良 5%-9%未成熟细胞 无奥氏小体
难治性贫血伴未成熟细胞过多-1 (RAEB-2)	血细胞减少 5%-19%未成熟细胞	多系或单系发育不良 10%-19%未成熟细胞

	奥氏小体± $<1 \times 10^9$每L单核细胞	奥氏小体±
骨髓增生异常综合征-未分类的(MDS-U) **	血细胞减少 <1%未成熟细胞	在一个或多个髓系的<10%细胞明确的发育不良，此时伴有视为MDS诊断的推定证据的细胞遗传学异常 <5%未成熟细胞
与分离的del(5q)有关的MDS	贫血 血小板计数通常正常或增加 无或很少未成熟细胞(<1%)	巨核细胞正常至增多伴分叶不良的神经核(hypolobated nuclei) <5%未成熟细胞 分离的del(5q)细胞遗传学异常 无奥氏小体

* 来自 Vardiman 等(2009) Blood 114:937-951

** 包括与血小板显著增多有关的难治性贫血伴环形铁粒幼红细胞增多(RARS-T)

[0100] 在本公开的一个实施方案中，ActRII拮抗剂用于在患者(包括MDS患者或患有铁粒幼红细胞性贫血的患者)治疗贫血，其中多于1%、2%、3%、4%、5%、6%、7%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%或95%的红细胞前体为环形铁粒幼红细胞，例如在难治性贫血伴环形铁粒幼红细胞增多(RARS)，与血小板显著增多有关的RARS (RARS-T)，或者难治性血细胞减少伴多系发育不良(RCMD，在环形铁粒幼红细胞增多突出的患者也称为RCMD-RS)。

[0101] 贫血经常发生于MDS。约80%的MDS患者存在贫血，并且其中相当大百分比变得在他们疾病期间依赖于输血[Steensma等. (2006) Mayo Clin Proc 81:104-130]。一些MDS亚型特征为“无效红细胞生成”，其中晚期红细胞前体细胞的分化减弱(成熟)，尽管早期红系祖细胞作为对组织缺氧的反应受EPO刺激而增生。因此，红细胞生成无效的一个关键迹象是持续性贫血，尽管内源性EPO的水平升高。红细胞生成无效通常发生于MDS的RARS亚型，但不发生于RAEB亚型，其特征为红系骨髓的增殖相对低下[Cazzola等. (1982) Br J Haematol 50:55-62]。铁调素的循环水平(一种铁稳态的关键调控因子)在RARS比RAEB低约一个数量级[Santini等. (2011) PLoS One 6:e23109]。因为铁调素水平低促进铁吸收，因此认为在障碍比如RARS和地中海贫血测量的不适当的低水平铁调素导致在这些障碍甚至不存在输

血时观察到的铁超负荷。

[0102] 慢性红细胞输注缓解贫血，但是使患者面临多重风险，包括传染性疾病、过敏或溶血反应及铁超负荷加重[Rawn (2008) Curr Opin Anaesthesiol 21:664-668;Özcan等. (2013) Expert Rev Hematol 6:165-189]。随着全身铁含量的增加，身体增加铁蛋白产生用于铁储存和减少转铁蛋白受体产生以减少铁进入细胞。当超过了循环转铁蛋白的铁结合能力时，发现铁在血浆中作为非转铁蛋白结合铁(NTBI)存在。在MDS，非转铁蛋白结合铁的水平在低风险比高风险亚型更高，并且在RARS最高[Santini等. (2011) PLoS One 6:e23109]。因为铁不能从身体中主动分泌出来，它最初积聚在网状内皮巨噬细胞中，并在后来主要沉积在心脏、肝脏和内分泌腺的实质细胞中[Siah等. (2006) Clin Biochem Rev 27:5-16]。在铁超负荷的情况下，非转铁蛋白结合铁转变成其称为不稳定性血浆铁的氧化还原活性形式，被运送到促进活性氧形成的细胞内。这些高毒性分子对造血作用产生不利影响，并且特别是损伤心脏、肝脏和内分泌组织。

[0103] MDS患者群体主要由患有共存疾病—包括心力衰竭、感染、出血和肝硬化的倾向的上了年纪的组成—并且铁超负荷可能会迅速加重这种预先存在的病症。与处于出现AML高风险下的MDS患者相比较，处于低-或中度-1风险下的患者可能由于其预期寿命更长而更易发生铁超负荷。由于这些原因，认为在患有低-或中度-1风险MDS亚型的患者铁螯合疗法减轻铁负荷是可取的，其有长的预期寿命并预计接受超过20次的RBC输注[Temraz等. (2014) Crit Rev Oncol Hematol 91:64-73]。

[0104] 新的测序技术在过去几年中已经导致识别了在MDS不断突变的数十种基因。此类基因按类型分类的2013列表显示在表3中。在几乎所有MDS患者可发现一种或多种这样突变，并且了解所涉及基因的性质改善了对MDS如何发展和演变的理解，尽管这尚未对治疗产生影响。应用于MDS患者样本的全基因组测序已确认了一类全新的编码mRNA剪接(剪接体)因子的癌症相关基因。在MDS确认的第一种这样的基因为*SF3B1*，其在RARS患者突变特别频繁[Papaemmanuil等. (2011) N Engl J Med 365:1384-1395]。突变基因的其他主要类别为表观遗传(DNA甲基化)调控因子、转录因子和信号分子[Cazzola等. (2013) Blood 122:4021-4034;Bejar等. (2014) Blood 124:2793-2803]。这些突变在MDS患者共现的程度似乎随基因类型而异。例如，约50%的MDS患者具有迄今为止确认编码突变剪接因子的十种基因之一，但是这些突变基因很少在同一患者身上共现[Bejar等. (2014) Blood 124:2793-2803]。因此，这些突变基因很少为用于同一个体的冗余标记(redundant markers)。编码突变表观遗传调控因子的基因更通常彼此和与突变剪接因子基因在同一患者身上共现。如本文公开的那样，突变基因的差异发生(比如在表3中列出的那些)提供了基因特征，可助于预测哪个患有MDS或铁粒幼红细胞性贫血的患者有可能对ActRII拮抗剂治疗上是反应性或非反应性的。

表 3. MDS 相关的体细胞突变

基因	在 MDS 中的频率 (%病例)
RNA 剪接	
SF3B1	14-28
SRSF2	15
U2AF1	8
ZRSR2	6
PRPF40B	1
SF3A1	1
SF1	1
U2AF65	< 1
LUC7L2	罕见
PRPF8	罕见
表观遗传调控因子	
TET2	19-26
ASXL1	10-20
DNMT3A	10
IDH1 / IDH2	4-12
EZH2	6
UTX	1
ATRX	< 1
转录因子	
RUNX1	10-20

TP53	4-14
ETV6	1-3
PHF6	罕见
WT1	罕见
信号传递	
NRAS	10
CBL	3
JAK2	3
FLT3	2-3
KRAS	1-2
c-KIT	1
BRAF	<1
CDKN2A	<1
GNAS	<1
PTEN	<1
PTPN11	<1
CBLB	罕见
MPL, CSF1R	罕见
其他	
NPM1	2-3

* 来自 Tothova 等. (2013) Clin Cancer Res 19:1637-1643。

[0105] 在表3列出的基因中，编码剪接因子的基因3B1 (*SF3B1*)最近在MDS，特别是在RARS、RARS-T和RCMD-RS亚型中起关键作用[Malcovati等. (2011) Blood 118:6239-6246; Dolatshad等. (2014) Leukemia doi: 10.1038/leu.2014.331电子版在线提前出版]。SF3B1的体细胞突变也发生在血液癌症，包括慢性淋巴细胞白血病(CLL)和急性髓性白血病(AML)以及乳腺癌、胰腺癌、胃癌、前列腺癌和葡萄膜黑色素瘤[Malcovati等. (2011) Blood 118:6239-6246;Wang等. (2011) N Engl J Med 365:2497-2506;The Cancer Genome Atlas Network (2012) Nature 490:61-70;Biankin等. (2012) Nature 491: 399-405;Chesnais等. (2012) Oncotarget 3:1284-1293;Furney等. (2013) Cancer Discov 3:1122-1129;Je等. (2013) Int J Cancer 133:260-266]。*SF3B1*突变谱(许多聚集在蛋白的几个位置)已在接触高浓度普拉二烯内酯的临床样本或细胞系被确认[Webb等. (2013) Drug Discov Today 18:43-49]。在MDS确认的*SF3B1*突变包括例如K182E、E491G、R590K、E592K、R625C、R625G、N626D、N626S、H662Y、T663A、K666M、K666Q、K666R、Q670E、G676D、V701I、I704N、I704V、G740R、A744P、D781G和A1188V。在癌症确认的*SF3B1*突变包括例如N619K、N626H、N626Y、R630S、I704T、G740E、K741N、G742D、D894G、Q903R、R1041H和I1241T。最后，在MDS和癌症两者确认的*SF3B1*突变包括例如G347V、E622D、Y623C、R625H、R625L、

H662D、H662Q、T663I、K666E、K666N、K666T、K700E和V701F。

[0106] 在本公开的上下文中以及在使用每个术语的特定背景下，本说明书中使用的术语通常具有其本领域的普通含义。在下文以及说明书的其它部分讨论了某些术语，以在描述本公开的组合物和方法以及如何制备和使用它们时为从业者提供另外的指导。术语的任何使用范围或含义从使用该术语的特定背景将是显而易见的。

[0107] 以其所有语法形式和拼写变体存在的“同源的”指的是具有“共同进化起源”的两种蛋白(包括来自相同物种生物的超家族的蛋白以及来自不同生物物种的同源蛋白)之间的关系。这种蛋白(及其编码核酸)具有序列同源性，如由其序列相似性所反映的那样，无论就相同度而言还是根据特定残基或基序以及保守位置的存在。

[0108] 以其所有语法形式存在的术语“序列相似性”指的是可共有或可不共有共同进化起源的核酸或氨基酸序列之间的同一性或对应程度。

[0109] 然而，在一般用法以及本申请中，当术语“同源的”用副词比如“高度”修饰时，可指序列相似性，并可或可不涉及共同进化起源。

[0110] “百分比(%)序列同一性”定义为在比对序列和引入间隙(如果必要的话)，以获得最大百分比序列同一性，并且不考虑任何保守性替换作为序列同一性的部分之后，相对于参考多肽(或核苷酸)序列，与参考多肽(核苷酸)序列中的氨基酸残基(或核酸)相同的候选序列中氨基酸残基(或核酸)的百分数。为了确定百分比氨基酸序列同一性的比对可以本领域技术范围内的各种方式实现，例如采用公开可用的计算机软件比如BLAST、BLAST-2、ALIGN或Megalign (DNASTAR)软件。本领域的技术人员可确定比对序列的适当参数，包括在所比较的整个序列长度实现最大限度比对需要的任何算法。然而，为了本文的目的，%氨基酸(核酸)序列同一性值采用序列比较计算机程序ALIGN-2产生。ALIGN-2序列比较计算机程序由Genentech, Inc.编写，并已用美国版权局(U.S. Copyright Office)，华盛顿特区(Washington D.C.)，20559的用户文档提交了源代码，其中以美国版权注册号TXU510087进行注册。ALIGN-2程序可公开得自Genentech, Inc.，南圣弗朗西斯科(South San Francisco)，加利福尼亚州(Calif.)，或者可从源代码编译。ALIGN-2程序应为在UNIX操作系统(包括数字UNIX V4.0D)上使用而进行编译。所有序列比较参数由ALIGN-2程序设定并且不改变。

[0111] 以其所有语法形式存在的“激动”指的是激活蛋白和/或基因(例如通过激活或放大所述蛋白的基因表达或者通过诱导非活性蛋白进入活性状态)或者增加蛋白和/或基因活性的过程。

[0112] 以其所有语法形式存在的“拮抗”指的是抑制蛋白和/或基因(例如通过抑制或减少所述蛋白的基因表达或者通过诱导活性蛋白进入非活性状态)或者降低蛋白和/或基因活性的过程。

[0113] 除非另外指明，本文使用的“基本上不结合于X”打算意指一种试剂对“X”具有大于约10^{-7}、10^{-6}、10^{-5}、10^{-4}或更大的K_D (例如通过用于测定K_D的试验不能检测的结合)，或者对“X”具有相对温和的结合，例如约1 x 10^{-8} M或约1 x 10^{-9} M。

[0114] 在整个说明书和权利要求中连同数值一起使用的术语“约”和“约”意指本领域技术人员熟悉和可接受的精确度区间。通常，这种精确度区间为± 10%。或者，并且具体地讲在生物系统中，术语“约”和“约”可意指在一个数量级范围内，优选地≤给定值的5倍和更优

选地≤ 2倍的数值。

[0115] 本文公开的数值范围包括定义所述范围的数值。

[0116] 术语“一个”和“一个”包括复数指涉，除非使用术语的上下文另外明确指明。术语“一个”(或“一个”)以及术语“一个或多个”和“至少一个”本文可互换使用。此外，其中本文使用的“和/或”被视为具体公开有或没有另一个的两个或多个具体特征或组件中每一个。因此，如在短语中使用的术语“和/或”比如本文的“A和/或B”打算包括“A和B”、“A或B”、“A”(单独)和“B”(单独)。同样地，如在短语中使用的术语“和/或”比如“A、B和/或C”打算包括以下方面中的每一个：A、B和C；A、B或C；A或C；A或B；B或C；A和C；A和B；B和C；A (单独)；B(单独)；及C (单独)。

[0117] 在整个该说明书中，词语“包含”或变体比如“包含”或“包含”应理解为意指包含指明的整体(integer)或整体(integer)组，但是不排除任何其他整体(integer)或整体(integer)组。

[0118] 2. ActRII拮抗剂

本文呈现的数据表明，ActRII拮抗剂(抑制剂) (例如ActRIIA和/或ActRIIB SMAD 2/3和/或SMAD 1/5/8信号传递的拮抗剂)可用于增加体内红细胞水平和提供给患者其他益处。具体地讲，这种ActRII拮抗剂本文显示有效治疗各种贫血以及MDS和铁粒幼红细胞性贫血的各种并发症(例如障碍/病症)。因此，本公开在某种程度上提供可单独或与一种或多种红细胞生成刺激剂(例如EPO)或其他支持疗法[例如造血生长因子(例如G-CSF或GM-CSF)、红细胞或全血输注、铁螯合疗法]组合使用的各种ActRII拮抗剂，在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍。

[0119] 在某些实施方案中，要按本文公开的方法使用的优选的ActRII拮抗剂为GDF-ActRII拮抗剂(例如GDF-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的拮抗剂)，特别是GDF11-和/或GDF8-介导的ActRII信号传递。在一些实施方案中，本公开优选的ActRII拮抗剂为可溶性ActRII多肽(例如可溶性ActRIIA和ActRIIB多肽)和GDF捕获多肽，比如ActRIIA-Fc融合蛋白、ActRIIB-Fc融合蛋白和GDF捕获物-Fc融合蛋白。

[0120] 尽管本公开的可溶性ActRII多肽和GDF捕获多肽可通过非GDF (例如GDF11和/或GDF8)拮抗的机制[例如GDF11和/或GDF8抑制作用可为试剂抑制一系列另外试剂，也许包括TGF-β超家族的其他成员(例如激活素B、激活素C、激活素E、BMP6、BMP7和/或Nodal)的活性的趋势的指示剂，并且这种叠加的抑制作用可对例如造血作用导致期望的效果]影响红细胞水平和/或MDS和铁粒幼红细胞性贫血的各种并发症，但是其他类型的GDF-ActRII拮抗剂预计是有用的，包括例如抗-GDF11抗体、抗-GDF8抗体；抗-激活素A、B、C和/或 E抗体；抗-ActRIIA抗体、抗-ActRIIB抗体、抗-ActRIIA/IIB抗体、反义、RNAi；或者抑制GDF11、GDF8、ActRIIA和/或ActRIIB中一种或多种的产生的核酶核酸；及GDF11、GDF8、ActRIIA和/或ActRIIB中一种或多种的其他抑制剂(例如小分子抑制剂)，特别是破坏GDF11-和/或GDF8-ActRIIA结合和/或GDF11-和/或GDF8-ActRIIB结合的试剂以及抑制GDF11、GDF8、ActRIIA

和/或ActRIIB中一种或多种的表达的试剂。任选地，本公开的GDF-ActRII拮抗剂可结合和/或抑制其他ActRII配体(包括例如激活素A、激活素AB、激活素B、激活素C、激活素E、BMP6、BMP7和/或Nodal)的活性(或表达)。任选地，本公开的GDF-ActRII拮抗剂可与至少一种另外的结合和/或抑制一种或多种另外的ActRII配体(包括例如激活素A、激活素AB、激活素B、激活素C、激活素E、BMP6、BMP7和/或Nodal)的活性(或表达)的ActRII拮抗剂组合使用。在一些实施方案中，要按本文公开的方法使用的ActRII拮抗剂基本上不结合和/或抑制激活素A(例如激活素A介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。

[0121] A. ActRII多肽和GDF捕获物

在某些方面，本公开涉及ActRII多肽。具体地讲，本公开提供单独或与一种或多种红细胞生成刺激剂(例如EPO)或其他支持疗法[例如造血生长因子(例如G-CSF或GM-CSF)、红细胞或全血输注、铁螯合疗法]组合使用ActRII多肽的方法，以例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍。本文使用的术语“ActRII”指的是II型激活素受体家族。该家族包括IIA型激活素受体和IIB型激活素受体两者。

[0122] 本文使用的术语“ActRIIB”指的是IIB型激活素受体(ActRIIB)蛋白家族，其来自于通过诱变或其他修饰源自这种ActRIIB蛋白的任何种类和变体。本文指涉ActRIIB应理解为指涉任何一种目前确认的形式。ActRIIB家族的成员通常为跨膜蛋白，由包含半胱氨酸富集区的细胞外配体结合域、跨膜域及具有预期的丝氨酸/苏氨酸激酶活性的胞浆域组成。

[0123] 术语“ActRIIB多肽”包括包含ActRIIB家族成员的任何天然存在的多肽及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式)的多肽。这种变体ActRIIA多肽的实例提供在整个本公开以及国际专利申请出版物第WO 2006/012627号中，其通过参照以其全部结合到本文中。任选地，本公开的ActRIIB多肽可用于在受试者增加红细胞水平。本文描述的全部ActRIIB相关多肽的氨基酸编号为基于以下提供的人ActRIIB前体蛋白序列(SEQ ID NO:1)的编号，除非另外具体指明。

[0124] 人ActRIIB前体蛋白序列如下：

```
1   MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE
51  GEQDKRLHCY ASWRNSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY
101 FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS
151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY
301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK
351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL
451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV
501 TNVDLPPKES SI                          (SEQ ID NO:1)
```

信号肽用单下划线表示;细胞外结构域用粗体字表示;和潜在的内源性N-连接的糖基化位点用双下划线表示。

[0125] 处理后的可溶性(细胞外)人ActRIIB多肽序列如下:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO:2)。

[0126] 在一些实施方案中,蛋白可在N-末端产生"SGR…"序列。细胞外结构域的C-末端"尾部"用单下划线表示。"尾部"缺失的序列(Δ15序列)如下:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO:3)。

[0127] 文献中还报道了在SEQ ID: 1的64位有丙氨酸的一种ActRIIB形式(A64) [参见例如Hilden等. (1994) Blood, 83(8): 2163-2170]。申请者已经确定,包含具有A64替换的ActRIIB细胞外结构域的ActRIIB-Fc融合蛋白对激活素和GDF11具有相对低的亲和力。相比之下,在64位具有精氨酸的相同ActRIIB-Fc融合蛋白(R64)在低纳摩尔至高皮摩尔范围对激活素和GDF11具有亲和力。因此,含R64的序列用作本公开中人ActRIIB的"野生型"参考序列。

[0128] 含有64位丙氨酸的ActRIIB形式如下:

```
1   MTAPWVALAL LWGSLCAGSG RGEAETRECI YYNANWELER TNQSGLERCE
51  GEQDKRLHCY ASWANSSGTI ELVKKGCWLD DFNCYDRQEC VATEENPQVY
101 FCCCEGNFCN ERFTHLPEAG GPEVTYEPPP TAPTLLTVLA YSLLPIGGLS
151 LIVLLAFWMY RHRKPPYGHV DIHEDPGPPP PSPLVGLKPL QLLEIKARGR
201 FGCVWKAQLM NDFVAVKIFP LQDKQSWQSE REIFSTPGMK HENLLQFIAA
251 EKRGSNLEVE LWLITAFHDK GSLTDYLKGN IITWNELCHV AETMSRGLSY
301 LHEDVPWCRG EGHKPSIAHR DFKSKNVLLK SDLTAVLADF GLAVRFEPGK
351 PPGDTHGQVG TRRYMAPEVL EGAINFQRDA FLRIDMYAMG LVLWELVSRC
401 KAADGPVDEY MLPFEEEIGQ HPSLEELQEV VVHKKMRPTI KDHWLKHPGL
451 AQLCVTIEEC WDHDAEARLS AGCVEERVSL IRRSVNGTTS DCLVSLVTSV
501 TNVDLPPKES SI                                    (SEQ ID NO:4)。
```

[0129] 信号肽用单下划线表示和细胞外结构域用粗体字表示。

[0130] 备选A64形式的处理后的可溶性(细胞外) ActRIIB多肽序列如下:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO:5)。

[0131] 在一些实施方案中,蛋白可在N-末端产生"SGR…"序列。细胞外结构域的C-末端"尾部"用单下划线表示。"尾部"缺失的序列(Δ15序列)如下:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWANSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEA (SEQ ID NO:6)。

[0132] 以下显示编码人ActRIIB前体蛋白的核酸序列(SEQ ID NO: 7),其由基因库参考序列NM_001106.3 (Genbank Reference Sequence NM_001106.3)的核苷酸25-1560组成,编码ActRIIB前体的氨基酸1-513。所显示的序列提供64位精氨酸,并可修饰为提供丙氨酸。信号序列为下划线的。

[0133] 1 ATGACGGCGC CCTGGGTGGC CCTCGCCCTC CTCTGGGGAT CGCTGTGCGC

51 CGGCTCTGGG CGTGGGGAGG CTGAGACACG GGAGTGCATC TACTACAACG

101 CCAACTGGGA GCTGGAGCGC ACCAACCAGA GCGGCCTGGA GCGCTGCGAA

151 GGCGAGCAGG ACAAGCGGCT GCACTGCTAC GCCTCCTGGC GCAACAGCTC

201 TGGCACCATC GAGCTCGTGA AGAAGGGCTG CTGGCTAGAT GACTTCAACT

251 GCTACGATAG GCAGGAGTGT GTGGCCACTG AGGAGAACCC CCAGGTGTAC

301 TTCTGCTGCT GTGAAGGCAA CTTCTGCAAC GAACGCTTCA CTCATTTGCC

351 AGAGGCTGGG GGCCCGGAAG TCACGTACGA GCCACCCCCG ACAGCCCCCA

401 CCCTGCTCAC GGTGCTGGCC TACTCACTGC TGCCCATCGG GGGCCTTTCC

451 CTCATCGTCC TGCTGGCCTT TTGGATGTAC CGGCATCGCA AGCCCCCCTA

501 CGGTCATGTG GACATCCATG AGGACCCTGG GCCTCCACCA CCATCCCCTC

551 TGGTGGGCCT GAAGCCACTG CAGCTGCTGG AGATCAAGGC TCGGGGGCGC

601 TTTGGCTGTG TCTGGAAGGC CCAGCTCATG AATGACTTTG TAGCTGTCAA

651 GATCTTCCCA CTCCAGGACA AGCAGTCGTG GCAGAGTGAA CGGGAGATCT

701 TCAGCACACC TGGCATGAAG CACGAGAACC TGCTACAGTT CATTGCTGCC

751 GAGAAGCGAG GCTCCAACCT CGAAGTAGAG CTGTGGCTCA TCACGGCCTT

801 CCATGACAAG GGCTCCCTCA CGGATTACCT CAAGGGGAAC ATCATCACAT

851 GGAACGAACT GTGTCATGTA GCAGAGACGA TGTCACGAGG CCTCTCATAC

901 CTGCATGAGG ATGTGCCCTG GTGCCGTGGC GAGGGCCACA AGCCGTCTAT

951 TGCCCACAGG GACTTTAAAA GTAAGAATGT ATTGCTGAAG AGCGACCTCA

1001 CAGCCGTGCT GGCTGACTTT GGCTTGGCTG TTCGATTTGA GCCAGGGAAA

1051 CCTCCAGGGG ACACCCACGG ACAGGTAGGC ACGAGACGGT ACATGGCTCC

1101 TGAGGTGCTC GAGGGAGCCA TCAACTTCCA GAGAGATGCC TTCCTGCGCA

1151 TTGACATGTA TGCCATGGGG TTGGTGCTGT GGGAGCTTGT GTCTCGCTGC

1201 AAGGCTGCAG ACGGACCCGT GGATGAGTAC ATGCTGCCCT TTGAGGAAGA

1251 GATTGGCCAG CACCCTTCGT TGGAGGAGCT GCAGGAGGTG GTGGTGCACA

1301 AGAAGATGAG GCCCACCATT AAAGATCACT GGTTGAAACA CCCGGGCCTG

1351 GCCCAGCTTT GTGTGACCAT CGAGGAGTGC TGGGACCATG ATGCAGAGGC

1401 TCGCTTGTCC GCGGGCTGTG TGGAGGAGCG GGTGTCCCTG ATTCGGAGGT

1451 CGGTCAACGG CACTACCTCG GACTGTCTCG TTTCCCTGGT GACCTCTGTC

1501 ACCAATGTGG ACCTGCCCCC TAAAGAGTCA AGCATC

(SEQ ID NO: 7)。

[0134] 编码处理后的可溶性(细胞外)人ActRIIB多肽的核酸序列如下(SEQ ID NO: 8)。所显示的序列提供64位精氨酸,并可修饰为提供丙氨酸。

[0135] 1 GGGCGTGGGG AGGCTGAGAC ACGGGAGTGC ATCTACTACA ACGCCAACTG

51 GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC GAAGGCGAGC

101 AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG CTCTGGCACC

151 ATCGAGCTCG TGAAGAAGGG CTGCTGGCTA GATGACTTCA ACTGCTACGA

201 TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG TACTTCTGCT

251 GCTGTGAAGG CAACTTCTGC AACGAACGCT TCACTCATTT GCCAGAGGCT

301 GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGCCC CCACC

(SEQ ID NO:8)。

[0136] 在某些实施方案中,本公开涉及ActRIIA多肽。本文使用的术语"ActRIIA"指的是IIA型激活素受体(ActRIIA)蛋白家族,其来自于通过诱变或其他修饰源自这种ActRIIA蛋白的任何种类和变体。本文指涉ActRIIA应理解为指涉任何一种目前确认的形式。ActRIIA家族的成员通常为跨膜蛋白,由包含半胱氨酸富集区的细胞外配体结合域、跨膜域及具有预期的丝氨酸/苏氨酸激酶活性的胞浆域组成。

[0137] 术语"ActRIIA多肽"包括包含ActRIIA家族成员的任何天然存在的多肽及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式)的多肽。这种变体ActRIIA多肽的实例提供在整个本公开以及国际专利申请出版物第WO 2006/012627号中,其通过参照以其全部结合到本文中。任选地,本公开的ActRIIA多肽可用于在受试者增加红细胞水平。本文描述的全部ActRIIA相关多肽的氨基酸编号为基于以下提供的人ActRIIA前体蛋白序列(SEQ ID NO:9)的编号,除非另外具体指明。

[0138] 人ActRIIA前体蛋白序列如下:

1 MGAAAKLAFA VFLISCSSGAILGRSETQEC LFFNANWEKD RTNQTGVEPC

51 YGDKDKRRHC FATWKNISGS IEIVKQGCWL DDINCYDRTD CVEKKDSPEV

101 YFCCCEGNMC NEKFSYFPEM EVTQPTSNPV TPKPPYYNIL LYSLVPLMLI

151 AGIVICAFWV YRHHKMAYPP VLVPTQDPGP PPPSPLLGLK PLQLLEVKAR

201 GRFGCVWKAQ LLNEYVAVKI FPIQDKQSWQ NEYEVYSLPG MKHENILQFI

251 GAEKRGTSVD VDLWLITAFH EKGSLSDFLK ANVVSWNELC HIAETMARGL

301 AYLHEDIPGL KDGHKPAISH RDIKSKNVLL KNNLTACIAD FGLALKFEAG

351 KSAGDTHGQV GTRRYMAPEV LEGAINFQRD AFLRIDMYAM GLVLWELASR

401 CTAADGPVDE YMLPFEEEIG QHPSLEDMQE VVVHKKKRPV LRDYWQKHAG

451 MAMLCETIEE CWDHDAEARL SAGCVGERIT QMQRLTNIIT TEDIVTVVTM

501 VTNVDFPPKE SSL (SEQ ID NO:9)

信号肽用单下划线表示;细胞外结构域用粗体字表示;和潜在的内源性N-连接的糖基化位点用双下划线表示。

[0139] 处理后的可溶性(细胞外)人ActRIIA多肽序列如下:

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPP (SEQ ID NO:10)

细胞外结构域的C-末端"尾部"用单下划线表示。"尾部"缺失的序列(Δ15序列)如下:

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEM (SEQ ID NO:11)

以下显示编码人ActRIIA前体蛋白的核酸序列(SEQ ID NO: 12),基因库参考序列NM_001106.4 (Genbank Reference Sequence NM_001106.4)的如下的核苷酸159-1700。信号序列为下划线的。

[0140] 1 atgggagctg ctgcaaagtt ggcgtttgcc gtctttctta tctcctgttc

51 ttcaggtgct atacttggta gatcagaaac tcaggagtgt cttttcttta

101 atgctaattg ggaaaaagac agaaccaatc aaactggtgt tgaaccgtgt
151 tatggtgaca aagataaacg gcggcattgt tttgctacct ggaagaatat
201 ttctggttcc attgaaatag tgaaacaagg ttgttggctg gatgatatca
251 actgctatga caggactgat tgtgtagaaa aaaaagacag ccctgaagta
301 tatttttgtt gctgtgaggg caatatgtgt aatgaaaagt tttcttattt
351 tccggagatg gaagtcacac agcccacttc aaatccagtt acacctaagc
401 caccctatta caacatcctg ctctattcct tggtgccact tatgttaatt
451 gcggggattg tcatttgtgc attttgggtg tacaggcatc acaagatggc
501 ctaccctcct gtacttgttc caactcaaga cccaggacca cccccacctt
551 ctccattact aggtttgaaa ccactgcagt tattagaagt gaaagcaagg
601 ggaagatttg gttgtgtctg gaaagcccag ttgcttaacg aatatgtggc
651 tgtcaaaata tttccaatac aggacaaaca gtcatggcaa aatgaatacg
701 aagtctacag tttgcctgga atgaagcatg agaacatatt acagttcatt
751 ggtgcagaaa aacgaggcac cagtgttgat gtggatcttt ggctgatcac
801 agcatttcat gaaaagggtt cactatcaga ctttcttaag gctaatgtgg
851 tctcttggaa tgaactgtgt catattgcag aaaccatggc tagaggattg
901 gcatatttac atgaggatat acctggccta aaagatggcc acaaacctgc
951 catatctcac agggacatca aaagtaaaaa tgtgctgttg aaaaacaacc
1001 tgacagcttg cattgctgac tttgggttgg ccttaaaatt tgaggctggc
1051 aagtctgcag gcgataccca tggacaggtt ggtacccgga ggtacatggc
1101 tccagaggta ttagagggtg ctataaactt ccaaagggat gcatttttga
1151 ggatagatat gtatgccatg ggattagtcc tatgggaact ggcttctcgc
1201 tgtactgctg cagatggacc tgtagatgaa tacatgttgc catttgagga
1251 ggaaattggc cagcatccat ctcttgaaga catgcaggaa gttgttgtgc
1301 ataaaaaaaa gaggcctgtt ttaagagatt attggcagaa acatgctgga
1351 atggcaatgc tctgtgaaac cattgaagaa tgttgggatc acgacgcaga
1401 agccaggtta tcagctggat gtgtaggtga aagaattacc cagatgcaga
1451 gactaacaaa tattattacc acagaggaca ttgtaacagt ggtcacaatg
1501 gtgacaaatg ttgactttcc tcccaaagaa tctagtcta

(SEQ ID NO:12)

编码处理后的可溶性(细胞外)人ActRIIA多肽的核酸序列如下：

1 atacttggta gatcagaaac tcaggagtgt cttttcttta atgctaattg
51 ggaaaaagac agaaccaatc aaactggtgt tgaaccgtgt tatggtgaca
101 aagataaacg gcggcattgt tttgctacct ggaagaatat ttctggttcc
151 attgaaatag tgaaacaagg ttgttggctg gatgatatca actgctatga
201 caggactgat tgtgtagaaa aaaaagacag ccctgaagta tatttttgtt
251 ctgtgaggg caatatgtgt aatgaaaagt tttcttattt tccggagatg
301 gaagtcacac agcccacttc aaatccagtt acacctaagc caccc

(SEQ ID NO:13)。

[0141] 在图1中图解说明了人ActRIIB可溶性细胞外结构域和人ActRIIA可溶性细胞外结构域的氨基酸序列比对。该比对表明认为直接接触ActRII配体的两种受体内的氨基酸残基。图2描绘了各种脊椎动物ActRIIB蛋白和人ActRIIA的多序列比对。从这些比对能够预测对于正常的ActRII-配体结合活性重要的配体结合域中的关键氨基酸位置,以及预测有可能容忍替换而不显著改变正常ActRII-配体结合活性的氨基酸位置。

[0142] 在其他方面,本公开涉及GDF捕获多肽(也称为"GDF捕获物"),其可例如单独或与一种或多种红细胞生成刺激剂(例如EPO)或其他支持疗法[例如造血生长因子(例如G-CSF或GM-CSF)、红细胞或全血输注、铁螯合疗法]组合使用,以例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或在有需要的受试者治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)。

[0143] 在一些实施方案中,本公开的GDF捕获物为可溶性的ActRII多肽(例如ActRIIA和ActRIIB多肽)变体,其在ActRII多肽(例如"野生型" ActRII多肽)的细胞外结构域(也称为配体结合域)包含一个或多个突变(例如氨基酸添加、缺失、替换及其组合),使得ActRII多肽变体比相应的野生型ActRII多肽具有一种或多种改变的配体结合活性。在优选的实施方案中,本公开的GDF捕获多肽保留至少一种与相应的野生型ActRII多肽(例如ActRIIA或ActRIIB多肽)相似的活性。例如,GDF捕获物可结合和/或抑制(例如拮抗)一种或多种ActRII配体的功能(例如抑制ActRII配体介导的ActRIIA和/或ActRIIB信号转导,比如SMAD 2/3和/或SMAD 1/5/8信号通路的激活)。在一些实施方案中,本公开的GDF捕获物结合和/或抑制激活素A、激活素B、激活素AB、激活素C、激活素E、Nodal、GDF8、GDF11、BMP6和/或BMP7中的一种或多种)。

[0144] 在某些实施方案中,本公开的GDF捕获多肽对一种或多种特定的ActRII配体(例如GDF8、GDF11、BMP6、Nodal和/或BMP7)的亲和力升高。在其他的实施方案中,本公开的GDF捕获多肽对一种或多种特定的ActRII配体(例如激活素A、激活素B、激活素AB、激活素C和/或激活素E)的亲和力降低。在仍然其他的实施方案中,本公开的GDF捕获多肽对一种或多种特定的ActRII配体的亲和力升高和对一种或多种不同/其他的ActRII配体的亲和力降低。因此,本公开提供对一种或多种ActRII配体结合特异性改变的GDF捕获多肽。

[0145] 在某些优选的实施方案中,例如与野生型ActRII多肽相比较,本公开的GDF捕获物被设计为优先结合和拮抗GDF11和/或GDF8 (也称为肌肉生长抑制素)。任选地,这种GDF11和/或GDF8-结合捕获物可进一步结合和/或拮抗Nodal、GDF8、GDF11、BMP6和/或BMP7中的一种或多种。任选地,这种GDF11和/或GDF8-结合捕获物可进一步结合和/或拮抗激活素B、激活素C、激活素E、Nodal、GDF8、GDF11、BMP6和/或BMP7中的一种或多种。任选地,这种GDF11和/或GDF8-结合捕获物可进一步结合和/或拮抗激活素A、激活素A/B、激活素B、激活素C、激活素E、Nodal、GDF8、GDF11、BMP6和/或BMP7中的一种或多种。在某些实施方案中,例如与野生型ActRII多肽相比较,本公开的GDF捕获物对激活素(例如激活素A、激活素A/B、激活素B、激活素C、激活素E)的亲和力减小。在某些优选的实施方案中,本公开的GDF捕获多肽对激活素A的亲和力减小。

[0146] 例如,本公开提供相对于激活素A优先结合和/或拮抗GDF8/GDF11的GDF捕获多肽。

如通过本公开的实例证实的那样，与对激活素A保留高亲和力的ActRII多肽相比较，这种GDF捕获多肽为体内红细胞生成的更有效激活剂。此外，这些非激活素A结合的GDF捕获多肽显示对其他组织的影响减小。因此，这种GDF捕获物可用于在受试者增加红细胞水平，同时减小与结合/拮抗激活素A有关的潜在偏离目标效应。然而，这种选择性GDF捕获多肽可能在其中对于治疗效果需要红细胞水平较为温和增长和其中某种程度的偏离目标效果是可接受的(或者甚至合乎需要的)一些应用方面不太令人满意。

[0147] ActRIIB蛋白的氨基酸残基(例如E39、K55、Y60、K74、W78、L79、D80和F101)处于ActRIIB配体结合口袋，并帮助介导与其配体(包括例如激活素A、GDF11和GDF8)的结合。因此本公开提供包含ActRIIB受体改变的配体结合域(例如GDF8/GDF11-结合域)(在那些氨基酸残基包含一个或多个突变)的GDF捕获多肽。

[0148] 任选地，相对于ActRIIB受体的野生型配体结合域，改变的配体结合域可增加对配体比如GDF11和/或GDF8的选择性。为了说明起见，可选择一种或多种突变，这种突变增加改变的配体结合域对GDF11和/或GDF8超过一种或多种激活素(激活素A、激活素B、激活素AB、激活素C和/或激活素A)，特别是激活素A的选择性。任选地，改变的配体结合域对激活素结合K_d与GDF11和/或GDF8结合K_d的比率，比相对于野生型配体结合域的比率大至少2-、5-、10-、20、50-、100-或者甚至1000-倍。任选地，改变的配体结合域抑制激活素的IC_{50}与抑制GDF11和/或GDF8的IC_{50}的比率，比相对于野生型配体结合域大至少2-、5-、10-、20-、50-、100-或者甚至1000-倍。任选地，改变的配体结合域抑制GDF11和/或GDF8的IC_{50}比抑制激活素的IC_{50}小至少2-、5-、10-、20-、50-、100-或者甚至1000-倍。

[0149] 作为一个具体实例，ActRIIB的配体结合域的荷正电氨基酸残基Asp (D80)可突变成不同的氨基酸残基，以产生优先结合GDF8而不是激活素的GDF捕获多肽。优选地，SEQ ID NO:1的D80残基变成选自以下的氨基酸残基：不荷电的氨基酸残基、负氨基酸残基和疏水性氨基酸残基。作为一个更具体的实例，SEQ ID NO:1的疏水性残基L79可被改变成赋予改变的激活素-GDF11/GDF8结合性能。例如，L79P替换在很大程度上比激活素结合减小GDF11结合。相比之下，用酸性氨基酸替代L79 [天冬氨酸或谷氨酸；L79D或L79E替换]极大降低激活素A亲和力同时保留GDF11亲和力。在示例性的实施方案中，本文描述的方法采用GDF捕获多肽，其为ActRIIB多肽变体，任选地与一种或多种另外的氨基酸替换、添加或缺失组合，在相应于SEQ ID NO: 1的79位的位置包含酸性氨基酸(例如D或E)。

[0150] 如本领域技术人员认识到的那样，本文描述的大多数所描述的突变、变体或修饰可在核酸水平进行，或者在一些情况下通过翻译后修饰或化学合成。这种技术为本领域熟知的，并且其中一些在本文得到描述。

[0151] 在某些实施方案中，本公开涉及其为可溶性ActRII多肽的ActRII多肽(ActRIIA和ActRIIB多肽)。如本文描述的那样，术语“可溶性ActRII多肽”通常指的是包含ActRII蛋白的细胞外结构域的多肽。本文使用的术语“可溶性ActRII多肽”包括ActRII蛋白的任何天然存在的细胞外结构域及其保留有用活性的任何变体(包括突变体、片段和拟肽形式) (例如本文描述的GDF捕获多肽)。可溶性ActRII多肽的其他实例包含除ActRII或GDF捕获物蛋白的细胞外结构域之外的信号序列。例如，信号序列可为ActRIIA或ActRIIB蛋白的天然信号序列或者选自以下另一种蛋白的信号序列：包括例如组织纤溶酶原激活剂(TPA)信号序列或蜜蜂蜂毒肽(HBM)信号序列。

[0152] 在某种程度上，本公开确认ActRII多肽的功能活动部分和变体，可用作本文描述的方法范围内生成和使用ActRIIA多肽、ActRIIB多肽和GDF捕获多肽的指导。

[0153] 本领域已就ActRII多肽的结构和功能特性而言，特别是关于配体结合进行了表征[参见例如Attisano等. (1992) Cell 68(1):97-108;Greenwald等. (1999) Nature Structural Biology 6(1): 18-22;Allendorph等. (2006) PNAS 103(20: 7643-7648; Thompson等. (2003) The EMBO Journal 22(7): 1555-1566;和美国专利号: 7709605、7612041和7842663]。

[0154] 例如，Attisano等显示ActRIIB的细胞外结构域C-末端的脯氨酸结缺失降低受体对激活素的亲和力。含有本SEQ ID NO:1的氨基酸20-119的ActRIIB-Fc融合蛋白(“ActRIIB(20-119)-Fc”)，相对于ActRIIB (20-134)-Fc减少了与GDF-11和激活素的结合，其包括脯氨酸结区域和完整的近膜结构域(参见例如美国专利第7842663号)。然而，ActRIIB (20-129)-Fc蛋白相对于野生型保留类似但略有减少的活性，即使脯氨酸结区域被破坏。因此，在氨基酸134、133、132、131、130和129 (相对于本SEQ ID NO:1)终止的ActRIIB细胞外结构域预计全部是活性的，但是在133或134终止的构建体可能最具活性。类似地，残基129-134(相对于SEQ ID NO:1)中任何一个的突变预计不会大幅度改变配体亲和力。支持这一点，P129和P130 (相对于SEQ ID NO:1)的突变不显著减少配体结合。因此，本公开的ActRIIB多肽或基于ActRIIB的GDF捕获多肽可早在氨基酸109 (最后的半胱氨酸)结尾，然而，在或于109和119之间(例如109、110、111、112、113、114、115、116、117、118或119)结尾的形式预计配体结合减少。氨基酸119 (相对于本SEQ ID NO:1)不太保守，并因此容易改变或截短。在128 (相对于本SEQ ID NO:1)或稍后结尾的ActRIIB多肽或基于ActRIIB的GDF捕获物应保留配体结合活性。在或于119和127之间(例如119、120、121、122、123、124、125、126或127)(相对于SEQ ID NO:1)结尾的ActRIIB多肽或基于ActRIIB的GDF捕获物将具有中等结合能力。可期望采用这些形式中的任何一种，取决于临床或试验环境。

[0155] 在ActRIIB的N-末端，预计在氨基酸29或之前(相对于本SEQ ID NO:1)开始的蛋白将保留配体结合活性。氨基酸29代表最初的半胱氨酸。24位(相对于本SEQ ID NO:1)的丙氨酸到天冬酰胺突变引入一种不显著影响配体结合的N-连接的糖基化序列(参见例如美国专利第7842663号)。这证实了在信号切割肽(signal cleavage peptide)与半胱氨酸交联区域之间(对应于氨基酸20-29)区域的突变耐受性良好。具体地讲，在20、21、22、23和24位(相对于本SEQ ID NO:1)开始的ActRIIB多肽和基于ActRIIB的GDF捕获物应保留一般配体结合活性，和在25、26、27、28和29位(相对于本SEQ ID NO:1)开始的ActRIIB多肽和基于ActRIIB的GDF捕获物预计也保留配体结合活性。本文以及在例如美国专利第7842663号中显示的数据令人惊讶地表明，在22、23、24或25开始的ActRIIB构建体具有最多的活性。

[0156] 总起来讲，ActRIIB的活性部分(例如配体结合活性)包含SEQ ID NO:1的氨基酸29-109。因此本公开的ActRIIB多肽和基于ActRIIB的GDF捕获物可例如包含一种氨基酸序列，这种序列与ActRIIB的一部分至少80%、85%、90%、95%、96%、97%、98%、99%或100%相同，所述ActRIIB的部分在相应于SEQ ID NO:1的氨基酸20-29的残基开始(例如在氨基酸20、21、22、23、24、25、26、27、28或29开始)，和在相应于SEQ ID NO:1的氨基酸109-134的位置结尾(例如在氨基酸109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134结尾)。在一些实施方案中，本公开基于

ActRIIB的GDF捕获多肽不包含或由SEQ ID NO:1的氨基酸29-109组成。其他实例包括在SEQ ID NO: 1的20-29 (例如20、21、22、23、24、25、26、27、28或29位)或21-29 (例如21、22、23、24、25、26、27、28或29位)中的位置开始和在119-134 (例如119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134)、119-133 (例如119、120、121、122、123、124、125、126、127、128、129、130、131、132或133)、129-134 (例如129、130、131、132、133或134)或129-133 (例如129、130、131、132或133)中的位置结尾的多肽。其他实例包括在SEQ ID NO: 1的20-24 (例如20、21、22、23或24)、21-24 (例如21、22、23或24)或22-25 (例如22、22、23或25)中的位置开始和在109-134 (例如109、110、111、112、113、114、115、116、117、118、119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134)、119-134 (例如119、120、121、122、123、124、125、126、127、128、129、130、131、132、133或134)或129-134 (例如129、130、131、132、133或134)中的位置结尾的构建体。还考虑了这些范围内的变体,特别是与SEQ ID NO: 1的相应部分具有至少80%、85%、90%、95%、96%、97%、98%、99%或100%同一性的那些变体。在一些实施方案中,ActRIIB多肽和基于ActRIIB的GDF捕获物包含具有与SEQ ID NO: 1的氨基酸残基25-131至少80%、85%、90%、95%、96%、97%、98%、99%或100%相同的氨基酸序列的多肽。在某些实施方案中,基于ActRIIB的GDF捕获多肽不包含或由SEQ ID NO:1的氨基酸25-131组成。

[0157] 本公开包括在图1中显示的复合ActRIIB结构的分析结果,表明在某种程度上由残基Y31、N33、N35、L38直到T41、E47、E50、Q53直到K55、L57、H58、Y60、S62、K74、W78直到N83、Y85、R87、A92和E94直到F101确定了配体结合口袋。在这些位置上,预计应容忍保守性突变,尽管K74A突变容忍良好,如同R40A、K55A、F82A和L79位的突变那样。非洲爪蟾蜍的R40为K,表明该位置容忍碱性氨基酸。牛ActRIIB的Q53为R和非洲爪蟾蜍ActRIIB的为K,并且因此该位置容忍包括R、K、Q、N和H在内的氨基酸。因此,本公开的ActRIIB多肽和基于ActRIIB的GDF捕获多肽的通式为包含与SEQ ID NO: 1的氨基酸29-109至少80%、85%、90%、95%、96%、97%、98%、99%或100%相同的氨基酸序列的多肽,其任选地在20-24 (例如20、21、22、23或24)或22-25 (例如22、23、24或25)范围内的位置开始和在129-134 (例如129、130、131、132、133或134)范围内的位置结尾,并且在配体结合口袋包含不多于1、2、5、10或15个保守性氨基酸变化和在配体结合口袋的40、53、55、74、79和/或82位包含0、1或更多个非保守性改变。变异可以特别良好容忍的结合口袋外面的位点包括细胞外结构域(如上面所提到的)及42-46和65-73位(相对于SEQ ID NO:1)的氨基和羧基末端。65位的天冬酰胺改变为丙氨酸(N65A)实际上改善了A64背景下的配体结合,并因此预计对R64背景下的配体结合没有不利影响(参见例如美国专利第7842663号)。这种变化可能消除A64背景下的N65糖基化,因此表明可能会容忍该区域的显著变化。尽管R64A变化不太容忍,R64K容忍良好,并且因此64位的另一种碱性残基比如H是可以容忍的(参见例如美国专利第7842663号)。

[0158] ActRIIB在几乎所有脊椎动物都很保守,细胞外结构域大的延伸完全保守。许多结合于ActRIIB的配体也是高度保守的。因此,比较各种脊椎动物生物的ActRIIB序列能够洞察可改变的残基。因此,按本公开的方法有用的活性人ActRIIB变体多肽和基于ActRIIB的GDF捕获物可能在另一种脊椎动物ActRIIB序列的相应位置包括一个或多个氨基酸,或者可包括与人或其他脊椎动物序列相似的残基。以下实例说明这种确定活性ActRIIB变体的方法。非洲爪蟾蜍ActRIIB的L46为缬氨酸,并因此该位置可以改变,且任选地可改变成另一种

疏水性残基比如V、I或F，或者非极性残基比如A。非洲爪蟾蜍的E52为K，表明该位点可容忍广泛种类的变化，包括极性残基，比如E、D、K、R、H、S、T、P、G、Y和可能为A。非洲爪蟾蜍的T93为K，表明该位置容忍广泛的结构变化，极性残基有利，比如S、K、R、E、D、H、G、P、G和Y。非洲爪蟾蜍的F108为Y，并因此应该容忍Y或其他疏水性基团，比如I、V或L。非洲爪蟾蜍的E111为K，表明该位置容忍荷电的残基，包括D、R、K和H以及Q和N。非洲爪蟾蜍的R112为K，表明该位置容忍碱性残基，包括R和H。119位相对不太保守，并且在啮齿动物显示为P和在非洲爪蟾蜍显示为V，因此基本上该位置应该容忍任何氨基酸。

[0159] 先前已经证实，相对于ActRIIB (R64)-Fc形式添加另外的N-连接糖基化位点(N-X-S/T)容忍良好(参见例如美国专利第7842663号)。因此，N-X-S/T序列可通常在本公开ActRIIB多肽和基于ActRIIB的GDF捕获物的图1中确定的配体结合口袋外面的位置引入。用于引入非内源性N-X-S/T序列的特别合适位点包括氨基酸20-29、20-24、22-25、109-134、120-134或129-134 (相对于SEQ ID NO:1)。N-X-S/T序列也可在ActRIIB序列和Fc结构域或其他融合组件之间引入到连接子中。这样的位点可通过在相对于先已存在的S或T的正确位置引入N或通过在相应于先已存在的N的位置引入S或T而用最少的努力来引入。因此，产生N-链接的糖基化位点的理想改变是：A24N、R64N、S67N (可能与N65A改变相结合)、E105N、R112N、G120N、E123N、P129N、A132N、R112S和R112T (相对于SEQ ID NO:1)。可把预计要糖基化的任何S改变为T而不产生免疫原性位点，由于糖基化提供保护。同样地，可把预计要糖基化的任何T改变为S。因此考虑改变S67T和S44T (相对于SEQ ID NO:1)。同样地，在A24N变体可采用S26T改变。因此，本公开的ActRIIB多肽和基于ActRIIB的GDF捕获多肽可为具有如以上描述的一种或多种另外的非内源性N-链接的糖基化共有序列的变体。

[0160] 本文描述的变化可以各种方式组合在一起。另外，本文描述的诱变程序的结果表明，ActRIIB中存在通常有利于保守的氨基酸位置。对于SEQ ID NO:1，这些位置包括64位(碱性氨基酸)、80位(酸性或疏水性氨基酸)、78位(疏水性的，并且特别是色氨酸)、37位(酸性的，并且特别是天冬氨酸或谷氨酸)、56位(碱性氨基酸)、60位(疏水性氨基酸，并且特别是苯丙氨酸或酪氨酸)。因此，在本文公开的ActRIIB多肽和基于ActRIIB的GDF捕获物中，本公开提供可能保守的氨基酸骨架。可能期望保守的其他位置如下：52位(酸性氨基酸)、55位(碱性氨基酸)、81位(酸性的)、98 (极性或荷电的，特别是E、D、R或K)，其全部为相对于SEQ ID NO:1。

[0161] 活性(例如配体结合) ActRIIA多肽的通式为包含在SEQ ID NO:9的氨基酸30开始和在氨基酸110结尾的多肽的通式。因此，本公开的ActRIIA多肽和基于ActRIIA的GDF捕获物可包含与SEQ ID NO:9的氨基酸30-110至少80%、85%、90%、95%、96%、97%、98%、99%或100%相同的多肽。在一些实施方案中，本公开基于ActRIIA的GDF捕获物不包含或由SEQ ID NO:9的氨基酸30-110组成。任选地，本公开的ActRIIA多肽和基于ActRIIA的GDF捕获多肽包含与SEQ ID NO:9的氨基酸12-82至少80%、85%、90%、95%、96%、97%、98%、99%或100%相同的多肽，其任选地分别在1-5 (例如1、2、3、4或5)或3-5 (例如3、4或5)范围内的位置开始和在110-116 (例如110、111、112、113、114、115或116)或110-115 (例如110、111、112、113、114或115)范围内的位置结尾，并且在SEQ ID NO:9的配体结合口袋包含不多于1、2、5、10或15个保守性氨基酸变化，和在配体结合口袋的40、53、55、74、79和/或82位包含0、1或多个非保守性改变。

[0162] 在某些实施方案中，本公开的ActRII多肽(例如ActRIIA和ActRIIB多肽)和GDF捕获多肽的功能活性片段，可通过筛选自编码ActRII多肽或GDF捕获多肽的核酸相应片段(例如SEQ ID NOs: 7、8、12、13、27、32、39、40、42、46和48)重组产生的多肽得到。另外，片段可采用本领域已知的技术比如常规的梅里菲尔德(Merrifield)固相f-Moc或t-Boc化学进行化学合成。这种片段可生产(重组或通过化学合成)和测试以确认那些肽片段可起ActRII受体和/或一种或多种ActRII配体(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和/或Nodal)的拮抗剂(抑制剂)作用。

[0163] 在一些实施方案中，本公开的ActRIIA多肽为包含与选自SEQ ID NOs: 9、10、11、22、26和28的氨基酸序列至少75%相同的氨基酸序列的多肽。在某些实施方案中，ActRIIA多肽包含与选自SEQ ID NOs: 9、10、11、22、26和28的氨基酸序列至少80%、85%、90%、95%、97%、98%、99%或100%相同的氨基酸序列。在某些实施方案中，ActRIIA多肽基本上由、或由与选自SEQ ID NOs: 9、10、11、22、26和28的氨基酸序列至少80%、85%、90%、95%、97%、98%、99%或100%相同的氨基酸序列组成。

[0164] 在一些实施方案中，本公开的ActRIIB多肽为包含与选自SEQ ID NOs: 1、2、3、4、5、6、29、31和49的氨基酸序列至少75%相同的氨基酸序列的多肽。在某些实施方案中，ActRIIB多肽包含与选自SEQ ID NOs: 1、2、3、4、5、6、29、31和49的氨基酸序列至少80%、85%、90%、95%、97%、98%、99%或100%相同的氨基酸序列。在某些实施方案中，ActRIIB多肽基本上由、或由与选自SEQ ID NOs: 1、2、3、4、5、6、29、31和49的氨基酸序列至少80%、85%、90%、95%、97%、98%、99%或100%相同的氨基酸序列组成。

[0165] 在一些实施方案中，本公开的GDF捕获多肽为包含与选自SEQ ID NOs: 1、2、3、4、5、6、29、30、31、36、37、38、41、44、45、49、50和51的氨基酸序列至少75%相同的氨基酸序列的ActRIIB多肽变体。在某些实施方案中，GDF捕获物包含与选自SEQ ID NOs: 1、2、3、4、5、6、29、30、31、36、37、38、41、44、45、49、50和51的氨基酸序列至少80%、85%、90%、95%、97%、98%、99%或100%相同的氨基酸序列。在某些实施方案中，GDF捕获物包含与选自SEQ ID NOs: 1、2、3、4、5、6、29、30、31、36、37、38、41、44、45、49、50和51的氨基酸序列至少80%、85%、90%、95%、97%、98%、99%或100%相同的氨基酸序列，其中相应于SEQ ID NO:1、4或49的L79的位置为酸性氨基酸(D或E氨基酸残基)。在某些实施方案中，GDF捕获物基本上由、或由与选自36、37、38、41、44、45、50和51的氨基酸序列至少80%、85%、90%、95%、97%、98%、99%或100%相同的氨基酸序列组成。在某些实施方案中，GDF捕获物不包含或由选自SEQ ID NOs: 1、2、3、4、5、6、29和31的氨基酸序列组成。

[0166] 在一些实施方案中，本公开的GDF捕获多肽为包含与选自SEQ ID NOs: 9、10、11、22、26、28、29和31的氨基酸序列至少75%相同的氨基酸序列的ActRIIA多肽变体。在某些实施方案中，GDF捕获物包含与选自SEQ ID NOs: 9、10、11、22、26、28、29和31的氨基酸序列至少80%、85%、90%、95%、97%、98%、99%或100%相同的氨基酸序列。在某些实施方案中，GDF捕获物基本上由、或由与选自SEQ ID NOs: 9、10、11、22、26、28、29和31的氨基酸序列至少80%、85%、90%、95%、97%、98%、99%或100%相同的氨基酸序列组成。在某些实施方案中，GDF捕获物不包含或由选自SEQ ID NOs: 9、10、11、22、26、28、29和31的氨基酸序列组成。

[0167] 在一些实施方案中，本公开考虑通过修饰ActRII多肽(例如和ActRIIA或ActRIIB多肽)或GDF捕获物的结构制备功能性变体，为了比如提高疗效或稳定性(例如保质期和体

内对蛋白水解降解的抵抗力)。变体可通过氨基酸替换、缺失、添加或其组合产生。例如,可以合理地预期,孤立地用异亮氨酸或缬氨酸替换亮氨酸、用谷氨酸替换天冬氨酸、用丝氨酸替换苏氨酸或者氨基酸用结构相关的氨基酸类似替换(例如保守性突变),对生成分子的生物活性没有重大影响。保守性替换为在其侧链相关的氨基酸家族内发生的那些替换。通过与未修饰的或野生型多肽相比较,评价多肽变体以与野生型多肽类似的方式在细胞中产生反应或者结合于一种或多种配体比如GDF11、激活素A、激活素B、激活素AB、激活素C、激活素E、GDF8、BMP6和BMP7的能力,可易于确定本公开多肽的氨基酸序列的变化是否导致生成功能同系物。

[0168] 在某些实施方案中,本公开考虑本公开的ActRII多肽和GDF捕获多肽的特定突变,从而改变多肽的糖基化。这种突变可进行选择,以至引入或消除一种或多种糖基化位点,比如O-连接或N-连接的糖基化位点。天冬酰胺-连接的糖基化识别位点通常包含三肽序列,天冬酰胺-X-苏氨酸或天冬酰胺-X-丝氨酸(其中"X"为任何氨基酸),其用适当的细胞糖基化酶特异性识别。也可通过对多肽序列(对O-连接的糖基化位点)用一个或多个丝氨酸或苏氨酸残基进行添加或替换进行改变。糖基化识别位点的第一或第三个氨基酸位置中的一个或两者的各种氨基酸替换或缺失(和/或第二个位置的氨基酸缺失),导致在修饰后的三肽序列非糖基化。增加多肽的碳水化合物部分数目的另一种手段为通过把糖苷化学或酶偶联于多肽。依所采用的偶联模式而定,糖可附着于(a) 精氨酸和组氨酸;(b) 游离羧基;(c) 游离巯基比如半胱氨酸的巯基;(d) 游离羟基比如丝氨酸、苏氨酸或羟脯氨酸的羟基;(e) 芳族残基比如苯丙氨酸、酪氨酸或色氨酸的芳族残基;或(f) 谷氨酰胺的酰胺基。去除存在于多肽上的一个或多个碳水化合物部分可化学和/或酶法完成。化学去糖基化可包括例如使多肽接触化合物三氟甲磺酸或等效化合物。这种处理导致除了连接糖(N-乙酰葡糖胺或N-乙酰半乳糖胺)外的大部分或全部糖解离,同时留下完整的氨基酸序列。多肽上碳水化合物部分的酶促裂解可如Thotakura等[Meth. Enzymol. (1987) 138:350]描述的那样,通过采用各种内切和外切糖苷酶实现。多肽的序列可视情况而定进行调整,这取决于所采用的表达系统类型,因为哺乳动物、酵母、昆虫和植物细胞都可引入不同的糖基化模式,其模式可能受肽的氨基酸序列影响。通常,本公开用于人的ActRII多肽和GDF捕获多肽可在提供适当糖基化的哺乳动物细胞系比如HEK293或CHO细胞系中表达,尽管其他哺乳动物表达细胞系也有望发挥作用。

[0169] 本公开进一步考虑产生突变体的方法,特别是本公开的ActRII多肽和GDF捕获多肽的组合突变体组以及截短突变体。组合突变体库(Pools)对于识别ActRII和GDF捕获物序列尤其有用。筛选这种组合库的目的可能是产生例如具有改变的性质比如改变的药代动力学或改变的配体结合的多肽变体。以下提供各种筛选试验,并且这种试验可用于评价变体。例如,可筛选ActRII多肽和GDF捕获多肽结合ActRII受体,以防止ActRII配体(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP7、BMP6和/或Nodal)结合ActRII多肽或干扰ActRII配体造成的信号传递的能力。

[0170] ActRII多肽或GDF捕获多肽的活性也可以基于细胞的或体内试验进行测试。例如,可评价ActRII多肽或GDF捕获多肽对参与造血作用的基因表达的作用。这可根据需要在一种或多种重组ActRII配体蛋白(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP7、BMP6和/或Nodal)存在下进行,并可转染细胞,从而产生ActRII多肽或GDF捕获

多肽,和任选地产生ActRII配体。同样,ActRII多肽或GDF捕获多肽可给予小鼠或其他动物,并可采用领域公认的方法评价一个或多个血液指标比如RBC计数、血红蛋白或网织红细胞计数。

[0171] 可产生组合衍生的变体,其相对于参考ActRII多肽或GDF捕获多肽具有选择性或通常增加效力。这种变体当自重组DNA构建体表达时,可用于基因治疗方案。同样,诱变可产生细胞内半衰期显著不同于相应未修饰的ActRII多肽或GDF捕获多肽的变体。例如,改变的蛋白可使得对蛋白水解降解或者导致未修饰的多肽破坏或失活的其他细胞过程更稳定或更不稳定。这种变体及编码它们的基因可用于通过调节多肽的半衰期改变ActRII多肽或GDF捕获多肽水平。例如,半衰期短会产生更短暂的生物效应,并当诱导表达系统的一部分时,可使得能够严格控制细胞内的重组ActRII多肽或GDF捕获多肽水平。在Fc融合蛋白,突变可在连接子(如果有任何)和/或Fc蛋白进行,以改变蛋白的半衰期。

[0172] 组合库可通过编码多肽库的简并基因库产生,其每一个包括潜在ActRII或GDF捕获物序列的至少一部分。例如,合成寡核苷酸的混合物可酶促连接于基因序列,使得编码核苷酸序列的潜在的ActRII或GDF捕获多肽的简并组可作为单个多肽或者作为一组更大的融合蛋白(例如噬菌体展示)表达。

[0173] 存在许多方法可自简并寡核苷酸序列产生潜在的同系物库。简并基因序列的化学合成可在DNA自动合成仪进行,并然后可把合成的基因连接到适当的表达载体。简并寡核苷酸的合成为本领域熟知的。参见例如Narang, SA (1983) Tetrahedron 39:3;Itakura等. (1981) Recombinant DNA, Proc (重组DNA,程序). 第3版. Cleveland Sympos. Macromolecules (大分子), AG Walton编辑, Amsterdam: Elsevier (阿姆斯特丹:爱思唯尔) 第273-289页;Itakura等. (1984) Annu. Rev. Biochem. 53:323;Itakura等. (1984) Science 198:1056;Ike等. (1983) Nucleic Acid Res. 11:477。这种技术已被用于其他蛋白的定向进化。参见例如Scott等, (1990) Science 249:386-390;Roberts等. (1992) PNAS USA 89:2429-2433;Devlin等. (1990) Science 249: 404-406;Cwirla等, (1990) PNAS USA 87: 6378-6382;以及美国专利号: 5223409、5198346和5096815。

[0174] 或者,其他形式的诱变可用于产生组合库。例如,通过采用例如丙氨酸扫描诱变进行筛选[参见例如Ruf等. (1994) Biochemistry 33:1565-1572;Wang等. (1994) J. Biol. Chem. 269:3095-3099;Balint等. (1993) Gene 137:109-118;Grodberg等. (1993) Eur. J. Biochem. 218:597-601;Nagashima等. (1993) J. Biol. Chem. 268: 2888-2892;Lowman等. (1991) Biochemistry 30:10832-10838;和Cunningham等. (1989) Science 244:1081-1085]、通过接头分区诱变[参见例如Gustin等. (1993) Virology 193:653-660;和Brown等. (1992) Mol. Cell Biol. 12:2644-2652;McKnight等. (1982) Science 232:316]、通过饱和诱变[参见例如Meyers等, (1986) Science 232:613]、通过PCR诱变[参见例如Leung等. (1989) Method Cell Mol Biol 1:11-19]或者通过随机诱变,包括化学诱变[参见例如Miller等. (1992) A Short Course in Bacterial Genetics (一门细菌遗传学的短期课程), CSHL Press (CSHL出版社), Cold Spring Harbor (科尔德斯普林港), NY (纽约);和Greener等. (1994) Strategies in Mol Biol (分子生物学策略) 7:32-34],可自库产生和分离本公开的ActRII多肽或GDF捕获多肽。特别是在组合环境的情况下,接头分区诱变是确认ActRII多肽的截短(生物活性)形式的有吸引力的方法。

[0175] 本领域已知广泛范围的技术用于筛选通过点突变和截短制备的组合库基因产物，并且就此而言，用于筛选具有某种性能的基因产物的cDNA库。这种技术通常适合于快速筛选通过组合诱变本公开的ActRII多肽或GDF捕获多肽产生的基因库。用于筛选大基因库的最广泛使用的技术一般包括将基因库克隆到可复制的表达载体，用生成的载体库转化适当的细胞，并在其中检测期望的活性便于相对易于分离编码基因(检测其产物)的载体条件下表达组合基因。优选的试验包括ActRII配体(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP7、BMP6和/或Nodal)结合试验和/或ActRII配体介导的细胞信号检测。

[0176] 在某些实施方案中，除ActRII (例如ActRIIA或ActRIIB多肽)或GDF 捕获多肽中天然存在的任何结构外，本公开的ActRII多肽或GDF捕获多肽可进一步包含翻译后修饰。这种修饰非限制性地包括乙酰化、羧化、糖基化、磷酸化、脂化和酰化。结果，ActRII多肽或GDF捕获多肽可含有非氨基酸元素，比如聚乙二醇、脂质、多糖或单糖及磷酸酯。这种非氨基酸元素对配体捕获多肽功能性的影响，可如本文对其他ActRII或GDF捕获物变体描述的那样进行测试。当本公开的多肽通过裂解一种新生形式多肽在细胞中产生时，翻译后加工对蛋白的正确折叠和/或功能可能也很重要。不同细胞(例如CHO、HeLa、MDCK、293、WI38、NIH-3T3或HEK293)对这种翻译后的活动具有特定的细胞机制和特性机制，并可进行选择以确保ActRII多肽或GDF捕获多肽的正确修饰与加工。

[0177] 在某些方面，本公开的ActRII多肽或GDF捕获多肽包括具有ActRII多肽(例如ActRIIA或ActRIIB多肽)或GDF捕获多肽的至少一部分(结构域)和一个或多个异源性部分(结构域)的融合蛋白。这种融合结构域的熟知实例非限制性地包括多组氨酸、Glu-Glu、谷胱苷肽S-转移酶(GST)、硫氧还蛋白、蛋白质A、蛋白质G、免疫球蛋白重链恒定区(Fc)、麦芽糖结合蛋白(MBP)或人血清白蛋白。融合结构域可进行选择以赋予期望的性能。例如，一些融合结构域对于通过亲和色谱法分离融合蛋白特别有用。为了亲和纯化，采用用于亲和色谱法的相关基质，比如谷胱甘肽-、淀粉酶-及镍-或钴-共轭树脂。许多这种基质可以“试剂盒(kit)”形式得到，比如与(HIS_6)融合配偶体一起使用的法玛西亚(Pharmacia) GST纯化系统和QIAexpress™系统(Qiagen)。作为另一个实例，融合结构域可进行选择以便于配体捕获多肽的检测。这种检测结构域的实例包括各种荧光蛋白(例如GFP)以及“表位标签”，其通常为特异性抗体可用的短肽序列。特异性单克隆抗体易于得到的熟知的表位标签包括FLAG、流感病毒血凝素(HA)和c-myc标签。在一些情况下，融合结构域具有比如因子Xa或凝血酶的蛋白酶裂解位点，使得相关蛋白酶能够部分消化融合蛋白，并从而由此释放重组蛋白。然后可自融合结构域经随后的色谱分离来分离所释放的蛋白。在某些优选的实施方案中，ActRII多肽或GDF捕获多肽与体内稳定多肽的结构域(“稳定元件”结构域(“stabilizer”domain))融合。所谓的“稳定”意味着增加血清半衰期的任何作用，不管这是否是因为破坏减少、肾清除率降低或其他药代动力学的影响。已知与免疫球蛋白的Fc部分融合赋予广泛范围的蛋白以合乎需要的药代动力学性质。同样，与人血清白蛋白融合可赋予合乎需要的性能。可选择的其他类型融合结构域包括多聚化(例如二聚化、四聚化)结构域和功能域(赋予另外的生物功能，比如进一步刺激肌肉生长)。

[0178] 在某些实施方案中，本公开提供包含以下IgG1 Fc区域序列的ActRII或GDF捕获物融合蛋白：

1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE

51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK

101 VSNKALPVPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF

151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV

201 FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO:14)。

[0179] 在其他的实施方案中,本公开提供包含以下IgG1 Fc结构域变体的ActRII或GDF捕获物融合蛋白:

1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSHEDPE

51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK

101 VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF

151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV

201 FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO:64)

在仍然其他的实施方案中,本公开提供包含以下IgG1 Fc结构域变体的ActRII或GDF捕获物融合蛋白:

1 THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV VVD(A)VSHEDPE

51 VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD WLNGKEYKCK(A)

101 VSNKALPVPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ VSLTCLVKGF

151 YPSDIAVEWE SNGQPENNYK TTPPVLDSDG PFFLYSKLTV DKSRWQQGNV

201 FSCSVMHEAL HN(A)HYTQKSLS LSPGK (SEQ ID NO:15)。

[0180] 任选地,IgG1 Fc结构域在残基比如Asp-265、赖氨酸322和Asn-434有一个或多个突变。在某些情况下,相对于野生型Fc结构域,具有一个或多个这些突变(例如Asp-265突变)的突变IgG1 Fc结构域结合Fcγ受体的能力降低。在其他情况下,相对于野生型IgG1 Fc结构域,具有一个或多个这些突变(例如Asn-434突变)的突变Fc结构域结合MHC类I-相关的Fc-受体(FcRN)的能力增强。

[0181] 在某些其他的实施方案中,本公开提供包含包括以下的IgG2 Fc结构域变体的ActRII或GDF捕获物融合蛋白:

1 VECPPCPAPP VAGPSVFLFP PKPKDTLMIS RTPEVTCVVV DVSHEDPEVQ

51 FNWYVDGVEV HNAKTKPREE QFNSTFRVVS VLTVVHQDWL NGKEYKCKVS

101 NKGLPAPIEK TISKTKGQPR EPQVYTLPPS REEMTKNQVS LTCLVKGFYP

151 SDIAVEWESN GQPENNYKTT PPMLDSDGSF FLYSKLTVDK SRWQQGNVFS

201 CSVMHEALHN HYTQKSLSLS PGK (SEQ ID NO:65)

应该理解,融合蛋白的不同元素可以与期望的功能性一致的任何方式排列。例如,ActRII多肽结构域或GDF捕获多肽结构域可置于异源结构域的C-末端,或者异源结构域可置于ActRII多肽结构域或GDF捕获多肽结构域的C-末端。ActRII多肽结构域或GDF捕获多肽结构域及异源结构域在融合蛋白中不需要相邻,并且另外的结构域或氨基酸序列可包括在结构域或结构域之间的C-或N-端。

[0182] 例如,ActRII或GDF捕获物融合蛋白可包含如在式A-B-C中阐述的氨基酸序列。B部分对应于ActRII多肽结构域或GDF捕获多肽结构域。A和C部分可独立地为0、1或多于1个氨基酸,并且A和C两部分(当存在时)与B异源。A和/或C部分可经连接序列附着于B部分。示例

性的连接子包括短的连接肽比如2-10、2-5、2-4、2-3甘氨酸残基，比如Gly-Gly-Gly连接子。上文描述了其他合适的连接子[例如TGGG连接子(SEQ ID NO: 53)]。在某些实施方案中，ActRII或GDF捕获物融合蛋白包含如在式A-B-C中阐述的氨基酸序列，其中A为前导(信号)序列，B由ActRII或GDF多肽结构域组成，和C为增强体内稳定性、体内半衰期、摄取/给予、组织定位或分布、蛋白复合物的形成和/或纯化中一种或多种的多肽部分。在某些实施方案中，ActRII或GDF捕获物融合蛋白包含如在式A-B-C中阐述的氨基酸序列，其中A为TPA前导序列，B由ActRII或GDF多肽结构域组成，和C为免疫球蛋白Fc结构域。优选的融合蛋白包含以SEQ ID NOs: 22、26、29、31、36、38、41、44和51中的任何一个阐述的氨基酸序列。

[0183] 在某些实施方案中，本公开的ActRII多肽或GDF捕获多肽含有能够稳定多肽的一种或多种修饰。例如，这种修饰提高多肽的体外半衰期、提高多肽的循环半衰期和/或减少多肽的蛋白水水解降解。这种稳定化修饰非限制性地包括融合蛋白(包括例如包含ActRII多肽结构域或GDF捕获多肽结构域及稳定元件结构域(stabilizer domain)的融合蛋白)、糖基化位点的修饰(包括例如向本公开的多肽添加糖基化位点)和碳水化合物部分的修饰(包括例如自本公开的多肽去除碳水化合物部分)。本文使用的术语“稳定元件结构域(stabilizer domain)”不仅指如在融合蛋白情况下的融合结构域(例如免疫球蛋白Fc结构域)，而且包括非蛋白修饰比如碳水化合物部分或非蛋白部分比如聚乙二醇。

[0184] 在优选的实施方案中，要按本文描述的方法使用的ActRII多肽和GDF捕获物为分离的多肽。本文使用的分离蛋白或多肽为已自其自然环境的组成部分分离的蛋白或多肽。在一些实施方案中，本公开的多肽被纯化为如通过例如电泳(例如SDS-PAGE、等电点聚焦(IEF)、毛细管电泳)或色谱(例如离子交换或反相HPLC)测定的那样大于95%、96%、97%、98%或99%纯度。用于评价抗体纯度的方法为本领域熟知的[参见例如Flatman等., (2007) J. Chromatogr. B 848:79-87]。

[0185] 在某些实施方案中，本公开的ActRII多肽和GDF捕获物可通过各种领域已知技术产生。例如，本公开的多肽可采用标准蛋白质化学技术比如在Bodansky, M. Principles of Peptide Synthesis (肽合成原理), Springer Verlag (施普林格), Berlin (柏林) (1993)和Grant G.A. (编辑), Synthetic Peptides: A User's Guide (合成肽:用户指南), W.H. Freeman and Company (W•H•弗里曼公司), New York (纽约) (1992)中描述的那些技术合成。另外，自动化肽合成仪可市售得到(参见例如Advanced ChemTech Model 396; Milligen/Biosearch 9600)。或者，本公开的多肽(包括其片段或变体)可采用本领域熟知的各种表达系统[例如大肠杆菌、中国仓鼠卵巢(CHO)细胞、COS细胞、杆状病毒]重组产生。在进一步的实施方案中，通过采用例如蛋白酶比如胰蛋白酶、嗜热菌蛋白酶、胰凝乳蛋白酶、胃蛋白酶或成对碱性氨基酸转化酶(PACE)，经消化重组产生的全长ActRII或GDF捕获多肽，可产生本公开的修饰或未修饰多肽。计算机分析(采用商品化软件，例如MacVecto、Omega、PCGene、Molecular Simulation, Inc.)可用于确认蛋白水解位点。或者，采用化学裂解(例如溴化氰、羟胺等)，自重组产生的全长ActRII或GDF捕获多肽可产生这种多肽。

[0186] 任何一种本文公开的ActRII多肽(例如ActRIIA或ActRIIB多肽)可与一种或多种另外的本公开ActRII拮抗剂组合以达到预期效果(例如在有需要的受试者增加红细胞水平和/或血红蛋白、治疗或预防贫血、治疗MDS或铁粒幼红细胞性贫血、治疗或预防MDS或铁粒

幼红细胞性贫血的一种或多种并发症)。例如,本文公开的ActRII多肽可与以下组合使用:i) 一种或多种另外的本文公开的ActRII多肽;ii) 一种或多种本文公开的GDF捕获物;iii) 一种或多种本文公开的ActRII拮抗剂抗体(例如抗-激活素A抗体、抗-激活素B抗体、抗-激活素C抗体、抗-激活素E抗体、抗-GDF11抗体、抗-GDF8抗体、抗-BMP6抗体、抗-BMP7抗体、抗-ActRIIA抗体和/或抗-ActRIIB抗体);iv) 一种或多种本文公开的小分子ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的小分子拮抗剂);v) 一种或多种本文公开的多核苷酸ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的多核苷酸拮抗剂);vi) 一种或多种本文公开的卵泡抑素多肽;和/或vii) 一种或多种本文公开的FLRG多肽。

[0187] 类似地,任何一种本文公开的GDF捕获物可与一种或多种另外的本公开ActRII拮抗剂组合以达到预期效果(例如在有需要的患者增加红细胞水平和/或血红蛋白、治疗或预防贫血、治疗MDS或铁粒幼红细胞性贫血、治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症)。例如,本文公开的GDF捕获物可与以下组合使用:i) 一种或多种另外的本文公开的GDF捕获物;ii) 本文公开的一种或多种本文公开的ActRII多肽(例如ActRIIA或ActRIIB多肽);iii) 一种或多种本文公开的ActRII拮抗剂抗体(例如抗-激活素A抗体、抗-激活素B抗体、抗-激活素C抗体、抗-激活素E抗体、抗-GDF11抗体、抗-GDF8抗体、抗-BMP6抗体、抗-BMP7抗体、抗-ActRIIA抗体和/或抗-ActRIIB抗体);iv) 一种或多种本文公开的小分子ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的小分子拮抗剂);v) 一种或多种本文公开的多核苷酸ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的多核苷酸拮抗剂);vi) 一种或多种本文公开的卵泡抑素多肽;和/或vii) 一种或多种本文公开的FLRG多肽。

[0188] B. 编码ActRII多肽和GDF捕获物的核酸

在某些实施方案中,本公开提供编码本文公开的ActRII多肽和GDF捕获多肽(包括其片段、功能变体和融合蛋白)的分离和/或重组核酸。例如,SEQ ID NO:12编码天然存在的人ActRIIA前体多肽,而SEQ ID NO:13编码处理后的ActRIIA细胞外结构域。另外,SEQ ID NO:7编码天然存在的人ActRIIB前体多肽(以上描述的R64变体),而SEQ ID NO:8编码处理后的ActRIIB细胞外结构域(以上描述的R64变体)。研究对象核酸可为单链或双链的。这种核酸可为DNA或RNA分子。这些核酸可用于例如制备本公开基于ActRII的配体捕获多肽的方法。

[0189] 本文使用的分离的核酸指的是已自其自然环境的组成部分分离的核酸分子。分离的核酸包括在通常含有核酸分子的细胞中含有的核酸分子,但是核酸分子存在于染色体外或在不同于其天然染色体定位的染色体位置。

[0190] 在某些实施方案中,编码本公开的ActRII多肽和GDF捕获物的核酸应理解为包括其为SEQ ID NOs: 7、8、12、13、27、32、39、40、42、43、46、47和48中任何一种的变体的核酸。核苷酸序列变体包括因一个或多个核苷酸替换、添加或缺失(包括等位基因变异)而不同的序列,并因此包括不同于以SEQ ID NOs: 7、8、12、13、27、32、39、40、42、43、46、47和48中的任何一种指定的核苷酸序列的编码序列。

[0191] 在某些实施方案中,本公开的ActRII多肽和GDF捕获物由与SEQ ID NOs: 7、8、12、

13、27、32、39、40、42、43、46、47和48至少80%、85%、90%、95%、97%、98%或99%相同的分离或重组核酸序列编码。在一些实施方案中，本公开的GDF捕获物不由包含或由任何一种核苷酸序列(对应SEQ ID NOs: 7、8、12、13、27和32中的任何一种)组成的核酸序列编码。本领域的普通技术人员应意识到，与同SEQ ID NOs: 7、8、12、13、27、32、39、42、47和48互补的序列至少80%、85%、90%、95%、97%、98%或99%相同的核酸序列及其变体也处于本公开的范围内。在进一步的实施方案中，本公开的核酸序列可分离、重组和/或与异源核苷酸序列融合，或在DNA库中。

[0192] 在其他的实施方案中，本公开的核酸也包括在非常严格的条件下杂交到以下的核苷酸序列：以SEQ ID NOs: 7、8、12、13、27、32、39、40、42、43、46、47和48指定的核苷酸序列；SEQ ID NOs: 7、8、12、13、27、32、39、40、42、43、46、47和48的互补序列，或其片段。如以上讨论的那样，本领域的普通技术人员应易于理解，促进DNA杂交的适当严格的条件可以变化。本领域的普通技术人员应该易于理解，促进DNA杂交的适当严格的条件可以变化。例如，可在约45℃下以6.0 x氯化钠/枸橼酸钠(SSC)实施杂交，随后在50℃下洗涤2.0 x SSC。例如，洗涤步骤的盐浓度可从50℃下约2.0 x SSC的低严格度到50℃下约0.2 x SSC的高严格度进行选择。另外，洗涤步骤的温度可从室温(约22℃)的低严格条件增加至约65℃的高严格条件。温度和盐两者可以变化，或者温度或盐浓度可保持不变而改变其他变量。在一个实施方案中，本公开提供在室温6 x SSC的低严格条件下杂交，随后在室温下以2 x SSC洗涤的核酸。

[0193] 由于遗传密码的简并性不同于以SEQ ID NOs: 7、8、12、13、27、32、39、40、42、43、46、47和48阐述的核酸的分离核酸也处于本公开的范围内。例如，一些氨基酸用多于1个三联体指定。详细说明相同氨基酸的密码子或者同义词(例如CAU和CAC为组氨酸的同义词)可能导致不影响蛋白氨基酸序列的“沉默”突变。然而，预期在哺乳动物细胞当中存在确实会导致研究对象蛋白的氨基酸序列变化的DNA序列多态性。本领域的技术人员应意识到，在给定物种的个体当中，由于自然等位基因变异而可能存在编码特定蛋白的核酸的一种或多种核苷酸(高达约3-5%的核苷酸)的这些变异。任何和所有这种核苷酸变异及生成的氨基酸多态性处于本公开的范围内。

[0194] 在某些实施方案中，本公开的重组核酸可在表达载体被切实可行地连接到一种或多种调控核苷酸序列。调控核苷酸序列通常适合用于表达的宿主细胞。本领域已知用于各种宿主细胞的许多类型合适的表达载体和合适的调控序列。一般地，所述一种或多种调控核苷酸序列可非限制性地包括启动子序列、前导或信号序列、核糖体结合位点、转录起始和终止序列、翻译起始和终止序列及增强子或激活序列。本公开考虑本领域已知的组成型或诱导型启动子。启动子可为天然存在的启动子或组合多于一种启动子元素的杂合启动子。表达载体可存在于细胞中附加体比如质粒上，或者表达载体可插入染色体。在一些实施方案中，表达载体含有选择标记基因，使得能够选择转化的宿主细胞。选择标记基因为本领域熟知的并随着采用的宿主细胞变化。

[0195] 在本公开的某些方面，在表达载体中提供研究对象核酸，其包含编码ActRII多肽或GDF捕获物的核苷酸序列，并被切实可行地连接到至少一种调控序列。调控序列为领域认可的，并被选择直接表达ActRII或GDF捕获多肽。因此，术语调控序列包括启动子、增强子及其他表达调控元件。示例性的调控序列描述在Goeddel, *Gene Expression Technology*:

Methods in Enzymology (基因表达技术:酶学方法), Academic Press (学术出版社), San Diego (圣地亚哥), CA (1990)中。例如,调控DNA序列表达的广泛种类表达调控序列中的任何一种,当与之有效连接时,可用于这些载体以表达编码ActRII或GDF捕获多肽的DNA序列。这种有用的表达调控序列包括例如SV40的早期和晚期启动子、*tet*启动子、腺病毒或巨细胞病毒立即早期启动子、RSV启动子、lac系统、*trp*系统、TAC或TRC系统、其表达由T7 RNA聚合酶引导的T7启动子、噬菌体λ的主要启动子和启动子区、fd外壳蛋白的调控区、3-磷酸甘油酸酯激酶或其他糖酵解酶的启动子、酸性磷酸酶例如Pho5的启动子、酵母α-交配因子的启动子、杆状病毒系统的多面体启动子及已知调控原核或真核细胞或其他病毒基因表达的其他序列及其各种组合。应该理解,表达载体的设计可能取决于要转化的宿主细胞选择和/或期望表达的蛋白类型等因素。此外,还应考虑载体的复制数目、控制复制数目的能力及由载体编码的任何其他蛋白的表达比如抗生素标记。

[0196] 本公开的重组核酸可通过把克隆的基因或其部分连接到适合于在原核细胞、真核细胞(酵母、鸟类、昆虫或哺乳类)或两者表达的载体产生。用于产生重组ActRII或GDF捕获多肽的表达载体包括质粒和其他载体。例如,合适的载体包括以下类型的质粒:pBR322-衍化质粒、pEMBL-衍化质粒、pEX-衍化质粒、pBTac-衍化质粒及用于在原核细胞比如大肠杆菌表达的pUC-衍化质粒。

[0197] 一些哺乳动物表达载体含有促进载体在细菌中传播的原核序列和在真核细胞表达的一种或多种真核转录单位两者。pcDNAI/amp、pcDNAI/neo、pRc/CMV、pSV2gpt、pSV2neo、pSV2-dhfr、pTk2、pRSVneo、pMSG、pSVT7、pko-neo和pHyg衍化的载体为适合于真核细胞转染的哺乳动物表达载体的实例。这些载体中的一些用细菌质粒比如pBR322的序列修饰,以促进在原核和真核细胞两者的复制和耐药性选择。或者,衍生病毒比如牛乳头状瘤病毒(BPV-1)或EB病毒(Epstein-Barr virus) (pHEBo、pREP-derived和p205)可用于蛋白在真核细胞中的瞬时表达。其他病毒(包括逆转录病毒)表达系统的实例可见以下基因治疗传递系统的描述。用于制备质粒和寄助物转化的各种方法为本领域熟知的。对于用于原核和真核细胞两者的其他合适的表达系统及一般重组程序,参见例如Molecular Cloning A Laboratory Manual (分子克隆实验室手册), 第3版, Sambrook, Fritsch and Maniatis编辑 (Cold Spring Harbor Laboratory Press (冷泉港实验室出版社), 2001)。在一些情况下,通过采用杆状病毒表达系统表达重组多肽可能是可取的。这种杆状病毒表达系统的实例包括pVL-衍化载体(比如pVL1392、pVL1393和pVL941)、pAcUW-衍化载体(比如pAcUW1)和pBlueBac-衍化载体(比如含有pBlueBac III的β-gal)。

[0198] 在一个优选的实施方案中,把载体设计成用于在CHO细胞产生研究对象ActRII 或GDF捕获多肽,比如Pcmv-Script载体(Stratagene, La Jolla (拉贺亚市), Calif. (加利福尼亚州))、pcDNA4载体(Invitrogen, Carlsbad (卡尔斯巴德), Calif. (加利福尼亚州))和pCI-neo载体(Promega, Madison (麦迪逊), Wisc. (威斯康星州))。显而易见,研究对象基因构建体(gene construct)可用于在培养中繁殖的细胞中引起表达研究对象ActRII多肽,例如产生用于纯化的蛋白,包括融合蛋白或变异蛋白。

[0199] 本公开还涉及用重组基因(包括一种或多种研究对象ActRII或GDF捕获多肽的编码序列)转染的宿主细胞。宿主细胞可为任何原核或真核细胞。例如,本公开的ActRII或GDF捕获多肽可在细菌细胞比如大肠杆菌、昆虫细胞(例如采用杆状病毒表达系统)、酵母或哺

乳动物细胞[例如中国仓鼠卵巢(CHO)细胞系]表达。其他合适的宿主细胞为本领域技术人员已知的。

[0200] 因此,本公开进一步涉及产生研究对象ActRII和GDF捕获多肽的方法。例如,用编码ActRII或GDF捕获多肽的表达载体转染的宿主细胞可在使得能够发生ActRII或GDF捕获多肽表达的适当条件下进行培养。多肽可自细胞与含多肽培养基的混合物分泌和分离。或者,ActRII或GDF捕获多肽可保留在细胞质或膜部分中,并收获细胞,细胞溶解和分离蛋白。细胞培养物包括宿主细胞、培养基及其他副产物。适合细胞培养的培养基为本领域熟知的。采用本领域已知用于纯化蛋白的技术,包括离子交换色谱、凝胶过滤色谱、超滤、电泳、用对ActRII或GDF捕获多肽的特定表位特异性的抗体进行免疫亲和纯化及用结合于与ActRII或GDF捕获多肽融合的结构域的试剂进行亲和纯化(例如蛋白A柱可用于纯化ActRII-Fc或GDF Trap-Fc融合蛋白),可自细胞培养基、宿主细胞或两者分离研究对象多肽。在一些实施方案中,ActRII或GDF捕获多肽为含有便于其纯化的结构域的融合蛋白。

[0201] 在一些实施方案中,通过一系列柱层析步骤,包括例如以下的三个或多个步骤(以任何顺序)实现纯化:蛋白A色谱、Q 琼脂糖色谱、苯基琼脂糖凝胶色谱法、尺寸排阻色谱法和阳离子交换色谱法。纯化可用病毒过滤和缓冲交换完成。ActRII-Fc或GDF捕获物-Fc蛋白可纯化为如经尺寸排阻色谱测定的那样纯度>90%、>95%、>96%、>98%或>99%,和如经SDS PAGE测定的那样纯度>90%、>95%、>96%、>98%或>99%。目标纯度水平应足以在哺乳动物系统,特别是非人灵长类动物、啮齿动物(小鼠)和人获得期望的结果。

[0202] 在另一个实施方案中,编码纯化前导序列的融合基因,比如重组ActRII或GDF捕获多肽所需部分的N-末端poly-(His)/肠激酶裂解位点序列,可使得能够采用Ni^{2+}金属树脂经亲和色谱纯化所表达的融合蛋白。纯化前导序列可然后接着通过用肠激酶处理去除,提供纯化的ActRII或GDF捕获多肽。参见例如Hochuli等. (1987) *J. Chromatography* 411:177;和Janknecht等. (1991) *PNAS USA* 88:8972。

[0203] 用于制备融合基因的技术为熟知的。本质上,按照常规技术,采用钝端或错端末端连接、限制酶解作用以提供适当的末端、视情况而定填满粘性末端、碱性磷酸酶处理以避免不期望的连接和酶催化连接,实施编码不同多肽序列的各种DNA片段的连接。在另一个实施方案中,融合基因可通过常规技术(包括DNA自动合成仪)合成。或者,可采用随后退火产生嵌合基因序列的在两个连续基因片段之间产生互补突出的锚定引物,实施基因片段的PCR扩增。参见例如Current Protocols in Molecular Biology (分子生物学现代方法), Ausubel等编辑, John Wiley & Sons: 1992。

[0204] C. 抗体拮抗剂

在某些方面,本公开涉及拮抗ActRII活性(例如抑制ActRIIA和/或ActRIIB信号转导,比如SMAD 2/3和/或SMAD 1/5/8信号传递)的抗体或抗体组合。这种抗体可结合和抑制一种或多种配体(例如GDF8、GDF11、激活素A、激活素B、激活素C、激活素E、BMP6、BMP7或Nodal)或者一种或多种受体(例如ActRIIA、ActRIIB、ALK4、ALK5)。具体地讲,本公开提供单独或与一种或多种红细胞生成刺激剂(例如EPO)或其他支持疗法[例如造血生长因子(例如G-CSF或GM-CSF)、红细胞或全血输注、铁螯合疗法]组合使用抗体ActRII拮抗剂或抗体ActRII拮抗剂组合的方法,以例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治

疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍。

[0205] 在某些实施方案中,优选的本公开抗体ActRII拮抗剂为结合和/或抑制至少GDF11的活性(例如GDF11-介导的ActRIIA和/或ActRIIB信号转导,比如SMAD 2/3信号传递的激活)的抗体或抗体组合。任选地,抗体或抗体组合进一步结合和/或抑制GDF8的活性(例如GDF8-介导的ActRIIA和/或ActRIIB信号转导,比如SMAD 2/3信号传递的激活),特别是在对GDF11和GDF8两者具有亲和力的多特异性抗体的情况下,或者在一种或多种抗-GDF11抗体和一种或多种抗-GDF8抗体组合的情况下。任选地,本公开的抗体或抗体组合基本上不结合和/或抑制激活素A的活性(例如激活素A-介导的ActRIIA或ActRIIB信号转导,比如SMAD 2/3信号传递的激活)。在一些实施方案中,结合和/或抑制GDF11和/或GDF8的活性的本公开抗体或抗体组合进一步结合和/或抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和Nodal中一种或多种的活性(例如ActRIIA或ActRIIB SMAD 2/3和/或SMAD 1/5/8信号通路的激活),特别是在对多种ActRII配体具有亲和力的多特异性抗体的情况下,或者在多种抗体组合-每一种对不同的ActRII配体具有亲和力的情况下。

[0206] 在某些方面,本公开的ActRII拮抗剂为结合和/或抑制至少GDF8的活性(例如GDF8-介导的ActRIIA和/或ActRIIB信号转导,比如SMAD 2/3信号传递的激活)的抗体或抗体组合。任选地,抗体或抗体组合进一步结合和/或抑制GDF11的活性(例如GDF11-介导的ActRIIA和/或ActRIIB信号转导,比如SMAD 2/3信号传递的激活),特别是在对GDF8和GDF11两者具有亲和力的多特异性抗体的情况下,或者在一种或多种抗-GDF8抗体和一种或多种抗-GDF11抗体组合的情况下。任选地,本公开的抗体或抗体组合基本上不结合和/或抑制激活素A的活性(例如激活素A-介导的ActRIIA或ActRIIB信号转导,比如SMAD 2/3信号传递的激活)。在一些实施方案中,结合和/或抑制GDF8和/或GDF11的活性的本公开抗体或抗体组合进一步结合和/或抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和Nodal中一种或多种的活性(例如ActRIIA或ActRIIB信号转导,比如SMAD 2/3和/或SMAD 1/5/8信号通路的激活),特别是在对多种ActRII配体具有亲和力的多特异性抗体的情况下,或者在组合多种抗体-每一种对不同的ActRII配体具有亲和力的情况下。

[0207] 在另一方面,本公开的ActRII拮抗剂为结合和/或抑制ActRII受体(例如ActRIIA或ActRIIB受体)活性的抗体或抗体组合。在优选的实施方案中,本公开的抗-ActRII受体抗体(例如抗-ActRIIA或抗-ActRIIB受体抗体)或抗体组合结合ActRII受体并防止ActRII受体被至少GDF11结合和/或激活(例如GDF11-介导的ActRIIA和/或ActRIIB信号转导,比如SMAD 2/3信号传递的激活)。任选地,本公开的抗-ActRII受体抗体或抗体组合进一步防止ActRII受体被GDF8结合和/或激活。任选地,本公开的抗-ActRII受体抗体或抗体组合基本上不抑制激活素A结合和/或激活ActRII受体。在一些实施方案中,结合ActRII受体并防止ActRII受体被GDF11和/或GDF8结合和/或激活的本公开抗-ActRII受体抗体或抗体组合,进一步防止ActRII受体被激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和Nodal中的一种或多种结合和/或激活。

[0208] 术语抗体本文在最广泛的意义上使用并包括各种抗体结构,非限制性地包括单克隆抗体、多克隆抗体、多特异性抗体(例如双异性抗体)和抗体片段(只要它们呈现期望的抗

原结合活性)。抗体片段指的是非完整抗体的分子,其包含完整抗体的一部分,结合完整抗体结合的抗原。抗体片段的实例非限制性地包括Fv、Fab、Fab'、Fab'-SH、F(ab')$_2$;双价抗体(diabodies)、线性抗体、单链抗体分子(例如scFv)及自抗体片段形成的多特异性抗体。参见例如Hudson等. (2003) Nat. Med. 9:129-134;Plückthun, 在The Pharmacology of Monoclonal Antibodies (单克隆抗体的药理学)中, 第113卷, Rosenburg and Moore编辑, (Springer-Verlag (斯普林格出版社), New York (纽约)), 第269-315页(1994);WO 93/16185;及美国专利第5571894、5587458和5869046号。本文公开的抗体可为多克隆抗体或单克隆抗体。在某些实施方案中,本公开的抗体包含附在上面并能检测的标记(例如标记可为放射性同位素、荧光化合物、酶或酶辅助因子)。在优选的实施方案中,本公开的抗体为分离的抗体。

[0209] 双价抗体(diabodies)为有两个抗原结合位点可为二价或双特异性的抗体片段。参见例如EP 404097、WO 1993/01161;Hudson等. (2003) Nat. Med. 9:129-134 (2003);和Hollinger等. (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448。在Hudson等. (2003) Nat. Med. 9:129-134中还描述了三链抗体(Triabodies)和四链抗体(tetrabodies)。

[0210] 单域抗体为包含抗体的重链可变结构域的全部或部分或者轻链可变结构域的全部或部分的抗体片段。在某些实施方案中,单域抗体为人单域抗体。参见例如美国专利第6248516号。

[0211] 抗体片段可通过各种技术制备,非限制性地包括完整抗体的蛋白水解消化及用重组宿主细胞(例如大肠杆菌或噬菌体)产生,如本文描述的那样。

[0212] 本文的抗体可属于任何种类。抗体种类指其重链具有的恒定域或恒定区的类型。存在5大类抗体:IgA、IgD、IgE、IgG和IgM,并且这些种类中的几种可进一步分为亚类(同种型(isotypes)),例如IgG_1、IgG_2、IgG_3、IgG_4、IgA_1和IgA_2。对应不同种类免疫球蛋白的重链恒定域称为α、δ、ε、γ和μ。

[0213] 通常,用于本文公开的方法的抗体具体地讲结合于其靶抗原,优选地具有高亲和力。亲和力可表示为K_D值,并反映内在的亲和力(例如最小的亲和力影响)。一般地,亲和力进行体外测量,无论是在无细胞还是在细胞相关的环境下。许多本领域已知试验中的任何一种(包括本文公开的那些试验)可用于获得亲和力测量,包括例如表面等离子体共振(Biacore™试验)、放射性标记抗原结合试验(RIA)和ELISA。在一些实施方案中,本公开的抗体结合于其靶抗原(例如GDF11、GDF8、ActRIIA、ActRIIB等),至少K_D为$1x\ 10^{-7}$或更强、$1x10^{-8}$或更强、$1x10^{-9}$或更强、$1x10^{-10}$或更强、$1x10^{-11}$或更强、$1x10^{-12}$或更强、$1x10^{-13}$或更强或者$1x10^{-14}$或更强。

[0214] 在某些实施方案中,K_D通过用感兴趣的Fab版抗体及其靶抗原实施的RIA测量,如用以下试验描述的那样。通过在滴定系列的非标记抗原存在下,用最小浓度的放射性标记抗原(例如^{125}I-标记的)平衡Fab,然后用抗-Fab抗体包被的酶标板捕获结合的抗原,测量Fabs对抗原的溶液结合亲和力[参见例如Chen等. (1999) J. Mol. Biol. 293:865-881]。为了建立试验条件,用捕获抗-Fab抗体(例如得自Cappel Labs (卡佩尔实验室))涂布(例如过夜)多孔板(例如来自Thermo Scientific (赛默科技)的MICROTITER®),并随后用牛血清白蛋白阻断,优选地在室温(约23℃)下。在非吸附板,放射性标记抗原与感兴趣的Fab

系列稀释液混合[例如与抗-VEGF抗体、Fab-12的评价一致，在Presta等，(1997) Cancer Res. 57:4593-4599]。然后温育感兴趣的Fab，优选地过夜，但是温育可持续更长一段时间(例如约65小时)，以确保达到平衡。之后，把混合物转移至捕获板温育，优选地在室温下约1小时。然后去除溶液，并优选地用聚山梨醇酯20和PBS混合物把板洗涤几次。当板已干燥时，加入闪烁剂(scintillant)(例如来自Packard的MICROSCINT®)，并把板在γ计数器(例如来自Packard的TOPCOUNT®)计数。

[0215] 根据另一个实施方案，K_D采用表面等离子体共振试验，用例如具有以约10个反应单位(RU)存在的固定化抗原CM5芯片的BIACORE® 2000或BIACORE® 3000 (Biacore, Inc., Piscataway(皮斯卡塔韦), N.J. (新泽西州))测量。简短地，根据供应商的用法说明，羧甲基化葡聚糖生物传感器芯片(CM5, Biacore, Inc.)用N-乙基-N'-(3-二甲基氨基丙基)-碳二亚胺盐酸盐(EDC)和N-羟基琥珀酰亚胺(NHS)激活。例如，抗原可用10 mM乙酸钠(pH 4.8)稀释至5 μg/ml (约0.2 μM)，之后以5 μl/分钟的流速注射，以获得约10个反应单位(Ru)的偶联蛋白。在注射抗原后，注射1 M乙醇胺以阻断未反应基团。对于动力学测量，以约25 μl/分钟的流速注射Fab在含有0.05%聚山梨醇酯20 (TWEEN-20®)表面活性剂(PBST)的PBS中的2倍连续稀释液(0.78 nM-500 nM)。采用例如简单的一对一朗格缪尔结合模型(BIACORE® Evaluation Software (BIACORE®评估软件) 3.2版)，经同时拟合缔合与解离传感图，计算缔合速率(k_{on})与解离速率(k_{off})。

[0216] 作为比率k_{off}/k_{on}计算平衡离解常数(K_D) [参见例如Chen等，(1999) J. Mol. Biol. 293:865-881]。如果通过以上表面等离子体共振试验缔合速率(on-rate)超过例如10^6 M^{-1} s^{-1}，那么缔合速率(on-rate)可通过采用荧光猝灭技术测定，该技术测量在抗原浓度增加的情况下，PBS中的20 nM 抗-抗原抗体(Fab形式)的荧光发射强度的增加或减少(例如激发=295 nm；发射=340 nm, 16 nm带通)，如以光谱仪比如停流式分光光度计(Aviv Instruments)或带混合容器的8000-系列SLM-AMINCO®分光光度计(ThermoSpectronic)测量的那样。

[0217] 本文使用的抗-GDF11抗体通常指能够以足够亲和力结合GDF11，使得抗体可用作靶向GDF11的诊断和/或治疗剂的抗体。在某些实施方案中，抗-GDF11抗体与不相关的非GDF11蛋白结合的程度低于抗体与GDF11结合的约10%、9%、8%、7%、6%、5%、4%、3%、2%或低于1%，如例如经放射性免疫测定(RIA)测量的那样。在某些实施方案中，本公开的抗-GDF11抗体结合于GDF11的表位(其在不同种类的GDF11当中为保守的)。在某些优选的实施方案中，本公开的抗-GDF11抗体为可抑制GDF11活性的拮抗剂抗体。例如，本公开的抗-GDF11抗体可抑制GDF11结合于同源受体(例如ActRIIA或ActRIIB受体)和/或抑制GDF11-介导的同源受体的信号转导(激活)，比如经ActRIIA和/或ActRIIB受体的SMAD2/3信号传递。在一些实施方案中，本公开的抗-GDF11抗体基本上不结合和/或抑制激活素A的活性。应该注意的是，GDF11与GDF8的序列同源性高，并因此结合和/或抑制GDF11的抗体在一些情况下也可结合和/或抑制GDF8。

[0218] 抗-GDF8抗体指能够以足够亲和力结合GDF8，使得抗体可用作靶向GDF8的诊断和/或治疗剂的抗体。在某些实施方案中，抗-GDF8抗体与不相关的非GDF8蛋白结合的程度低于抗体与GDF8结合的约10%、9%、8%、7%、6%、5%、4%、3%、2%或低于1%，如例如经放射性免疫测定(RIA)测量的那样。在某些实施方案中，抗-GDF8抗体结合于GDF8的表位(其在不同种类的

GDF8当中为保守的)。在优选的实施方案中,本公开的抗-GDF8抗体为可抑制GDF8活性的拮抗剂抗体。例如,本公开的抗-GDF8抗体可抑制GDF8结合于同源受体(例如ActRIIA或ActRIIB受体)和/或抑制GDF8-介导的同源受体的信号转导(激活),比如经ActRIIA和/或ActRIIB受体的SMAD2/3信号传递。在一些实施方案中,本公开的抗-GDF8抗体基本上不结合和/或抑制激活素A的活性。应该注意的是,GDF8与GDF11的序列同源性高,并因此结合和/或抑制GDF8的抗体在许多情况下也可结合和/或抑制GDF11。

[0219] 抗-ActRIIA抗体指能够以足够亲和力结合ActRIIA,使得抗体可用作靶向ActRIIA的诊断和/或治疗剂的抗体。在某些实施方案中,抗-ActRIIA抗体与不相关的非ActRIIA蛋白结合的程度低于抗体与ActRIIA结合的约10%、9%、8%、7%、6%、5%、4%、3%、2%或低于1%,如例如经放射性免疫测定(RIA)测量的那样。在某些实施方案中,抗-ActRIIA抗体结合于ActRIIA的表位(其在不同种类的ActRIIA当中为保守的)。在优选的实施方案中,本公开的抗-ActRIIA抗体为可抑制ActRIIA活性的拮抗剂抗体。例如,本公开的抗- ActRIIA抗体可抑制一种或多种选自激活素A、激活素B、激活素AB、激活素C、激活素E、GDF11、GDF8、激活素A、BMP6和BMP7的ActRIIA配体结合于ActRIIA受体和/或抑制这些配体之一激活ActRIIA信号传递(例如SMAD2/3和/或SMAD 1/5/8 ActRIIA信号传递)。在优选的实施方案中,本公开的抗- ActRIIA抗体抑制GDF11结合于ActRIIA受体和/或抑制GDF11激活ActRIIA信号传递。任选地,本公开的抗- ActRIIA抗体进一步抑制GDF8结合于ActRIIA受体和/或抑制GDF8激活ActRIIA信号传递。任选地,本公开的抗- ActRIIA抗体基本上不抑制激活素A结合于ActRIIA受体和/或基本上不抑制激活素A介导的ActRIIA信号传递的激活。在一些实施方案中,抑制GDF11和/或GDF8结合和/或激活ActRIIA受体的本公开抗-ActRIIA抗体进一步抑制激活素A、激活素B、激活素AB、激活素C、激活素E、激活素A、GDF8、BMP6和BMP7中的一种或多种结合和/或激活ActRIIA受体。

[0220] 抗-ActRIIB抗体指能够以足够亲和力结合ActRIIB,使得抗体可用作靶向ActRIIB的诊断和/或治疗剂的抗体。在某些实施方案中,抗-ActRIIB抗体与不相关的非ActRIIB蛋白结合的程度低于抗体与ActRIIB结合的约10%、9%、8%、7%、6%、5%、4%、3%、2%或低于1%,如例如经放射性免疫测定(RIA)测量的那样。在某些实施方案中,抗-ActRIIB抗体结合于ActRIIB的表位(其在不同种类的ActRIIB当中为保守的)。在优选的实施方案中,本公开的抗-ActRIIB抗体为可抑制ActRIIB活性的拮抗剂抗体。例如,本公开的抗- ActRIIB抗体可抑制一种或多种选自激活素A、激活素B、激活素AB、激活素C、激活素E、GDF11、GDF8、激活素A、BMP6和BMP7的ActRIIB配体结合于ActRIIB受体和/或抑制这些配体之一激活ActRIIA信号传递(例如SMAD2/3和/或SMAD 1/5/8 ActRIIB信号传递)。在优选的实施方案中,本公开的抗- ActRIIB抗体抑制GDF11结合于ActRIIB受体和/或抑制GDF11激活ActRIIB信号传递。任选地,本公开的抗- ActRIIB抗体进一步抑制GDF8结合于ActRIIB受体和/或抑制GDF8激活ActRIIB信号传递。任选地,本公开的抗- ActRIIB抗体基本上不抑制激活素A结合于ActRIIB受体和/或基本上不抑制激活素A介导的ActRIIB信号传递的激活。在一些实施方案中,抑制GDF11和/或GDF8结合和/或激活ActRIIB受体的本公开抗-ActRIIB抗体进一步抑制激活素A、激活素B、激活素AB、激活素C、激活素E、激活素A、GDF8、BMP6和BMP7中的一种或多种结合和/或激活ActRIIB受体。

[0221] 人GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、GDF8、BMP6、BMP7、

ActRIIB和ActRIIA的核酸和氨基酸序列为本领域熟知的，并因此用于本公开用途的抗体拮抗剂可由技术人员基于本领域的知识和本文提供的讲授常规制备。

[0222] 在某些实施方案中，本文提供的抗体(例如抗-GDF11抗体、抗-GDF8抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)为嵌合抗体。嵌合抗体指其中重和/或轻链的一部分源自特定的来源或物种，而重和/或轻链的其余部分源自不同的来源或物种的抗体。例如在美国专利第4816567号;和Morrison等, (1984) Proc. Natl. Acad. Sci. USA, 81:6851-6855中描述了某些嵌合抗体。在一些实施方案中，嵌合抗体包含非人可变区(例如源自小鼠、大鼠、仓鼠、兔或非人灵长类动物比如猴子的可变区)和人恒定区。在一些实施方案中，嵌合抗体为其中种类或亚类已自母源抗体改变的“类别转换”抗体。通常，嵌合抗体包括其抗原结合片段。

[0223] 在某些实施方案中，本文提供的嵌合抗体(例如抗-GDF11抗体、抗-GDF8抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)为人源化抗体。人源化抗体指包含非人高变区(HVRs)的氨基酸残基和人框架区(FRs)的氨基酸残基的嵌合抗体。在某些实施方案中，人源化抗体基本上全部包含至少一种，并且一般地两种可变域，其中全部或基本上全部的HVRs (例如CDRs)对应非人抗体的那些结构域，和全部或基本上全部的FRs对应人抗体的那些结构域。人源化抗体任选地可包含源自人抗体的抗体恒定区的至少一部分。抗体例如非人抗体的“人源化形式”指的是已经历人源化的抗体。

[0224] 例如在Almagro and Fransson (2008) Front. Biosci. 13:1619-1633中综述了人源化抗体及制备它们的方法，并且例如在Riechmann等, (1988) Nature 332:323-329; Queen等. (1989) Proc. Nat'l Acad. Sci. USA 86:10029-10033;美国专利第5821337、7527791、6982321和7087409号;Kashmiri等. (2005) Methods 36:25-34 [描述SDR (a-CDR)嫁接];Padlan, Mol. Immunol. (1991) 28:489-498 (描述“表面重修(resurfacing)”);Dall'Acqua等. (2005) Methods 36:43-60 (描述“FR替换(shuffling)”);Osbourn等. (2005) Methods 36:61-68;和Klimka等. Br. J. Cancer (2000) 83:252-260 (描述FR替换(shuffling)的“定向选择”方法)中得到进一步描述。

[0225] 可用于人源化的人框架区非限制性地包括:采用“最优满足”法选择的框架区[参见例如Sims等. (1993) J. Immunol. 151:2296]、源自轻链或重链可变区特定亚群的人抗体共有序列的框架区[参见例如Carter等. (1992) Proc. Natl. Acad. Sci. USA, 89: 4285;和Presta等. (1993) J. Immunol., 151:2623]、人成熟(体细胞突变)框架区或人生殖系框架区[参见例如Almagro and Fransson (2008) Front. Biosci. 13:1619-1633]及源自筛选FR库的框架区[参见例如Baca等. (1997) J. Biol. Chem. 272:10678-10684;和Rosok等., (1996) J. Biol. Chem. 271:22611-22618]。

[0226] 在某些实施方案中，本文提供的抗体(例如抗-GDF11抗体、抗-GDF8抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)为人抗体。人抗体可采用本领域已知的各种技术产生。人抗体通常在van Dijk and van de Winkel (2001) Curr. Opin. Pharmacol. 5: 368-74和Lonberg (2008) Curr. Opin. Immunol. 20:450-459中得到描述。

[0227] 人抗体可通过把免疫原(例如GDF11多肽、GDF8多肽、ActRIIA多肽或ActRIIB多肽)给予转基因动物制备，这种动物已经过修饰产生有响应抗原刺激(antigenic challenge)的人可变区的完整人抗体或完整抗体。这种动物一般地含有全部或部分的人免疫球蛋白基

因座，其替代内源性的免疫球蛋白基因座，或者其存在染色体外或随机整合到动物染色体中。在这种转基因动物，内源性的免疫球蛋白基因座通常已失活。对于自转基因动物获得人抗体方法的综述参见例如Lonberg (2005) Nat. Biotechnol. 23:1117-1125；美国专利第6075181和6150584号(描述XENOMOUSE™技术)；美国专利第5770429号(描述HuMab®技术)；美国专利第7041870号(描述K-M MOUSE®技术)；和美国专利申请出版物第2007/0061900号(描述VelociMouse®技术)。用这种动物产生的完整抗体的人可变区可例如通过组合不同的人恒定区进一步修饰。

[0228] 本文提供的人抗体也可通过基于杂交瘤的方法制备。已描述了用于产生人单克隆抗体的人骨髓瘤和小鼠-人异源骨髓瘤细胞系[参见例如Kozbor J. Immunol., (1984) 133: 3001；Brodeur等. (1987) Monoclonal Antibody Production Techniques and Applications (单克隆抗体生产技术和应用), 第51-63页, Marcel Dekker, Inc. (马塞尔·德克尔公司), New York (纽约)；和Boerner等. (1991) J. Immunol., 147: 86]。在Li等. (2006) Proc. Natl. Acad. Sci. USA, 103:3557-3562中也描述了经人B细胞杂交瘤技术产生的人抗体。另外的方法包括例如在美国专利第7189826号(描述自杂交瘤细胞系产生单克隆人IgM抗体)和Ni, Xiandai Mianyixue (2006) 26(4):265-268 (2006) (描述人-人杂交瘤)中描述的那些方法。在Vollmers and Brandlein (2005) Histol. Histopathol., 20(3):927-937 (2005)和Vollmers and Brandlein (2005) Methods Find Exp. Clin. Pharmacol., 27(3):185-91中也描述了人杂交瘤技术(三源杂交瘤技术)。

[0229] 本文提供的人抗体(例如抗-GDF11抗体、抗-激活素B抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)也可通过分离选自人源噬菌体展示库的Fv克隆可变域序列产生。这种可变域序列然后可与期望的人恒定域组合。本文描述了用于自抗体库选择人抗体的技术。

[0230] 例如，本公开的抗体可通过筛选组合库具有期望的活性或多种活性的抗体进行分离。本领域已知用于产生噬菌体展示库和筛选这种库具有期望结合特性的抗体的各种方法。在例如Hoogenboom等. (2001)在Methods in Molecular Biology (分子生物学方法) 178:1-37, O'Brien等编辑, Human Press, Totowa, N.J. (新泽西州)中综述了这种方法，并且例如在McCafferty等. (1991) Nature 348:552-554；Clackson等. (1991) Nature 352: 624-628；Marks等. (1992) J. Mol. Biol. 222:581-597；Marks and Bradbury (2003)在Methods in Molecular Biology (分子生物学方法) 248:161-175, Lo编辑, Human Press, Totowa, N.J. (新泽西州)；Sidhu等. (2004) J. Mol. Biol. 338(2):299-310；Lee等. (2004) J. Mol. Biol. 340(5):1073-1093；Fellouse (2004) Proc. Natl. Acad. Sci. USA 101(34):12467-12472；和Lee等. (2004) J. Immunol. Methods 284(1-2): 119-132中得到进一步描述。

[0231] 在某些噬菌体展示方法中，VH和VL基因的抗体库分别经聚合酶链反应(PCR)克隆并在噬菌体库中随机重组，然后可如在Winter等. (1994) Ann. Rev. Immunol., 12: 433-455中描述的那样筛选抗原结合的噬菌体。噬菌体通常显示作为单链Fv (scFv)片段或作为Fab片段的抗体片段。来自免疫源的库为免疫原(例如GDF11、激活素B、ActRIIA或ActRIIB)提供高亲和力抗体而不需要构建杂交瘤。或者，可以克隆天然抗体库(例如自人)，以提供单一来源的针对广泛范围的非自体及自体抗原而没有任何免疫的抗体，如由

Griffiths等. (1993) EMBO J, 12: 725-734描述的那样。最后,如由Hoogenboom and Winter (1992) J. Mol. Biol., 227: 381-388描述的那样,通过克隆来自干细胞的未重新排列的(un-rearranged) V-基因节段和采用含有随机序列的PCR引物,以编码高度可变的CDR3区和完成体外重排,也可合成制备天然抗体库。描述人噬菌体抗体库的专利出版物包括例如:美国专利第5750373号和美国专利出版物第2005/0079574、2005/0119455、2005/0266000、2007/0117126、2007/0160598、2007/0237764、2007/0292936和2009/0002360号。

[0232] 在某些实施方案中,本文提供的抗体为多特异性抗体,例如双特异性抗体。多特异性抗体(一般地为单克隆抗体)对一种或多种(例如2、3、4、5、6或多种)抗原上的至少两个不同表位(例如2、3、4、5或6或多个)具有结合特异性。

[0233] 在某些实施方案中,本公开的多特异性抗体包含两种或多种结合特异性,其中至少一种结合特异性为对GDF11表位的特异性,和任选地一种或多种另外的结合特异性为对不同ActRII配体(例如GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和/或Nodal)和/或ActRII受体(例如ActRIIA和/或ActRIIB受体)上表位的特异性。在某些实施方案中,多特异性抗体可结合于GDF11的两个或多个不同表位。优选地在某种程度上对GDF11表位具有亲和力的本公开多特异性抗体可用于抑制GDF11活性(例如结合和/或激活ActRIIA和/或ActRIIB受体的能力),和任选地抑制一种或多种不同的ActRII配体(例如GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和/或Nodal)和/或ActRII受体(例如ActRIIA和/或ActRIIB受体)的活性。在某些优选的实施方案中,结合和/或抑制GDF11的本公开多特异性抗体至少进一步结合和/或抑制GDF8。任选地,结合和/或抑制GDF11的本公开多特异性抗体基本上不结合和/或基本上不抑制激活素A。在一些实施方案中,结合和/或抑制GDF11和GDD8的本公开多特异性抗体进一步结合和/或抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和/或Nodal中的一种或多种。

[0234] 在某些实施方案中,本公开的多特异性抗体包含两种或多种结合特异性,其中至少一种结合特异性为对GDF8表位的特异性,和任选地一种或多种另外的结合特异性为对不同ActRII配体(例如GDF11、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和/或Nodal)和/或ActRII受体(例如ActRIIA和/或ActRIIB受体)上表位的特异性。在某些实施方案中,多特异性抗体可结合于GDF8的两个或多个不同表位。优选地在某种程度上对GDF8表位具有亲和力的本公开多特异性抗体可用于抑制GDF8活性(例如结合和/或激活ActRIIA和/或ActRIIB受体的能力),和任选地抑制一种或多种不同的ActRII配体(例如GDF11、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和/或Nodal)和/或ActRII受体(例如ActRIIA和/或ActRIIB受体)的活性。在某些优选的实施方案中,结合和/或抑制GDF8的本公开多特异性抗体至少进一步结合和/或抑制GDF11。任选地,结合和/或抑制GDF8的本公开多特异性抗体基本上不结合和/或基本上不抑制激活素A。在一些实施方案中,结合和/或抑制GDF8和GDD11的本公开多特异性抗体进一步结合和/或抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和/或Nodal中的一种或多种。

[0235] 本文也包括具有三个或多个功能性抗原结合位点的工程抗体,包括“章鱼抗体”(参见例如US 2006/0025576A1)。

[0236] 在某些实施方案中,本文公开的抗体(例如抗-GDF11抗体、抗-激活素B抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)为单克隆抗体。单克隆抗体指的是得自基本上均质性抗体

的抗体群,即包含该群的单个抗体相同和/或结合相同的表位,除了可能的变异抗体,例如含有天然发生的突变或在单克隆抗体的制备生产过程中出现,这种变体一般以少量存在。与多克隆抗体(其一般地包括针对不同表位的不同抗体)制备形成对照,单克隆抗体制备的每种单克隆抗体针对抗原上的单个表位。因此,修饰语"单克隆"表明因为得自基本上均质性的抗体群并且不能解释为需要通过任何特定的方法产生抗体的抗体特性。例如,要按本方法使用的单克隆抗体可通过各种技术制备,技术非限制性地包括杂交瘤法、重组DNA法、噬菌体展示法以及利用含有全部或部分人免疫球蛋白基因座的转基因动物的方法,这种方法和其他示例性方法用于制备本文描述的单克隆抗体。

[0237] 例如,通过采用源自GDF11或GDF8的免疫原,抗-蛋白/抗-肽抗血清或单克隆抗体可通过标准方案制备[参见例如Harlow and Lane编辑的Antibodies: A Laboratory Manual (抗体:实验室手册) (1988), Cold Spring Harbor Press (冷泉港出版社)]。哺乳动物比如小鼠、仓鼠或兔可用GDF11或GDF8多肽的免疫原性形式、能够引起抗体反应的抗原片段或融合蛋白进行免疫。用于赋予蛋白或肽免疫原性的技术包括缀合于载体或本领域熟知的其他技术。GDF11或GDF8多肽的免疫原性部分可在佐剂存在下给予。免疫的进程可通过检测血浆或血清中的抗体滴度来监测。可使用标准ELISA或其他免疫测定法,免疫原作为抗原评价抗体产生水平和/或亲和力水平。

[0238] 在用GDF11或GDF8的抗原制剂免疫接种动物后,可得到抗血清(如果需要),可自血清分离多克隆抗体。为了产生单克隆抗体,可自免疫动物收获抗体产生细胞(淋巴细胞),并经标准体细胞融合过程融合,用永生化细胞比如骨髓瘤细胞产生杂交瘤细胞。这种技术为本领域熟知的,并且包括例如杂交瘤技术[参见例如Kohler and Milstein (1975) Nature, 256: 495-497]、人B细胞杂交瘤技术[参见例如Kozbar等. (1983) Immunology Today, 4:72]及产生人单克隆抗体的EBV-杂交瘤技术[Cole等. (1985) Monoclonal Antibodies and Cancer Therapy (单克隆抗体与肿瘤治疗), Alan R. Liss, Inc. 第77-96页]。杂交瘤细胞可进行免疫化学筛选产生与GDF11或GDF8多肽特异性反应的抗体,并自包含这种杂交瘤细胞的培养物分离单克隆抗体。

[0239] 在某些实施方案中,可向本文提供的抗体(例如抗-GDF11抗体、抗-激活素B抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)的Fc区引入一种或多种氨基酸修饰,从而产生Fc区变体。Fc区变体可包含在一个或多个氨基酸位置包含氨基酸修饰(例如替换、缺失和/或添加)的人Fc区序列(例如人IgG1、IgG2、IgG3或IgG4 Fc区)。

[0240] 例如,本公开考虑具有一些但不是所有效应功能的抗体变体,这些效应功能使其成为其中抗体的体内半衰期是重要的然而某些效应功能[例如补体依赖性细胞毒性(CDC)和抗体依赖性细胞毒性(ADCC)]是不必要或有害的应用的理想候选者。可实施体外和/或体内细胞毒性试验以确认CDC和/或ADCC活性的减少/消耗。例如,可实施Fc受体(FcR)结合实验以确保抗体缺乏Fcγ结合(因此可能缺乏ADCC活性),但是保留FcRn结合能力。介导ADCC的原代细胞(NK细胞)仅表达Fcγ RIII,而单核细胞表达Fcγ RI、Fcγ RII和Fcγ RIII。在例如Ravetch and Kinet (1991) Annu. Rev. Immunol. 9:457-492中概述了FcR在造血细胞的表达。在美国专利第5500362号;Hellstrom, I.等. (1986) Proc. Nat'l Acad. Sci. USA 83:7059-7063;Hellstrom, I等. (1985) Proc. Nat'l Acad. Sci. USA 82:1499-1502;美国专利第5821337号;和Bruggemann, M.等. (1987) J. Exp. Med. 166:1351-

1361中描述了评价感兴趣分子的ADCC活性的体外试验的非限制性实例。或者，可采用非放射性检测方法(例如ACTI™, non-radioactive cytotoxicity assay for flow cytometry (流式细胞仪的非放射性细胞毒性试验); CellTechnology, Inc (细胞工程公司). Mountain View (山景城), Calif. (加利福尼亚州)；和CytoTox 96® non-radioactive cytotoxicity assay (非放射性细胞毒性试验), Promega, Madison (麦迪逊), Wis. (威斯康星州))。这种试验有用的效应细胞包括外周血单核细胞(PBMC)和自然杀手(NK)细胞。或者，或另外，可例如以动物模型比如在Clynes等. (1998) Proc. Nat'l Acad. Sci. USA 95:652-656中公开的动物模型体内评价感兴趣分子的ADCC活性。也可实施C1q结合试验，以确认抗体不能结合C1q并因此缺乏CDC活性[参见例如WO 2006/029879和WO 2005/100402中的C1q和C3c结合ELISA]。为了评价补体激活，可实施CDC试验[参见例如Gazzano-Santoro等. (1996) J. Immunol. Methods 202:163;Cragg, M.S.等. (2003) Blood 101:1045-1052;和Cragg, M.S, and M.J. Glennie (2004) Blood 103:2738-2743]。也可采用本领域已知的方法实施FcRn结合和体内清除率/半衰期测定[参见例如Petkova, S.B.等. (2006) Int. Immunol. 18(12):1759-1769]。

[0241] 效应功能减少的本公开抗体(例如抗-GDF11抗体、抗-激活素B抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)包括具有一种或多种Fc区残基238、265、269、270、297、327和329替换的那些抗体(美国专利第6737056号)。这种Fc突变体包括在氨基酸位置265、269、270、297和327中的两个或多个有替换的Fc突变体，包括残基265和297替换为丙氨酸的所谓"DANA"Fc突变体(美国专利第7332581号)。

[0242] 在某些实施方案中，产生半胱氨酸工程化抗体可能是可取的，例如"thioMAbs"，其中抗体的一个或多个残基用半胱氨酸残基替换。在具体的实施方案中，替换的残基出现在抗体的可及位点上。通过用半胱氨酸替换那些残基，活性的硫醇基从而位于抗体的可及位点上并可用于使抗体缀合于其他部分，比如药物部分或连接子-药物部分，以产生免疫缀合物，如本文进一步描述的那样。在某些实施方案中，以下残基中的任何一种或多种可用半胱氨酸替换：轻链的V205 (Kabat编号)、重链的A118 (EU编号)和重链Fc区的S400 (EU编号)。半胱氨酸工程化抗体可如例如在美国专利第7521541号中描述的那样产生。

[0243] 另外，用于筛选抗体以确认合乎需要的抗体的技术可能影响所得抗体的性能。例如，如果抗体是用于溶液中结合抗原，测试溶液结合可能是可取的。有各种不同的技术可用于测试抗体与抗原之间的相互作用，以确认特别合乎需要的抗体。这种技术包括ELISAs、表面等离子体共振结合试验(例如Biacore™ binding assay (Biacore™结合试验)、Biacore AB, Uppsala (乌普萨拉), Sweden (瑞典))、夹心法分析(例如the paramagnetic bead system of IGEN International, Inc. (IGEN国际公司的顺磁珠系统), Gaithersburg (盖瑟斯堡), Maryland (马里兰))、蛋白质印迹法、免疫沉淀技术和免疫组织化学。

[0244] 在某些实施方案中，考虑本文提供的抗体和/或结合多肽的氨基酸序列变体。例如，可能可取的是改善抗体和/或结合多肽的亲和力和/或其他生物学性质。通过向编码抗体和/或结合多肽的核苷酸序列引入适当的修饰或者通过肽合成，可制备抗体和/或结合多肽的氨基酸序列变体。这种修饰包括例如抗体和/或结合多肽的氨基酸序列中残基的缺失和/或插入和/或替换。可进行缺失、插入和替换的任何组合，以获得最终构建体 ，条件是最

终构建体 具有期望的特性,例如靶标结合(GDF11、GDF8、ActRIIA和/或ActRIIB结合)。

[0245] 可在HVRs进行改变(例如替换),例如改善抗体亲和力。这种改变可在HVR“热点区域(hotspots)”进行,即由体细胞成熟过程中经历高频率突变的密码子编码的残基(参见例如Chowdhury (2008) Methods Mol. Biol. 207:179-196 (2008))和/或SDRs (a-CDRs),测试生成变体VH或VL的亲和力。本领域已描述了通过从二级库(secondary libraries)构建和重新选择的亲和力成熟[参见例如Hoogenboom等., 在Methods in Molecular Biology (分子生物学方法) 178:1-37, O'Brien等编辑, Human Press, Totowa, N.J. (新泽西州), (2001)]。在亲和力成熟的一些实施方案中,通过各种方法中的任何一种向为成熟而选择的可变基因引入多样性(diversity is introduced into the variable genes chosen for maturation) (例如易错PCR、链替换(chain shuffling)或寡核苷酸定点诱变)。然后创建二级库(secondary library)。然后筛选库以确认具有期望亲和力的任何抗体变体。引入多样性的另一种方法包括HVR定向法(HVR-directed approaches),其中几个HVR残基(例如一次4-6个残基)随机化。参与抗原结合的HVR残基可例如采用丙氨酸扫描诱变或建模进行具体确认。具体地讲通常靶向CDR-H3和CDR-L3。

[0246] 在某些实施方案中,替换、插入或缺失可出现在一种或多种HVRs中,只要这种改变不显著降低抗体结合抗原的能力。例如,可在HVRs进行不显著降低亲和力的保守性改变(例如本文提供的保守性替换)。这种改变可在HVR“热点区域(hotspots)”或SDRs外进行。在以上提供的变异VH和VL序列的某些实施方案中,每一种HVR不变或者含有多于1、2或3种氨基酸替换。

[0247] 用于确认可靶向诱变的抗体和/或结合多肽的残基或区域的有用方法称为“丙氨酸扫描诱变”,如由Cunningham and Wells (1989) Science, 244:1081-1085描述的那样。在该方法中,目标残基中的残基或基团(例如荷电残基比如arg、asp、his、lys和glu)被确认并用中性或荷负电的氨基酸(例如丙氨酸或聚丙氨酸)替代,以确定抗体或结合多肽与抗原的相互作用是否受到影响。可在氨基酸位置引入进一步的替换来证实对初始替换的功能灵敏度(functional sensitivity)。或者,或另外,抗原-抗体复合物的晶体结构可用于确认抗体与抗原之间的接触点。这种接触残基与邻近残基可作为替换的候选者靶向或消除。可筛选变体来确定它们是否含有期望的性质。

[0248] 氨基酸序列插入包括长度从1个残基到含有一百或更多残基的多肽范围内的氨基-和/或羧基-末端融合,以及单个或多个氨基酸残基的序列内插入。末端插入的实例包括具有N-末端甲硫氨酰残基的抗体。抗体分子的其他插入变异包括抗体N-或C-末端与酶(例如ADEPT)或增加抗体血清半衰期的多肽的融合物。

[0249] 在某些实施方案中,本文提供的抗体和/或结合多肽可被进一步修饰,以含有另外的本领域已知和易于得到的非蛋白质部分。适合于抗体和/或结合多肽衍生化的部分非限制性地包括水溶性聚合物。水溶性聚合物的非限制性实例非限制性地包括聚乙二醇(PEG)、乙二醇/丙二醇共聚物、羧甲基纤维素、葡聚糖、聚乙烯醇、聚乙烯吡咯烷酮、聚(1,3-二氧戊环)、聚(1,3,6-三氧六环)、乙烯/马来酸酐共聚物、聚氨基酸(均聚物或无规共聚物)和葡聚糖或聚(n-乙烯基吡咯烷酮)聚乙二醇(poly(n-vinyl pyrrolidone)polyethylene glycol)、聚丙二醇(propropylene glycol)均聚物、聚环氧丙烷/环氧乙烷共聚物(prolypropylene oxide/ethylene oxide co-polymers)、聚氧乙基化多元醇

(polyoxyethylated polyols)(例如甘油)、聚乙烯醇及其混合物。聚乙二醇丙醛由于其水中稳定性而可具有制备的有利条件。聚合物可具有任何分子量,并可为分支或未分支的。附着于抗体和/或结合多肽的聚合物数目可以变化,并且如果附着多于一种聚合物,它们可为相同或不同的分子。通常,用于衍生化的聚合物数目和/或类型可基于非限制性地包括要改善的抗体和/或结合多肽的具体性质或功能的考虑来确定,无论抗体衍生物和/或结合多肽衍生物是否在确定的条件下用于治疗。

[0250] 本文公开的任何一种ActRII拮抗剂抗体(例如抗-激活素A抗体、抗-激活素B抗体、抗-激活素C抗体、抗-激活素E抗体、抗-GDF11抗体、抗-GDF8抗体、抗-BMP6抗体、抗-BMP7抗体、抗-ActRIIA抗体和/或抗-ActRIIB抗体)可与一种或多种另外的本公开ActRII拮抗剂组合,以达到预期的效果。例如,本文公开的ActRII拮抗剂抗体(例如抗-GDF11抗体、抗-激活素B抗体、抗-激活素C抗体、抗-激活素E抗体、抗-GDF11抗体、抗-GDF8抗体、抗-BMP6抗体、抗-BMP7抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)可与以下组合使用:i) 一种或多种另外的本文公开的ActRII拮抗剂抗体,ii) 一种或多种本文公开的ActRII多肽(例如ActRIIA和/或ActRIIB多肽),iii) 一种或多种本文公开的GDF捕获物;iv) 一种或多种本文公开的小分子ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的小分子拮抗剂);v) 一种或多种本文公开的多核苷酸ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的多核苷酸拮抗剂);vi) 一种或多种本文公开的卵泡抑素多肽;和/或vii) 一种或多种本文公开的FLRG多肽。

[0251] D. 小分子拮抗剂

在另一方面,本公开涉及拮抗ActRII活性(例如抑制ActRIIA和/或ActRIIB信号转导,比如SMAD 2/3和/或SMAD 1/5/8信号传递)的小分子或小分子的组合。具体地讲,本公开提供单独或与一种或多种红细胞生成刺激剂(例如EPO)或其他支持疗法[例如造血生长因子(例如G-CSF或GM-CSF)、红细胞或全血输注、铁螯合疗法]组合,使用ActRII的小分子拮抗剂或抗体拮抗剂的组合的方法,以例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍。

[0252] 在一些实施方案中,优选的本公开ActRII拮抗剂为直接或间接抑制至少GDF11活性的小分子拮抗剂或小分子拮抗剂的组合。任选地,这种小分子拮抗剂或小分子拮抗剂的组合可进一步抑制(直接或间接地) GDF8。任选地,本公开的小分子拮抗剂或小分子拮抗剂的组合基本上不抑制激活素A活性。在一些实施方案中,抑制(直接或间接地) GDF11和/或GDF8活性的本公开小分子拮抗剂或小分子拮抗剂的组合进一步抑制(直接或间接地)激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB中一种或多种的活性。

[0253] 在某些实施方案中,本公开的小分子拮抗剂或小分子拮抗剂的组合为GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、Nodal、ActRIIA和ActRIIB中一

种或多种的间接抑制剂。例如，本公开的小分子拮抗剂或小分子拮抗剂的组合可抑制至少GDF11的表达(例如转录、翻译、细胞分泌或其组合)。任选地，这种小分子拮抗剂或小分子拮抗剂的组合可进一步抑制GDF8的表达。任选地，本公开的小分子拮抗剂或小分子拮抗剂的组合基本上不抑制激活素A的表达。在一些实施方案中，抑制GDF11和/或GDF8表达的本公开小分子拮抗剂或小分子拮抗剂的组合可进一步抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB中一种或多种的表达。

[0254] 在其他的实施方案中，本公开的小分子拮抗剂或小分子拮抗剂的组合为GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB中一种或多种的直接抑制剂。例如，优选的本公开小分子拮抗剂或小分子拮抗剂的组合直接结合并抑制至少GDF11活性(例如抑制GDF11结合ActRIIA和/或ActRIIB受体的能力；抑制GDF11-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。任选地，本公开的小分子拮抗剂或小分子拮抗剂的组合可进一步结合并抑制GDF8活性(例如抑制GDF8结合ActRIIA和/或ActRIIB受体的能力；抑制GDF8-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。任选地，本公开的小分子拮抗剂或小分子拮抗剂的组合基本上不结合或抑制激活素A活性(例如激活素A结合ActRIIA和/或ActRIIB受体的能力；激活素A介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号通路的激活)。在一些实施方案中，结合并抑制GDF11和/或GDF8活性的本公开小分子拮抗剂或小分子拮抗剂的组合进一步结合并抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB中一种或多种的活性。

[0255] 在一些实施方案中，本公开的小分子拮抗剂或小分子拮抗剂的组合直接结合并抑制至少GDF8活性(例如抑制GDF8结合ActRIIA和/或ActRIIB受体的能力；抑制GDF8-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。任选地，本公开的小分子拮抗剂或小分子拮抗剂的组合可进一步结合并抑制GDF11活性(例如抑制GDF11结合ActRIIA和/或ActRIIB受体的能力；抑制GDF11-介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。任选地，本公开的小分子拮抗剂或小分子拮抗剂的组合基本上不结合或抑制激活素A活性(例如激活素A结合ActRIIA和/或ActRIIB受体的能力；激活素A介导的ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。在一些实施方案中，结合并抑制GDF8和/或GDF11活性的本公开小分子拮抗剂或小分子拮抗剂的组合进一步结合并抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB中一种或多种的活性。

[0256] 在一些实施方案中，本公开的小分子拮抗剂或小分子拮抗剂的组合直接结合并抑制至少ActRIIA活性(例如ActRII配体-介导的ActRIIA信号转导，比如SMAD 2/3信号传递的激活)。例如，优选的本公开小分子拮抗剂或小分子拮抗剂的组合结合ActRIIA受体并抑制至少GDF11结合和/或激活ActRIIA受体。任选地，这种小分子拮抗剂或小分子拮抗剂的组合可进一步抑制GDF8结合和/或激活ActRIIA受体。任选地，本公开的小分子拮抗剂或小分子拮抗剂的组合基本上不抑制激活素A结合和/或激活ActRIIA受体。在一些实施方案中，抑制GDF11和/或GDF8结合和/或激活ActRIIA受体的本公开小分子拮抗剂或小分子拮抗剂的组合进一步抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和Nodal中的一种或多种结合和/或激活ActRIIA受体。

[0257] 在一些实施方案中，本公开的小分子拮抗剂或小分子拮抗剂的组合直接结合并抑制至少ActRIIB活性(例如ActRII配体-介导的ActRIIB信号转导，比如SMAD 2/3信号传递的激活)。例如，优选的本公开小分子拮抗剂或小分子拮抗剂的组合结合ActRIIB受体并抑制至少GDF11结合和/或激活ActRIIB受体。任选地，这种小分子拮抗剂或小分子拮抗剂的组合可进一步抑制GDF8结合和/或激活ActRIIB受体。任选地，本公开的小分子拮抗剂或小分子拮抗剂的组合基本上不抑制激活素A结合和/或激活ActRIIB受体。在一些实施方案中，抑制GDF11和/或GDF8结合和/或激活ActRIIB受体的本公开小分子拮抗剂或小分子拮抗剂的组合进一步抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7和Nodal中的一种或多种结合和/或激活ActRIIB受体。

[0258] 本公开的结合有机小分子拮抗剂可采用已知方法学确认和化学合成(参见例如PCT出版物 第WO 00/00823和WO 00/39585号)。通常，本公开的小分子拮抗剂通常大小小于约2000道尔顿，或者大小小于约1500、750、500、250或200道尔顿，其中这种有机小分子能够结合，优选地特异性地结合于本文描述的多肽(例如GDF11、GDF8、ActRIIA和ActRIIB)。这种小分子拮抗剂可采用熟知的技术而不需过度试验来确认。在这方面，需要指出的是，用于筛选有机小分子库能够结合于多肽靶标的分子的技术为本领域熟知的(参见例如国际专利出版物第WO00/00823和WO00/39585号)。

[0259] 本公开的结合有机小分子可为例如醛、酮、肟、腙、缩氨基脲、卡巴肼、伯胺、仲胺、叔胺、N-取代的肼、酰肼、醇、醚、硫醇、硫醚、二硫化物、羧酸、酯、酰胺、脲、氨基甲酸酯、碳酸酯、缩酮、酮缩硫醇、缩醛、硫缩醛、芳基卤化物、芳基磺酸酯、烷基卤化物、烷基磺酸酯、芳族化合物、杂环化合物、苯胺、链烯烃、炔烃、二醇、氨基醇、噁唑烷、噁唑啉、噻唑烷、噻唑啉、烯胺、磺酰胺、环氧化物、氮杂环丙烷、异氰酸酯、磺酰氯、重氮化合物和酰氯。

[0260] 本文公开的任何一种小分子ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的小分子拮抗剂)可与一种或多种另外的本公开ActRII拮抗剂组合，以达到预期的效果(例如在有需要的受试者增加红细胞水平和/或血红蛋白、治疗或预防贫血、治疗MDS或铁粒幼红细胞性贫血、治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症)。例如，本文公开的小分子ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的小分子拮抗剂)可与以下组合使用：i) 一种或多种另外的本文公开的小分子ActRII拮抗剂，ii) 一种或多种本文公开的ActRII多肽(例如ActRIIA和/或ActRIIB多肽)，iii) 一种或多种本文公开的GDF捕获物；iv) 一种或多种本文公开的ActRII拮抗剂抗体(例如抗-GDF11抗体、抗-激活素B抗体、抗-激活素C抗体、抗-激活素E抗体、抗-GDF11抗体、抗-GDF8抗体、抗-BMP6抗体、抗-BMP7抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)；v) 一种或多种本文公开的多核苷酸ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的多核苷酸拮抗剂)；vi) 一种或多种本文公开的卵泡抑素多肽；和/或vii) 一种或多种本文公开的FLRG多肽。

[0261] E. 拮抗剂多核苷酸

在另一方面，本公开涉及拮抗ActRII活性(例如抑制ActRIIA和/或ActRIIB信号转导，比如SMAD 2/3和/或SMAD 1/5/8信号传递)的多核苷酸或多核苷酸的组合。具体地讲，本公

开提供单独或与一种或多种红细胞生成刺激剂(例如EPO)或其他支持疗法[例如造血生长因子(例如G-CSF或GM-CSF)、红细胞或全血输注、铁螯合疗法]组合,使用多核苷酸ActRII拮抗剂或多核苷酸ActRII拮抗剂的组合的方法,以例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍。

[0262] 在一些实施方案中,本公开的多核苷酸ActRII拮抗剂或多核苷酸ActRII拮抗剂的组合可用于抑制GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的活性和/或表达。在某些优选的实施方案中,本公开的多核苷酸ActRII拮抗剂或多核苷酸ActRII拮抗剂的组合为GDF-ActRII拮抗剂。

[0263] 在一些实施方案中,本公开的多核苷酸拮抗剂或多核苷酸拮抗剂的组合抑制至少GDF11的活性和/或表达(例如转录、翻译、分泌或其组合)。任选地,这种多核苷酸拮抗剂或多核苷酸拮抗剂的组合可进一步抑制GDF8的活性和/或表达。任选地,本公开的多核苷酸拮抗剂或多核苷酸拮抗剂的组合基本上不抑制激活素A的活性和/或表达。在一些实施方案中,抑制GDF11和/或GDF8活性和/或表达的本公开多核苷酸拮抗剂或多核苷酸拮抗剂的组合可进一步抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的活性和或表达。

[0264] 在一些实施方案中,本公开的多核苷酸拮抗剂或多核苷酸拮抗剂的组合抑制至少GDF8的活性和/或表达(例如转录、翻译、分泌或其组合)。任选地,这种多核苷酸拮抗剂或多核苷酸拮抗剂的组合可进一步抑制GDF11的活性和/或表达。任选地,本公开的多核苷酸拮抗剂或多核苷酸拮抗剂的组合基本上不抑制激活素A的活性和/或表达。在一些实施方案中,抑制GDF8和/或GDF11活性和/或表达的本公开多核苷酸拮抗剂或多核苷酸拮抗剂的组合可进一步抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的活性和或表达。

[0265] 在一些实施方案中,本公开的多核苷酸拮抗剂或多核苷酸拮抗剂的组合抑制至少ActRIIA的活性和/或表达(例如转录、翻译、分泌或其组合)。任选地,本公开的多核苷酸拮抗剂或多核苷酸拮抗剂的组合基本上不抑制激活素A的活性和/或表达。在一些实施方案中,抑制ActRIIA的活性和/或表达的本公开多核苷酸拮抗剂或多核苷酸拮抗剂的组合可进一步抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal和/或ActRIIB中一种或多种的活性和或表达。

[0266] 在一些实施方案中,本公开的多核苷酸拮抗剂或多核苷酸拮抗剂的组合抑制至少ActRIIB的活性和/或表达(例如转录、翻译、分泌或其组合)。任选地,本公开的多核苷酸拮抗剂或多核苷酸拮抗剂的组合基本上不抑制激活素A的活性和/或表达。在一些实施方案中,抑制ActRIIB的活性和/或表达的本公开多核苷酸拮抗剂或多核苷酸拮抗剂的组合可进一步抑制激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal和/或ActRIIA中一种或多种的活性和或表达。

[0267] 本公开的多核苷酸拮抗剂可为反义核酸、RNAi分子[例如小干扰RNA (siRNA)、小发夹RNA (shRNA)、微RNA (miRNA)]、适体和/或核酶。人GDF11、GDF8、激活素A、激活素B、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB的核酸和氨基酸序列为本领域已知的,并因此用于本公开方法用途的多核苷酸拮抗剂可由技术人员基于本领域的知识和本文提供的讲授常规制备。

[0268] 例如,反义技术可用于通过反义DNA或RNA或者通过三重螺旋形成调控基因表达。例如在Okano (1991) J. Neurochem. 56:560; Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression (寡脱氧核苷酸作为基因表达的反义抑制剂), CRC Press (CRC出版社), Boca Raton (波卡拉顿), Fla. (佛罗里达州) (1988)中讨论了反义技术。在例如Cooney等. (1988) Science 241:456;和Dervan等. (1991) Science 251:1300中讨论了三重螺旋形成。方法基于多核苷酸与互补DNA或RNA的结合。在一些实施方案中,反义核酸包含与本文公开的基因(例如GDF11、GDF8、激活素A、激活素B、激活素C、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB)的RNA转录本的至少一部分互补的单链RNA或DNA序列。然而,绝对的互补性尽管优选,但不需要。

[0269] “与RNA的至少一部分互补的”序列本文指的是,意指有足够的互补性,能够与RNA杂交形成稳定的双螺旋的序列,在本文公开的基因(例如GDF11、GDF8、激活素A、激活素B、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB)的双链反义核酸情况下,可因此测试双螺旋DNA的单链,或者可试验三螺旋形成。杂交的能力取决于互补性程度和反义核酸的长度两方面。通常,杂交的核酸越大,与RNA碱基错配越多,但其含有并仍然形成稳定的双螺旋(或三螺旋(视情况而定))。本领域技术人员可通过采用测定杂交复合物熔点的标准程序来确定错配容忍度。

[0270] 与遗传信息的5’端(例如5’-非翻译序列直到并包括AUG起始密码子)互补的多核苷酸,应最有效地抑制翻译。然而,与mRNAs的3’-非翻译序列互补的序列也已显示有效抑制mRNAs的翻译[参见例如Wagner, R. (1994) Nature 372:333-335]。因此,与本公开的基因(例如GDF11、GDF8、激活素A、激活素B、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB)的5’-或3’-非翻译、非编码区互补的寡核苷酸,可用于反义技术以抑制内源性mRNA的翻译。与mRNA的5’-非翻译区互补的多核苷酸应包括AUG起始密码子的互补染色体。与mRNA编码区互补的反义多核苷酸为翻译的不太有效抑制剂,但是可按照本公开的方法使用。无论设计成与本公开mRNA (例如GDF11、GDF8、激活素A、激活素B、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB mRNA)的5’-非翻译、3’-非翻译还是编码区杂交,反义核酸长度应为至少6个核苷酸,并且优选地为长度在6-约50个核苷酸范围内的寡核苷酸。在特定方面,寡核苷酸为至少10个核苷酸、至少17个核苷酸、至少25个核苷酸或至少50个核苷酸。

[0271] 在一个实施方案中,本公开的反义核酸(例如GDF11、GDF8、激活素A、激活素B、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA或ActRIIB反义核酸)通过自外源序列转录在细胞内产生。例如,转录载体或其一部分,产生本公开基因的反义核酸(RNA)。这种载体含有编码所期望反义核酸的序列。这种载体可保持游离或者变为染色体整合,只要其可转录产生期望的反义RNA。这种载体可通过本领域的重组DNA技术方法标准构建。载体可为质粒、病毒或本领域已知用于在脊椎动物细胞复制和表达的其他种类。可通过本领域已知在脊椎动物,

优选地在人细胞起作用的任何启动子，表达编码本公开所期望的基因序列或其片段。这种启动子可为诱导型或组成型的。这种启动子非限制性地包括SV40早期启动子区[参见例如Benoist and Chambon (1981) Nature 29:304-310]、包含在劳斯氏肉瘤病毒3'长末端重复序列的动子子[参见例如Yamamoto等. (1980) Cell 22:787-797]、疱疹胸苷启动子[参见例如Wagner等. (1981) Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445]和金属硫蛋白基因的调控序列[参见例如Brinster等. (1982) Nature 296:39-42]。

[0272] 在一些实施方案中，多核苷酸拮抗剂为靶向GDF11、GDF8、激活素A、激活素B、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB中一种或多种的表达的干扰RNA或RNAi分子。RNAi指的是干扰被靶向的mRNA表达的RNA表达。具体地讲，RNAi经通过siRNA (小干扰RNA)与特定mRNA相互作用使所靶向的基因沉默。然后靶向ds RNA复合物的细胞退化。siRNA分子为10-50个核苷酸长度的双链RNA双螺旋，其干扰充分互补的靶基因表达(例如与基因至少80%同一性)。在一些实施方案中，siRNA分子包含与靶基因的核苷酸序列至少85、90、95、96、97、98、99或100%相同的核苷酸序列。

[0273] 另外的RNAi分子包括短发夹RNA (shRNA)，还有短干扰发夹和微RNA (miRNA)。shRNA分子含有经环形连接的靶基因的有义和反义序列。shRNA从细胞核运送到细胞质中，并与mRNA一起降解。Pol III或U6启动子可用于对RNAi表达RNAs (express RNAs for RNAi)。Paddison等. [Genes & Dev. (2002) 16:948-958, 2002]已采用折叠成发夹的小RNA分子作为一种手段来影响RNAi。因此，这种短发夹RNA (shRNA)分子也被有利地用于本文描述的方法。功能性shRNAs的茎环长度会变化，茎的长度范围可在约25-约30 nt的任何地方，和环大小范围可在4-约25 nt之间而不影响沉默活性。尽管不希望收到任何特定理论的束缚，确信这些shRNAs类似于DICER RNase的双链RNA (dsRNA)产物，并且在任何情况下，有相同的抑制特定基因表达的能力。shRNA可自慢病毒载体表达。miRNA为长度约10-70个核苷酸的单链RNA，其最初转录为特征为"茎环"结构的miRNA前体，并在通过RISC进一步加工后随后加工成成熟的miRNA。

[0274] 介导RNAi (非限制性地包括siRNA)的分子可通过化学合成(Hohjoh, FEBS Lett 521:195-199, 2002)、dsRNA水解(Yang等. Proc Natl Acad Sci USA 99:9942-9947, 2002)、通过用T7 RNA聚合酶体外转录(Donzeet等. Nucleic Acids Res 30:e46, 2002;Yu等. Proc Natl Acad Sci USA 99:6047-6052, 2002)、和通过采用核酶比如大肠杆菌RNase III的双链RNA水解(Yang等. Proc Natl Acad Sci USA 99:9942-9947, 2002)产生。

[0275] 根据另一方面，本公开提供非限制性地包括以下的多核苷酸拮抗剂：陷阱DNA (decoy DNA)、双链DNA、单链DNA、DNA复合物(complexed DNA)、包被的DNA (encapsulated DNA)、病毒DNA、质粒DNA、裸露RNA、包被的RNA (encapsulated RNA)、病毒RNA、双链RNA、能够产生RNA干扰的分子或其组合。

[0276] 在一些实施方案中，本公开的多核苷酸拮抗剂为适体。适体为核酸分子，包括双链DNA和单链RNA分子，其结合并形成三级结构，特异性结合靶分子比如GDF11、GDF8、激活素A、激活素B、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和ActRIIB多肽。本领域很好建立了适体的产生和治疗用途。参见例如美国专利第5475096号。关于适体的另外信息可见于美国专利申请出版物第20060148748号。核酸适体采用本领域已知的方法，例如经指数富集配基

的系统进化(SELEX)方法进行选择。SELEX为用于高度特异性结合于靶标分子的核酸分子体外进化的方法,如在例如美国专利第5475096、5580737、5567588、5707796、5763177、6011577和6699843号中描述的那样。确认适体的另一种筛选方法描述在美国专利第5270163号中。SELEX法为基于核酸形成各种二-和三-维结构的能力,以及起配体作用(与几乎任何化学化合物形成特异性结合对)的核苷酸单体(无论单体还是聚合物,包括其他核酸分子和多肽)范围内可得到的化学多功能性。任何大小或组成的分子可用作靶标。SELEX法包括采用相同的一般选择方案自候选寡核苷酸的混合物进行选择,并逐步反复进行结合、分离和扩增,以达到期望的亲和力和选择性。从其可包含一段随机序列的核酸混合物开始,SELEX法包括在对结合有利的条件下使混合物与靶标接触、分离(partitioning)未结合的核酸与特异性结合于靶标分子的那些核酸、使核酸-靶标复合物解离、扩增自核酸-靶标复合物解离的核酸以得到配体富集的核酸混合物的步骤。把结合、分离(partitioning)、解离和扩增步骤根据需要重复尽可能多的周期,得到靶标分子的高特异性高亲和力的核酸配体。

[0277] 一般地,这种结合分子单独给予动物[参见例如O'Connor (1991) J. Neurochem. 56:560],但是这种结合分子也可由宿主细胞自摄取的多核苷酸体内表达和体内表达[参见例如Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression (寡脱氧核苷酸作为基因表达的反义抑制剂), CRC Press (CRC 出版社), Boca Raton (波卡拉顿), Fla. (佛罗里达州) (1988)]。

[0278] 任何一种本文公开的多核苷酸ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的多核苷酸拮抗剂)可与一种或多种另外的文公开ActRII拮抗剂组合,以达到预期的效果(例如在有需要的受试者增加红细胞水平和/或血红蛋白、治疗或预防贫血、治疗MDS或铁粒幼红细胞性贫血、治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症)。例如,本文公开的多核苷酸ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的多核苷酸拮抗剂)可与以下组合使用:i) 一种或多种另外的本文公开的多核苷酸ActRII拮抗剂,ii) 一种或多种本文公开的ActRII多肽(例如ActRIIA和/或ActRIIB多肽),iii) 一种或多种本文公开的GDF捕获物;iv) 一种或多种本文公开的ActRII拮抗剂抗体(例如抗-GDF11抗体、抗-激活素B抗体、抗-激活素C抗体、抗-激活素E抗体、抗-GDF11抗体、抗-GDF8抗体、抗-BMP6抗体、抗-BMP7抗体、抗-ActRIIA抗体或抗-ActRIIB抗体);v) 一种或多种本文公开的小分子ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的小分子拮抗剂);vi) 一种或多种本文公开的卵泡抑素多肽;和/或vii) 一种或多种本文公开的FLRG多肽。

[0279] F. 其他拮抗剂

在其他方面,用于本文公开的方法用途的试剂为卵泡抑素多肽,其可单独或与一种或多种红细胞生成刺激剂(例如EPO)或其他支持疗法[例如造血生长因子(例如G-CSF或GM-CSF)、红细胞或全血输注、铁螯合疗法]组合使用,以例如在有需要的受试者增加红细胞水平、在有需要的受试者治疗或预防贫血(包括例如降低输注负担)、在有需要的受试者治疗MDS或铁粒幼红细胞性贫血和/或治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发

症(例如贫血、输血需求、嗜中性白血球减少症、铁超负荷、急性心肌梗塞、肝衰竭、肝肿大、脾肿大、进展为急性髓细胞性淋巴瘤)和或在有需要的受试者治疗或预防与*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍。术语“卵泡抑素多肽”包括包含任何天然存在的卵泡抑素多肽及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式)的多肽,并且进一步包括卵泡抑素的任何功能性单体或多聚体。在某些优选的实施方案中,本公开的卵泡抑素多肽结合和/或抑制激活素活性,特别是激活素A (例如激活素介导的ActRIIA和/或ActRIIB SMAD 2/3信号传递的激活)。保留激活素结合性能的卵泡抑素多肽变体可基于涉及卵泡抑素和激活素相互作用的先前研究来确认。例如,WO2008/030367公开了显示对激活素结合重要的特异性卵泡抑素结构域(“FSDs”)。如以下在SEQ ID NOs: 18-20显示的那样,卵泡抑素N-末端结构域(“FSND”SEQ ID NO:18)、FSD2 (SEQ ID NO: 20)及在较小程度上FSD1 (SEQ ID NO: 19)代表卵泡抑素中对激活素结合重要的示例性结构域。另外,以上在ActRII多肽上下文中描述了用于制备和测试多肽库的方法,并且这种方法也涉及制备和测试卵泡抑素的变体。卵泡抑素多肽包括源自任何已知卵泡抑素序列的多肽,其具有与卵泡抑素多肽序列至少约80%相同,和任选地具有至少85%、90%、95%、96%、97%、98%、99%或更大同一性的序列。卵泡抑素多肽的实例包括如例如在WO2005/025601中描述的人卵泡抑素前体多肽(SEQ ID NO: 16)的成熟卵泡抑素多肽或较短同种型或其他变体。

[0280] 人卵泡抑素前体多肽同种型FST344如下:

1 mvrarhqpgg lclllllcq fmedrsaqag ncwlrqakng rcqvlyktel
51 skeeccstgr lstswteedv ndntlfkwmi fnggapncip cketcenvdc
101 gpgkkcrmnk knkprcvcap dcsnitwkgp vcgldgktyr necallkarc
151 keqpelevqy qgrckktcrd vfcpgsstcv vdqtnnaycv tcnricpepa
201 sseqylcgnd gvtyssachl rkatcllgrs iglayegkci kakscediqc
251 tggkkclwdf kvgrgrcslc delcpdsksd epvcasdnat yasecamkea
301 acssgvllev khsgscnsis edteeeeede dqdysfpiss ilew
(SEQ ID NO: 16; NCBI参考号 NP_037541.1)

信号肽为下划线的;而且以上下划线的为最后27个残基,其代表区分此种卵泡抑素同种型与以下显示的较短卵泡抑素同种型FST317的C-末端延伸。

[0281] 人卵泡抑素前体多肽同种型FST317如下:

1 MVRARHQPGG LCLLLLLLCQ FMEDRSAQAG NCWLRQAKNG RCQVLYKTEL
51 SKEECCSTGR LSTSWTEEDV NDNTLFKWMI FNGGAPNCIP CKETCENVDC
101 GPGKKCRMNK KNKPRCVCAP DCSNITWKGP VCGLDGKTYR NECALLKARC
151 KEQPELEVQY QGRCKKTCRD VFCPGSSTCV VDQTNNAYCV TCNRICPEPA
201 SSEQYLCGND GVTYSSACHL RKATCLLGRS IGLAYEGKCI KAKSCEDIQC
251 TGGKKCLWDF KVGRGRCSLC DELCPDSKSD EPVCASDNAT YASECAMKEA
301 ACSSGVLLEV KHSGSCN
(SEQ ID NO: 17; NCBI参考号 NP_006341.1)

信号肽为下划线的。

[0282] 卵泡抑素N-末端结构域(FSND)序列如下:

gncwlrqakngrcqvlyktelskeeccstgrlstswteedvndnt

lfkwmifnggapncipck (SEQ ID NO: 18; FSND)

FSD1和FSD2序列如下：

etcenvdcgpgkkcrmnkknkprcv (SEQ ID NO: 19; FSD1)

ktcrdvfcpgsstcvvdqtnnaycvt (SEQ ID NO: 20; FSD2)

在其他方面，用于本文公开的方法用途的试剂为卵泡抑素样相关基因(FLRG)，也称为卵泡抑素相关蛋白3 (FSTL3)。术语“FLRG多肽”包括包含任何天然存在的FLRG多肽及其保留有用活性的任何变体(包括突变体、片段、融合物和拟肽形式)的多肽。在某些优选的实施方案中，本公开的FLRG多肽结合和/或抑制激活素活性，特别是激活素A (例如激活素介导的ActRIIA和/或ActRIIB SMAD 2/3信号传递的激活)。保留激活素结合性能的FLRG多肽变体可采用测定FLRG和激活素相互作用的常规方法确认(参见例如US 6537966)。另外，以上在ActRII多肽上下文中描述了用于制备和测试多肽库的方法，并且这种方法也涉及制备和测试FLRG的变体。FLRG多肽包括源自任何已知FLRG序列的多肽，其具有与FLRG多肽序列至少约80%相同，和任选地具有至少85%、90%、95%、97%、99%或更大同一性的序列。

[0283] 人FLRG前体(卵泡抑素相关蛋白3前体)多肽如下：

1 mrpgapgplw plpwgalawa vgfvssmgsg npapggvcwl qqgqeatcsl

51 vlqtdvtrae ccasgnidta wsnlthpgnk inllgflglv hclpckdscd

101 gvecgpgkac rmlggrprce capdcsglpa rlqvcgsdga tyrdecelra

151 arcrghpdls vmyrgrcrks cehvvcprpq scvvdqtgsa hcvvcraapc

201 pvpsspgqel cgnnnvtyis schmrqatcf lgrsigvrha gscagtpeep

251 pggesaeeee nfv

(SEQ ID NO:21; NCBI参考号 NP_005851.1)

信号肽为下划线的。

[0284] 在某些实施方案中，卵泡抑素多肽和FLRG多肽的功能性变体或修饰形式包括具有卵泡抑素多肽或FLRG多肽的至少一部分和一个或多个融合结构域(比如便于多肽的分离、检测、稳定化或多聚化的结构域)的融合蛋白。以上详细讨论了ActRII多肽的合适的融合结构域。在一些实施方案中，本公开的拮抗剂为包含融合于Fc结构域的卵泡抑素多肽的激活素-结合部分的融合蛋白。在另一个实施方案中，本公开的拮抗剂为包含融合于Fc结构域的FLRG多肽的激活素结合部分的融合蛋白。

[0285] 任何一种本文公开的卵泡抑素多肽可与一种或多种另外的本公开ActRII拮抗剂组合，以达到预期的效果(例如在有需要的受试者增加红细胞水平和/或血红蛋白、治疗或预防贫血、治疗MDS或铁粒幼红细胞性贫血、治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症)。例如，本文公开的卵泡抑素多肽可与以下组合使用：i) 一种或多种另外的本文公开的卵泡抑素多肽，ii) 一种或多种本文公开的ActRII多肽(例如ActRIIA和/或ActRIIB多肽)，iii) 一种或多种本文公开的GDF捕获物；iv) 一种或多种本文公开的ActRII拮抗剂抗体(例如抗-GDF11抗体、抗-激活素B抗体、抗-激活素C抗体、抗-激活素E抗体、抗-GDF11抗体、抗-GDF8抗体、抗-BMP6抗体、抗-BMP7抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)；v) 一种或多种本文公开的小分子ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的小分子拮抗剂)；vi) 一种或多种本文公开的多核苷酸ActRII拮抗剂(例如GDF11、

GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的多核苷酸拮抗剂)；和/或一种或多种本文公开的FLRG多肽。

[0286] 类似地，任何一种本文公开的FLRG多肽可与一种或多种另外的本公开ActRII拮抗剂组合，以达到预期的效果。例如本文公开的FLRG多肽可与以下组合使用：i) 一种或多种另外的本文公开的FLRG多肽，ii) 一种或多种本文公开的ActRII多肽(例如ActRIIA和/或ActRIIB多肽)，iii) 一种或多种本文公开的GDF捕获物；iv) 一种或多种本文公开的ActRII拮抗剂抗体(例如抗-GDF11抗体、抗-激活素B抗体、抗-激活素C抗体、抗-激活素E抗体、抗-GDF11抗体、抗-GDF8抗体、抗-BMP6抗体、抗-BMP7抗体、抗-ActRIIA抗体或抗-ActRIIB抗体)；v) 一种或多种本文公开的小分子ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的小分子拮抗剂)；vi) 一种或多种本文公开的多核苷酸ActRII拮抗剂(例如GDF11、GDF8、激活素A、激活素B、激活素AB、激活素C、激活素E、BMP6、BMP7、Nodal、ActRIIA和/或ActRIIB中一种或多种的多核苷酸拮抗剂)；和/或一种或多种本文公开的卵泡抑素多肽。

[0287] 3．筛选试验

在某些方面，本公开涉及研究对象ActRII多肽(例如ActRIIA和ActRIIB多肽)和GDF捕获多肽确认化合物(试剂)为ActRIIB多肽的激动剂或拮抗剂的用途。可测试通过该筛选确认的化合物，以评价其体内或体外调控红细胞、血红蛋白和/或网织红细胞水平的能力。可例如以动物模型测试这些化合物。

[0288] 有许多方法筛选通过靶向ActRII信号传递(例如ActRIIA和/或ActRIIB SMAD 2/3和/或SMAD 1/5/8信号传递)增加红细胞或血红蛋白水平的治疗剂。在某些实施方案中，可实施化合物的高通量筛选，以确认干扰ActRII-介导的影响所选择细胞系的试剂。在某些实施方案中，试验筛选并确认特异性抑制或减少ActRII多肽或GDF捕获多肽与其结合伙伴比如ActRII配体(例如激活素A、激活素B、激活素AB、激活素C、Nodal、GDF8、GDF11或BMP7)的结合的化合物。或者，试验可用于确认增强ActRII多肽或GDF捕获多肽与其结合伙伴比如ActRII配体的结合的化合物。在进一步的实施方案中，化合物可通过其与ActRII多肽或GDF捕获多肽相互作用的能力来确认。

[0289] 各种试验格式(assay formats)足够用，并且鉴于本公开，本文未明确描述的那些仍将被本领域的普通技术人员所理解。如本文描述的那样，本发明的研究对象化合物(试剂)可通过任何组合化学方法产生。或者，研究对象化合物可为体内或体外合成的天然存在的生物分子。要测试其起组织生长调节剂作用能力的化合物(试剂)可例如通过细菌、酵母、植物或其他生物产生(例如天然产物)、化学产生(例如小分子，包括拟肽)或重组产生。本发明设想的受试化合物包括非肽有机分子、肽、多肽、拟肽、糖、激素和核酸分子。在某些实施方案中，受试试剂为具有少于约2000道尔顿分子量的有机小分子。

[0290] 本公开的受试化合物可作为单一的、离散性实体提供，或者以更复杂的库提供，比如通过组合化学制备的库。这些库可包含例如醇、烷基卤化物、胺、酰胺、酯、醛、醚及其他类有机化合物。受试化合物可以分离形式或作为化合物的混合物呈现于测试系统，尤其是在初始筛选步骤。任选地，化合物可用其他化合物任选衍生化并具有便于化合物分离的衍生化基团。衍生化基团的非限制性实例包括生物素、荧光素、洋地黄毒苷、绿色荧光蛋白、同位素、多组氨酸、磁珠、谷胱苷肽S-转移酶(GST)、光敏交联剂或其任何组合。

[0291] 在测试化合物和天然提取物库的许多药物筛选程序中，高通量分析是合乎需要的，以便在给定的一段时间内使被测化合物的数量达到最大。以无细胞系统进行的试验(比如可用纯化或半纯化的蛋白衍化)作为“初步”筛选通常是优选的，其中它们可产生以容许快速显现和相对易于检测由受试化合物介导的分子靶标的变化。此外，受试化合物的细胞毒性或生物利用度的影响在体外系统中通常可被忽视，试验反而主要集中在药物对分子靶标的作用，如同ActRII多肽或GDF捕获多肽与其结合配偶体(例如ActRII配体)之间的亲和力改变可证实的那样。

[0292] 只是为了说明，在本公开的一个示例性筛选试验中，为了试验视情况而定，使感兴趣的化合物与通常能够结合ActRIIB配体的分离和纯化的ActRIIB多肽接触。然后向化合物和ActRIIB多肽的混合物加入含有ActRIIB配体(例如GDF11)的组成。检测和定量ActRIIB/ActRIIB配体复合物提供了一种手段，用于确定化合物抑制(或增强) ActRIIB多肽与其结合蛋白之间形成复合物的效力。化合物的效力可通过自使用各种浓度的受试化合物得到的数据产生剂量反应曲线来评估。此外，还可实施对照试验以提供比较基准。例如，在对照试验中，把分离和纯化的ActRIIB配体加入到含有ActRIIB多肽的组成中，并在不存在受试化合物情况下量化ActRIIB /配体复合物的形成。应该理解，通常，混合反应物的顺序可以改变，并可同时混合。此外，代替纯化蛋白，细胞抽提物和溶解产物可用于提供合适的无细胞试验系统。

[0293] ActRII多肽或GDF捕获多肽与其结合蛋白之间的复合物形成可通过各种技术检测。例如，使用例如可检测标记的蛋白比如放射性标记的(例如^{32}P、^{35}S、^{14}C或^{3}H)、荧光标记的(例如FITC)或酶标记的ActRII多肽或GDF捕获多肽和/或其结合蛋白，通过免疫测定或通过色谱检测，可对复合物形成的调控进行定量。

[0294] 在某些实施方案中，本公开考虑使用荧光偏振试验和荧光共振能量转移(FRET)试验测量(直接或间接地) ActRII多肽或GDF捕获多肽与其结合蛋白之间的相互作用程度。进一步地，其他检测模式，比如基于光波导(参见例如PCT出版物WO 96/26432和美国专利第5677196号)、表面等离子体共振(SPR)、表面电荷传感器和表面力传感器的那些检测模式，可与本公开的多种实施方案相适配。

[0295] 此外，本公开考虑一种相互作用捕集试验(也称为“双杂交试验”)，用于确认破坏或增强ActRII多肽或GDF捕获多肽与其结合配偶体之间相互作用的试剂的用途。参见例如美国专利第5283317号;Zervos等. (1993) Cell 72:223-232;Madura等. (1993) J Biol Chem 268:12046-12054;Bartel等. (1993) Biotechniques 14:920-924;和Iwabuchi等. (1993) Oncogene 8:1693-1696)。在一个具体的实施方案中，本公开考虑反向双杂交系统确认使ActRII多肽或GDF捕获物与其结合蛋白之间的相互作用解离的化合物(例如小分子或肽类)的用途[参见例如Vidal and Legrain, (1999) Nucleic Acids Res 27:919-29;Vidal and Legrain, (1999) Trends Biotechnol 17:374-81;和美国专利第5525490、5955280和5965368号]。

[0296] 在某些实施方案中，研究对象化合物通过其与ActRII多肽或GDF捕获多肽的相互作用的能力来确认。化合物与ActRII多肽或GDF捕获多肽之间的相互作用可为共价或非共价的。例如，这种相互作用可采用体外生物化学法(包括光致交联、放射性标记配体结合和亲和色谱法)以蛋白水平确认[参见例如Jakoby WB等. (1974) Methods in Enzymology

46:1]。在某些情况下，化合物可以基于机制的试验进行筛选，比如检测化合物结合ActRII多肽或GDF捕获多肽的试验。这可能包括固相或流体相结合事件。或者，编码ActRII多肽或GDF捕获多肽的基因可用报告系统(例如β-半乳糖苷酶、荧光素酶或绿色荧光蛋白)转染进入细胞，并对库优选用高通量筛选或者用库的单个成员进行筛选。可使用其他基于机制的结合试验，例如检测自由能变化的结合试验。结合试验可用固定在孔、珠或芯片上，或者用固定化抗体捕获的或用毛细管电泳解析的靶标进行。结合的化合物通常可采用比色终点或荧光或表面等离子体共振检测。

[0297] 4．示例性的治疗用途

在某些方面，本公开提供用一种或多种ActRII拮抗剂治疗MDS和铁粒幼细胞性贫血，特别是治疗或预防MDS的一种或多种亚型或并发症，包括治疗患有特征为存在环形铁粒幼红细胞和/或*SF3B1*、*DNMT3A*和/或*TET2*基因的一种或多种突变的患者的方法。具体地讲，本公开提供使用ActRII拮抗剂或ActRII拮抗剂的组合，治疗或预防MDS和铁粒幼红细胞性贫血的一种或多种并发症(包括例如贫血、嗜中性白血球减少症、脾肿大、输血需求、急性髓性白血病的发展(development of acute myeloid leukemia)、铁超负荷及铁超负荷的并发症，其中有充血性心力衰竭、心律失常、心肌梗死、其他形式的心脏病、糖尿病、呼吸困难、肝脏疾病以及铁螯合疗法的不良反应)的方法。

[0298] 具体地讲，本公开提供使用ActRII拮抗剂或ActRII拮抗剂的组合，在MDS的亚型治疗或预防贫血或其他并发症的方法，包括骨髓中成红细胞数目升高(细胞过多)的MDS患者；在骨髓中铁粒幼红细胞多于1%、2%、3%、4%、5%、6%、7%、8%、9%、10%、11%、12%、13%、14%、15%、16%、17%、18%、19%、20%、25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%或95%的MDS患者；在难治性贫血伴环形铁粒幼红细胞增多(RARS)的MDS患者；在难治性贫血伴环形铁粒幼红细胞和血小板增多(RARS-T)的MDS患者；在难治性血细胞减少伴单系发育不良(RCUD)的MDS患者；在难治性血细胞减少伴多系发育不良和环形铁粒幼红细胞增多(RCMD-RS)的MDS患者；在伴*SF3B1*、*SRSF2*、*DNMT3A*或*TET2*体细胞突变的MDS患者；在没有*ASXL1*或*ZRSR2*体细胞突变的MDS患者；在伴铁超负荷的MDS患者及在伴嗜中性白血球减少症的MDS患者。

[0299] 还具体地讲，本公开提供使用ActRII拮抗剂或ActRII拮抗剂的组合，治疗或预防贫血或铁粒幼红细胞性贫血的其他并发症的方法，非限制性地包括难治性贫血伴环形铁粒幼红细胞增多(RARS)、难治性贫血伴环形铁粒幼红细胞和血小板增多(RARS-T)、难治性血细胞减少伴多系发育不良和环形铁粒幼红细胞增多(RCMD-RS)、与酗酒有关的铁粒幼细胞性贫血、药物诱发的铁粒幼细胞性贫血、铜缺乏引起的铁粒幼细胞性贫血(锌毒性)、低温引起的铁粒幼细胞性贫血、X连锁铁粒幼细胞性贫血(XLSA)、SLC25A38缺乏、谷氧还蛋白5缺乏、红细胞生成性原卟啉病、X连锁铁粒幼细胞性贫血伴共济失调(XLSA/A)、铁粒幼细胞性贫血伴B细胞免疫缺陷、发热及发育迟缓(SIFD)；皮尔森骨髓胰腺综合征、肌病、乳酸性酸中毒和铁粒幼红细胞性贫血(MLASA)；硫胺素反应性巨幼细胞性贫血(TRMA)以及不明原因的综合征性/非综合征性铁粒幼细胞性贫血。

[0300] 在某些方面，本公开提供治疗或预防与生殖系或体细胞*SF3B1*、*DNMT3A*和/或*TET2*突变有关的障碍或障碍的并发症，比如骨髓增生异常综合征、慢性淋巴细胞白血病(CLL)和急性髓性白血病(AML)以及乳腺癌、胰腺癌、胃癌、前列腺癌和葡萄膜黑色素瘤的方法。在某

些方面，障碍可发生在具有对*SF3B1*、*DNMT3A*和/或*TET2*突变检测呈阳性的骨髓细胞，特别是骨髓增生异常综合征、CLL和AML的受试者。任选地，*SF3B1*基因突变在外显子、内含子或5’或3’非翻译区。任选地，*SF3B1*、*DNMT3A*和/或*TET2*突变造成基因编码蛋白质的氨基酸序列发生变化，或者不造成氨基酸序列发生改变。任选地，*SF3B1*基因的突变造成基因编码蛋白质的氨基酸发生选自以下的变化：K182E、E491G、R590K、E592K、R625C、R625G、N626D、N626S、H662Y、T663A、K666M、K666Q、K666R、Q670E、G676D、V701I、I704N、I704V、G740R、A744P、D781G、A1188V、N619K、N626H、N626Y、R630S、I704T、G740E、K741N、G742D、D894G、Q903R、R1041H、I1241T、G347V、E622D、Y623C、R625H、R625L、H662D、H662Q、T663I、K666E、K666N、K666T、K700E和V701F。任选地，*DNMT3A*基因的突变造成基因编码蛋白质的氨基酸发生选自以下的变化：R882C、R882H、P904L和P905P。任选地，*DNMT3A*基因的突变引入提前终止密码子。例如，在一些实施方案中，引入提前终止密码子的*DNMT3A*基因的突变选自以下位置：Y436X和W893X。任选地，*TET2*基因的突变造成基因编码蛋白质的氨基酸发生选自以下的变化：E47Q、Q1274R、W1291R、G1370R、N1387S和Y1724H。任选地，*TET2*基因的突变引入提前终止密码子。例如，在一些实施方案中，引入提前终止密码子的*TET2*基因的突变选自以下位置：R550X、Q1009X、Y1337X、R1404X、R1516X和Q1652X。

[0301] 术语“受试者”、“个体”或“患者”在整个说明书中可互换并且通常指的是哺乳动物。哺乳动物非限制性地包括饲养动物(例如牛、羊、猫、狗和马)、灵长类动物(例如人和非人灵长类动物比如猴子)、兔和啮齿类动物(例如小鼠和大鼠)。

[0302] 本文使用的“预防”障碍或症状的治疗药物指的是化合物，其在统计样本中相对于未治疗的对照样本减少治疗样本的障碍或病症的发生，或者相对于未治疗的对照样本延迟或减少障碍或症状的一种或多种症状的严重程度。

[0303] 本文使用的术语“治疗”包括一旦已经建立时病症的改善或消除。无论发生哪种情况，预防或治疗可以医生或其他医护人员提供的诊断和给予治疗剂的预期结果进行辨别。

[0304] 通常，治疗或预防本公开描述的疾病或病症通过以有效量给予一种或多种本公开的ActRII拮抗剂(例如ActRIIA和/或ActRIIB拮抗剂)达到。试剂的有效量指的是以必要的剂量和时间段达到期望的治疗或预防效果有效的量。本公开试剂的“治疗有效量”可根据因素比如个体的疾病状态、年龄、性别和体重、以及试剂在个体引起期望的反应的能力而变化。“预防有效量”指的是以必要的剂量和时间段达到期望的预防效果有效的量。

[0305] 骨髓增生异常综合征(MDS)为多种血液疾病的集合，其特征为骨髓血细胞产生无效和转化为急性髓性白血病的风险。在MDS患者，造血干细胞未发育成健康的红细胞、白细胞或血小板。MDS障碍包括例如难治性贫血、难治性血细胞减少伴单系发育不良(RCUD)、难治性贫血伴环形铁粒幼红细胞增多(RARS)、与血小板显著增多有关的难治性贫血伴环形铁粒幼红细胞增多(RARS-T)、难治性贫血伴未成熟细胞过多(RAEB-1)、难治性贫血伴未成熟细胞过多转变型(RAEB-2)、难治性血细胞减少伴多系发育不良(RCMD)、未分类的MDS (MDS-U)和与分离的5q染色体异常有关的骨髓增生异常综合征[具有del (5q)的MDS]。

[0306] 同种异体干细胞移植是MDS唯一已知的潜在治疗方法。然而，由于高龄、医疗并发症和合适的干细胞供体获得有限，只有少数患者接受该方法。即使对于那些进行同种异体干细胞移植的患者，明显的治疗相关性死亡率和发病率，包括急性和慢性移植物抗宿主病，以及高复发率都会危及长期无病生存[Zeidan等. (2013) Blood Rev 27:243-259]。由于

这些原因，大多数MDS患者仍基于非治愈性意图治疗模式进行处理。治疗基于预测急性髓性白血病的生存或进展的预后因素。低风险MDS患者的生存期范围在几个月到十年以上，并且大多数这些患者死于与MDS并发症直接相关的原因[Dayyani等. (2010) Cancer 116:2174-2179]。因此，低风险患者的治疗策略适合特定患者的情况，包括血细胞减少的严重程度和类型及预期生存。较低风险患者有多种治疗选择，包括用生长因子治疗、del (5q)综合征情况下的来那度胺、沙利度胺、泊马度胺、低甲基化剂比如阿扎胞苷或地西他滨以及潜在的试验性试剂。与此相反，不适合同种异体干细胞移植的较高风险MDS患者通常用低甲基化剂、密集化疗或试验性试剂[Garcia-Manero等. (2011) 29:516-523]治疗。

[0307] 因为MDS表现为造血细胞数量和质量两方面的不可逆性缺陷，大多数MDS患者都受到慢性贫血困扰。大约80%-90%的MDS患者在其疾病过程中出现贫血，其中至少40%成为RBC输注依赖性的[Santini (2011) Oncologist 16:35-42;Malcovati等. (2005) J Clin Oncol 23:7594-7603;Leitch (2011) Blood Rev 25:17-31]。较高风险MDS组的患者(根据IPSS分类)甚至更可能变为输注依赖性的；例如，在一项研究中79%的高风险类别患者和(versus)39%的低风险患者需要长期输注以治疗或预防严重贫血[Öscan等. (2013) Expert Rev Hematol 6:165-189]。血红蛋白水平低会导致大脑和外周器官的氧合作用不佳，结果，MDS患者通常会嗜睡、精神警觉性下降、身体虚弱和注意力低下。这些症状与健康相关的生活质量降低有关。另外，血红蛋白阈值与MDS的显著发病率和死亡率独立相关。在血红蛋白水平分别低于8 g/dL和9 g/dL的女性和男性患者，发病率和死亡率的风险增加，主要是由于心脏并发症的风险增加所致[Malcovati等. (2011) Haematologica 96:1433-1440]。血红蛋白水平低和依赖于红细胞输注与MDS患者的心血管结果低下和死亡率增加有关，这代表积极治疗MDS贫血的有力依据[Goldberg等. (2010) J Clin Oncol 28:2847-2852;Leitch (2011) Blood Rev 25:17-31;Oliva等. (2011) Am J Blood Res 1:160-166;Özcan等. (2013) Expert Rev Hematol 6:165-189]。

[0308] MDS患者最终需要输血和/或用红细胞生成生长因子(例如ESAs比如EPO)单独或与集落刺激因子[例如粒细胞集落刺激因子(G-CSF)的类似物比如非格司亭或粒细胞-巨噬细胞集落刺激因子(GM-GSF)的类似物比如沙格司亭]组合治疗，以增加红细胞水平。输注的次数取决于疾病的程度和并发症的存在。已知长期输注增加血红蛋白水平，进而改善大脑和外周组织的氧合作用，从而改善体力活动和精神警觉性。然而，许多MDS患者由于使用这种疗法而出现副作用。例如，接受频繁红细胞输注的患者可能由于铁蓄积和产生有毒活性氧而出现组织和器官损伤。因此，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选与EPO受体激活剂组合，可用于治疗患有MDS或铁粒幼红细胞性贫血的患者。在某些实施方案中，患有MDS或铁粒幼红细胞性贫血的患者可使用一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)治疗(任选与EPO受体激活剂组合)。在其他的实施方案中，患有MDS或铁粒幼红细胞性贫血的患者可使用一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)与一种或多种另外的用于治疗MDS的治疗剂的组合进行治疗，所述治疗剂包括例如ESAs (包括例如依泊汀α、依泊汀β (例如NeoRecormon)、依泊汀δ (例如Dynepo)、依泊汀ω、达贝泊汀α (例如Aranesp)、甲氧基聚乙二醇依泊汀β (例如Micera)和合成的红细胞生成蛋白(SEP))、G-CSF类似物(包括非格司

亭)、GM-CSF类似物(包括沙格司亭)、来那度胺、沙利度胺、泊马度胺、低甲基化剂(包括阿扎胞苷和地西他滨)、铁螯合剂(包括去铁胺(又名去铁敏B、去铁胺B、DFO-B、DFOA、DFB或去铁敏甲磺酸)、去铁酮(又名奥贝安可)和地拉罗司(又名双羟基苯基三氮唑、ICL670、或Exjade™)、促血小板生成素拟似物(包括罗米司亭和艾曲波帕)、化学治疗剂(包括阿糖胞苷(ara-C),单独或与伊达比星、拓扑替康或氟达拉滨组合)、免疫抑制剂(包括抗胸腺细胞球蛋白、阿仑单抗和环孢霉素)、组蛋白去乙酰化酶抑制剂(HDAC抑制剂,包括伏立诺他、丙戊酸、丁酸苯酯、恩替诺特、MGCD0103和其他I类核HDAC抑制剂、II类非核HDAC抑制剂、全面(pan)HDAC抑制剂和同种型特异性HDAC抑制剂)、法尼基转移酶抑制剂(包括替吡法尼和洛那法尼)、肿瘤坏死因子α (TNF-α)抑制剂(包括依那西普或英夫利昔单抗)、谷胱甘肽-S-转移酶(GST)抑制剂P1-1 (包括ezatiostat)和CD33抑制剂(包括吉妥单抗)。

[0309] 一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等),任选地与EPO受体激活剂和/或一种或多种另外的疗法组合,可用于增加患有贫血(MDS或铁粒幼红细胞性贫血)患者的红细胞水平、血红蛋白水平和/或血细胞比容水平。在监测人体血红蛋白和/或血细胞比容水平时,适当年龄和性别类别的低于正常水平可能表明贫血,尽管考虑到个体差异。例如,10-12.5 g/dl的血红蛋白水平,并且通常约11.0 g/dl认为是处于健康成年人的正常范围,尽管在治疗方面,目标水平较低可能会造成心血管副作用较少。参见例如,Jacobs等. (2000) Nephrol Dial Transplant 15, 15-19。或者,血细胞比容水平(细胞所占血液样本体积的百分数)可用作贫血的量度。健康个体的血细胞比容水平对成年男性在约41-51%范围内和对成年女性在35-45%范围内。在某些实施方案中,患者可用打算把患者恢复到红细胞、血红蛋白和/或血细胞比容的目标水平或者允许减少或消除红细胞输注同时保持红细胞、血红蛋白和/或血细胞比容在可接受的水平的给药方案进行治疗。因为血红蛋白和血细胞比容水平因人而异,目标血红蛋白和/或血细胞比容水平最好为每个患者个体化。

[0310] 嗜中性白血球减少症意指循环粒细胞水平异常低的病症,约占MDS患者的40%[Steensma等. (2006) Mayo Clin Proc 81:104-130]。嗜中性白血球减少症患者的常见不适包括疲劳和频繁细菌感染,尤其是皮肤。然而,嗜中性白血球减少症也可导致MDS患者的严重并发症,感染是MDS相关死亡的最常见原因。用粒细胞集落刺激因子(非格司亭)治疗可帮助保持严重嗜中性白血球减少患者的嗜中性粒细胞计数超过1 × 10^9/L[Akhtari (2011) Oncology (Williston Park) 25:480-486],但髓系生长因子没有明显改变疾病史,并且可能只增加产生缺乏杀菌能力的功能上有缺陷的嗜中性粒细胞[Dayyani等. (2010) Cancer 116:2174-2179;Steensma (2011) Semin Oncol 38:635-647]。预防性抗生素在MDS患者没有得到证实的作用,并且不推荐给MDS相关的嗜中性白血球减少症患者。然而,MDS患者的嗜中性白血球减少性发热应视为一种医疗紧急情况,需要立即给予经验性广谱抗生素和经常住院治疗[Barzi等. (2010) Cleve Clin J Med 77:37-44]。在一些实施方案中,一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等),任选地与EPO受体激活剂和/或一种或多种另外的疗法比如G-CSF或GM-CSF疗法组合,可用于治疗MDS患者的嗜中性白血球减少症。一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等),任选与EPO受体激活剂和/或一种或多种另外的疗法组合,可用于减少MDS患者的粒细胞输注的次

数。

[0311] 接受频繁的红细胞或全血输注的患有MDS或铁粒幼红细胞性贫血的患者易于出现输血性铁超负荷，这可部分解释为什么在MDS的输注依赖性与生存的可能性降低有关。然而，在输注依赖的MDS患者使用铁螯合疗法仍存在争议，因为回顾和注册表数据表明螯合的患者可能比未螯合的患者存活时间更长，然而尚无前瞻性的随机试验数据证实螯合作用的发病率或死亡率获益，并且目前批准的试剂对许多患者不方便给药(去铁胺)或者昂贵和耐受性差(地拉罗司) [Steensma等. (2013) Best Pract Res Clin Haematol 26:431-444; Lyons等. (2014) Leuk Res 38:149-154]。

[0312] 在某些实施方案中，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选地与EPO受体激活剂和/或一种或多种另外的疗法组合，可用于预防或逆转MDS或铁粒幼红细胞性贫血患者的铁超负荷的并发症。在某些方面，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选地与EPO受体激活剂和/或一种或多种另外的疗法组合，可用于预防或逆转铁超负荷的心脏并发症，包括例如心输出量增加、心脏肥大、心肌病、左心室肥大、急性心肌梗塞、心律失常和充血性心力衰竭。在某些方面，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选地与EPO受体激活剂和/或一种或多种另外的疗法组合，可用于减少肝铁含量和/或预防或逆转铁超负荷的肝脏并发症，包括例如肝脏肿大(肝肿大)、肝纤维化(疤痕组织增加)和肝硬化(广泛的疤痕形成)。在某些方面，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选地与EPO受体激活剂和/或一种或多种另外的疗法组合，可用于预防或逆转铁超负荷的内分泌并发症，包括例如糖尿病。

[0313] 在某些方面，本公开的ActRII拮抗剂可与一种或多种另外的试剂[例如ESAs、G-CSF类似物(包括非格司亭)、GM-CSF类似物(包括沙格司亭)、来那度胺、沙利度胺、泊马度胺、低甲基化剂(包括阿扎胞苷和地西他滨)、铁螯合剂(包括去铁胺和地拉罗司)、促血小板生成素拟似物(包括罗米司亭和艾曲波帕)、化学治疗剂(包括阿糖胞苷(ara-C)，单独或与伊达比星、拓扑替康或氟达拉滨组合)、免疫抑制剂(包括抗胸腺细胞球蛋白、阿仑单抗和环孢霉素)、组蛋白去乙酰化酶抑制剂(HDAC抑制剂，包括伏立诺他、丙戊酸、丁酸苯酯、恩替诺特、MGCD0103和其他I类核HDAC抑制剂、II类非核HDAC抑制剂、全面(pan) HDAC抑制剂和同种型特异性HDAC抑制剂)、法尼基转移酶抑制剂(包括替吡法尼和洛那法尼)、肿瘤坏死因子α (TNF-α)抑制剂(包括依那西普或英夫利昔单抗)、谷胱甘肽-S-转移酶(GST)抑制剂P1-1(包括ezatiostat)和CD33抑制剂(包括吉妥单抗)]或支持疗法[例如红细胞输注、粒细胞输注、凝血细胞(血小板)输注]组合，给予有需要的受试者，用于治疗MDS和铁粒幼红细胞性贫血或者MDS和铁粒幼红细胞性贫血的一种或多种并发症。

[0314] 本文使用的“组合”或“联合给予”指的是任何形式的给予，以至另外的疗法(例如第二、第三、第四种等)在人体仍然有效(例如多种化合物在患者同时有效，可包括这些化合物的协同效应)。有效性可能与血液、血清或血浆中试剂的可测量浓度无关。例如，不同的治疗化合物可以相同的制剂或以单独的制剂给予(同时或依序给予)，也可按不同的时间表给予。因此，接受这种治疗的个体可得益于不同疗法的联合效应。一种或多种本公开的GDF11和/或激活素B拮抗剂(任选地进一步为GDF8、激活素A、激活素C、激活素E和BMP6中一种或多

种中拮抗剂)可与一种或多种其他另外的试剂或支持疗法同时、在之前或在之后给予。通常,每种治疗剂应以对特定试剂确定的剂量和/或时间表给予。用于给药方案的特定组合应考虑到本公开的拮抗剂与疗法和/或要达到的期望的治疗效果的相容性。

[0315] 在某些实施方案中,一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物、抗体等),任选地与EPO受体激活剂和/或一种或多种另外的疗法组合,可与红细胞或全血输注组合用于治疗患有MDS或铁粒幼红细胞性贫血的患者的贫血。在接受频繁的全血或红细胞输注的患者,铁稳态的正常机制可能被压倒,最终导致铁在重要组织比如心脏、肝脏和内分泌腺中的毒性和潜在致命性蓄积。常规的红细胞输注需要接触各种血液供体单位并因此具有较高风险的同种异体免疫。铁螯合的血管通路困难、可获得性和依从性以及高成本是为什么限制红细胞输注数量可能有益的其中一些原因。在一些实施方案中,本公开的方法涉及通过给予本公开的ActRII拮抗剂和一种或多种红细胞输注的组合,在有需要的受试者治疗MDS或铁粒幼红细胞性贫血。在一些实施方案中,本公开的方法涉及通过给予本公开的ActRII拮抗剂和一种或多种红细胞输注的组合,在有需要的受试者治疗或预防MDS或铁粒幼红细胞性贫血的一种或多种并发症。在一些实施方案中,用一种或多种本公开的ActRII拮抗剂治疗在减少患有MDS或铁粒幼红细胞性贫血患者的输血需求,例如减少有效治疗MDS或铁粒幼红细胞性贫血或者一种或多种其并发症需要的输血次数和/或量方面是有效的。

[0316] 在某些实施方案中,一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等),任选地与EPO受体激活剂和/或一种或多种另外的疗法组合,可与一种或多种铁螯合分子组合使用,以促进尿和/或粪便中的铁排泄,并从而预防或逆转患有MDS或铁粒幼红细胞性贫血患者的组织铁超负荷。有效的铁螯合剂应能够选择性地结合和中和三价铁,非转铁蛋白结合铁的氧化形式,这可能是通过催化产生羟基自由基和氧化产物造成大多数铁中毒的原因[参见例如Esposito等. (2003) Blood 102:2670-2677]。这些试剂为结构多样性的,但都含有能以1:1 (六齿)、2:1 (三齿)或3:1 (二齿)的化学计量与单个铁原子形成中性的八面体配位化合物的氧或氮供体原子[Kalinowski等. (2005) Pharmacol Rev 57:547-583]。通常,有效的铁螯合剂也是相对低分子量(例如小于700道尔顿),在水和脂质两者中具有溶解性,使得能够接近受影响的组织。铁螯合分子的具体实例包括去铁胺(一种细菌来源的需要每天非肠道给予的六齿试剂)和口服活性的合成试剂去铁酮(二齿)和地拉罗司(三齿)。包含同一天给予两种铁螯合剂的联合疗法对单一螯合疗法不反应的患者显示出希望,并且还克服了单独给予去铁胺患者依从性差的问题[Cao等. (2011) Pediatr Rep 3(2):e17;和Galanello等. (2010) Ann NY Acad Sci 1202:79-86]。

[0317] 一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等),任选地与EPO受体激活剂和/或一种或多种另外的疗法组合,可用于增加患有MDS或铁粒幼红细胞性贫血患者的红细胞水平、血红蛋白水平和/或血细胞比容水平。当观察人体内血红蛋白和/或血细胞比容水平时,对于适当年龄和性别类别低于正常水平可能表明贫血,尽管考虑到个体差异。例如,在健康成年人10-12.5 g/dl的血红蛋白水平,并且通常约11.0 g/dl认为是处于正常范围内,尽管在治疗方面,目标水平较低可能会造成心血管副作用较少。参见例如Jacobs等. (2000) Nephrol Dial Transplant 15,

15-19。或者，血细胞比容水平(细胞所占血液样本体积的百分数)可用作贫血的量度。健康个体的血细胞比容水平在约41-51% (成年男性)和(35-45%)成年女性范围内。在某些实施方案中，患者可用打算把患者恢复到红细胞、血红蛋白和/或血细胞比容的目标水平的给药方案进行治疗。因为血红蛋白和血细胞比容水平因人而异，目标血红蛋白和/或血细胞比容水平最好为每个患者个体化。

[0318] 一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)可与EPO受体激活剂和/或一种或多种另外的疗法组合用于治疗贫血。一些MDS患者的次优促红细胞生成素反应被认为是一种用ESAs治疗MDS相关贫血的生物基本原理[Greenberg等. (2011) J Natl Compr Canc Netw 9:30-56;Santini (2012) Semin Hematol 49:295-303]。尽管没有被FDA批准用于MDS相关贫血，ESAs在临床广泛应用并且是MDS的最常用疗法[Casadevall等. (2004) Blood 104:321-327; Greenberg等. (2009) Blood 114:2393-2400]。2001-2005年之间SEER-Medicare有关数据的分析发现，62%患有MDS的医疗保险受益人接受ESAs[Davidoff 等. (2013) Leuk Res 37:675-680]。Greenberg等. (2009) Blood 114:2393-2400]。一些临床前和临床研究提示，粒细胞集落刺激因子(G-CSF)可与ESAs具有协同效应，并且小剂量G-CSF可尝试在一些患者改善红细胞反应，尤其是那些患有RARS的患者(初始或在单一ESA疗法缺乏应答的情况下) [Negrin等. (1996) Blood 87:4076-4081]。另外，具有低风险MDS和较低水平血清EPO (< 200-500 mU/mL)的患者和具有较低RBC输注需求(< 2单位/月)的那些患者具有更高可能性用ESAs获得红细胞反应[Hellstrom-Lindberg等. (2003) Br J Haematol 120:1037-1046;Park等. (2008) 111:574-582]。因此，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等) 可与粒细胞集落刺激因子(例如非格司亭)或粒细胞巨噬细胞集落刺激因子(例如沙格司亭)组合用于治疗贫血。

[0319] 在某些实施方案中，本公开提供通过给予个体治疗有效量的一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)和EPO受体激活剂，在需要它的个体治疗或预防贫血的方法。在某些实施方案中，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)可与EPO受体激活剂组合，用于在易受ESAs副作用影响的患者减少这些激活剂需要的剂量。这些方法可用于患者的治疗性和预防性治疗。

[0320] 一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)可与EPO受体激活剂组合，用于实现增加红细胞，特别是以较低剂量范围。这可有益于减少已知的偏离目标效应和与高剂量EPO受体激活剂有关的风险。ESAs的主要不良反应包括例如血细胞比容或血红蛋白水平和红细胞增多的过度增加。血细胞比容水平升高可导致高血压(更具体地讲是高血压加重)和血管栓塞。ESAs的其他不良反应已被报道，其中一些涉及高血压的为头痛、流感样综合征，梗阻分流、由于血栓形成引起的心肌梗塞和脑抽搐、高血压性脑病和红细胞发育不全。参见例如Singibarti (1994) J. Clin Investig 72(增刊6), S36-S43;Horl等. (2000) Nephrol Dial Transplant 15(增刊4), 51-56;Delanty等. (1997) Neurology 49, 686-689;和Bunn (2002) N Engl J Med 346(7), 522-523)。

[0321] 如果本公开的拮抗剂通过ESAs不同的机制起作用，这些拮抗剂可用于在不很好应

答ESAs或其他EPO受体激活剂的患者增加红细胞和血红蛋白水平。例如，本公开的ActRII拮抗剂对其中给予正常至增加剂量的ESA (> 300 IU/kg/周)不导致血红蛋白水平增加至目标水平的患者可能是有益的。在所有类型的贫血中发现了对ESAs反应不充分的患者，但是已观察到更高数量的非应答者，在患有癌症的患者和患有终末期肾病的患者特别频繁。对ESAs反应不充分可以是组成型(用ESA第一次治疗时观察到)或获得(用ESA反复治疗时观察到)。

[0322] 来那度胺为一种被批准用于伴有del5q和输注依赖性贫血的较低风险MDS患者的沙利度胺衍生物。泊马度胺为另一种沙利度胺衍生物。来那度胺在美国批准是基于Ⅱ期试验的结果，其中在研究的约三分之二的患者中达到脱离输注，脱离输注的平均持续时间为2.2年[List等. (2006) N Engl J Med 355:1456-1465]。随后在欧洲也获得批准[Giagounidis等. (2014) Eur J Haematol 93:429-438]，来那度胺被视为伴有del5q和贫血的较低风险MDS患者的一线治疗。在某些实施方案中，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选与EPO受体激活剂和/或一种或多种另外的疗法组合，可与来那度胺组合用于治疗患有MDS的患者。

[0323] DNA甲基化异常是MDS预后不良的特征[Shen等. (2010) J Clin Oncol 28:605-613]。阿扎胞苷和地西他滨是两种DNA低甲基化活性的试剂，目前用于治疗主要患有高风险MDS的患者[Kantarjian等. (2007) Cancer 109:1133-1137;Fenaux等. (2009) Lancet Oncol 10:223-232]。因为其治疗作用的机制不确定，这些试剂有时根据其化学结构(氮杂核苷类)或已知的体外活性(DNA甲基转移酶抑制剂)进行分类。尽管阿扎胞苷和地西他滨在较低风险MDS治疗经验不足，但研究表明阿扎胞苷和地西他滨可在30%-40%的ESA抗性的较低风险MDS患者产生红细胞应答[Lyons等. (2009) J Clin Oncol 27:1850-1856]。在血小板减少症患者也观察到血小板应答。基于这些结果，阿扎胞苷和地西他滨在美国被批准用于治疗患有症状性血细胞减少的较低风险MDS。在某些实施方案中，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选地与EPO受体激活剂和/或一种或多种另外的疗法组合，可与阿扎胞苷、地西他滨或另一种DNA甲基转移酶抑制剂组合用于治疗患有MDS的患者。

[0324] 血小板减少症发生在约35%-45%的MDS患者，患者可能抱怨容易瘀伤或频繁的轻微皮肤粘膜出血，并可显示紫癜或瘀点[Steensma等. (2006) Mayo Clin Proc 81:104-130]。在更极端的情况下，可能会出现胃肠出血或颅内出血的风险增加。对血小板减少的患者，当血小板水平下降至低于10000血小板/μL时一般表明输注血小板[Slichter (2007) Hematology Am Soc Hematol Educ Program 2007:172-178]。由于长期输注依赖性MDS患者的致敏频率，支持使用血小板输注是暂时的，并且不总是有效的。有相当大的兴趣在MDS疗法中使用具有血小板生成活性的生长因子。罗米司亭和艾曲波帕为在美国被批准用于特发性血小板减少性紫癜的促血小板生成药物，并且罗米司亭在MDS患者被广泛研究[Kantarjian等. (2010) J Clin Oncol 28:437-444;Santini (2012) Semin Hematol 49:295-303]。当用作单一试剂(药物)时，罗米司亭能显著改善约50%患伴有血小板减少症的较低风险MDS患者的血小板计数。然而，在15%的患者可观察到短暂的骨髓未成熟细胞百分数增加，有时大于20%，与MDS的未成熟细胞上存在促血小板生成素受体相符。罗米司亭也能显著减少接受阿扎胞苷、地西他滨或来那度胺、沙利度胺或泊马度胺的MDS患者的血小板

减少症和/或血小板输注，并可成为这些治疗的重要佐剂[Kantarjian等. (2010) Blood 116:3163-3170]。艾曲波帕也正在被开发用于MDS。在某些实施方案中，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选地与EPO受体激活剂和/或一种或更多种另外的疗法组合，可与促血小板生成药物比如罗米司亭或艾曲波帕组合，用于治疗患有MDS或铁粒幼红细胞性贫血的患者。

[0325] 在某些实施方案中，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选地与EPO受体激活剂和/或一种或多种另外的疗法组合，可与铁调素、铁调素类似物或铁调素受体激活剂组合，用于治疗患有MDS或铁粒幼红细胞性贫血的患者，特别是用于与铁超负荷有关的并发症。循环多肽主要在肝脏产生，铁调素被认为是借助其诱导铁转运蛋白降解的能力进行铁代谢的主要调控因子，其为一种位于吸收性肠上皮细胞、肝细胞和巨噬细胞的铁输出蛋白。从广义上说，铁调素减少胞外铁的可用性，因此铁调素、铁调素类似物或铁调素受体激活剂可有利于治疗患有MDS或铁粒幼红细胞性贫血的患者，特别是用于与铁超负荷有关的并发症。

[0326] 用于MDS的试验性药物处于开发中。这些包括用于满足基于免疫抑制的疗法标准的患者的单一药物组蛋白去乙酰化酶抑制剂、p38MAPK抑制剂、谷胱甘肽S-转移酶π抑制剂和阿仑单抗[Garcia-Manero等. (2011) J Clin Oncol 29:516-523]。例如，已报道在用包括抗胸腺细胞球蛋白或阿仑单抗在内的免疫抑制疗法治疗的MDS患者呈现高应答率[Sloand等. (2010) J Clin Oncol 28:5166-5173]。然而，这种患者比MDS患者整体通常更年轻并具有更高的正常染色体组型频率，这限制这些结果的普适性。试验性疗法包括例如组蛋白去乙酰化酶抑制剂(HDAC抑制剂，包括伏立诺他、丙戊酸、丁酸苯酯、恩替诺他、MGCD0103和其他I类核HDAC抑制剂、II类非核类HDAC抑制剂、全面(pan) HDAC抑制剂和同种型特异性HDAC抑制剂)；法尼基转移酶抑制剂(包括替吡法尼和洛那法尼)、肿瘤坏死因子α(TNF-α)抑制剂(包括依那西普或英夫利昔单抗)、谷胱甘肽-S-转移酶(GST)抑制剂P1-1(包括ezatiostat)和CD33抑制剂(包括吉妥单抗)]。在某些实施方案中，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选地与EPO受体激活剂和/或一种或多种另外的疗法组合，可与一种或多种这些试验性MDS疗法组合使用。

[0327] 越来越多的证据表明，组合使用具有不同作用机制的MDS治疗剂对急性髓性白血病以副作用减少、改善总生存期和延缓病程进展的形式提供实质性的益处。多项研究指明，当与传统的单一疗法比较时，组合各种作用机制和毒性不重叠的药物可对MDS患者带来显著益处。与生长因子、DNA甲基转移酶抑制剂、组蛋白去乙酰化酶抑制剂和免疫抑制剂治疗的各种联合疗法提供令人鼓舞的数据[Ornstein等. (2012) Int J Hematol 95:26-33]。因此，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)，任选地与EPO受体激活剂组合，可用于增加另外用一种或两种试剂(药物)(非限制性地包括生长因子、DNA甲基转移酶抑制剂、组蛋白去乙酰化酶抑制剂或免疫抑制剂治疗)的组合治疗的MDS患者的红细胞数目。

[0328] 在某些实施方案中，本公开提供通过测量患者的一个或多个血液学参数，用于治疗已用一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)治疗或为所述治疗的候选者的患者的方法。血液学参数可用于评价对

于的患者(其为要用本公开的拮抗剂治疗的候选者)的适当给予、监测治疗期间的血液学参数、评价是否在用一种或多种本公开的拮抗剂治疗期间调节剂量和/或评价一种或多种本公开的拮抗剂的适当维持剂量。如果血液学参数中的一个或多个在正常水平以外,一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)的给予可减少、延迟或终止。

[0329] 可按照本文提供的方法测量的血液学参数包括例如红细胞水平、血压、铁储存及与红细胞水平增加相关的体液中发现的其他试剂(采用领域公认的方法)。这种参数可采用患者的血样确定。红细胞水平、血红蛋白水平和/或血细胞比容水平的增加可造成血压增加。

[0330] 在一个实施方案中,如果一个或多个血液学参数在要用一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)治疗的患者候选者处于正常范围之外或在正常范围高侧,那么可延迟开始给予一种或多种本公开的拮抗剂,直至血液学参数已自然或经治疗干预回复到正常或可接受的水平。例如如果候选患者是高血压或高血压前期,那么患者可用降压药治疗以便降低患者的血压。适用于个体患者病症的任何降压药都可使用,包括例如利尿剂、肾上腺素能抑制剂(包括α受体阻滞剂和β受体阻滞剂)、血管扩张剂、钙通道阻滞剂、血管紧张素转换酶(ACE)抑制剂或血管紧张素II受体阻滞剂。血压或者可采用饮食和锻炼方案进行治疗。类似地,如果候选患者具有低于正常或正常低侧的铁储存,那么患者可用适当的饮食和/或铁补充方案治疗,直至患者的铁储存回复到正常或可接受的水平。对于具有高于正常红细胞水平和/或血红蛋白水平的患者,则可延迟给予一种或多种本公开的拮抗剂,直至水平回复到正常或可接受的水平。

[0331] 在某些实施方案中,如果一个或多个血液学参数在要用一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)治疗的患者候选者处于正常范围之外或在正常范围高侧,那么不可延迟开始给予。然而,给予一种或多种本公开拮抗剂的剂量或次数可设置为一定量,这种量会降低给予一种或多种本公开的拮抗剂时出现的血液学参数不可接受的增加的风险。或者,治疗方案可针对合并有一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)和解决不合乎需要水平的血液学参数的治疗剂的患者开发。例如,如果患者具有升高的血压,则可设计包括给予一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)和降压药的治疗方案。对于具有低于期望的铁储存的患者,可开发包括一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)和铁补充剂的治疗方案。

[0332] 在一个实施方案中,可对要用一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)治疗的患者候选者建立一个或多个血液学参数的基线参数,并且基于基线值对该患者确立合适的给药方案。或者,基于患者的医疗史建立的基线参数可用于告知对患者合适的拮抗剂给药方案。例如,如果健康患者具有高于定义的正常范围的所建立的基线血压读数,那么在用一种或多种本公开的拮抗剂治疗前可能不必要使患者的血压处于对一般群体认为正常的范围。在用一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)治疗前,患者的一个或多个血液学参数的基线值也可用作相关比较值来监测在用一种或多种本

公开的拮抗剂治疗期间血液学参数的任何变化。

[0333] 在某些实施方案中，在正用一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)治疗的患者测量一个或多个血液学参数。这些血液学参数可用于在治疗期间监测患者并允许调整或终止给予一种或多种本公开的拮抗剂或另外给予另一种治疗剂。例如，如果给予一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)导致血压、红细胞水平或血红蛋白水平增加或者铁储存减少，那么一种或多种本公开拮抗剂的剂量可在量或次数方面减少，以降低一种或多种本公开的拮抗剂对一个或多个血液学参数的影响。如果给予一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)导致不利于患者的一个或多个血液学参数变化，那么给予一种或多种本公开的拮抗剂可临时中断直至血液学参数回复到可接受的水平，或者永久性地终止。类似地，如果一个或多个血液学参数在减少给予一种或多种本公开拮抗剂的剂量或次数后未回到可接受的范围内，那么可终止给予。作为减少或终止给予一种或多种本公开的拮抗剂的备选或附加，患者可给予解决血液学参数的不合乎需要水平的另外治疗剂，比如降压药或铁补充剂。例如，如果用一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)治疗的患者具有升高的血压，那么可在相同水平持续给予一种或多种本公开的拮抗剂并将降压药加入治疗方案，可减少给予一种或多种本公开的拮抗剂(例如量和/或次数)并将降压药加入治疗方案，或者可终止给予一种或多种本公开的拮抗剂并且患者可用降压药治疗。

[0334] 6．药用组合物

在某些方面中，一种或多种本公开的ActRII拮抗剂(例如GDF-ActRII拮抗剂、ActRIIA多肽、ActRIIB多肽、GDF捕获物等)可单独或作为药用制剂(也称为治疗组合物或药用组合物)的组分给予。药用制剂指的是容许其中含有的活性成分(例如本公开的试剂)的生物活性是有效的，并且其不含有对给予制剂的受试者具有不可接受的毒性的另外组分的制剂。研究对象化合物可配制成以用于人或兽医任何便利的方式给予。例如，一种或多种本公开的试剂可用药学上可接受的载体配制。药学上可接受的载体指的是药用制剂中除活性成分以外，对受试者通常非毒性的成分。药学上可接受的载体非限制性地包括缓冲剂、赋形剂、稳定剂和/或防腐剂。通常，用于本公开的药用制剂在给予受试者时以无热原的、生理学上可接受的形式存在。除本文描述的那些以外的治疗有用的试剂(其可任选地包含在以上描述的制剂中)，可以本公开的方法与研究对象试剂组合给予。

[0335] 一般地，化合物非肠道给予[例如经静脉内(I.V.)注射、动脉内注射、骨内注射、肌肉注射、鞘内注射、皮下注射或皮内注射]。适合于非肠道给予的药用组合物可包含一种或多种本公开的试剂与一种或多种药学上可接受的无菌等渗水或非水溶液、分散液、混悬液或乳液，或可在临用前重构成无菌的可注射溶液或分散液的无菌粉末。可注射溶液剂或分散剂可含有抗氧化剂、缓冲剂、抑菌剂、悬浮剂、增稠剂或使制剂与打算的接受者的血液等渗的溶质。可在本公开的药用制剂中采用的合适的水和非水载体的实例包括水、乙醇、多元醇(例如甘油、丙二醇、聚乙二醇等)、植物油(例如橄榄油)、可注射的有机酯(例如油酸乙酯)及其合适的混合物。例如通过使用包衣材料(例如卵磷脂)，在分散液的情况下通过维持需要的粒度和通过使用表面活性剂，可维持适当的流动性。

[0336] 在一些实施方案中，本公开的治疗方法包括从植入物或装置，全身或局部给予药用组合物。另外，药用组合物可以传递至靶组织位点(例如骨髓或肌肉)的形式包封或注射。在某些实施方案中，本公开的组合物可包括能够将一种或多种本公开的试剂传递至靶组织位点(例如骨髓或肌肉)的基质，为发育中的组织提供结构且最好能够重吸收到体内。例如，基质可提供一种或多种本公开试剂的缓慢释放。这种基质可由目前其他植入医学申请使用的材料形成。

[0337] 基质材料的选择可基于以下中的一种或多种：生物相容性、生物可降解性、机械性质、外观和界面性质。研究对象组合物的具体应用将决定合适的制剂。用于组合物的可能基质可为生物可降解的和化学定义的硫酸钙、磷酸三钙、羟基磷灰石、聚乳酸和聚酸酐。其它可能的材料为生物可降解的且在生物学上充分定义的，包括例如骨或真皮胶原。其他基质包含纯蛋白或细胞外基质组分。其它可能的基质为不可生物降解且经化学定义的，包括例如烧结的羟基磷灰石、生物玻璃、铝酸盐或其他陶瓷。基质可包含任何一种以上提及类型的材料的组合，例如聚乳酸与羟基磷灰石或者胶原蛋白与磷酸三钙的组合。生物陶瓷可在组成上(如钙-铝酸盐-磷酸盐)被改变并进行加工，以改变孔径、粒度、粒子形状和生物可降解性中的一种或多种。

[0338] 在某些实施方案中，本公开的药用组合物可例如以胶囊剂、扁囊剂、丸剂、片剂、糖锭剂(使用矫味基质例如蔗糖和阿拉伯树胶或黄蓍胶)、粉剂、颗粒剂、在水或非水液体中的溶液剂或混悬剂、水包油或油包水液体乳剂、或者酏剂或糖浆剂、或者锭剂(采用惰性基质，比如明胶和甘油，或者蔗糖和阿拉伯树胶)和/或漱口剂的形式口服给予，每种形式含预定量的本公开化合物和任选地一种或多种其他活性成分。本公开的化合物和任选地一种或多种其他活性成分也可作为大丸剂、药糖剂(electuary)或糊剂给予。

[0339] 在用于口服给予的固体剂型(例如胶囊剂、片剂、丸剂、糖衣剂、粉剂和颗粒剂)中，一种或多种本公开的化合物可与一种或多种药学上可接受的载体混合，包括例如枸橼酸钠、磷酸二钙、填充剂或增量剂(extender)(例如淀粉、乳糖、蔗糖、葡萄糖、甘露醇和硅酸)、粘合剂(例如羟甲基纤维素、藻酸盐、明胶、聚乙烯吡咯烷酮、蔗糖和阿拉伯树胶)、湿润剂(例如甘油)、崩解剂(例如琼脂、碳酸钙、马铃薯或木薯淀粉、海藻酸、硅酸盐和碳酸钠)、溶液阻滞剂(例如石蜡)、吸收促进剂(例如季铵化合物)、湿润剂(例如鲸蜡醇和单硬脂酸甘油酯)、吸附剂(例如高岭土和膨润土)、润滑剂(例如滑石、硬脂酸钙、硬脂酸镁、固体聚乙二醇、十二烷基硫酸钠)、着色剂及其混合物。在胶囊剂、片剂和丸剂的情况下，药用制剂(组合物)也可包含缓冲剂。相似类型的固体组合物也可在软和硬填充明胶胶囊中用作填充剂，所述胶囊使用一种或多种赋形剂，包括例如乳糖或奶糖以及高分子量聚乙二醇。

[0340] 用于口服给予的药用组合物的液体剂型可包括药学上可接受的乳剂、微乳剂、溶液剂、混悬剂、糖浆剂和酏剂。除活性成分外，液体剂型可含有本领域通常使用的惰性稀释剂(包括例如水或其它溶剂)、增溶剂和/或乳化剂[例如乙醇、异丙醇、碳酸乙酯、乙酸乙酯、苯甲醇、苯甲酸苄酯、丙二醇或1,3-丁二醇、油(例如棉籽油、花生油、玉米油、胚芽油、橄榄油、蓖麻油和芝麻油)、甘油、四氢呋喃甲醇、聚乙二醇、失水山梨糖醇的脂肪酸酯及其混合物]。除惰性稀释剂以外，口服制剂也可包含佐剂，包括例如润湿剂、乳化剂和悬浮剂、甜味剂、矫味剂、着色剂、香料、防腐剂及其组合。

[0341] 除活性化合物，悬浮液可含有悬浮剂，包括例如乙氧基化异硬脂醇、聚氧乙烯山梨

糖醇、脱水山梨糖醇酯、微晶纤维素、偏氢氧化铝、膨润土、琼脂、黄蓍胶及其组合。

[0342] 预防微生物的作用和/或生长可通过包含各种抗菌和抗真菌试剂(包括例如尼泊金、氯丁醇和山梨酸苯酚)确保。

[0343] 在某些实施方案中,可能合乎需要的是在组合物中包含等渗剂(包括例如糖或氯化钠)。另外,可注射药用形式的延长吸收可通过包含延迟吸收的试剂(包括例如单硬脂酸铝和明胶)造成。

[0344] 应该理解,给药方案将由主治医生考虑到改变一种或多种本公开试剂作用的各种因素来确定。所述各种因素非限制性地包括:患者的红细胞计数、血红蛋白水平、期望的目标红细胞计数、患者年龄、患者性别、患者饮食、可能助于降低红细胞水平的任何一种疾病的严重程度、给予时间及其他临床因素。向最后的组合物加入其他已知的活性剂也可能影响剂量。通过定期评估红细胞水平、血红蛋白水平、网织红细胞水平及其他造血过程指标中的一个或多个可监测进展情况。

[0345] 在某些实施例中,本公开还提供用于体内产生一种或多种本公开试剂的基因疗法。这种疗法通过将试剂序列引入到具有以上列出的一种或多种障碍的细胞或组织来实现其治疗效果。例如,通过使用重组表达载体比如嵌合病毒或胶态分散系统,可实现试剂序列的传递。一种或多种本公开试剂序列的优选治疗传递是使用靶向脂质体。

[0346] 可用于本文讲授的基因疗法的各种病毒载体包括腺病毒、疱疹病毒、牛痘或RNA病毒(例如逆转录病毒)。逆转录病毒载体可能是鼠或禽逆转录病毒的衍生物。其中可插入单一外源基因的逆转录病毒载体的实例非限制性地包括:莫洛尼(氏)鼠白血病病毒(MoMuLV)、哈维鼠肉瘤病毒(HaMuSV)、鼠类乳腺肿瘤病毒(MuMTV)和劳斯氏肉瘤病毒(RSV)。许多另外的逆转录病毒载体可包含多个基因。所有这些载体都可转移或整合一个可选择标记的基因,以至于可识别和产生转导的细胞。逆转录病毒载体可通过附着例如糖、糖脂或蛋白靶向特异性地制备。优选的靶向通过使用抗体完成。本领域的技术人员将认识到,特定的多核苷酸序列可插入到逆转录病毒基因组中或附着于病毒包膜上,以使得含有一种或多种本公开试剂的逆转录病毒载体能够靶标特异性地传递。

[0347] 或者,通过常规的磷酸钙转染,组织培养细胞可直接用编码逆转录病毒结构基因(gag、pol和env)的质粒转染。然后用含有感兴趣基因的载体质粒转染这些细胞。所生成的细胞将逆转录病毒载体释放到培养基中。

[0348] 一种或多种本公开试剂的另一种靶向传递系统为胶态分散系统。胶体分散系统包括例如大分子复合物、纳米胶囊、微球、珠和基于脂质的系统,包括水包油乳液、胶束、混合胶束和脂质体。在某些实施例中,本公开优选的胶体系统是脂质体。脂质体为体外和体内都可用作传递载体的人工膜囊。RNA、DNA和完整的病毒粒子可被包封在水内,并以生物活性形式传递至细胞[参见例如Fraley等. (1981) Trends Biochem. Sci., 6:77]。采用脂质体载体进行有效基因转移的方法为本领域已知的[参见例如Mannino等. (1988) Biotechniques, 6:682, 1988]。

[0349] 脂质体的组成通常为磷脂的组合,其可包括类固醇(例如胆固醇)。脂质体的物理特性取决于pH、离子强度和二价阳离子的存在。其他磷脂或其他脂质也可使用,包括例如磷脂酰化合物(例如磷脂酰甘油、磷脂酰胆碱,磷脂酰丝氨酸、磷脂酰乙醇胺、鞘脂类、脑苷脂或神经节苷脂)、蛋磷脂酰胆碱、二棕榈酰磷脂酰胆碱和二硬脂酰磷脂酰胆碱。脂质体的靶

向性也可能基于例如器官特异性、细胞特异性和细胞器特异性，并且为本领域已知的。

实施例

[0350] 本发明现在被一般性描述，其通过参照以下实施例将更易于理解，包括这些实施例仅为了说明本发明的某些实施方案和实施方案的目的，并且不打算限制本发明。

[0351] 实施例1: ActRIIa-Fc融合蛋白

申请者构建了 可溶性ActRIIA融合蛋白，其具有融合于人或小鼠Fc结构域的人ActRIIa细胞外结构域(之间具有最小连接子)。构建体 分别称为ActRIIA-hFc和ActRIIA-mFc。

[0352] 以下显示ActRIIA-hFc，为从CHO细胞系纯化(SEQ ID NO: 22)：

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

ActRIIA-hFc和ActRIIA-mFc蛋白在CHO细胞系表达。考虑3种不同的前导序列：

(i) 蜜蜂蜂毒肽(HBML)：MKFLVNVALVFMVVYISYIYA (SEQ ID NO: 23)

(ii) 组织纤溶酶原激活剂(TPA)：MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 24)

(iii) 天然的：MGAAAKLAFAVFLISCSSGA (SEQ ID NO: 25)。

[0353] 所选择的形式采用TPA前导序列并具有以下未加工的氨基酸序列：

MDAMKRGLCCVLLLCGAVFVSPGAAILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMEVTQPTSNPVTPKPPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 26)

这种多肽由以下核酸序列(SEQ ID NO: 12)编码：

ATGGATGCAATGAAGAGAGGGCTCTGCTGTGTGCTGCTGCTGTGTGGAGCAGTCTTCGTTTCGCCCGGCGCCGCTATACTTGGTAGATCAGAAACTCAGGAGTGTCTTTTTTTAATGCTAATTGGGAAAAAGACAGAACCAATCAAACTGGTGTTGAACCGTGTTATGGTGACAAAGATAAACGGCGGCATTGTTTTGCTACCTGGAAGAATATTTCTGGTTCCATTGAATAGTGAAACAAGGTTGTTGGCTGGATGATATCAACTGCTATGACAGGACTGATTGTGTAGAAAAAAAAGACAGCCCTGAAGTATATTTCTGTTGCTGTGAGGGCAATATGTGTAATGAAAAGTTTTCTTATTTTCCGGAGATGGAAGTCACACAGCCCACTTCAAATCCAGTTACACCTAAGCCACCCACCGGTGGTGGAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGTCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC

AGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTATAGCAAGCTCACC GTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACAC GCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGAATTC (SEQ ID NO:27)

ActRIIA-hFc和ActRIIA-mFc两者非常适合重组表达。如在图3中显示的那样,该蛋白被纯化为单一的、峰态轮廓分明的蛋白。N-末端测序揭示单一序列的-ILGRSETQE (SEQ ID NO: 34)。纯化可通过一系列柱层析步骤实现,包括例如三个或多个以下步骤(以任何顺序):蛋白A色谱、Q 琼脂糖色谱、苯基琼脂糖凝胶色谱法,尺寸排阻色谱法和阳离子交换色谱法。纯化可用病毒过滤和缓冲交换完成。ActRIIA-hFc蛋白被纯化为如经尺寸排阻色谱测定的那样纯度>98%,和如经SDS PAGE测定的那样纯度>95%。

[0354] ActRIIA-hFc和ActRIIA-mFc对配体,特别是激活素A显示高亲和力。在Biacore™ CM5芯片上使用标准胺偶联法固定化GDF-11或激活素A。将ActRIIA-hFc和ActRIIA-mFc蛋白加载到系统上,并测量结合。ActRIIA-hFc结合于激活素的解离常数(K_D)为5 x 10^{-12},结合于GDF11的解离常数K_D为9.96 x 10^{-9}。参见图4。ActRIIA-mFc行为类似。

[0355] ActRIIA-hFc在药代动力学研究中非常稳定。给予大鼠1 mg/kg、3 mg/kg或10 mg/kg的ActRIIA-hFc蛋白,并在24、48、72、144和168小时测量蛋白的血浆水平。在一项分开的研究中,给予大鼠1 mg/kg、10 mg/kg或30 mg/kg。在大鼠,ActRIIA-hFc具有11-14天的血清半衰期,并且药物的循环水平在两周后相当高(对初始分别给予1 mg/kg、10 mg/kg或30 mg/kg为11 μg/ml、110 μg/ml或304 μg/ml)。在食蟹猴,血浆半衰期显著大约14天,并且药物的循环水平对初始分别给予1 mg/kg、10 mg/kg或30 mg/kg为25 μg/ml、304 μg/ml或1440 μg/ml。

[0356] 实施例2:ActRIIA-hFc蛋白的表征

使用SEQ ID NO: 9的组织纤溶酶原前导序列,ActRIIA-hFc融合蛋白在稳定转染的CHO-DUKX B11细胞自pAID4载体(SV40 ori/增强子,CMV启动子)表达。如以上在实施例1中描述的那样纯化,蛋白具有SEQ ID NO: 22的序列。Fc部分为人IgG1 Fc序列,如在SEQ ID NO: 22显示的那样。蛋白分析揭示ActRIIA-hFc融合蛋白作为含二硫键的同源二聚体形成。

[0357] CHO细胞表达的材料对激活素B配体比已报道在人293细胞表达的ActRIIA-hFc融合蛋白具有更高亲和力[参见del Re等. (2004) J Biol Chem. 279(51): 53126-53135]。另外,使用TPA前导序列比其他前导序列提供更高的产生,并且不像用天然前导序列表达的ActRIIA-Fc那样,提供高纯度的N-末端序列。使用天然前导序列导致两个主要种类的ActRIIA-Fc,每个具有不同的N-末端序列。

[0358] 实施例3:ActRIIA-hFc增加非人灵长类动物的红细胞水平

研究使用4组食蟹猴,每组5只雄性和5只雌性,每组每一性别3只计划在第29天终止研究,并且每组每一性别2只计划在第57天终止研究。每只动物在1、8、15和22天经静脉内(IV)注射以1、10或30 mg/kg的剂量给予媒介物(组1)或ActRIIA-Fc (分别为组2、3和4)。剂量体积维持在3 mL/kg。在第一次给予前2天和第一次给予后的第15、29和57天(剩下2只动物)评价红细胞水平的各种测量。

[0359] 在整个研究的所有剂量水平下和时间点上,ActRIIA-hFc引起雄性和雌性动物的平均红细胞参数[红细胞计数(RBC)、血红蛋白(HGB)和血细胞比容(HCT)]的统计学上的显著增加,伴随绝对和相对网织红细胞计数(ARTC;RTC)升高。参见图5-8。

[0360] 在基线时，相对于治疗组的平均值计算每个治疗组的统计显著性。

[0361] 值得注意的是，红细胞计数和血红蛋白水平的增加幅度大致相当于促红细胞生成素报告的效果。这些影响的发生，ActRIIA-Fc比促红细胞生成素更快速。

[0362] 对大鼠和小鼠观察到类似的结果。

[0363] 实施例4：ActRIIA-hFc增加人患者的红细胞水平和骨形成标记物

在一项随机，双盲，安慰剂对照的研究中，把在实施例1中描述的ActRIIA-hFc融合蛋白给予人受试者，研究主要是评价这种蛋白在健康的绝经后妇女的安全性。48名受试者被随机分配在6组(cohort)中接受单剂量的ActRIIA-hFc或安慰剂(5个为活性物:1个为安慰剂)。剂量水平在0.01-3.0 mg/kg静脉内(IV)注射和0.03-0.1 mg/kg皮下(SC)注射范围内。所有受试者随访120天。除了药代动力学(PK)分析以外，ActRIIA-hFc的生物活性也通过测量骨形成的生化标志物和重吸收以及FSH水平进行评价。

[0364] 为了寻找潜在的变化，在研究过程中对所有研究对象详细检查血红蛋白和RBC数目，并与基线水平进行比较。血小板计数在相同时间与对照组比较。血小板计数自基线值随着时间的推移没有明显的临床变化。

[0365] ActRIIA-hFc的药代动力学(PK)分析揭示随着剂量的线性分布，和平均半衰期约为25-32天。ActRIIA-hFc的曲线下面积(AUC)与剂量呈线性相关，并且SC给予后的吸收基本上完全。参见图9和10。这些数据表明，SC为合乎需要的给予方法，因为其提供了药物的等效生物利用度和血清半衰期，同时避免了与iv给予的最初几天相关的药物血清浓度的急剧上升(参见图10)。ActRIIA-hFc造成骨特异性碱性磷酸酶(BAP)的血清水平快速、持续的剂量依赖性增加，其为合成骨生长的标志，并且引起C-末端1型胶原蛋白端肽和抗酒石酸酸性磷酸酶5b水平呈剂量依赖性下降，它们是骨吸收标志物。其他标记比如P1NP显示不确定的结果。在药物的最高剂量下，BAP水平显示出接近饱和的效果，表明在0.3 mg/kg的剂量下可实现对这种骨合成代谢的生物标记物的半最大效应，剂量范围最多可达3 mg/kg。计算药物的药效学作用与AUC的关系，EC50为51465 (天* ng/ml) (参见图11)。这些骨生物标志物的变化在所测试的最高剂量水平下持续了大约120天。血清FSH水平也呈剂量依赖性下降，与激活素的抑制作用一致。

[0366] 总的来说，无论IV还是SC给予，在0.01和0.03 mg/kg组，可能与研究放血有关的第一周研究中存在非常小的非药物相关的血红蛋白减少。到第8-15天，0.1 mg/kg SC和IV血红蛋白结果是稳定的或显示呈适度增长。在0.3 mg/kg IV剂量水平下，早在第2天观察到HGB水平明显增加，并且通常在第15-29天达到峰值，而这在安慰剂治疗的受试者未观察到。在1.0 mg/kg IV剂量和3.0 mg/kg IV剂量下，观察到响应于单次给予的血红蛋白平均增加大于1 g/dl，RBC计数和血细胞比容相应增加。这些血液学参数在给予后约60天达到峰值，到第120天显著下降。这表明如果以间隔少于120天(即在返回基线前)进行，给予增加红细胞水平可能更有效，90天或更少或者60天或更少的给予间隔可能是可取的。对于血液学变化的概述参见图12-15。

[0367] 总的来说，ActRIIA-hFc对红细胞计数和网织红细胞计数显示剂量依赖效应。

[0368] 实施例5：用ActRIIA-hFc治疗贫血患者

一项临床研究被设计成以0.1 mg/kg、0.3 mg/kg和1.0 mg/kg 3个剂量水平，用多剂量给予ActRIIA-hFc治疗患者，给予每30天发生一次。正常的健康受试者在试验中呈现血红蛋

白和血细胞比容增加,这与实施例4中报道的I期临床试验中观察到的增加相一致,除了在一些情况下血红蛋白(Hg)和血细胞比容(Hct)升高超出正常范围。血红蛋白水平约为7.5 g/dL的贫血患者也以1 mg/kg水平接受两次给予,导致两个月后血红蛋白水平约为10.5 g/dL。患者的贫血是小红细胞的贫血,认为是慢性缺铁造成的。

[0369] ActRIIA-Fc融合蛋白已进一步证实在各种贫血模型(包括例如化学治疗诱导的贫血和与慢性肾病有关的贫血)增加红细胞水平是有效性(参见例如美国专利申请出版物第2010/0028331号)。

[0370] 实施例6:备选的ActRIIA-Fc蛋白

可按本文描述的方法使用的各种ActRIIA变体在作为WO2006/012627 (参见例如第55-58页)发表的国际专利申请中得到描述,其通过参照以其全部结合到本文中。备选的构建体可能有C-末端尾部缺失(ActRIIA细胞外结构域的最后15个氨基酸)。以下呈现这种构建体的序列(Fc部分下划线) (SEQ ID NO: 28):

ILGRSETQECLFFNANWEKDRTNQTGVEPCYGDKDKRRHCFATWKNISGSIEIVKQGCWLDDINCYDRTDCVEKKDSPEVYFCCCEGNMCNEKFSYFPEMTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

实施例7:ActRIIB-Fc融合蛋白的产生

申请者构建了 可溶性ActRIIB融合蛋白,其具有融合于人或小鼠Fc结构域的人ActRIIB的细胞外结构域(之间具有最小连接子(三个甘氨酸))。构建体 分别称为ActRIIB-hFc和ActRIIB-mFc。

[0371] 以下显示ActRIIB-hFc,为从CHO细胞系纯化(SEQ ID NO: 29):

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

ActRIIB-hFc和ActRIIB-mFc蛋白在CHO细胞系表达。考虑3种不同的前导序列:

(i) 蜜蜂蜂毒肽(HBML):MKFLVNVALVFMVVYISYIYA (SEQ ID NO: 23)

(ii) 组织纤溶酶原激活剂(TPA):MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 24)

(iii) 天然的:MGAAAKLAFAVFLISCSSGA (SEQ ID NO: 30)。

[0372] 所选择的形式利用TPA前导序列并具有以下未加工氨基酸序列(SEQ ID NO: 31):

MDAMKRGLCCVLLLCGAVFVSPGASGRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

这种多肽由以下核酸序列(SEQ ID NO: 32)编码:

A TGGATGCAAT GAAGAGAGGG CTCTGCTGTG TGCTGCTGCT

GTGTGGAGCA GTCTTCGTTT CGCCCGGCGC CTCTGGGCGT

GGGGAGGCTG AGACACGGGA GTGCATCTAC TACAACGCCA ACTGGGAGCT GGAGCGCACC AACCAGAGCG GCCTGGAGCG CTGCGAAGGC GAGCAGGACA AGCGGCTGCA CTGCTACGCC TCCTGGCGCA ACAGCTCTGG CACCATCGAG CTCGTGAAGA AGGGCTGCTG GCTAGATGAC TTCAACTGCT ACGATAGGCA GGAGTGTGTG GCCACTGAGG AGAACCCCCA GGTGTACTTC TGCTGCTGTG AAGGCAACTT CTGCAACGAG CGCTTCACTC ATTTGCCAGA GGCTGGGGGC CCGGAAGTCA CGTACGAGCC ACCCCCGACA GCCCCCACCG GTGGTGGAAC TCACACATGC CCACCGTGCC CAGCACCTGA ACTCCTGGGG GGACCGTCAG TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCCAG TCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAG GAGATGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGCTGGACT CCGACGGCTC CTTCTTCCTC TATAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAATGA

CHO细胞产生的材料的N-末端测序揭示了-GRGEAE (SEQ ID NO: 33)的主要序列。值得注意的是,文献中报道的其他构建体 从-SGR…序列开始。

[0373] 纯化可通过一系列柱层析步骤实现,包括例如三个或多个以下步骤(以任何顺序):蛋白A色谱、Q 琼脂糖色谱、苯基琼脂糖凝胶色谱法,尺寸排阻色谱法和阳离子交换色谱法。纯化可用病毒过滤和缓冲交换完成。

[0374] ActRIIB-Fc融合蛋白也在HEK293细胞和COS细胞表达。尽管来自所有细胞系的材料和合理的培养条件提供具有体内肌肉生长活性的蛋白,或许可观察到与细胞系选择和/或培养条件相关的效力变化。

[0375] 申请者在ActRIIB的细胞外结构域产生了一系列突变,并且产生的这些突变蛋白为细胞外ActRIIB和Fc结构域之间的可溶性融合蛋白。背景ActRIIB-Fc融合物具有SEQ ID NO: 29的序列。

[0376] 把包括N-和C-末端截短的各种突变引入到背景ActRIIB-Fc蛋白。基于在实施例1中给出的数据,预计这些构建体 如果用TPA前导表达将缺乏N-末端丝氨酸。通过PCR诱变在ActRIIB细胞外结构域产生突变。PCR之后,通过Qiagen柱纯化片段,用SfoI和AgeI消化和凝胶纯化。这些片段连接到表达载体pAID4 (参见WO2006/012627),以至于在连接时建立与人IgG1的融合合体。在转化到大肠杆菌DH5α时,收集菌落并分离DNAs。对小鼠构建体 (mFc),小鼠IgG2a取代人IgG1。所有突变体的序列被验证。

[0377] 所有的突变体在HEK293T细胞通过瞬时转染产生。总之,在500 ml旋转器上,HEK293T细胞设定在Freestyle (Invitrogen)培养基中6 x 10^5细胞/ml (体积250 ml)并生长过夜。第二天,以0.5 ug/ml最终DNA浓度用DNA:PEI (1:1)复合物处理这些细胞。4小时后,加入250 ml培养基,并使细胞生长7天。通过使细胞旋转减慢收获条件培养基并浓缩。

[0378] 使用各种技术纯化突变体,包括例如蛋白A柱,并用低pH (3.0)甘氨酸缓冲液洗脱。中和后,把这些对PBS透析。

[0379] 通过类似方法也在CHO细胞产生突变体。通过本文参照结合的WO 2008/097541和WO 2006/012627中描述的结合试验和/或生物试验，测试突变体。在一些情况下，用条件培养基而不是纯化蛋白进行试验。ActRIIB的另外变体在美国专利第7842663号中得到描述。

[0380] 实施例8:ActRIIB-Fc刺激非人灵长类动物的红细胞生成

食蟹猴分为7组(6只/性别/组)并以0.6、3或15 mg/kg的剂量每2周或每4周皮下注射给予ActRIIB (20-134)-hFc，连续9个月的时间。对照组(6只/性别/组)以与ActRIIB (20-134)-hFc处理的动物相同的剂量体积(0.5 ml/kg/剂量)接受媒介物。监测动物的常规临床病理学参数(例如血液学、临床化学、凝血和尿液分析)的变化。血液学、凝血和临床化学参数(包括铁参数、脂肪酶和淀粉酶)在给予开始之前和第59、143、199、227天和第267天(每4周给予的组)或281天(每2周给予的组)评价2次。最后一次给予后2周发生第267/281天的评价。

[0381] 给予ActRIIB (20-134)-hFc导致雄性和雌性猴的血液学参数呈非不良的、剂量相关的变化。这些变化包括红细胞计数、网织红细胞计数和红细胞分布宽度增加及平均红细胞容积、平均红细胞血红蛋白和血小板计数降低。在雄性体内，RBC计数在所有剂量水平下增加，并且增加的幅度通常与是否每2周或每4周给予ActRIIB (20-134)-hFcC类似。平均RBC计数在第59和267/281天之间的所有时间点均增加(除第281天第2组的雄性RBC计数未增加[0.6 mg/kg每2周])。在雌性体内，RBC计数每2周增加≥ 3 mg/kg，并且在第143和281天之间发生变化，在第59和267天之间平均RBC计数每4周增加15 mg/kg。

[0382] 这些作用与ActRIIB (20-134)-hFc对刺激红细胞生成的正面作用一致。

[0383] 实施例9:GDF捕获物的产生

申请者如下构建了 GDF捕获物。将具有修饰的ActRIIB细胞外结构域(具有L79D替换的SEQ ID NO:1的氨基酸20-134) (相对于GDF11和/或肌肉生长抑制素激活素A结合大大降低(由于SEQ ID NO:1中79位的亮氨酸-至-天冬氨酸替换的结果))的多肽与人或小鼠Fc结构域融合(之间具有最小连接子(三个甘氨酸))。该构建体 分别称为ActRIIB (L79D 20-134)-hFc和ActRIIB (L79D 20-134)-mFc。类似地实施在79位带有谷氨酸而不是天冬氨酸的备选形式(L79E)。也产生相对于以下SEQ ID NO: 36的226位带有丙氨酸而不是缬氨酸的备选形式并同样实施所有方面的测试。79位(相对于SEQ ID NO: 1，或相对于SEQ ID NO: 36的60位)的天冬氨酸以下以双下划线指明。相对于SEQ ID NO: 36的226位的缬氨酸以下也以双下划线指明。

[0384] 以下显示GDF捕获物ActRIIB (L79D 20-134)-hFc，为从CHO细胞系纯化(SEQ ID NO: 36)。

[0385] GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPVPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

GDF捕获物的ActRIIB衍生部分具有以下阐述的氨基酸序列(SEQ ID NO: 37)，并且该部分可用作单体，或用作作为单体、二聚体或更高级复合体的非Fc融合蛋白。

[0386]

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPT (SEQ ID NO: 37)

GDF捕获物蛋白在CHO细胞系表达。考虑3种不同的前导序列：

(i) 蜜蜂蜂毒肽(HBML)：MKFLVNVALVFMVVYISYIYA (SEQ ID NO: 23)

(ii) 组织纤溶酶原激活剂(TPA)：MDAMKRGLCCVLLLCGAVFVSP (SEQ ID NO: 24)

(iii) 天然的：MTAPWVALALLWGSLCAGS (SEQ ID NO: 30)。

[0387] 所选择的形式采用TPA前导序列并具有以下未加工的氨基酸序列：

MDAMKRGLCCVLLLCGAVFVSPGASGRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVKKGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEVTYEPPPTAPTGGGTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 38)

该多肽由以下核酸序列(SEQ ID NO: 39)编码：

A TGGATGCAAT GAAGAGAGGG CTCTGCTGTG TGCTGCTGCT GTGTGGAGCA GTCTTCGTTT CGCCCGGCGC CTCTGGGCGT GGGGAGGCTG AGACACGGGA GTGCATCTAC TACAACGCCA ACTGGGAGCT GGAGCGCACC AACCAGAGCG GCCTGGAGCG CTGCGAAGGC GAGCAGGACA AGCGGCTGCA CTGCTACGCC TCCTGGCGCA ACAGCTCTGG CACCATCGAG CTCGTGAAGA AGGGCTGCTG GGACGATGAC TTCAACTGCT ACGATAGGCA GGAGTGTGTG GCCACTGAGG AGAACCCCCA GGTGTACTTC TGCTGCTGTG AAGGCAACTT CTGCAACGAG CGCTTCACTC ATTTGCCAGA GGCTGGGGGC CCGGAAGTCA CGTACGAGCC ACCCCCGACA GCCCCCACCG GTGGTGGAAC TCACACATGC CCACCGTGCC CAGCACCTGA ACTCCTGGGG GGACCGTCAG TCTTCCTCTT CCCCCCAAAA CCCAAGGACA CCCTCATGAT CTCCCGGACC CCTGAGGTCA CATGCGTGGT GGTGGACGTG AGCCACGAAG ACCCTGAGGT CAAGTTCAAC TGGTACGTGG ACGGCGTGGA GGTGCATAAT GCCAAGACAA AGCCGCGGGA GGAGCAGTAC AACAGCACGT ACCGTGTGGT CAGCGTCCTC ACCGTCCTGC ACCAGGACTG GCTGAATGGC AAGGAGTACA AGTGCAAGGT CTCCAACAAA GCCCTCCCAG TCCCCATCGA GAAAACCATC TCCAAAGCCA AAGGGCAGCC CCGAGAACCA CAGGTGTACA CCCTGCCCCC ATCCCGGGAG GAGATGACCA AGAACCAGGT CAGCCTGACC TGCCTGGTCA AAGGCTTCTA TCCCAGCGAC ATCGCCGTGG AGTGGGAGAG CAATGGGCAG CCGGAGAACA ACTACAAGAC CACGCCTCCC GTGCTGGACT CCGACGGCTC CTTCTTCCTC TATAGCAAGC TCACCGTGGA CAAGAGCAGG TGGCAGCAGG GGAACGTCTT CTCATGCTCC GTGATGCATG AGGCTCTGCA CAACCACTAC ACGCAGAAGA GCCTCTCCCT GTCTCCGGGT AAATGA

纯化可通过一系列柱层析步骤实现，包括例如三个或多个以下步骤(以任何顺序)：蛋白A色谱、Q 琼脂糖色谱、苯基琼脂糖凝胶色谱法、尺寸排阻色谱法和阳离子交换色谱法。纯化可用病毒过滤和缓冲交换完成。在纯化方案的一个实例中，使细胞培养基通过蛋白A柱，以150 mM Tris/NaCl (pH 8.0)洗涤，然后以50 mM Tris/NaCl (pH 8.0)洗涤并用0.1 M甘氨酸(pH 3.0)洗脱。将低pH洗脱物在室温下保持30分钟，作为病毒清除步骤。然后中和洗脱物并使得通过Q琼脂糖离子交换柱，且以50 mM Tris pH 8.0、50 m1 NaCl洗涤，并以50 mM Tris pH 8.0 (含有150 mM-300 mM浓度的NaCl)洗脱。然后将洗脱物变更为50 mM Tris pH 8.0、1.1 M硫酸铵并通过苯基琼脂糖柱、洗涤并以50 mM Tris pH 8. 0 (含有150 mM-300

mM的硫酸铵)洗脱。将洗脱物透析并过滤使用。

[0388] 另外的GDF捕获物(修饰的ActRIIB-Fc融合蛋白,以至于降低激活素A结合相对于肌肉生长抑制素或GDF11结合的比率)描述于WO 2008/097541和WO 2006/012627中,本文通过参照结合。

[0389] 实施例10:对GDF-11-和激活素-介导的信号传递的生物试验

A-204报告基因试验用于评价ActRIIB-Fc蛋白和GDF捕获物对经GDF-11和激活素A的信号传递的作用。细胞系:人横纹肌肉瘤(衍生自肌肉)。报告载体:pGL3 (CAGA) 12 (描述在Dennler等,1998, EMBO 17: 3091-3100中)。CAGA12基序存在于TGF-β应答基因(例如PAI-1基因),因此这种载体通常用于通过SMAD2和3的因子信号传递。

[0390] 第1天:将A-204细胞分到48孔板中。

[0391] 第2天:将A-204细胞用10 ug pGL3 (CAGA) 12或pGL3 (CAGA) 12 (10 ug) + pRLCMV (1 μg)和Fugene转染。

[0392] 第3天:加入因子(稀释到培养基 + 0.1% BSA中)。抑制剂需要在加入到细胞之前与因子预温育1小时。6小时后,细胞用PBS漂洗并溶解细胞。

[0393] 随后进行萤光素酶试验。在不存在任何抑制剂的情况下,激活素A显示报告基因表达的10倍刺激和ED50 ~ 2 ng/ml。GDF-11:16倍刺激,ED50:~ 1.5 ng/ml。

[0394] ActRIIB (20-134)为该试验中激活素A、GDF-8和GDF-11活性的有效抑制剂。如以下描述的那样,ActRIIB变体也在该试验中测试。

[0395] 实施例11:ActRIIB-Fc变体,基于细胞的活性

用以上描述的基于细胞的试验测试ActRIIB-Fc蛋白和GDF捕获物的活性。结果概述在下表中。一些变体在不同的C-末端截短构建体 方面进行测试。如以上讨论的那样,截短5或15个氨基酸引起活性减少。GDF捕获物(L79D和L79E变体)显示激活素A基本上丧失抑制作用而几乎保留GDF-11的野生型抑制作用。

[0396] 结合于GDF11和激活素A的可溶性ActRIIB-Fc:

ActRIIB-Fc 变异	ActRIIB 部分(相应于 SEQ ID NO:1 的氨基酸)	GDF11 抑制活性	激活素抑制活性
R64	20-134	+++ (约 10^{-8} M K_I)	+++ (约 10^{-8} M K_I)
A64	20-134	+ (约 10^{-6} M K_I)	+ (约 10^{-6} M K_I)
R64	20-129	+++	+++
R64 K74A	20-134	++++	++++
R64 A24N	20-134	+++	+++
R64 A24N	20-119	++	++
R64 A24N K74A	20-119	+	+
R64 L79P	20-134	+	+
R64 L79P K74A	20-134	+	+
R64 L79D	20-134	+++	+
R64 L79E	20-134	+++	+
R64K	20-134	+++	+++
R64K	20-129	+++	+++
R64 P129S P130A	20-134	+++	+++
R64N	20-134	+	+

。

+ 活性差(大致 1 x 10^{-6} K_I)

++ 活性中等(大致 1 x 10^{-7} K_I)

+++ (野生型)活性良好(大致 1 x 10^{-8} K_I)

++++ 高于野生型活性

[0397] 已评价了几种变体在大鼠的血清半衰期。ActRIIB (20-134)-Fc具有约70小时的血清半衰期。ActRIIB (A24N 20-134)-Fc具有约100-150小时的血清半衰期。A24N变体在基于细胞的试验(上述)和体内分析(下文)中具有与野生型分子相当的活性。加上较长的半衰期,这意味着随着时间的推移A24N变体将产生比野生型分子更大的每单位蛋白的作用。A24N变体和以上测试的任何一种其他变体可与GDF捕获物分子结合,例如L79D或L79E变体。

[0398] 实施例12:GDF-11和激活素A结合

以Biacore™试验测试某些ActRIIB-Fc蛋白和GDF捕获物与配体的结合。

[0399] 采用抗-hFc抗体将ActRIIB-Fc变体或野生型蛋白捕获到系统中。注射配体并流经捕获的受体蛋白。结果概述在下表中。

[0400] 配体结合特异性IIB变体。

	GDF11		
蛋白	K_{on}(1/Ms)	K_{off}(1/s)	KD (M)
ActRIIB (20-134)-hFc	1.34e-6	1.13e-4	8.42e-11
ActRIIB (A24N 20-134)-hFc	1.21e-6	6.35e-5	5.19e-11
ActRIIB (L79D 20-134)-hFc	6.7e-5	4.39e-4	6.55e-10
ActRIIB (L79E 20-134)-hFc	3.8e-5	2.74e-4	7.16e-10
ActRIIB (R64K 20-134)-hFc	6.77e-5	2.41e-5	3.56e-11
	GDF8		
蛋白	K_{on} (1/Ms)	K_{off} (1/s)	KD (M)
ActRIIB (20-134)-hFc	3.69e-5	3.45e-5	9.35e-11
ActRIIB (A24N 20-134)-hFc			
ActRIIB (L79D 20-134)-hFc	3.85e-5	8.3e-4	2.15e-9
ActRIIB (L79E 20-134)-hFc	3.74e-5	9e-4	2.41e-9
ActRIIB (R64K 20-134)-hFc	2.25e-5	4.71e-5	2.1e-10
ActRIIB (R64K 20-129)-hFc	9.74e-4	2.09e-4	2.15e-9
ActRIIB (P129S, P130R 20-134)-hFc	1.08e-5	1.8e-4	1.67e-9
ActRIIB(K74A 20-134)-hFc	2.8e-5	2.03e-5	7.18e-11
	激活素 A		
蛋白质	K_{on} (1/Ms)	K_{off} (1/s)	KD (M)
ActRIIB (20-134)-hFc	5.94e6	1.59e-4	2.68e-11
ActRIIB (A24N 20-134)-hFc	3.34e6	3.46e-4	1.04e-10
ActRIIB (L79D 20-134)-hFc			低结合
ActRIIB (L79E 20-134)-hFc			低结合
ActRIIB (R64K 20-134)-hFc	6.82e6	3.25e-4	4.76e-11
ActRIIB (R64K 20-129)-hFc	7.46e6	6.28e-4	8.41e-11
ActRIIB (P129S, P130R 20-134)-hFc	5.02e6	4.17e-4	8.31e-11

[0401] 在无细胞试验得到的这些数据证实基于细胞的试验数据，表明A24N变体保持与ActRIIB (20-134)-hFc分子类似的配体-结合活性，并且L79D或L79E分子保持肌肉生长抑制素和GDF11结合但显示与激活素A的结合明显降低(不可计量)。

[0402] 已产生和测试了其他变体，如WO2006/012627 (通过参照以其全部结合到本文中)中报告的那样。参见例如第59-60页，采用偶联于该装置的配体并使受体流经这种偶联的配体。值得注意的是，K74Y、K74F、K74I (并可假定在K74的其他疏水性替换，比如K74L)和D80I造成激活素A (ActA)结合与GDF11结合的比率降低(相对于野生型K74分子)。以下重现关于这些变体的数据表：

与GDF11和激活素A结合的可溶性ActRIIB-Fc变体(Biacore™试验)

ActRIIB	ActA	GDF11
WT (64A)	KD=1.8e-7M (+)	KD= 2.6e-7M (+)
WT (64R)	na	KD= 8.6e-8M (+++)
+15 尾端	KD ~2.6 e-8M (+++)	KD= 1.9e-8M (++++)
E37A	*	*
R40A	-	-
D54A	-	*
K55A	++	*
R56A	*	*
K74A	KD=4.35e-9 M +++++	KD=5.3e-9M +++++
K74Y	*	--
K74F	*	--
K74I	*	--
W78A	*	*
L79A	+	*
D80K	*	*
D80R	*	*
D80A	*	*
D80F	*	*
D80G	*	*
D80M	*	*
D80N	*	*
D80I	*	--
F82A	++	-

* 未观察到结合
-- <1/5 WT 结合
- ~1/2 WT 结合
+ WT
++ <2x 结合增加
+++ ~5x 结合增加
++++ ~10x 结合增加
+++++ ~40x 结合增加

实施例13:GDF捕获物增加体内红细胞水平

将12周大的雄性C57BL/6NTac小鼠分配到2个治疗组中的1个(N=10)。通过皮下注射

(SC)以10 mg/kg给予小鼠媒介物或变体ActRIIB多肽(“GDF捕获物”)[ActRIIB (L79D 20-134)-hFc],每周2次,进行4周。在研究终止时,通过心脏穿刺把全血收集到含有EDTA的管中,并采用HM2血液学分析仪(Abaxis, Inc)分析细胞分布。

[0403] 组指定

组	N	小鼠	注射	剂量 (mg/kg)	途径	频率
1	10	C57BL/6	PBS	0	SC	2 次/周
2	10	C57BL/6	GDF 捕获物 [ActRIIB (L79D 20-134)-hFc]	10	SC	2 次/周

与媒介物对照组相比较,用GDF捕获物治疗对白细胞(WBC)数目没有统计学显著效果。相对于对照组,治疗组的红细胞(RBC)数目增加(参见下表)。血红蛋白含量(HGB)和血细胞比容(HCT)两者也都由于另外的红细胞而增加。红细胞的平均宽度(RDWc)在治疗动物较高,表明不成熟红细胞群增加。因此,用GDF捕获物治疗导致红细胞增加,对白细胞群没有可识别的效果。

[0404] 血液学结果

	RBC 10^{12}/L	HGB (g/dL)	HCT (%)	RDWc (%)
PBS	10.7 ± 0.1	14.8 ± 0.6	44.8 ± 0.4	17.0 ± 0.1
GDF 捕获物	12.4 ± 0.4**	17.0 ± 0.7*	48.8 ± 1.8*	18.4 ± 0.2**

*=p<0.05, **= p<0.01

实施例14:GDF捕获物对于增加体内红细胞水平优于ActRIIB-Fc

将19周龄的雄性C57BL/6NTac小鼠随机分配到3个组中的一个。小鼠通过皮下注射给予媒介物(10 mM Tris缓冲盐水,TBS)、野生型ActRIIB (20-134)-mFc或GDF捕获物ActRIIB (L79D 20-134) -hFc,每周2次,进行3周。在基线和给药3周后经颊部放血收集血液并采用血液学分析仪(HM2,Abaxis, Inc.)分析细胞分布。

[0405] 与媒介物对照组相比较,用ActRIIB-Fc或GDF捕获物治疗对白细胞(WBC)数目没有显著效果。与对照组或野生型构建体 相比较,红细胞计数(RBC)、血细胞比容(HCT)和血红蛋白水平在用GDF捕获物治疗的小鼠都升高(参见下表)。因此,在直接比较中,GDF捕获物促进红细胞增加至比野生型ActRIIB-Fc蛋白显著更高的程度。事实上,在本试验中,野生型ActRIIB-Fc蛋白不造成红细胞的统计学显著增加,表明需要较长或较高剂量以展现这种效果。

[0406] 给予3周后的血液学结果

	RBC (10^{12}/ml)	HCT %	HGB g/dL
TBS	11.06 ± 0.46	46.78 ± 1.9	15.7 ± 0.7
ActRIIB-mFc	11.64 ± 0.09	49.03 ± 0.3	16.5 ± 1.5
GDF 捕获物	13.19 ± 0.2**	53.04 ±0.8**	18.4 ± 0.3**

**= p<0.01

实施例15:带有截短的ActRIIB细胞外结构域的GDF捕获物的产生

如在实施例9中描述的那样,通过TPA前导序列与含有亮氨酸-至-天冬氨酸替换(在SEQ ID NO:1的残基79)的ActRIIB细胞外结构域(SEQ ID NO:1的残基20-134)的N-末端融合以及人Fc结构域与最小连接子(3个甘氨酸残基)的C-末端融合,产生称为ActRIIB (L79D 20-134)-hFc的GDF捕获物(图16)。相应于该融合蛋白的核苷酸序列显示在图17A和17B中。

[0407] 通过TPA前导序列与含有亮氨酸-至-天冬氨酸替换(在SEQ ID NO:1的残基79)的截短的细胞外结构域(SEQ ID NO:1的残基25-131)的N-末端融合以及人Fc结构域与最小连接子(3个甘氨酸残基)的C-末端融合,产生称为ActRIIB (L79D 25-131)-hFc的带有截短的ActRIIB细胞外结构域的GDF捕获物(图18)。相应于该融合蛋白的核苷酸序列显示在图19A和19B中。

[0408] 实施例16:带有双截短的ActRIIB细胞外结构域的GDF捕获物的选择性配体结合

用Biacore™仪器体外评价GDF捕获物和其他ActRIIB-hFc蛋白对几种配体的亲和力。结果概述在下表中。Kd值通过由于复合体的非常快速缔合和解离(其阻止K_{on}和K_{off}的准确确定)的稳态亲和力拟合获得。

[0409] ActRIIB-hFc变体的配体选择性:

融合构建体	激活素 A (Kd e-11)	激活素 B (Kd e-11)	GDF11 (Kd e-11)
ActRIIB (L79 20-134)-hFc	1.6	1.2	3.6
ActRIIB (L79D 20-134)-hFc	1350.0	78.8	12.3
ActRIIB (L79 25-131)-hFc	1.8	1.2	3.1
ActRIIB (L79D 25-131)-hFc	2290.0	62.1	7.4

与缺乏L79D替换的ActRIIB-hFc配对物相比较,带有截短的细胞外结构域的GDF捕获物ActRIIB (L79D 25-131)-hFc等于或胜过更长变体ActRIIB (L79D 25-134)-hFc呈现的配体选择性,伴有激活素A结合明显丢失,激活素B结合部分丢失和GDF11结合几乎完全保留。注意单独的截短(没有L79D替换)不改变此处呈现的配体间的选择性[比较ActRIIB (L79 25-131)-hFc与ActRIIB(L79 20-134)-hFc]。

[0410] 实施例17:具有备选核苷酸序列的ActRIIB (L79D 25-131)-hFc的产生

为了产生ActRIIB (L79D 25-131)-hFc,将在天然79位(SEQ ID NO:1)带有天冬氨酸替换和带有N-末端和C-末端截短(SEQ ID NO: 1的残基25-131)的人ActRIIB细胞外结构域在N-末端与TPA前导序列而不是天然ActRIIB前导序列和在C-末端与人Fc结构域经最小连接子(3个甘氨酸残基)融合(图18)。一种编码该融合蛋白的核苷酸序列显示在图19A和19B中

(SEQ ID NO: 42)，并且编码完全相同融合蛋白的备选核苷酸序列显示在图22A和22B中(SEQ ID NO: 46)。这种蛋白采用实施例9中描述的方法学表达和纯化。

[0411] 实施例18:带有截短的ActRIIB细胞外结构域的GDF捕获物增加小鼠红系祖细胞的增殖

评价ActRIIB (L79D 25-131)-hFc以确定其对红系祖细胞增殖的效果。雄性C57BL/6只小鼠(8周龄)在第1和4天用ActRIIB (L79D 25-131)-hFc (10 mg/kg，皮下;n=6)或媒介物(TBS;n=6)治疗，然后在第8天安乐死，以收集脾、胫骨、股骨和血液。分离脾和骨髓的细胞，以含有5%胎牛血清的Iscove改良的Dulbecco培养基稀释，悬浮于专门的基于甲基纤维素的培养基中，并培养2或12天，以分别评价集落形成单位-红细胞(CFU-E)和爆发形成单位-红细胞(BFU-E)期的克隆祖细胞水平。用于BFU-E测定的基于甲基纤维素的培养基(MethoCult M3434，Stem Cell Technologies (干细胞技术))包括重组鼠干细胞因子、白介素-3和白介素-6，其在用于CFU-测定的甲基纤维素培养基中不存在(MethoCult M3334，Stem CellTechnologies (干细胞技术))，同时两种培养基都含有促红细胞生成素等其他组分。对于BFU-E和CFU-E两者，克隆(colonies)数目以源自每种组织样品的两个培养板测定，并且结果的统计学分析基于每个治疗组的小鼠数目。

[0412] 来自用ActRIIB (L79D 25-131)-hFc治疗小鼠的脾-衍生的培养物具有的CFU-E克隆(colonies)数目是来自对照组小鼠的相应培养物的2倍(P<0.05)，而BFU-E克隆(colonies)数目与体内治疗组没有显著不同。来自骨髓培养物的CFU-E或BFU-E克隆(colonies)数目也没有显著不同于治疗组。如预期的那样，与对照组相比较，在用ActRIIB (L79D 25-131)-hFc治疗的小鼠安乐死下，在脾-衍生的培养物中CFU-E克隆(colonies)数目的增加伴随红细胞水平(增加11.6%)、血红蛋白浓度(增加12%)和血细胞比容水平(增加11.6%)的高度显著((P<0.001)变化。这些结果表明，体内给予带有截短的ActRIIB细胞外结构域的GDF捕获物可刺激红系祖细胞增殖，作为其增加红细胞水平的总体效果的一部分。

[0413] GDF捕获物融合蛋白已进一步证实在各种贫血模型(包括例如化学治疗诱导的贫血、肾切除术诱导的贫血和失血中的贫血)增加红细胞水平是有效性(参见例如国际专利申请出版物第WO 2010/019261号)。

[0414] 实施例19:带有截短的ActRIIB细胞外结构域的GDF捕获物增加非人灵长类动物的红细胞水平

评价两种GDF捕获物ActRIIB (L79D 20-134)-hFc和ActRIIB (L79D 25-131)-hFc其刺激食蟹猴红细胞产生的能力。猴在第1和8天用GDF捕获物(10 mg/kg;n = 4只雄性/4雌性)或媒介物(n = 2只雄性/2只雌性)皮下治疗。在第1 (治疗前基线)、3、8、15、29和44天收集血样，并分析红细胞水平(图24)、血细胞比容(图25)、血红蛋白水平(图26) 和网织红细胞水平(图27)。媒介物治疗的猴在所有治疗后时间点显示红细胞、血细胞比容和血红蛋白水平降低(反复采血的预期效果)。与此相反，用ActRIIB (L79D 20-134)-hFc或ActRIIB (L79D 25-131)-hFc治疗到第一个治疗后时间点(第3天)这些参数增加，并在研究持续期间内将其维持在显著升高的水平(图24-26)。重要的是，与媒介物相比较，用ActRIIB (L79D 20-134)-hFc或ActRIIB (L79D 25-131)-hFc治疗的猴网织红细胞水平在第8、15和29天显著增加(图27)。该结果说明，GDF捕获物治疗增加红细胞前体产生，导致红细胞水平升高。

[0415] 总之，这些数据说明截短的GDF捕获物以及全长变体可以用作GDF11的选择性拮抗

剂和潜在相关的配体以增加体内红细胞形成。

[0416] 实施例20:源自ActRIIB5的GDF捕获物

其他人报道了备选的可溶形式的ActRIIB (称为ActRIIB 5),其中外显子4 (包括ActRIIB跨膜域)已用不同的C-末端序列替换(参见例如WO 2007/053775)。

[0417] 不含其前导序列的天然人ActRIIB5的序列如下:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVK
KGCWLDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEGPWAST
TIPSGGPEATAAAGDQGSGALWLCLEGPAHE (SEQ ID NO:49)

可如描述的那样在天然79位(下划线的)实施亮氨酸-至-天冬氨酸替换或其他酸性替换,以构建 变体ActRIIB5 (L79D),其具有以下序列:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVK
KGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEGPWAST
TIPSGGPEATAAAGDQGSGALWLCLEGPAHE (SEQ ID NO:50)

这种变体可用TGGG连接子(单下划线)连接于人Fc (双下划线),以产生具有以下序列的人ActRIIB5 (L79D)-hFc融合蛋白:

GRGEAETRECIYYNANWELERTNQSGLERCEGEQDKRLHCYASWRNSSGTIELVK
KGCWDDDFNCYDRQECVATEENPQVYFCCCEGNFCNERFTHLPEAGGPEGPWAST
TIPSGGPEATAAAGDQGSGALWLCLEGPAHETGGGTHTCPPCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN
STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP
PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:51)。

[0418] 该构建体 可在CHO细胞表达。

[0419] 实施例21:用EPO和带有截短的ActRIIB细胞外结构域的GDF捕获物联合治疗在小鼠中的作用

EPO通过增加红细胞前体增殖诱导红细胞形成,而GDF捕获物可以补充或增强EPO作用的方式潜在地影响红细胞形成。因此,申请者研究用EPO和ActRIIB (L79D 25-131)-hFc联合治疗对红细胞生成参数的作用。雄性C57BL/6只小鼠(9周龄)给予单次腹膜(i.p.)内注射单独的重组人EPO (依泊汀α,1800单位/kg)、单独的ActRIIB (L79D 25-131)-hFc (10 mg/kg)、EPO和ActRIIB (L79D 25-131)-hFc两者或媒介物(Tris-缓冲盐水)。给药后72小时对小鼠安乐死来收集血液、脾和股骨。

[0420] 处理脾和股骨以获得红细胞前体细胞进行流式细胞检测分析。去除后,将脾在含5%胎牛血清的Iscove改良的Dulbecco培养基中切碎,并通过用无菌1-mL注射器的活塞推过70-μm细胞滤网机械离解。把股骨清除任何残留肌肉或结缔组织并修剪末端,以允许通过经连接于3-mL注射器的21号针用含有5%胎牛血清的Iscove改良的Dulbecco培养基冲洗残留的骨干来收集骨髓。将细胞悬液离心(2000 rpm,10分钟)并将细胞团块重新悬浮于含5%胎牛血清的PBS中。将来自每种组织的细胞(10^6)用抗小鼠IgG温育以阻滞非特异性结合,然后用针对小鼠细胞表面标记CD71 (转铁蛋白受体)和Ter119 (与细胞表面血型糖蛋白A有关的抗原)的荧光标记抗体温育,冲洗,并通过流式细胞计分析。通过用碘化丙啶复染将样品

中的死细胞自分析中排除。通过在分化过程中降低的CD71标记程度和在末端红细胞分化(起始于原成红细胞期)期间增加的Ter119标记程度，评价脾或骨髓中的红细胞分化(Socolovsky等，2001, Blood 98:3261-3273;Ying等，2006, Blood108:123-133)。因此，如所描述的那样，采用流式细胞术测定原成红细胞(CD71^{高}Ter119^{低})、嗜碱性成红细胞(CD71^{高}Ter119^{高})、多染色性+正染色成红细胞(CD71^{中}Ter119^{高})和晚期正染色成红细胞+网织红细胞(CD71^{低}Ter119^{高})的数目，如所述的。

[0421] 用EPO和ActRIIB (L79D 25-131)-hFc联合治疗导致红细胞令人惊讶地剧烈增加。在该试验的72-h时间框架中，单独的EPO和ActRIIB (L79D 25-131)-hFc与媒介物相比较都不显著增加血细胞比容，而用两种试剂联合治疗导致血细胞比容差不多增加25%，这是出乎意料地协同的，即大于其单独效果的总和(图28)。该类型的协同作用通常考虑为证明单个试剂通过不同的细胞机制起作用。对血红蛋白浓度(图29)和红细胞浓度(图30)也观察到类似结果，其每一个也通过联合治疗协同增加。

[0422] 分析红细胞前体水平显示更复杂的方式。在小鼠，认为脾是负责诱导性(“应激”)红细胞生成的主要的器官。脾组织在72 h的流式细胞检测分析显示EPO与媒介物相比较显著改变红细胞生成前体概况，增加嗜碱性成红细胞数目多余170%，代价是晚期前体(晚期正染色成红细胞+网织红细胞)，其降低超过三分之一(图31)。重要的是，联合治疗与媒介物相比较显著增加嗜碱性成红细胞，但是比单独EPO的程度更低，同时支持晚期前体的成熟不减少(图31)。因此，用EPO和ActRIIB (L79D 25-131)-hFc联合治疗通过平衡前体增殖和成熟的增强增加红细胞生成。与脾形成对照，联合治疗后骨髓中的前体细胞概况没有明显不同于单独EPO之后。申请者根据脾前体概况预测，联合治疗会导致网织红细胞水平增加并会伴有成熟红细胞水平的持续升高(如果试验延长超过72 h)。

[0423] 综上所述，这些发现说明带有截短的ActRIIB细胞外结构域的GDF捕获物可与EPO组合给予以协同增加体内红细胞形成。通过互补但未确定机制的作用，GDF捕获物可减缓单独EPO受体激活剂的强增殖效果，并仍然允许以较低剂量的EPO受体激活剂获得目标红细胞水平，从而避免与较高水平的EPO受体激活相关的潜在不良作用或其他问题。

[0424] 实施例22:GDF捕获物对MDS小鼠模型无效红细胞生成和贫血的作用

申请者研究了GDF捕获物ActRIIB (L79D 25-131)-mFc (RAP-536)在MDS的NUP98-HOXD13小鼠模型的作用，其特征为发育不全的前体成熟和无效造血。在这个模型上，疾病严重程度随着年龄而增加，最终在大多数小鼠发展成急性髓性白血病，它们的平均寿命约为14个月。在大约4个月龄开始，这些小鼠呈现贫血、白细胞减少、红细胞生成无效和骨髓从正常细胞至细胞过多[Lin等. (2005) Blood 106:287-295]。为了监测长期给予的影响，用RAP-536 (10 mg/kg，s.c.)或媒介物处理MDS小鼠，每周2次，在4个月龄时开始并持续7个月，同时在基线和之后每月1次收集血液样本(50 μL)用于全血计数分析。如所预期的那样，6个月龄MDS小鼠与野生型小鼠相比较出现严重的贫血(图32A)，并且骨髓分析揭示与年龄匹配的FVB野生型小鼠相比较，在MDS小鼠红细胞前体数目增加(图32A)和骨髓/红系(M/E)比率更低[Suragani等. (2014) Nat Med 20:408-414]，表明红细胞生成无效。在6个月龄MDS小鼠，用RAP-536处理显著增加RBC计数(达16.9%)和血红蛋白浓度(达12.5%)(图32A)，减少骨髓中的红细胞前体细胞计数(图32A)，以及使M/E比率与野生型小鼠归一化[Suragani等. (2014) Nat Med 20:408-414]。

[0425] 在12个月龄的MDS小鼠，与媒介物相比较，RAP-536处理显著增加RBC计数(达18.3%)和血红蛋白水平(达13.0%)(图32B)，减少红细胞前体细胞计数(图32B)，以及改善M:E比率[Suragani等. (2014) Nat Med 20:408-414]。RAP-536处理不影响髓系前体细胞的绝对数目。流式细胞术证实RAP-536处理减少两个年龄段MDS小鼠的红系增生。用RAP-536处理7个月的MDS小鼠的时间进程分析显示在研究期间RBC数目持续升高[Suragani等. (2014) Nat Med 20:408-414]。总起来，这些结果表明用GDF捕获物处理改善MDS小鼠的贫血、红系增生和无效红细胞生成而与疾病严重程度无关。

[0426] 实施例23:治疗上应答于GDF捕获物的MDS患者的细胞学和遗传特征

含有修饰的IIB型激活素受体和IgG Fc [ActRIIB (L79D 25-131)-hFc，也称为罗特西普或ACE-536]的重组融合蛋白被开发用于治疗由无效红细胞生成引起的贫血比如骨髓增生异常综合征(MDS)。患有MDS的患者经常具有EPO水平升高，并且可能对红细胞生成刺激剂(ESAs)是非响应性或是难治性的。MDS患者也已显示GDF11的血清水平增加[Suragani等. (2014) Nat Med 20:408-414]和骨髓Smad 2/3信号传递增加[Zhou等. (2008) Blood 112:3434-3443]。ActRIIB (L79D 25-131)-hFc结合于TGFβ超家族中的配体(包括GDF11)，抑制Smad 2/3信号传递和通过与ESAs不同的机制促进晚期红细胞分化。小鼠版本的ActRIIB (L79D 25-131)-mFc在MDS小鼠模型减少Smad 2信号传递，增加血红蛋白(Hb)水平，并减少的骨髓红系增多[Suragani等. (2014) Nat Med 20:408-414]。在一项健康志愿者研究中，ActRIIB (L79D 25-131)-hFc耐受良好并增加Hb水平[Attie等. (2014) Am J Hematol 89:766-770]。

[0427] 申请者进行持续2期、多中心、开放标签的剂量探索研究，以评价ActRIIB (179d 25-131)-hFc对低或Int-1风险MDS患者(有高输注负担(HTB，定义为基线前≥4个单位的RBC每8周)或低输注负担(LTB，定义为基线前＜4单位的RBC每8周))贫血的影响。研究结果包括红细胞反应(在LTB患者Hb增加或在HTB患者输注负担减小)、安全性、耐受性、药代动力学和药效学生物标志物。入选标准包括：低或Int-1风险MDS、年龄≥18岁、贫血(定义为HTB患者或在LTB患者基线Hb＜10.0 g/dL)、EPO ＞ 500 U/L、或者对ESAs无响应/难治的、事先没有阿扎胞苷或地西他滨；目前没有用ESA、粒细胞集落刺激因子(G-CSF)、粒细胞-巨噬细胞集落刺激因子(GM-CSF)或者来那度胺、沙利度胺或泊马度胺治疗。在剂量递增阶段，以0.125、0.25、0.5、0.75、1.0、1.33和1.75 mg/kg的剂量经皮下注射给予ActRIIB (179d 25-131)-hFc，每3周1次，连续七组(n = 3-6)，最多5个剂量，随访3个月。

[0428] 数据对26例患者是可以得到的(7例LTB/19例HTB)。平均年龄为71岁(范围：27-88岁)，50%为女性，54%先前使用EPO疗法，和15%先前使用来那度胺。患者按WHO分型分类如下：15% RARS、46% RCMD-RS、15% RCMD、15% RAEB-1 (包括两名有≥15%环形铁粒幼红细胞的患者)和8% del (5q)。LTB患者(n = 7)的平均(SD)基线Hgb为9.1 (0.4) g/dL。治疗前输注RBC 8周的平均(SD)单位对LTB患者为0.9 (1.1)单位和对HTB患者为6.3 (2.4)单位。7例LTB患者中有2例经8周与基线相比较平均Hb增加≥ 1.5 g/dL。在0.125 (n=1)、0.25 (n = 1)、0.75 (n = 3)和1.75 (n = 2) mg/kg剂量组，LTB患者的平均最大Hb增加分别为0.8、1.0、2.2和3.5 g/dL。在研究期间，7例LTB患者中有6例达到脱离RBC输注(RBC-TI) ≥ 8周。0.75 mg/kg-1.75 mg/kg范围内的剂量水平认为是有效剂量。在有效剂量组的5例患者中，4例(80%)达到预先指定终端，即Hgb增加≥ 1.5 g/dl ≥2周。2例患者(40%)达到HI-E响应

[International Working Group (国际工作组); Cheson等. (2000) Blood 96:3671-3674;Cheson等. (2006) Blood 108:419-425],定义为LTB患者Hgb增加≥ 1.5 g/dl ≥ 8周。在HTB患者,HI-E响应定义为与研究开始前8周相比较输注8周期间输注负担减少至少4个红细胞单位。在有效剂量组,12例HTB患者当中5例(42%)满足预先指定的终端,即与治疗前8周相比较,治疗期间经输注8周间隔减少≥ 4 RBC单位或RBC单位减少≥ 50%,并且相同的患者(12例当中5例,42%)达到HI-E响应;在有效剂量组,12例HTB患者当中3例(25%)在研究期间达到RBC-TI ≥ 8周。在一些患者观察到在给予研究药物后嗜中性粒细胞计数增加。最后,ActRIIB (L79D 25-131)-hFc通常耐受良好。相关的严重不良事件迄今未见报道。不考虑因果关系,最常见的不良事件为腹泻(n = 4,级别1/2)、骨痛、疲劳、肌肉痉挛、肌痛和鼻咽炎(n = 3每组,级别1/2)。

[0429] 骨髓穿刺评价证实了环形铁粒幼红细胞的存在(如果≥ 15%红细胞前体呈环形铁粒幼红细胞形态就认为阳性)与活性剂量组(0.75-1.75 mg/kg)中对ActRIIB (L79D 25-131)-hFc响应性之间的关联。LTB和HTB两者患者的总缓解率(使用上文描述的HI-E标准)为17例当中有7例(41%)。在基线对环形铁粒幼红细胞呈阳性的患者中,13例患者当中有7例(54%)达到HI-E应答,并且值得注意的是4例在基线下对环形铁粒幼红细胞呈阴性的患者当中没有1例达到HI-E响应。

[0430] 还对患者的骨髓穿刺抽出物评价与MDS有关的对港(harbor)突变(主要是体细胞突变)已知的21个不同基因中的突变的存在。自骨髓穿刺分离基因组DNA,通过聚合酶链反应扩增21个基因的选定编码区,采用新一代测序(Myeloid Molecular Profile 21-gene panel,Genoptix, Inc.,Carlsbad (卡尔斯巴德),CA (美国))测定这些区域的DNA序列。该分析检查激活的信号传递基因(*KIT*、*JAK2*、*NRAS*、*CBL*和*MPL*)、转录因子(*RUNX1*, *ETV6*)、表观遗传基因(*IDH1*、*IDH2*、*TET2*、*DNMT3A*、*EZH2*、*ASXL1*和*SETBP1*)、RNA剪接基因(*SF3B1*、*U2AF1*、*ZRSR2*和*SRSF2*)和肿瘤抑制基因/其他(*TP53*、*NPM1*、*PHF6*)。SF3B1的分析特异性靶向外显子13-16。在这些评价的与MDS有关的21种基因当中,在响应的患者骨髓细胞内比无响应的患者更频繁地检测到*SF3B1*突变。在这些患者检测到的单个*SF3B1*突变显示在下表中。相同的突变有时发生在多名患者。

核苷酸	核苷酸替换	氨基酸替换	外显子
1873	C → T	R625C	14
1874	G → T	R625L	14
1986	C → G	H662Q	14
2098	A → G	K700E	15
2342	A → G	D781G	16

[0431] 在活性剂量组具有*SF3B1*突变的患者中,9例当中有6例(67%)达到HI-E响应,包括所有3例脱离输注超过8周的患者。在没有*SF3B1*突变的患者,8例当中仅有1例(13%)达到HI-E响应。在具有环形铁粒幼红细胞和与无效红细胞生成有关的MDS患者经常观察到*SF3B1*突变。

[0432] 以上呈现的进行中的临床试验的初步分析在后面的分析中得到证实,其中数据得自44例患者(15 LTB/29 HTB)。平均年龄为71岁(范围:27-88岁),43%为女性,61%先前使用EPO疗法,和21%先前使用来那度胺。LTB患者(n = 15)的平均基线Hgb为9.0 (范围:6.8-

10.1) g/dL。治疗前输注8周的平均RBC单位对接受输注的6例LTB患者为2 (范围:2-2)单位和对HTB患者为6 (范围:4-14)单位。在0.75 mg/kg-1.75 mg/kg范围内的剂量水平认为是有效剂量。在有效剂量组的35例LTB和HTB患者当中,22例(63%)达到预先指定的终端,即对LTB患者Hgb增加≥ 1.5 g/dl ≥ 2周和对HTB患者输注减少≥ 4单位或50%超过8周。在有效剂量组中,35例患者当中19例(54%)达到HI-E响应[International Working Group (国际工作组); Cheson等. (2000) Blood 96:3671-3674;Cheson等. (2006) Blood 108:419-425],定义为LTB患者Hgb增加≥ 1.5 g/dl ≥ 8周和定义为在HTB患者与治疗开始前8周相比较,在治疗期间经输注8周间隔减少≥ 4 RBC单位或RBC单位减少≥ 50%。在有效剂量组中,28例具有基线输注的患者当中10例(36%)达到脱离输注至少8周的时间段。骨髓穿刺评价证实了环形铁粒幼红细胞(如果≥ 15%红细胞前体呈环形铁粒幼红细胞形态就认为阳性)或SF3B1基因突变的存在与活性剂量组(0.75-1.75 mg/kg)中对ActRIIB (L79D 25-131)-hFc响应性之间的关联。LTB和HTB两者患者的总缓解率(使用上文描述的HI-E标准)为19/35 (54%)。在基线对环形铁粒幼红细胞呈阳性的患者中,19/30 (63%)患者达到HI-E应答,并且值得注意的是4例在基线对环形铁粒幼红细胞呈阴性的患者当中没有1例达到HI-E响应。在基线对SF3B1突变呈阳性的患者中,16/22 (73%)患者达到HI-E应答,并且值得注意的是仅有在基线对SF3B1突变呈阴性的3/13 (23%)患者达到HI-E响应。最后,ActRIIB (L79D 25-131)-hFc通常耐受良好。不考虑因果关系,最常见的不良事件为腹泻、鼻咽炎、肌痛、骨痛、支气管炎、头痛和肌肉痉挛。两个可能相关的严重不良事件(SAEs)被报道:3级肌肉疼痛;一般病症的3级恶化。一个可能相关的非严重3级不良事件的未成熟细胞计数增加被报道。

[0433] 进一步的数据评价在稍后的时间进行,延伸并通常证实了以上结果。总的说来,在活性剂量组49例患者中的24例(49%)达到HI-E响应,定义在具有低输注负担(LTB)的患者血红蛋白浓度增加≥ 1.5 g/dL ≥8周,和定义在具有高输注负担(HTB)的患者,与治疗开始前8周相比较,在治疗期间经输注8周间隔减少≥ 4 RBC单位或减少≥ 50% RBC单位。具有基线输注的活性剂量组中40例患者中的14例(35%)脱离输注至少8周时间段。

[0434] 对活性剂量组的患者还实施了在某些体细胞基因突变存在下的缓解率评价。在应答的患者比无应答的患者骨髓细胞中更频繁地检测到*SF3B1*突变。在具有*SF3B1*突变的活性剂量组的30例患者当中有18例(60%)达到HI-E响应,而在该基因上没有检测到突变的19例这种患者当中仅有6例(32%)达到HI-E响应。在这些患者检测到的单个*SF3B1*突变显示在下表。相同的突变有时发生在多名患者。

核苷酸	核苷酸变化	AA变化	外显子
1868	A → G	Y623C	14
1873	C → T	R625C	14
1874	G → T	R625L	14
1986	C → G	H662Q	14
2098	A → G	K700E	15
2342	A → G	D781G	16
2347	G → A	E783K	16

[0435] 类似地,在活性剂量组应答的患者比无应答的患者骨髓细胞中更加频繁地检测到

*DNMT3A*突变。在具有*DNMT3A*突变的活性剂量组11例患者当中有7例(64%)达到HI-E响应，而在该基因上没有检测到突变的38例这种患者当中有17例(45%)达到HI-E响应。在这些患者检测到的单个*DNMT3A*突变显示在下表(IVS指的是内含子突变和X表示形成提前终止密码子)。

核苷酸	核苷酸变化	AA变化	外显子
1308	C → A	Y436X	10
IVS 2082	+2 T → C	--	--
2193_2195	del CTT	框内	18
2216	del A	移码	18
IVS 2322	+2 T → C	--	--
2644	C → T	R882C	22
2645	G → A	R882H	22
2678	G → A	W893X	22
2711	C → T	P904L	22
2714	T → C	L905P	22

[0436] 类似地，在活性剂量组应答的患者比无应答的患者骨髓细胞中更加频繁地检测到*TET2*突变。在具有*TET2*突变的活性剂量组20例患者当中有11例(55%)达到HI-E响应，而在该基因上没有检测到突变的29例这种患者当中有13例(45%)达到HI-E响应。在这些患者检测到的单个*TET2*基因突变(不包括已知的多态性)显示在下表中。

核苷酸	核苷酸变化	AA变化	外显子
73	del T	移码	1
139	G → C	E47Q	1
735	del C	移码	1
1201_1202	ins ACCACCACCAC	移码	1
1337	del T	移码	1
1588_1591	del CAGC	移码	1
1648	C → T	R550X	1
1842_1843	ins G	移码	1
2145	del C	移码	1
2305	del C	移码	1
2784	del T	移码	1
3025	C → T	Q1009X	1
3727_3729	del AAA	框内	4
3731_3738	del TCTACTCG	移码	4
3821	A → G	Q1274R	5
3854_3856	del TCT	框内	5
3871	T → A	W1291R	5
IVS 3955	-2 A → G	--	--
4011	T → A	Y1337X	6

4108	G → A	G1370R	7
4109	G → A	G1370E	7
4160	A → G	N1387S	7
4209	del T	移码	8
4210	C → T	R1404X	8
4211_4217	del GAGAATT	移码	8
4546	C → T	R1516X	9
4954	C → T	Q1652X	9
5168	del C	移码	9
5170	T → C	Y1724H	9
5576_5582	del TTGGGGG	移码	9

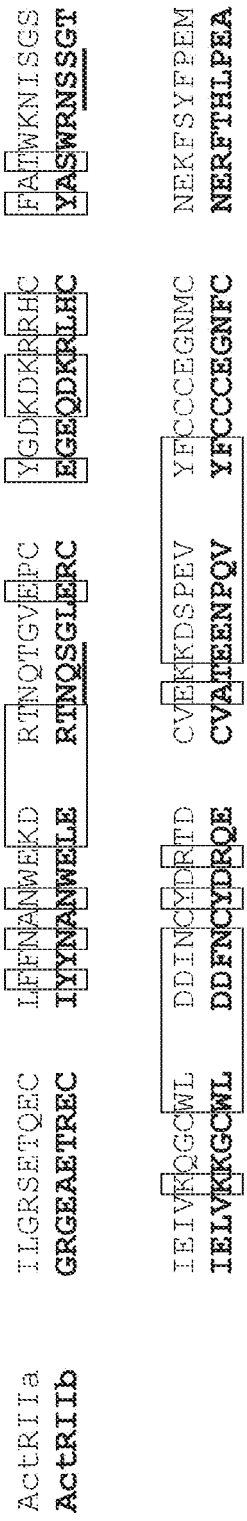
[0437] 与无应答患者的细胞类似频繁地，在应答患者的骨髓细胞检测到其他基因突变。例如，在具有*ASXL1*突变的活性剂量组的8位患者当中有4例(50%)达到HI-E响应，而类似百分比的这样患者(41例当中有20例，49%)在达到HI-E响应的该基因上没有检测到突变。

[0438] 这些结果表明，呈现≥ 15%环形铁粒幼红细胞(和患有其他形式铁粒幼红细胞性贫血的患者)和/或具有至少一个*SF3B1*突变的患有MDS的患者比具有＜15%环形铁粒幼红细胞和/或无*SF3B1*突变的MDS患者更可能治疗上应答于ActRIIB (L79D 25-131)-hFc。类似地，呈现≥ 15%环形铁粒幼红细胞(和患有其他形式的铁粒幼红细胞性贫血的患者)和/或至少一个*DNMT3A*或*TET2*突变的患者比具有< 15%环形铁粒幼红细胞和/或没有*DNMT3A*或*TET2*突变的MDS患者更可能治疗上应答于ActRIIB (L79D 25-131)-hFc。基于这些数据，任何这些患者亚型的选择性治疗有望大大增加用ActRII抑制剂治疗的效益/风险比。

[0439] 通过参照结合

本文提及的所有出版物和专利均通过参照以其全部结合到本文中，如同每一个单个出版物或专利均特别和单个地通过参照结合表明的那样。

[0440] 尽管已经讨论了主题的具体实施方案，以上说明书为说明性的，而不具有限制性。在阅读本说明书和以下权利要求后，许多变化对于本领域技术人员将是显而易见的。本发明的完整范围应参照权利要求及其所有的等同范围和说明书及这种变化一起得以确定。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ActRIIb
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GRGEAETREC
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IYYNANWELE
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EGEQDKRLHC
FATWKNISGS
YASWRNSSGT
IEIVKQGCWL
IELVKKGCWL
DDINCYDRTD
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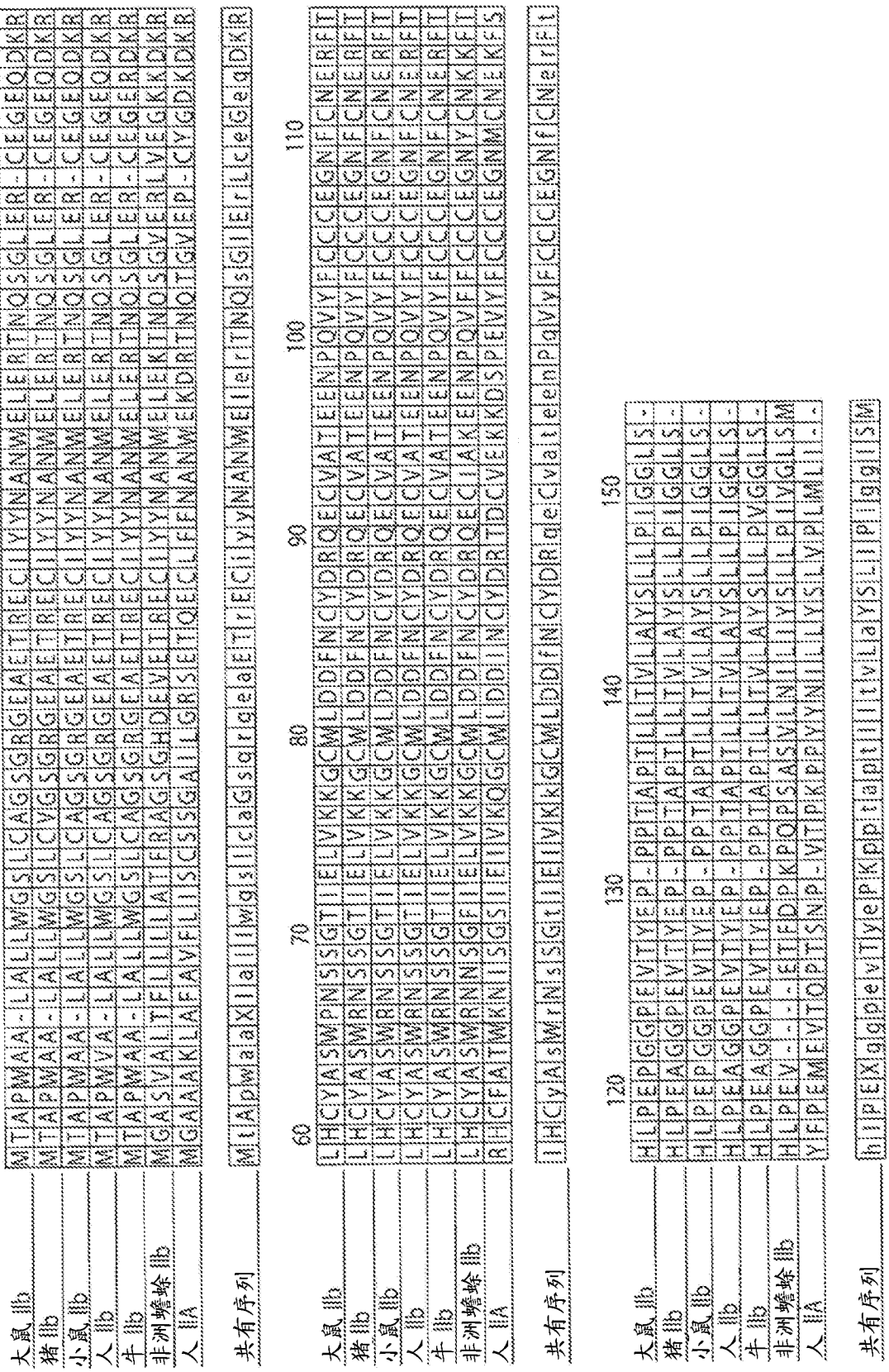
图 1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猪 IIb
小鼠 IIb
人 IIb
牛 IIb
非洲蟾蜍 IIb
人 IIA
共有序列
大鼠 IIb
猪 IIb
小鼠 IIb
人 IIb
牛 IIb
非洲蟾蜍 IIb
人 IIA
共有序列
大鼠 IIb
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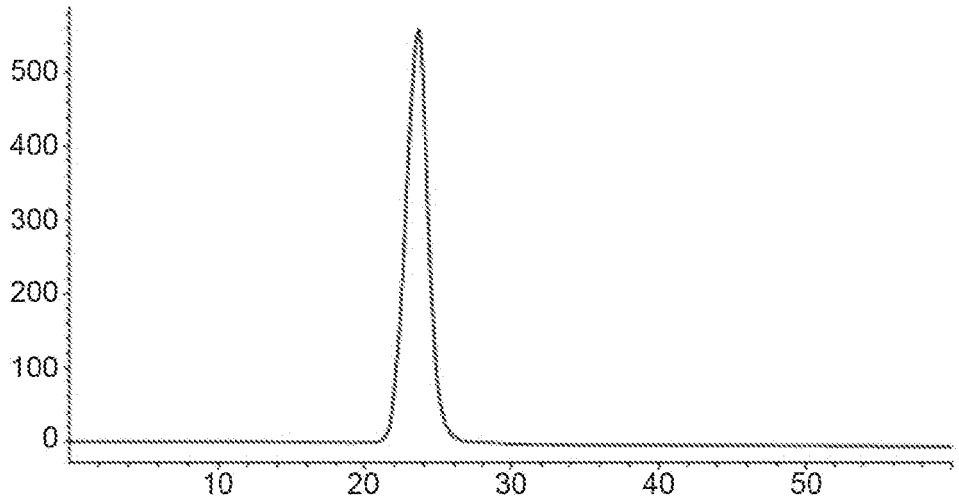
人 IIA
共有序列

图 2



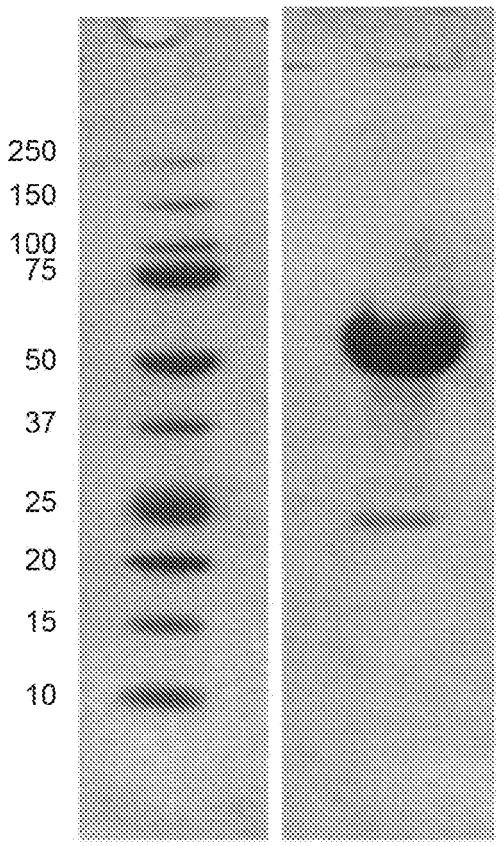
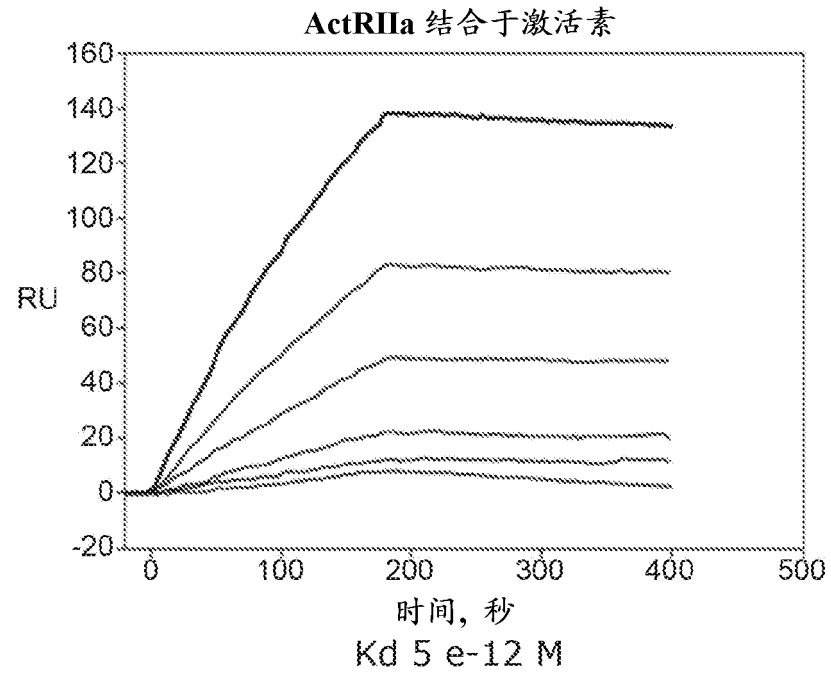


图 3


ActRIIa 结合于激活素
160
140
120
100
80
60
40
20
0
-20
RU
0
100
200
300
400
500
时间，秒
Kd 5 e-12 M

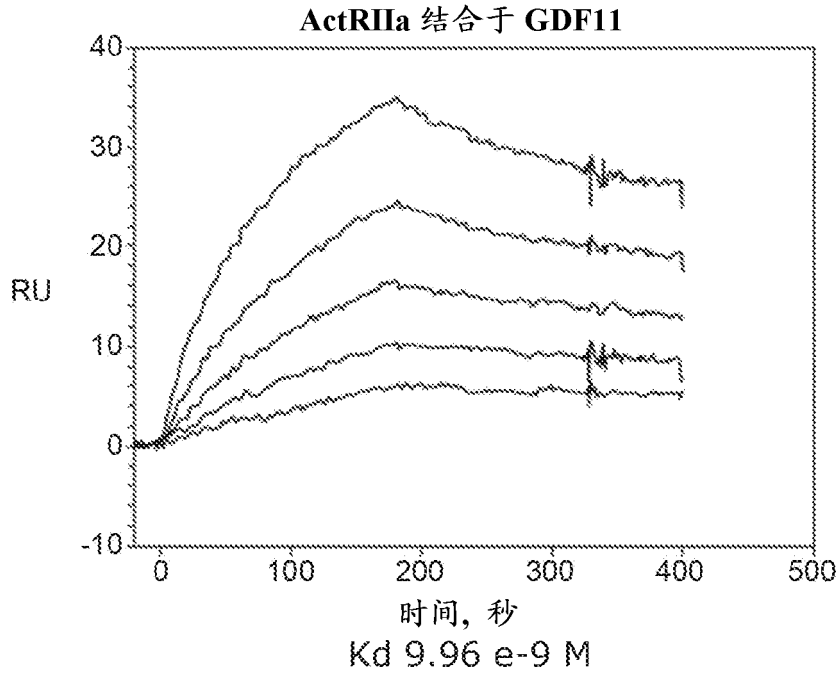

ActRIIa 结合于 GDF11
40
30
20
10
0
-10
RU
0
100
200
300
400
500
时间，秒
Kd 9.96 e-9 M

图 4

ActRIIA-Fc 对雌性 NHPs RBC 计数的影响

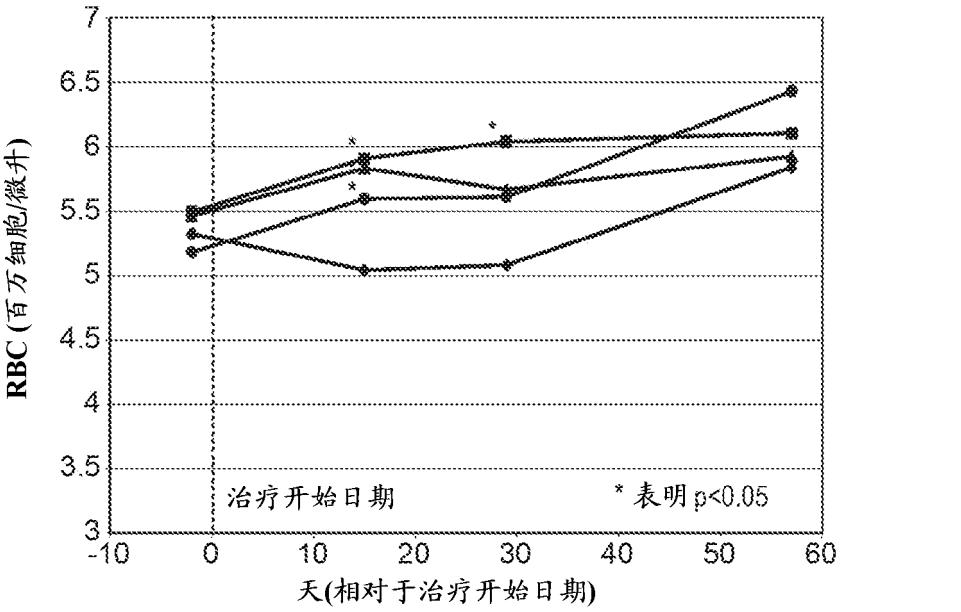


图 5A

ActRIIA-Fc 对雌性 NHPs 血红蛋白的影响

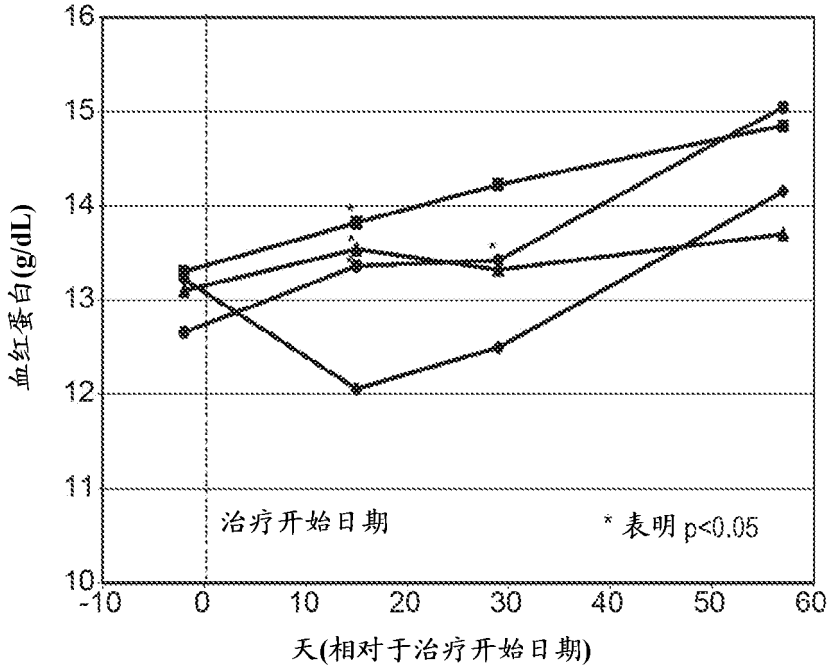


图 5B

ActRIIA-Fc 对雄性 NHPs RBC 计数的影响

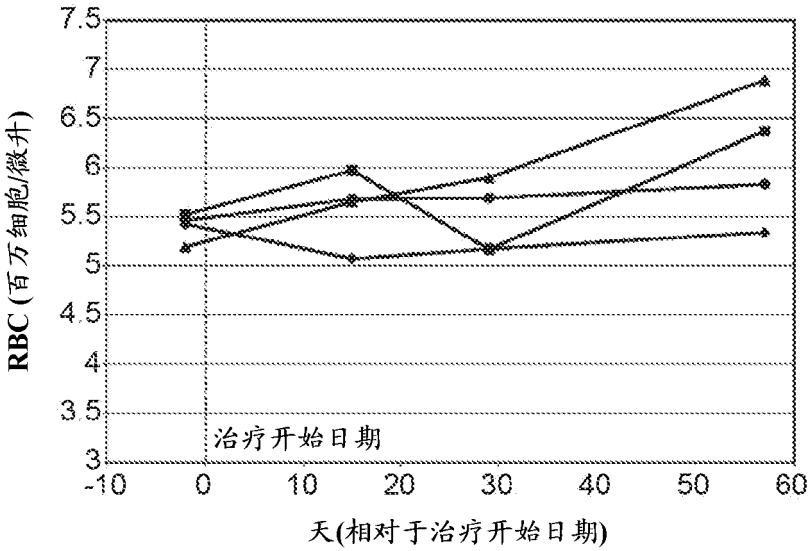


图 6A

ActRIIA-Fc 对雄性 NHPs 血红蛋白的影响

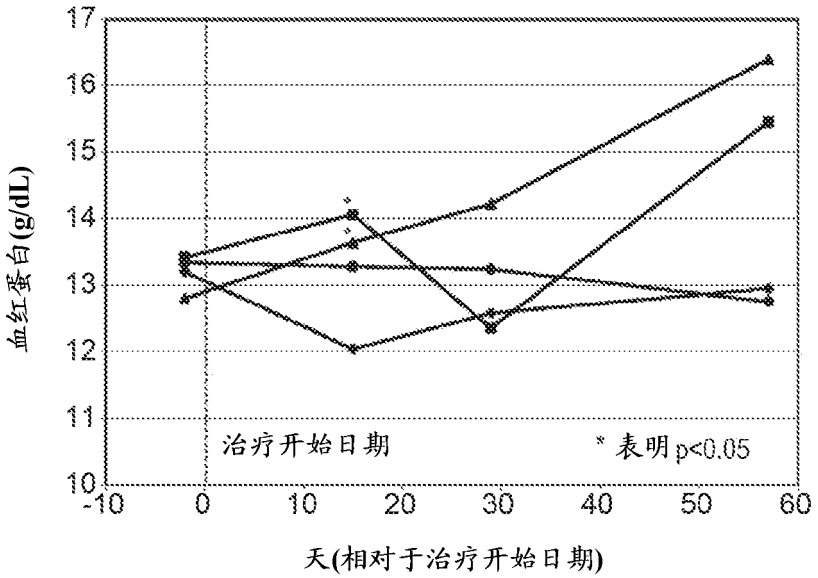


图 6B

ActRIIA-Fc 对雌性 NHPs 网织红细胞计数的影响

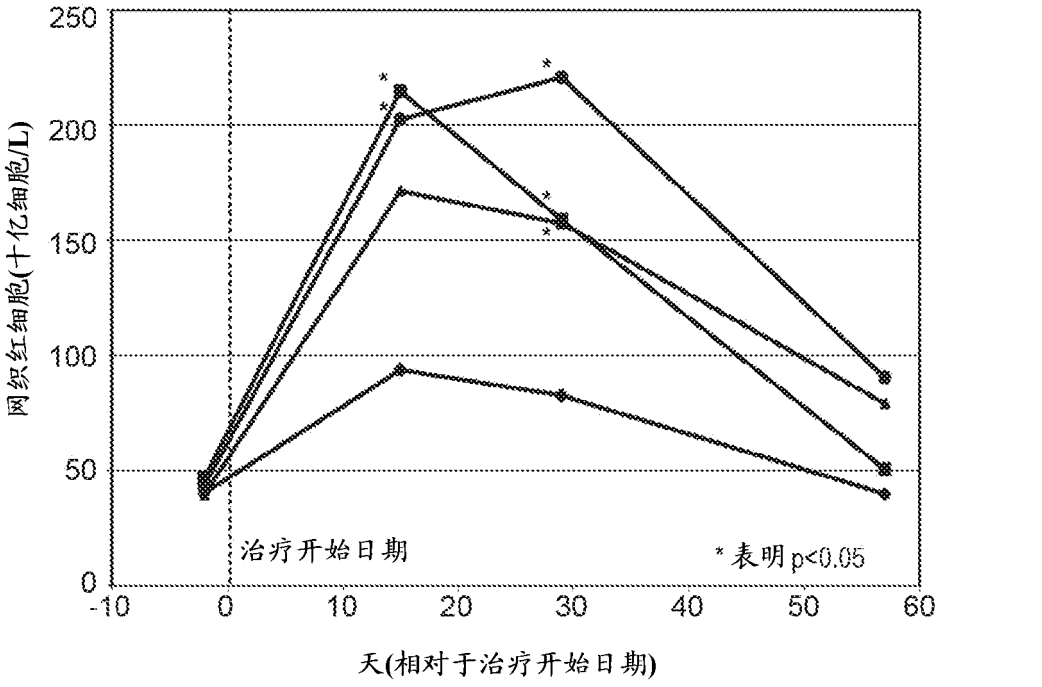


图 7A

ActRIIA-Fc 对雌性 NHPs 网织红细胞百分数的影响

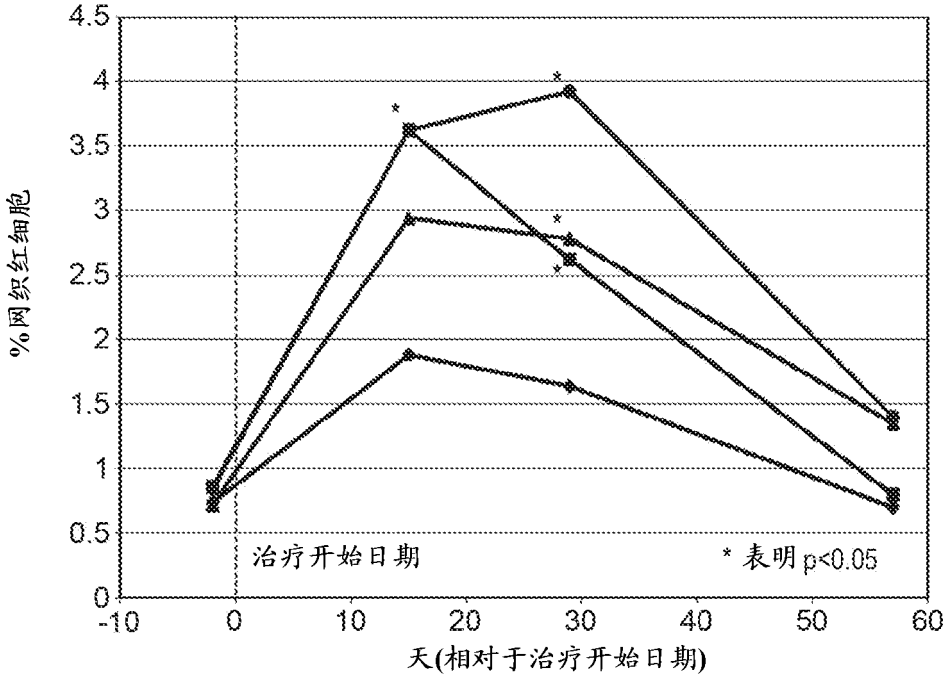


图 7B

ActRIIA-Fc 对雄性 NHPs 网织红细胞计数的影响

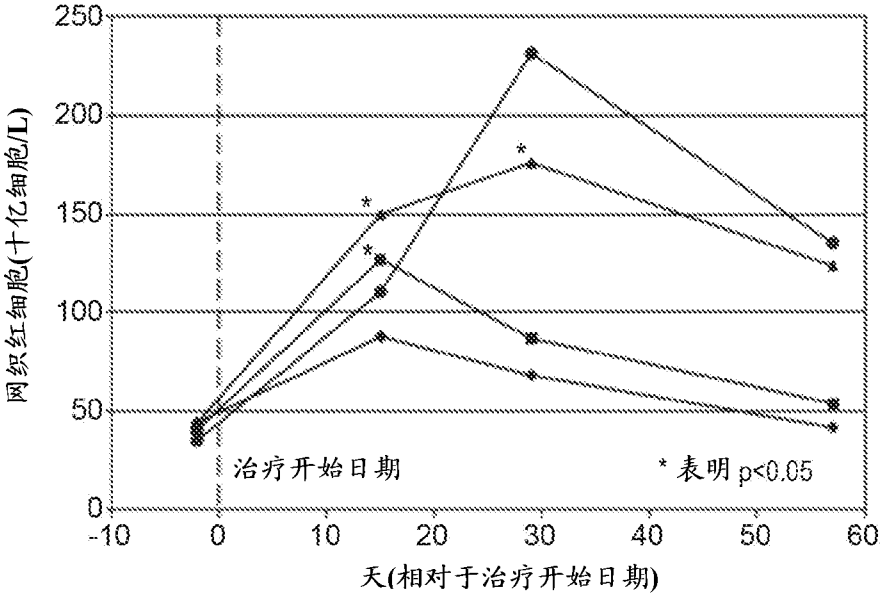
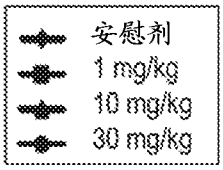


图 8A



ActRIIA-Fc 对雄性 NHPs 网织红细胞百分数的影响

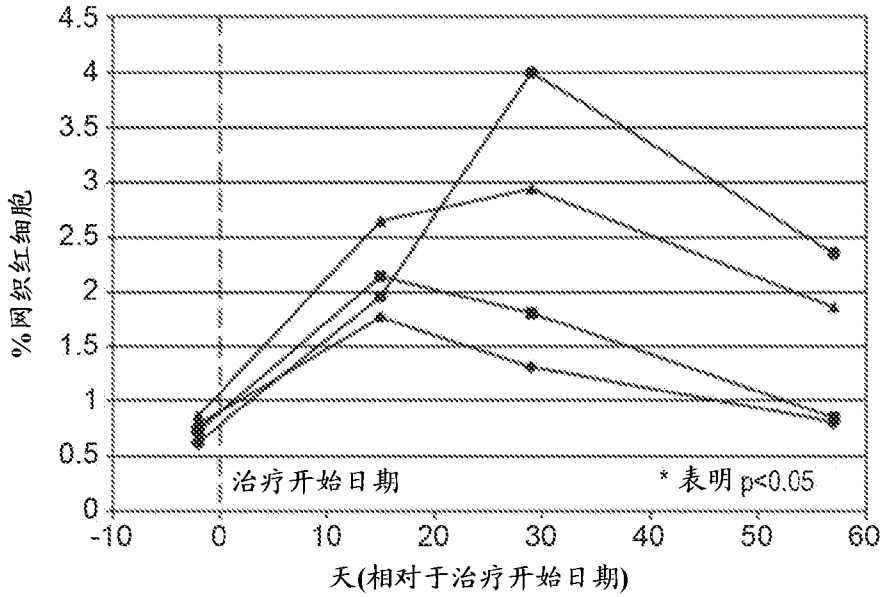


图 8B

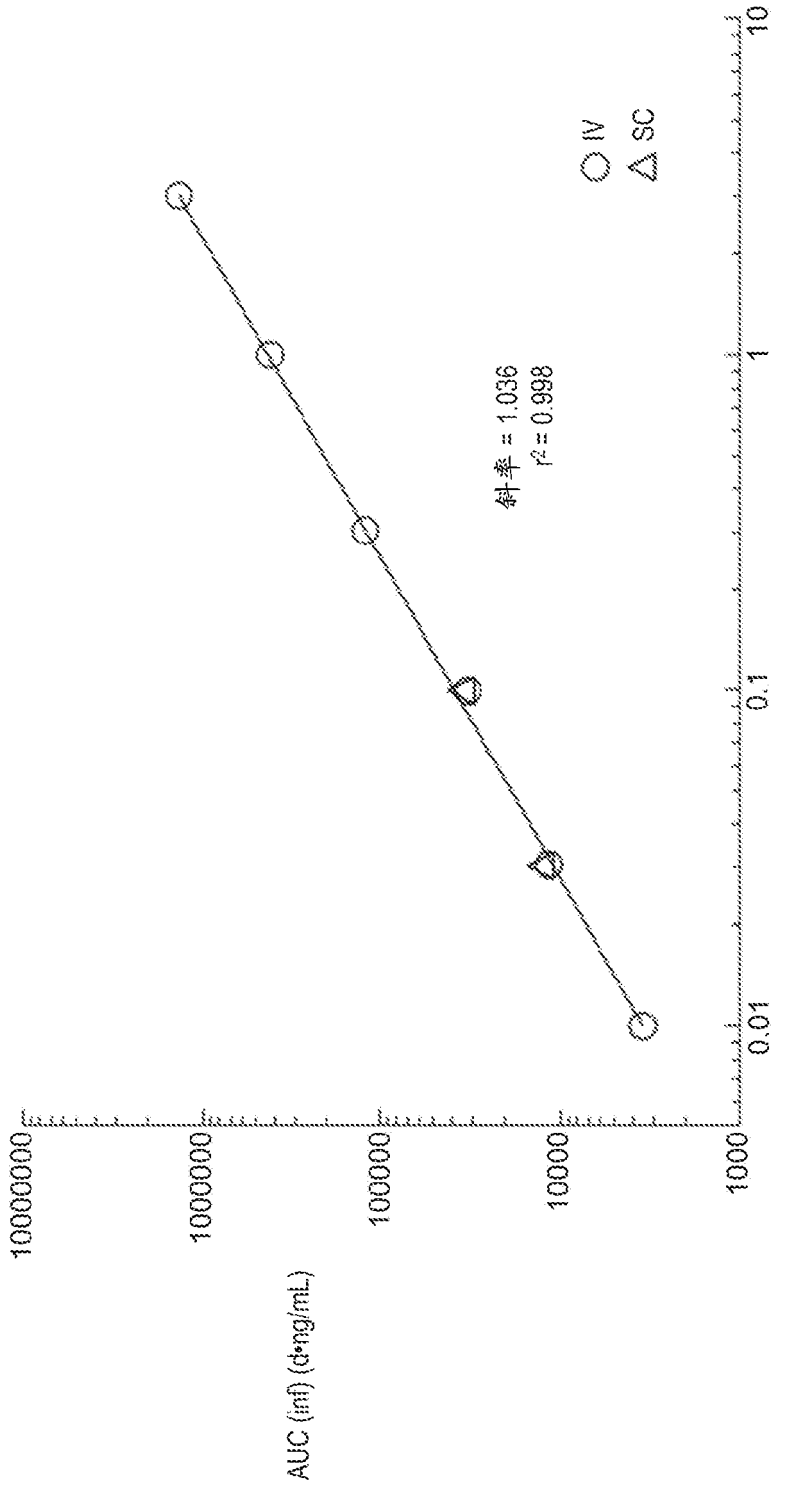

10000000
1000000
100000
10000
1000
AUC (inf) (d•ng/mL)
0.01
0.1
1
10
斜率 = 1.036
r² = 0.998
IV
SC

图 9

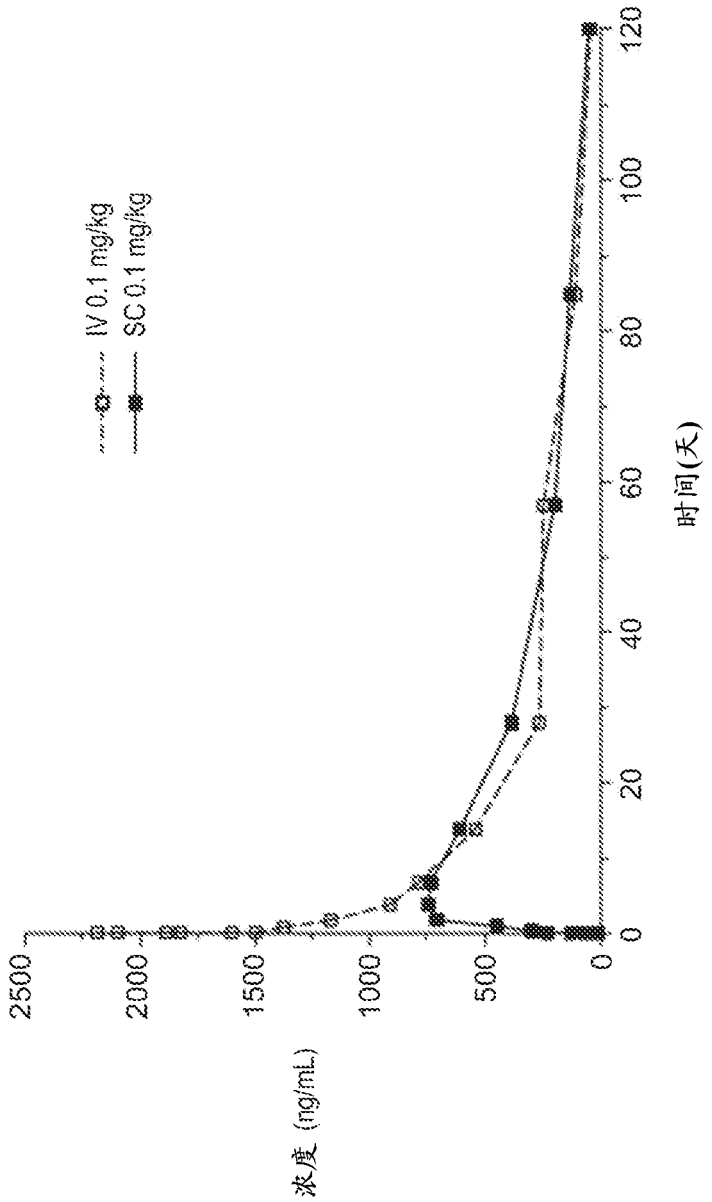

IV 0.1 mg/kg
SC 0.1 mg/kg
浓度 (ng/mL)
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
时间(天)

图 10

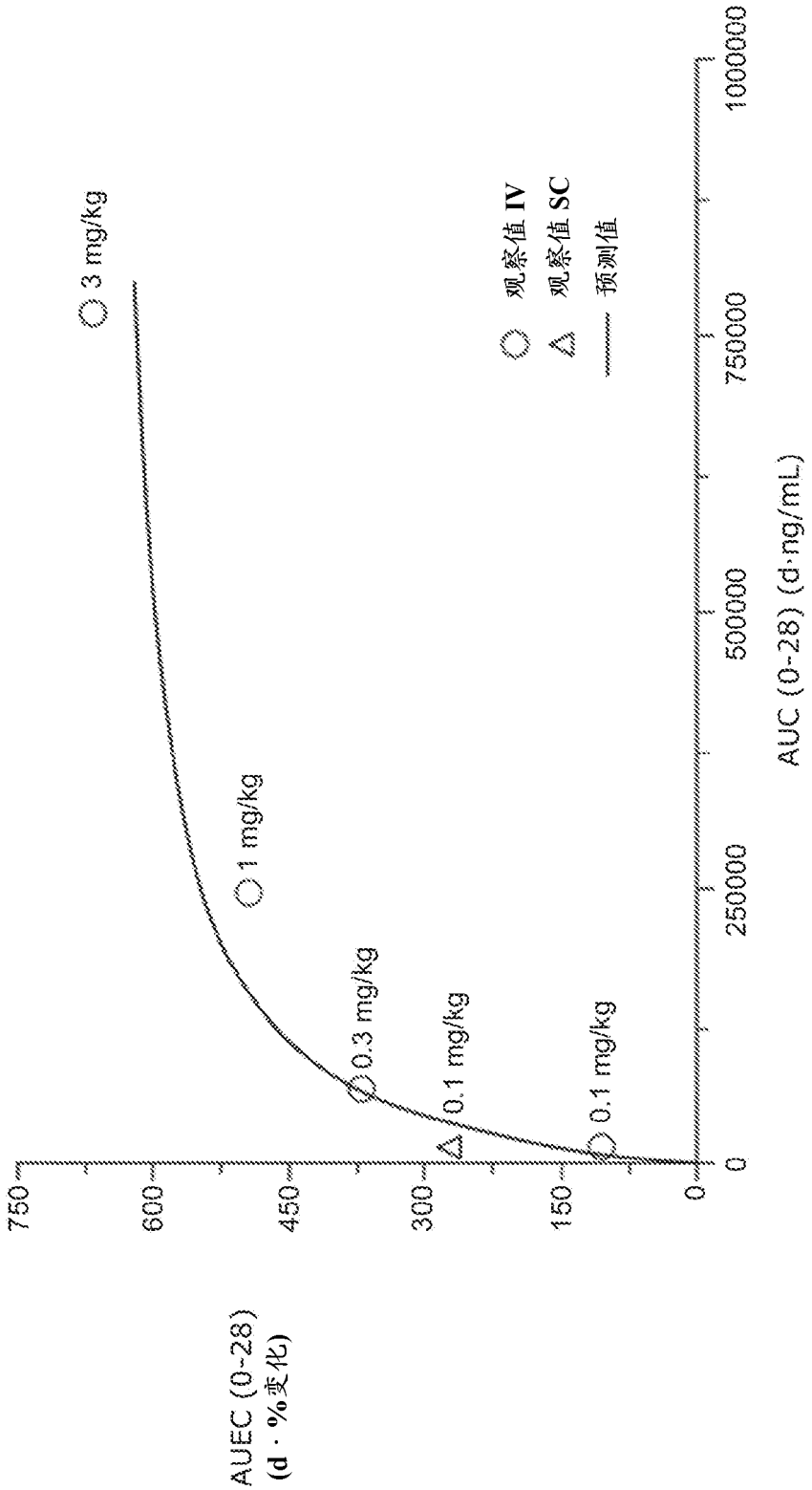

AUEC (0-28)
(d · %变化)
750
600
450
300
150
0
0
250000
500000
750000
1000000
AUC (0-28) (d·ng/mL)
观察值 IV
观察值 SC
预测值
3 mg/kg
1 mg/kg
0.3 mg/kg
0.1 mg/kg
0.1 mg/kg

图 11

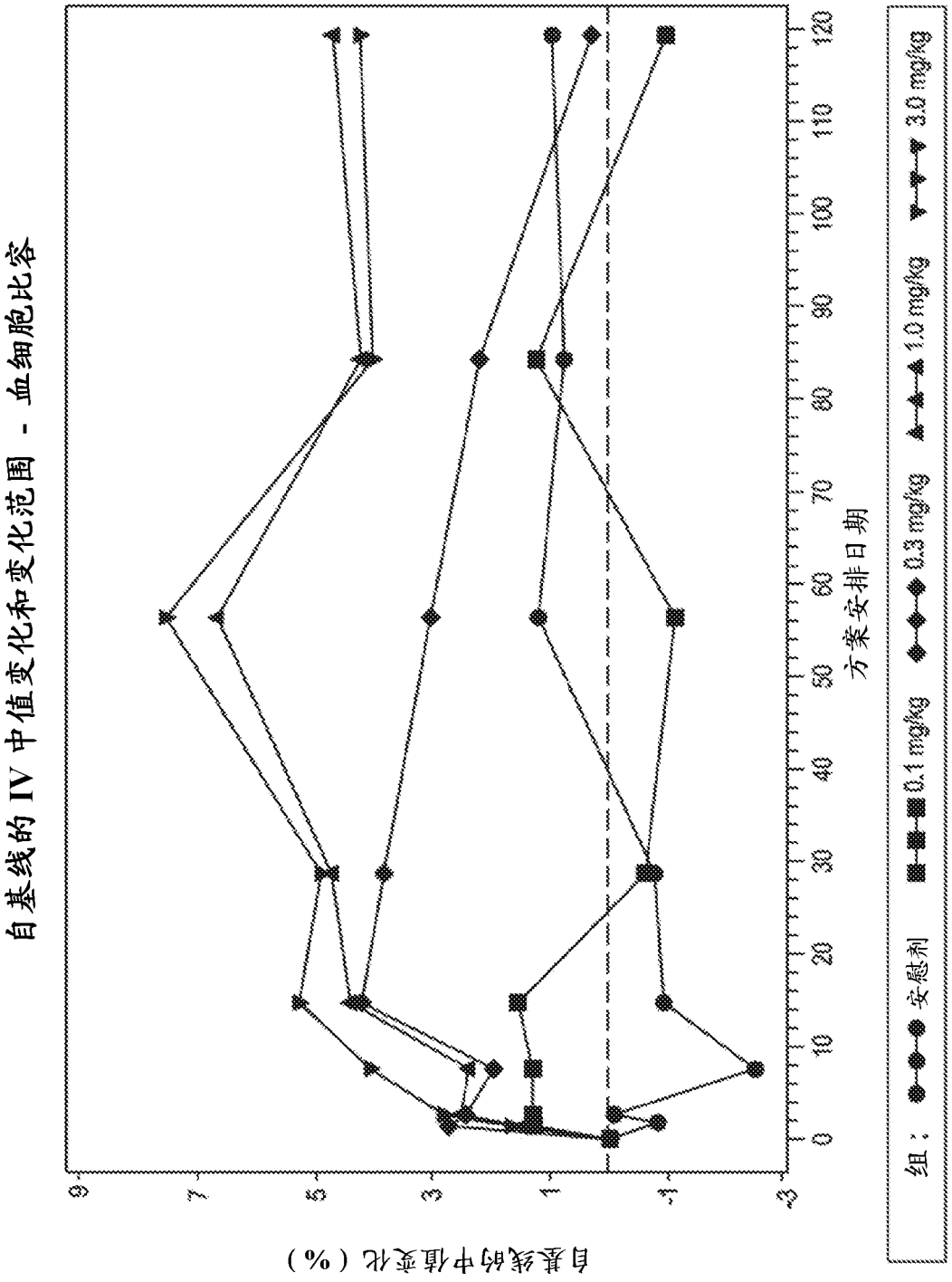

自基线的 IV 中值变化和变化范围 - 血细胞比容
自基线的中值变化（%）
9
7
5
3
1
-1
-3
0
10
20
30
40
50
60
70
80
90
100
110
120
方案安排日期
组：
安慰剂
0.1 mg/kg
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg

图 12

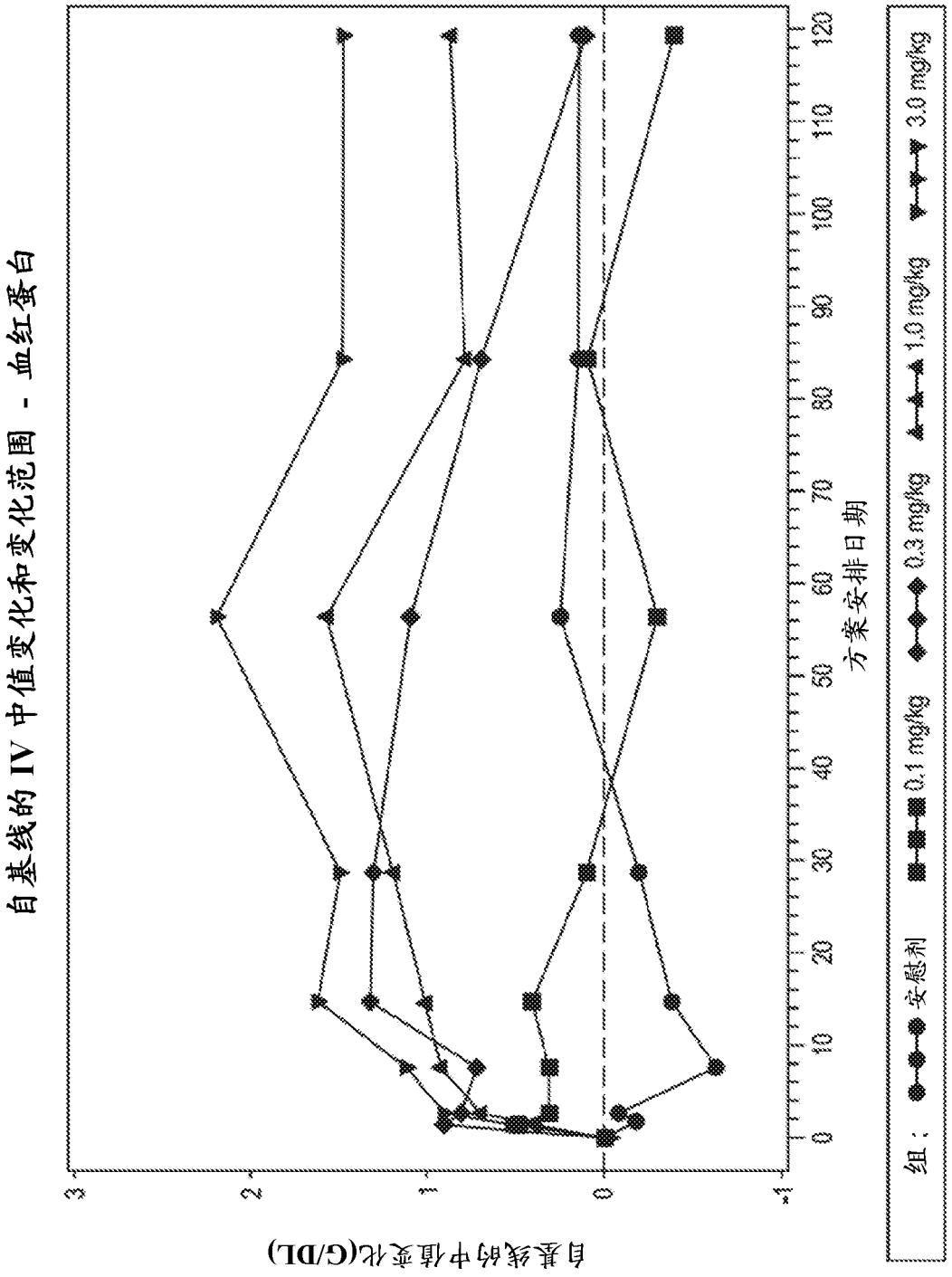

自基线的 IV 中值变化和变化范围 - 血红蛋白
自基线的中值变化(G/DL)
方案安排日期
组：
安慰剂
0.1 mg/kg
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg

图 13

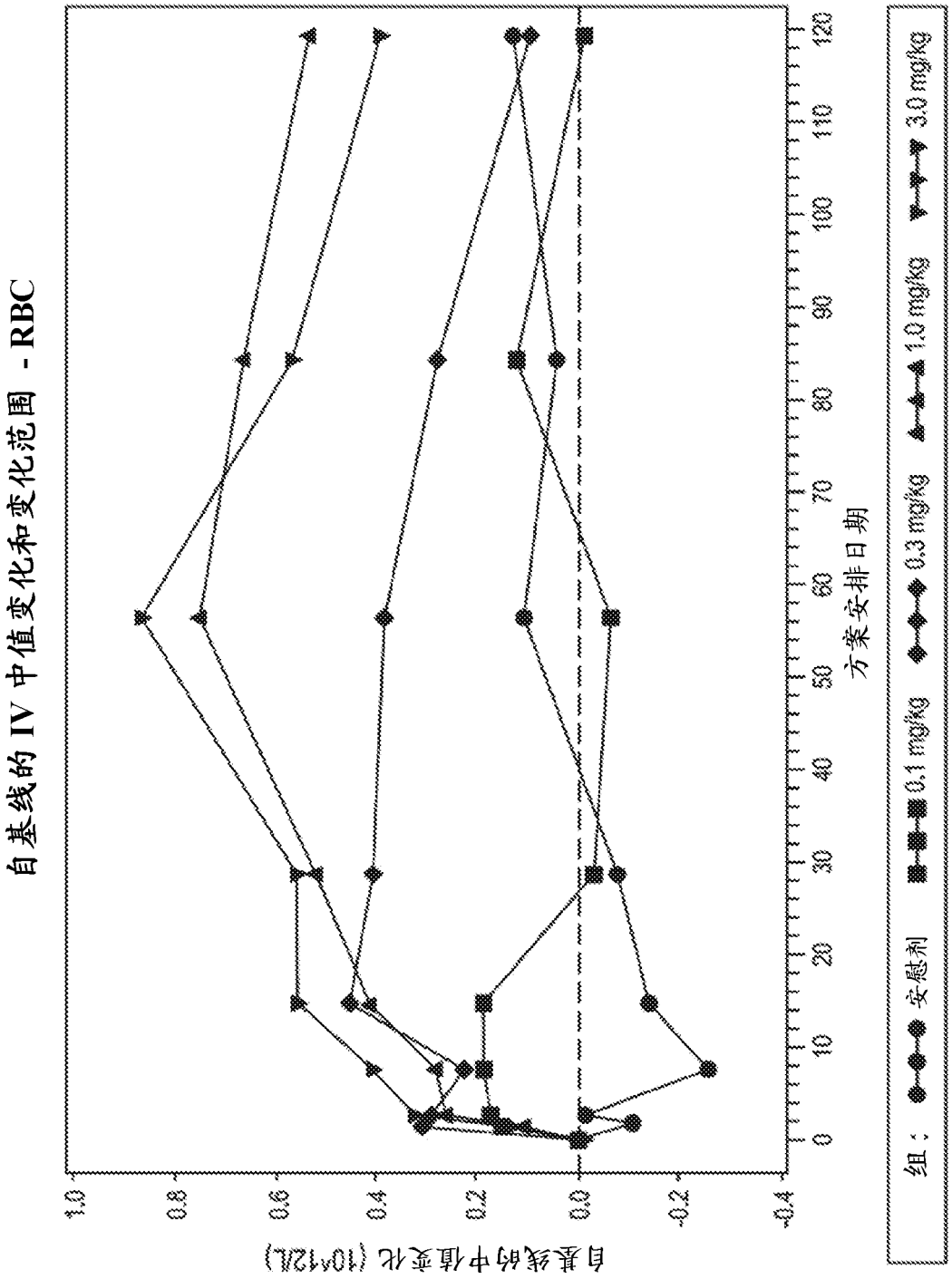

自基线的 IV 中值变化和变化范围 - RBC
自基线的中值变化 (10^12/L)
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
0
10
20
30
40
50
60
70
80
90
100
110
120
方案安排日期
组：
安慰剂
0.1 mg/kg
0.3 mg/kg
1.0 mg/kg
3.0 mg/kg

图 14

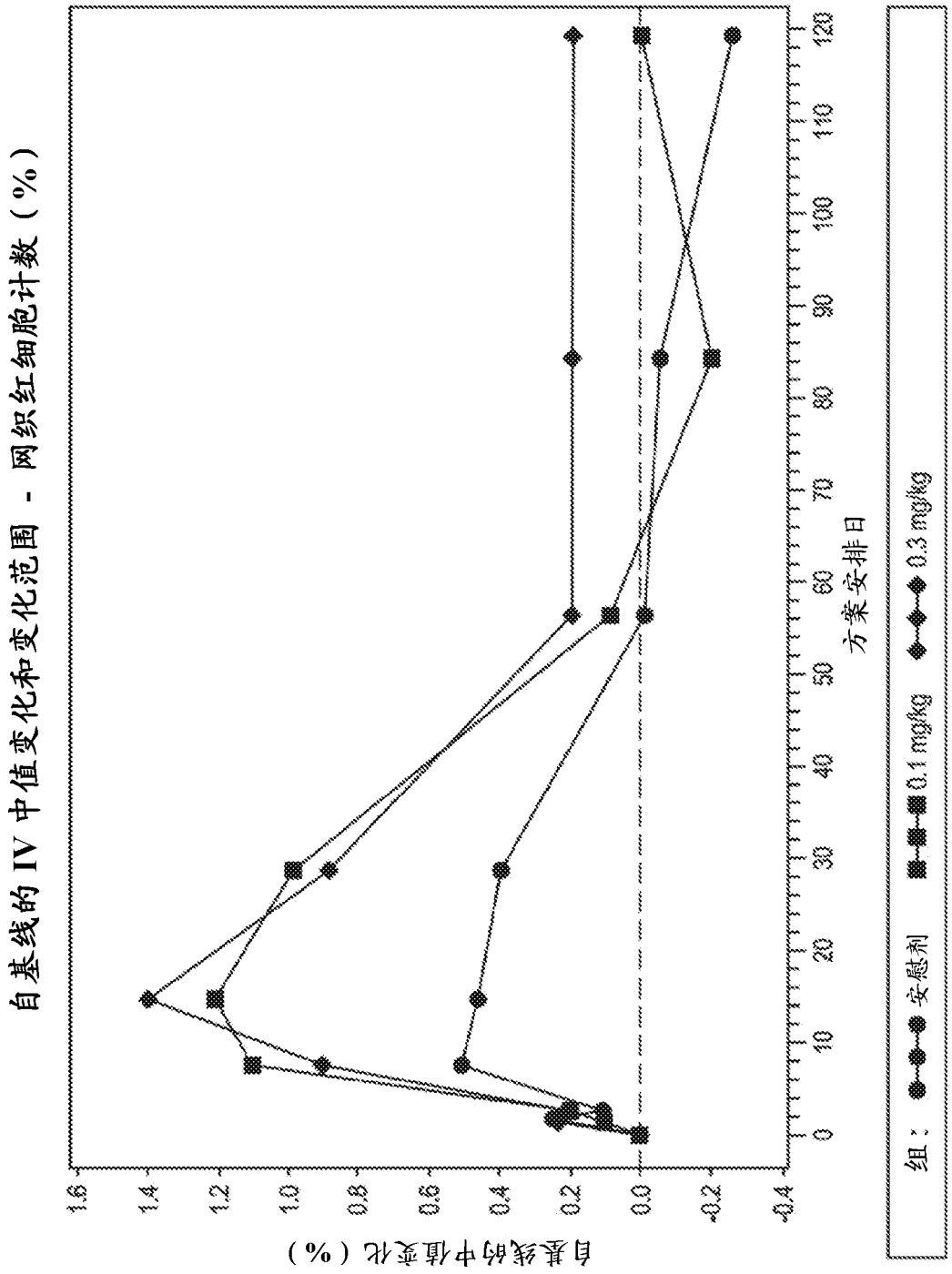

自基线的 IV 中值变化和变化范围 - 网织红细胞计数（%）
自基线的中值变化（%）
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
0
10
20
30
40
50
60
70
80
90
100
110
120
方案安排日
组：
安慰剂
0.1 mg/kg
0.3 mg/kg

图 15

```
  1  MDAMKRGLCC VLLLCGAVFV SPGASGRGEA ETRECIYYNA NWELERTNQS
 51  GLERCEGEQD KRLHCYASWR NSSGTIELVK KGCWDDDFNC YDRQECVATE
101  ENPQVYFCCC EGNFCNERFT HLPEAGGPEV TYEPPPTAPT GGGTHTCPPC
151  PAPELLGGPS VFLFPPKPKD TLMISRTPEV TCVVVDVSHE DPEVKFNWYV
201  DGVEVHNAKT KPREEQYNST YRVVSVLTVL HQDWLNGKEY KCKVSNKALP
251  APIEKTISKA KGQPREPQVY TLPPSREEMT KNQVSLTCLV KGFYPSDIAV
301  EWESNGQPEN NYKTTPPVLD SDGSFFLYSK LTVDKSRWQQ GNVFSCSVMH
351  EALHNHYTQK SLSLSPGK (SEQ ID NO:30)
```

图 16

```
   1  ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
      TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

  51  AGTCTTCGTT TCGCCCGGCG CCTCTGGGCG TGGGCAGGCT GAGACACGGG
      TCAGAAGCAA AGCGGGCCGC GGAGACCCGC ACCCCTCCGA CTCTGTGCCC

 101  AGTGCATCTA CTACAACGCC AACTGGGAGC TGGAGCGCAC CAACCAGAGC
      TCACGTAGAT GATGTTGCGG TTGACCCTCG ACCTCGCGTG GTTGGTCTCG

 151  GGCCTGGAGC GCTGCGAAGG CGAGCAGGAC AAGCGGCTGC ACTGCTACGC
      CCGGACCTCG CGACGCTTCC GCTCGTCCTG TTCGCCGACG TGACGATGCG

 201  CTCCTGGCGC AACAGCTCTG GCACCATCGA GCTCGTGAAG AAGGGCTGCT
      GAGGACCGCG TTGTCGAGAC CGTGGTAGCT CGAGCACTTC TTCCCGACGA

 251  GGGATGATGA CTTCAACTGC TACGATAGGC AGGAGTGTGT GGCCACTGAG
      CCCTACTACT GAAGTTGACG ATGCTATCCG TCCTCACACA CCGGTGACTC

 301  GAGAACCCCC AGGTGTACTT CTGCTGCTGT GAAGGCAACT TCTGCAACGA
      CTCTTGGGGG TCCACATGAA GACGACGACA CTTCCGTTGA AGACGTTGCT

 351  GCGCTTCACT CATTTGCCAG AGGCTGGGGG CCCGCAAGTC ACGTACGAGC
      CGCGAAGTGA GTAAACGGTC TCCGACCCCC GGGCCTTCAG TGCATGCTCG

 401  CACCCCCGAC AGCCCCCACC GGTGGTGGAA CTCACACATG CCCACCGTGC
      GTGGGGGCTG TCGGGGGTGG CCACCACCTT GAGTGTGTAC GGGTGGCACG

 451  CCAGCACCTG AACTCCTGGG GGGACCGTCA GTCTTCCTCT TCCCCCCAAA
      GGTCGTGGAC TTGAGGACCC CCCTGGCAGT CAGAAGGAGA AGGGGGGTTT

 501  ACCCAAGGAC ACCCTCATGA TCTCCCGGAC CCCTGAGGTC ACATGCGTGG
      TGGGTTCCTG TGGGAGTACT AGAGGGCCTG GGGACTCCAG TGTACGCACC

 551  TGGTGGACGT GAGCCACGAA GACCCTGAGG TCAAGTTCAA CTGGTACGTG
      ACCACCTGCA CTCGGTGCTT CTGGGACTCC AGTTCAAGTT GACCATGCAC

 601  GACGGCGTGG AGGTGCATAA TGCCAAGACA AAGCCGCGGG AGGAGCAGTA
      CTGCCGCACC TCCACGTATT ACGGTTCTGT TTCGGCGCCC TCCTCGTCAT

 651  CAACAGCACG TACCGTGTGG TCAGCGTCCT CACCGTCCTG CACCAGGACT
      GTTGTCGTGC ATGGCACACC AGTCGCAGGA GTGGCAGGAC GTGGTCCTGA

 701  GGCTGAATGG CAAGGAGTAC AAGTGCAAGG TCTCCAACAA AGCCCTCCCA
      CCGACTTACC GTTCCTCATG TTCACGTTCC AGAGGTTGTT TCGGGAGGGT

 751  GCCCCCATCG AGAAAACCAT CTCCAAAGCC AAAGGGCAGC CCCGAGAACC
      CGGGGGGTAGC TCTTTTGGTA GAGGTTTCGG TTTCCCGTCG GGGCTCTTGG
```

图 17A

```
 801   ACAGGTGTAC ACCCTGCCCC CATCCCGGGA GGAGATGACC AAGAACCAGG
       TGTCCACATG TGGGACGGGG GTAGGGCCCT CCTCTACTGG TTCTTGGTCC

 851   TCAGCCTGAC CTGCCTGGTC AAAGGCTTCT ATCCCAGCGA CATCGCCGTG
       AGTCGGACTG GACGGACCAG TTTCCGAAGA TAGGGTCGCT GTAGCGGCAC

 901   GAGTGGGAGA GCAATGGGCA GCCGGAGAAC AACTACAAGA CCACGCCTCC
       CTCACCCTCT CGTTACCCGT CGGCCTCTTG TTGATGTTCT GGTGCGGAGG

 951   CGTGCTGGAC TCCGACGGCT CCTTCTTCCT CTATAGCAAG CTCACCGTGG
       GCACGACCTG AGGCTGCCGA GGAAGAAGGA GATATCGTTC GAGTGGCACC

1001   ACAAGAGCAG GTGGCAGCAG GGGAACGTCT TCTCATGCTC CGTGATGCAT
       TGTTCTCGTC CACCGTCGTC CCCTTGCAGA AGAGTACGAG GCACTACGTA

1051   GAGGCTCTGC ACAACCACTA CACGCAGAAG AGCCTCTCCC TGTCCCCGGG
       CTCCGAGACG TGTTGGTGAT GTGCGTCTTC TCGGAGAGGG ACAGGGGCCC

1101   TAAATGA (SEQ ID NO:39)
       ATTTACT (SEQ ID NO:40)
```

图 17B

```
  1 MDAMKRGLCC VLLLCGAVFV SPGAAETREC IYYNANWELE RTNQSGLERC
 51 EGEQDKRLHC YASWRNSSGT IELVKKGCWD DDFNCYDRQE CVATEENPQV
101 YFCCCEGNFC NERFTHLPEA GGPEVTYEPP PTGGGTHTCP PCPAPELLGG
151 PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA
201 KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS
251 KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP
301 ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT
351 QKSLSLSPGK (SEQ ID NO: 41)
```

图 18

```
                                                                  E  T   R  E  C   I  Y  Y
  1 ATGGATGCAA TGAACAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
    TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG

    N  A  N  W   E  L  E   R  T  N   Q  S  G  L   E  R  C
 51 ACGCCAACTG GGAGCTGGAG CGCACCAACC AGAGCGGCCT GGAGCGCTGC
    TGCGGTTGAC CCTCGACCTC GCGTGGTTGG TCTCGCCGGA CCTCGCGACG

    E  G  E   Q  D  K  R   L  H  C   Y  A  S   W  R  N  S
101 GAAGGCGAGC AGGACAAGCG GCTGCACTGC TACGCCTCCT GGCGCAACAG
    CTTCCGCTCG TCCTGTTCGC CGACGTGACG ATGCGGAGGA CCGCGTTGTC

    S  G  T   I  E  L   V  K  K  G   C  W  D   D  D  F
151 CTCTGGCACC ATCGAGCTCG TGAAGAAGGG CTGCTGGGAC GATGACTTCA
    GAGACCGTGG TAGCTCGAGC ACTTCTTCCC GACGACCCTG CTACTGAAGT

    N  C  Y  D   R  Q  E   C  V  A   T  E  E  N   P  Q  V
201 ACTGCTACGA TAGGCAGGAG TGTGTGGCCA CTGAGGAGAA CCCCCAGGTG
    TGACGATGCT ATCCGTCCTC ACACACCGGT GACTCCTCTT GGGGGTCCAC

    Y  F  C   C  C  E  G   N  F  C   N  E  R   F  T  H  L
251 TACTTCTGCT GCTGTGAAGG CAACTTCTGC AACGAGCGCT TCACTCATTT
    ATGAAGACGA CGACACTTCC GTTGAAGACG TTGCTCGCGA AGTGAGTAAA

    P  E  A   G  G  P   E  V  T  Y   E  P  P   P  T
301 GCCAGAGGCT GGGGGCCCGG AAGTCACGTA CGAGCCACCC CCGACAGGTG
    CGGTCTCCGA CCCCCGGGCC TTCAGTGCAT GCTCGGTGGG GGCTGTCCAC

351 GTGGAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA
    CACCTTGAGT GTGTACGGGT GGCACGGGTC GTGGACTTGA GGACCCCCCT

401 CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
    GGCAGTCAGA AGGAGAAGGG GGGTTTTGGG TTCCTGTGGG AGTACTAGAG

451 CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC
    GGCCTGGGGA CTCCAGTGTA CGCACCACCA CCTGCACTCG GTGCTTCTGG

501 CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC
    GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG
```

图 19A

```
 601  AAGACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG
      TTCTGTTTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAGTC

 651  CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
      GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA

 701  GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
      CGTTCCAGAG GTTGTTTCGG GAGGGTCGGG GGTAGCTCTT TTGGTAGAGG

 751  AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC
      TTTCGGTTTC CCGTCGGGGC TCTTGGTGTC CACATGTGGG ACGGGGGTAG

 801  CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
      GGCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

 851  GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
      CGAAGATAGG GTCGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

 901  GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT
      CTCTTGTTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

 951  CTTCCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
      GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTCGTCCCCT

1001  ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
      TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051  CAGAAGAGCC TCTCCCTGTC CCCGGGTAAA TGA   (SEQ ID NO: 42)
      GTCTTCTCGG AGAGGGACAG GGGCCCATTT ACT   (SEQ ID NO: 43)
```

图 19B

```
   1   ETPECIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK

  51   KGCWQDDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV

 101   TYEPPPTGGG THTCPPCPAP ELLGGPSVFL FPPKPKDTLM ISRTPEVTCV

 151   VVDVSHEDPE VKFNWYVDGV EVHNAKTKPR EEQYNSTYRV VSVLTVLHQD

 201   WLNGKEYKCK VSNKALPAPI EKTISKAKGQ PREPQVYTLP PSREEMTKNQ

 251   VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV

 301   DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK (SEQ ID NO: 44)
```

图 20

```
  1   [E]TRRCIYYNA NWELERTNQS GLERCEGEQD KRLHCYASWR NSSGTIELVK
 51   KGCW[D]DDFNC YDRQECVATE ENPQVYFCCC EGNFCNERFT HLPEAGGPEV
101   TYEPPPT  (SEQ ID NO: 45)
```

图 21

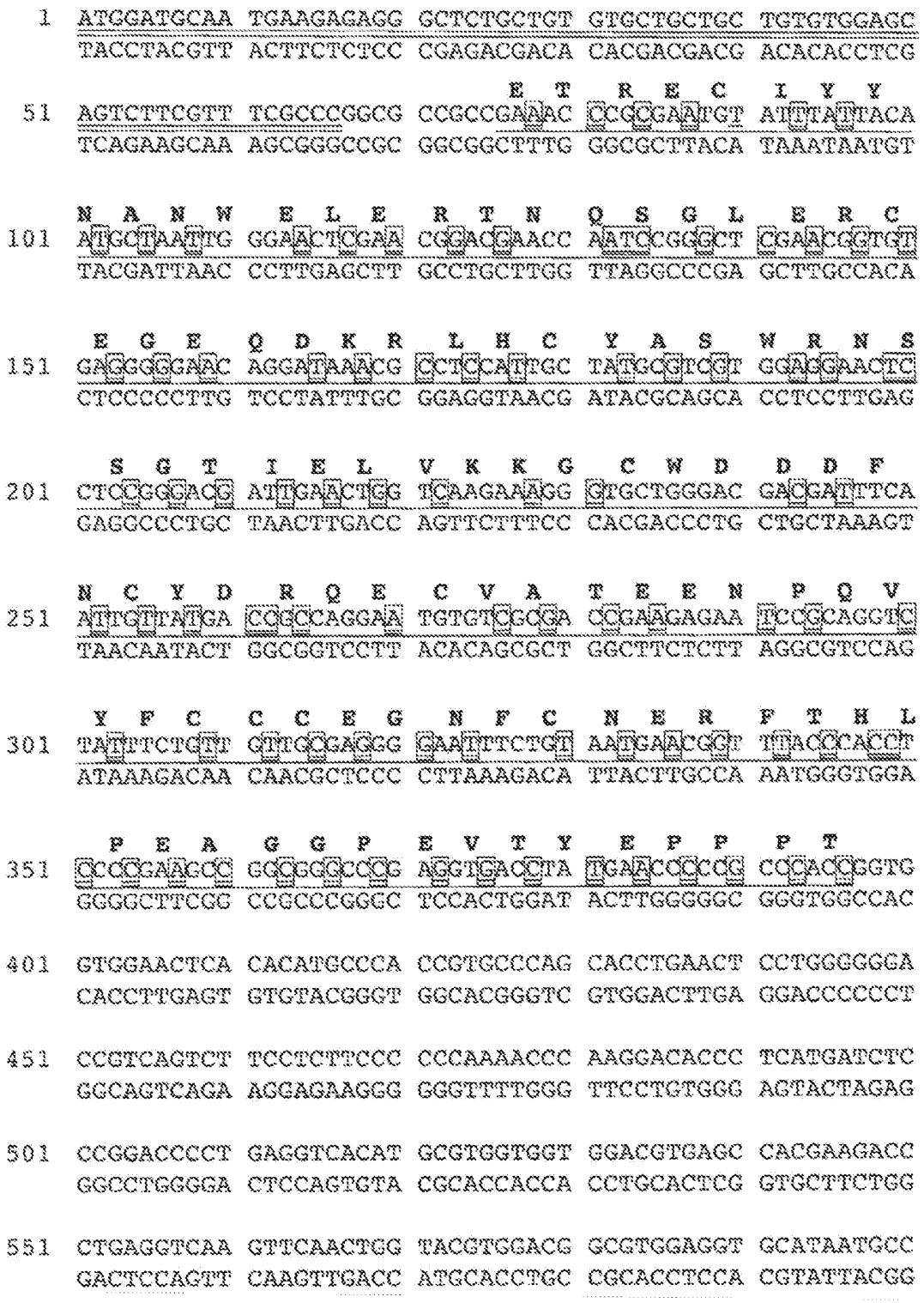

1 ATGGATGCAA TGAAGAGAGG GCTCTGCTGT GTGCTGCTGC TGTGTGGAGC
TACCTACGTT ACTTCTCTCC CGAGACGACA CACGACGACG ACACACCTCG
E T R E C I Y Y
51 AGTCTTCGTT TCGCCCGGCG CCGCCGAAAC CCGCGAATGT ATTTATTACA
TCAGAAGCAA AGCGGGCCGC GGCGGCTTTG GGCGCTTACA TAAATAATGT
N A N W E L E R T N Q S G L E R C
101 ATGCTAATTG GGACTCGAA CGGACGAACC AATCCGGGCT CGAACGGTGT
TACGATTAAC CCTTGAGCTT GCCTGCTTGG TTAGGCCCGA GCTTGCCACA
E G E Q D K R L H C Y A S W R N S
151 GAGGGGGAAC AGGATAAACG CCTCCATTGC TATGCGTCGT GGAGGAACTC
CTCCCCCTTG TCCTATTTGC GGAGGTAACG ATACGCAGCA CCTCCTTGAG
S G T I E L V K K G C W D D D F
201 CTCCGGCACG ATTGAACTGG TCAAGAAAGG GTGCTGGGAC GACGATTTCA
GAGGCCCTGC TAACTTGACC AGTTCTTTCC CACGACCCTG CTGCTAAAGT
N C Y D R Q E C V A T E E N P Q V
251 ATTGTTATGA CCGCCAGGAA TGTGTCGCGA CCGAAGAGAA TCCGCAGGTC
TAACAATACT GGCGGTCCTT ACACAGCGCT GGCTTCTCTT AGGCGTCCAG
Y F C C C E G N F C N E R F T H L
301 TATTTCTGTT GTTGCGAGGG GAATTTCTGT AATGAACGCT TTACCCACCT
ATAAAGACAA CAACGCTCCC CTTAAAGACA TTACTTGCCA AATGGGTGGA
P E A G G P E V T Y E P P P T
351 CCCCGAACCC GGCGGGCCCG AGGTGACCTA TGAACCCCCG CCCACCGGTG
GGGGCTTCGG CCGCCCGGGC TCCACTGGAT ACTTGGGGGC GGGTGGCCAC
401 GTGGAACTCA CACATGCCCA CCGTGCCCAG CACCTGAACT CCTGGGGGGA
CACCTTGAGT GTGTACGGGT GGCACGGGTC GTGGACTTGA GGACCCCCCT
451 CCGTCAGTCT TCCTCTTCCC CCCAAAACCC AAGGACACCC TCATGATCTC
GGCAGTCAGA AGGAGAAGGG GGGTTTTGGG TTCCTGTGGG AGTACTAGAG
501 CCGGACCCCT GAGGTCACAT GCGTGGTGGT GGACGTGAGC CACGAAGACC
GGCCTGGGGA CTCCAGTGTA CGCACCACCA CCTGCACTCG GTGCTTCTGG
551 CTGAGGTCAA GTTCAACTGG TACGTGGACG GCGTGGAGGT GCATAATGCC
GACTCCAGTT CAAGTTGACC ATGCACCTGC CGCACCTCCA CGTATTACGG

图 22A

```
 601 AACACAAAGC CGCGGGAGGA GCAGTACAAC AGCACGTACC GTGTGGTCAG
     TTCTGTTTCG GCGCCCTCCT CGTCATGTTG TCGTGCATGG CACACCAGTC

 651 CGTCCTCACC GTCCTGCACC AGGACTGGCT GAATGGCAAG GAGTACAAGT
     GCAGGAGTGG CAGGACGTGG TCCTGACCGA CTTACCGTTC CTCATGTTCA

 701 GCAAGGTCTC CAACAAAGCC CTCCCAGCCC CCATCGAGAA AACCATCTCC
     CGTTCCAGAG GTTGTTTCGG GAGGGTCGGG GGTAGCTCTT TTGGTAGAGG

 751 AAAGCCAAAG GGCAGCCCCG AGAACCACAG GTGTACACCC TGCCCCCATC
     TTTCGGTTTC CCGTCGGGGC TCTTGGTGTC CACATGTGGG ACGGGGGTAG

 801 CCGGGAGGAG ATGACCAAGA ACCAGGTCAG CCTGACCTGC CTGGTCAAAG
     GGCCCTCCTC TACTGGTTCT TGGTCCAGTC GGACTGGACG GACCAGTTTC

 851 GCTTCTATCC CAGCGACATC GCCGTGGAGT GGGAGAGCAA TGGGCAGCCG
     CGAAGATAGG GTCGCTGTAG CGGCACCTCA CCCTCTCGTT ACCCGTCGGC

 901 GAGAACAACT ACAAGACCAC GCCTCCCGTG CTGGACTCCG ACGGCTCCTT
     CTCTTGTTGA TGTTCTGGTG CGGAGGGCAC GACCTGAGGC TGCCGAGGAA

 951 CTTCCTCTAT AGCAAGCTCA CCGTGGACAA GAGCAGGTGG CAGCAGGGGA
     GAAGGAGATA TCGTTCGAGT GGCACCTGTT CTCGTCCACC GTCGTCCCCT

1001 ACGTCTTCTC ATGCTCCGTG ATGCATGAGG CTCTGCACAA CCACTACACG
     TGCAGAAGAG TACGAGGCAC TACGTACTCC GAGACGTGTT GGTGATGTGC

1051 CAGAAGAGCC TCTCCCTGTC CCCGGGTAAA TGA (SEQ ID NO: 46)
     GTCTTCTCGG AGAGGGACAG GGGCCCATTT ACT (SEQ ID NO: 47)
```

图 22B

```
      GAAAC CCGCGAATGT ATTTATTACA ATGCTAATTG GGAACTCGAA
CGGACGAACC AATCCGGGCT CGAACGGTGT GAGGGGGAAC AGGATAAACG
CCTCCATTGC TATGCGTCGT GGAGGAACTC CTCCGGCACG ATTGAACTGG
TCAAGAAAGG GTGCTGGGAC GACGATTTCA ATTGTTATGA CCGCCAGGAA
TGTGTCGCGA CCGAAGAGAA TCCGCAGGTC TATTTCTGTT GTTGCGAGGG
GAATTTCTGT AATGAACGGT TTACCCACCT CCCCGAAGCC GCCGGGCCCG
AGGTGACCTA TGAACCCCCG CCCACC    (SEQ ID NO: 48)
```

图 23

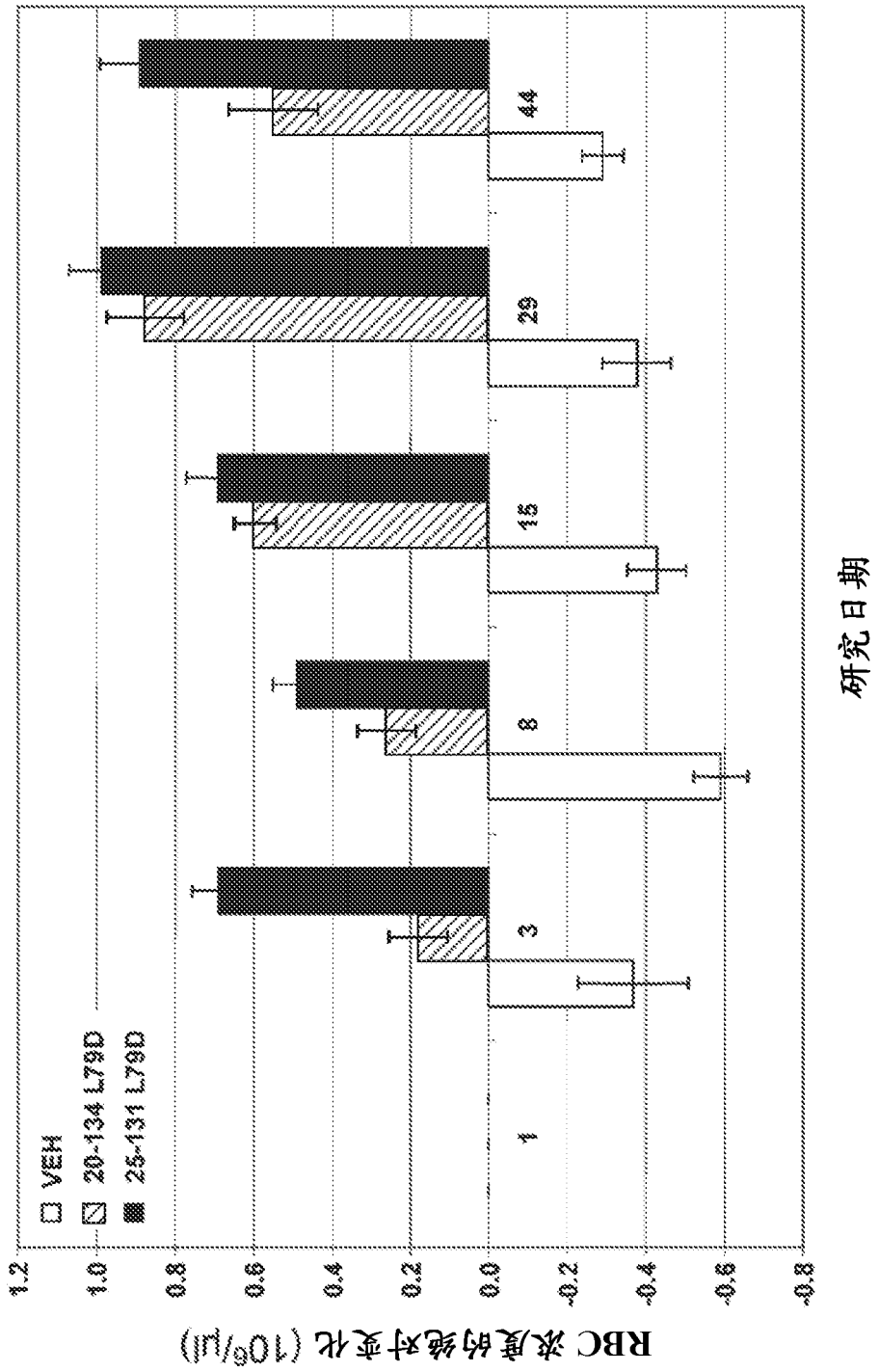

RBC 浓度的绝对变化 (10^6/μl)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
VEH
20-134 L79D
25-131 L79D
1
3
8
15
29
44
研究日期

图 24

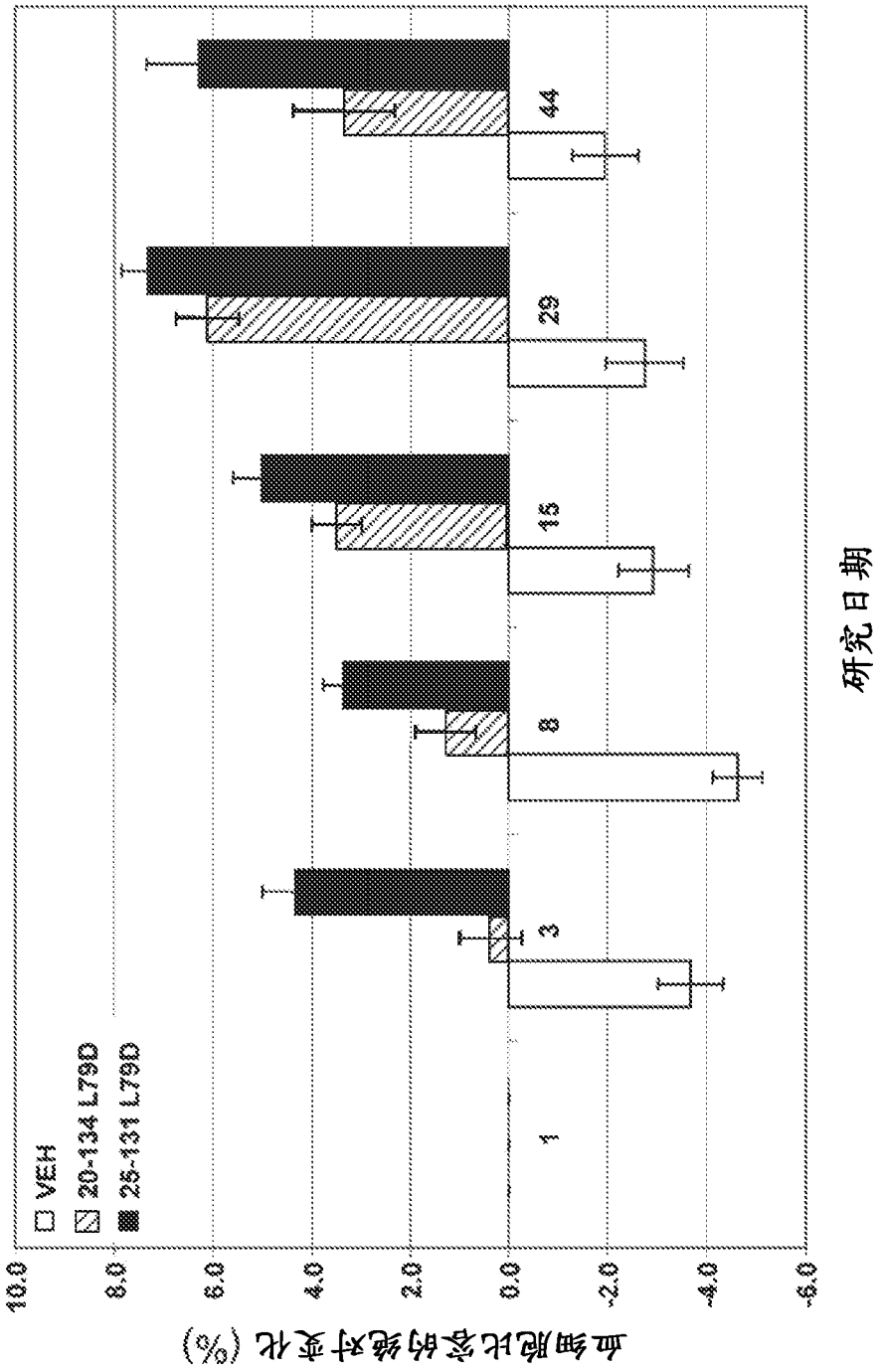

VEH
20-134 L79D
25-131 L79D
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
1
3
8
15
29
44
研究日期
血细胞比容的绝对变化 (%)

图 25

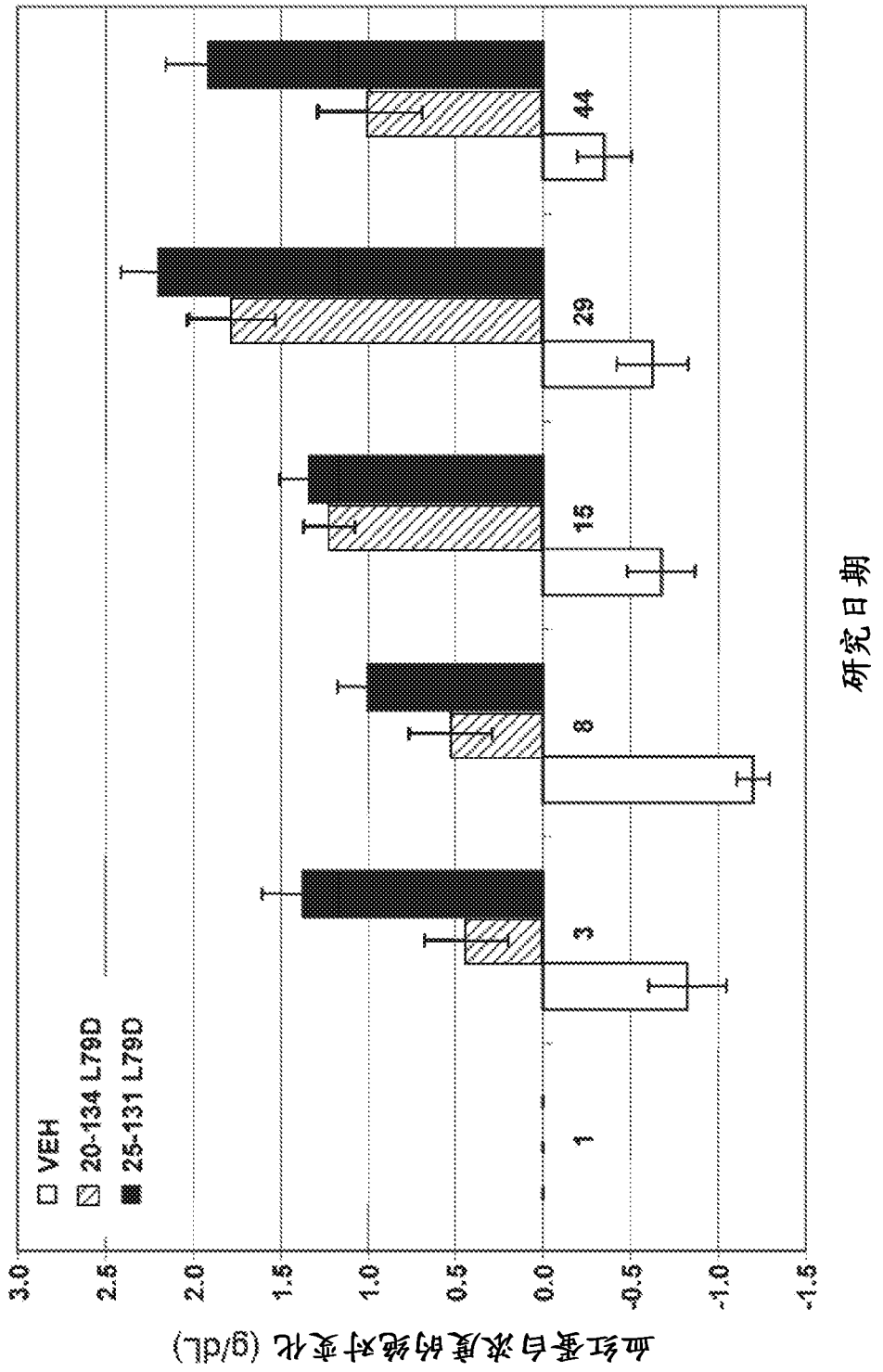

血红蛋白浓度的绝对变化 (g/dL)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
VEH
20-134 L79D
25-131 L79D
1
3
8
15
29
44
研究日期

图 26

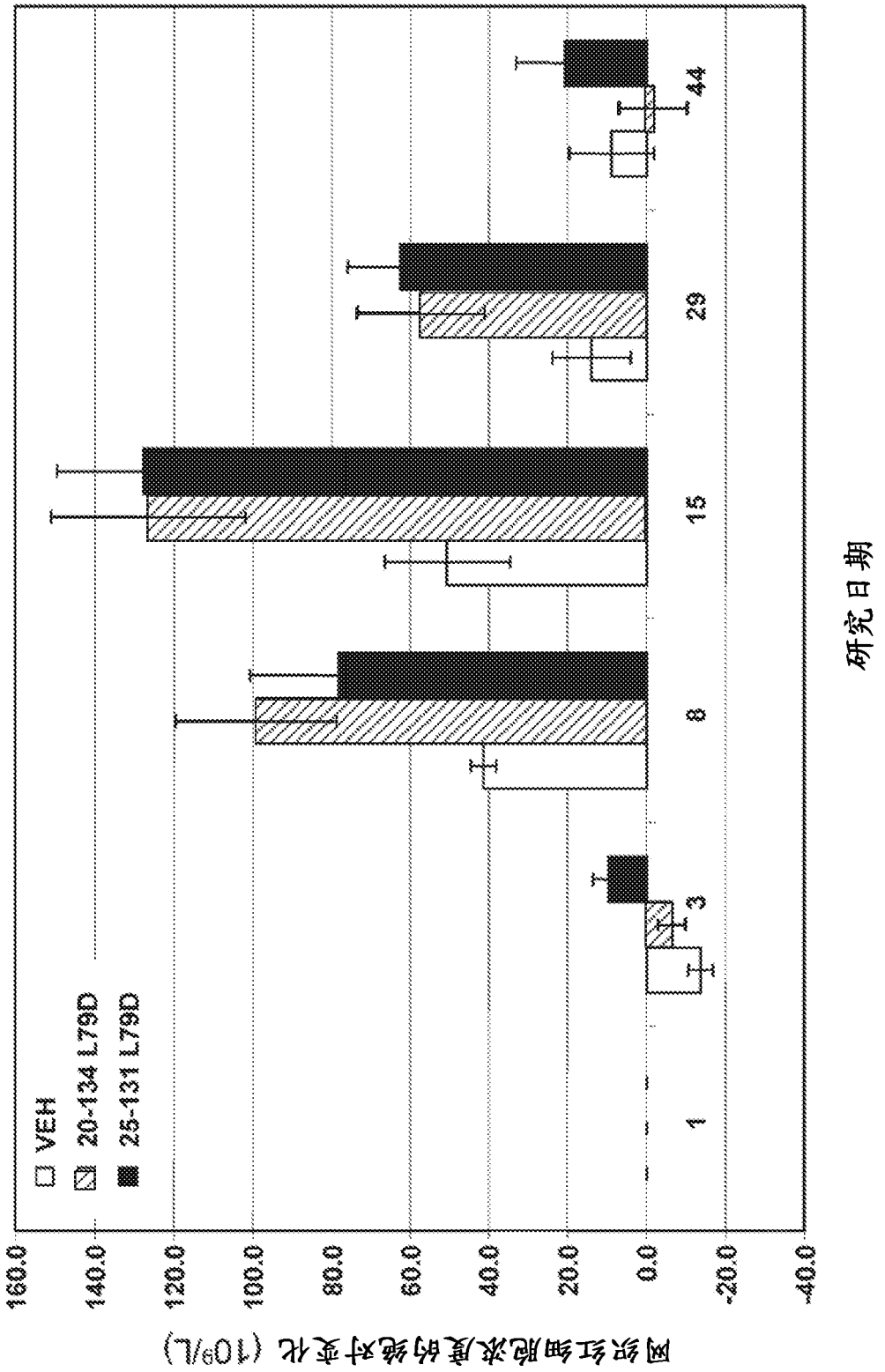

网织红细胞浓度的绝对变化 (10⁹/L)
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
-20.0
-40.0
VEH
20-134 L79D
25-131 L79D
1
3
8
15
29
44
研究日期

图 27

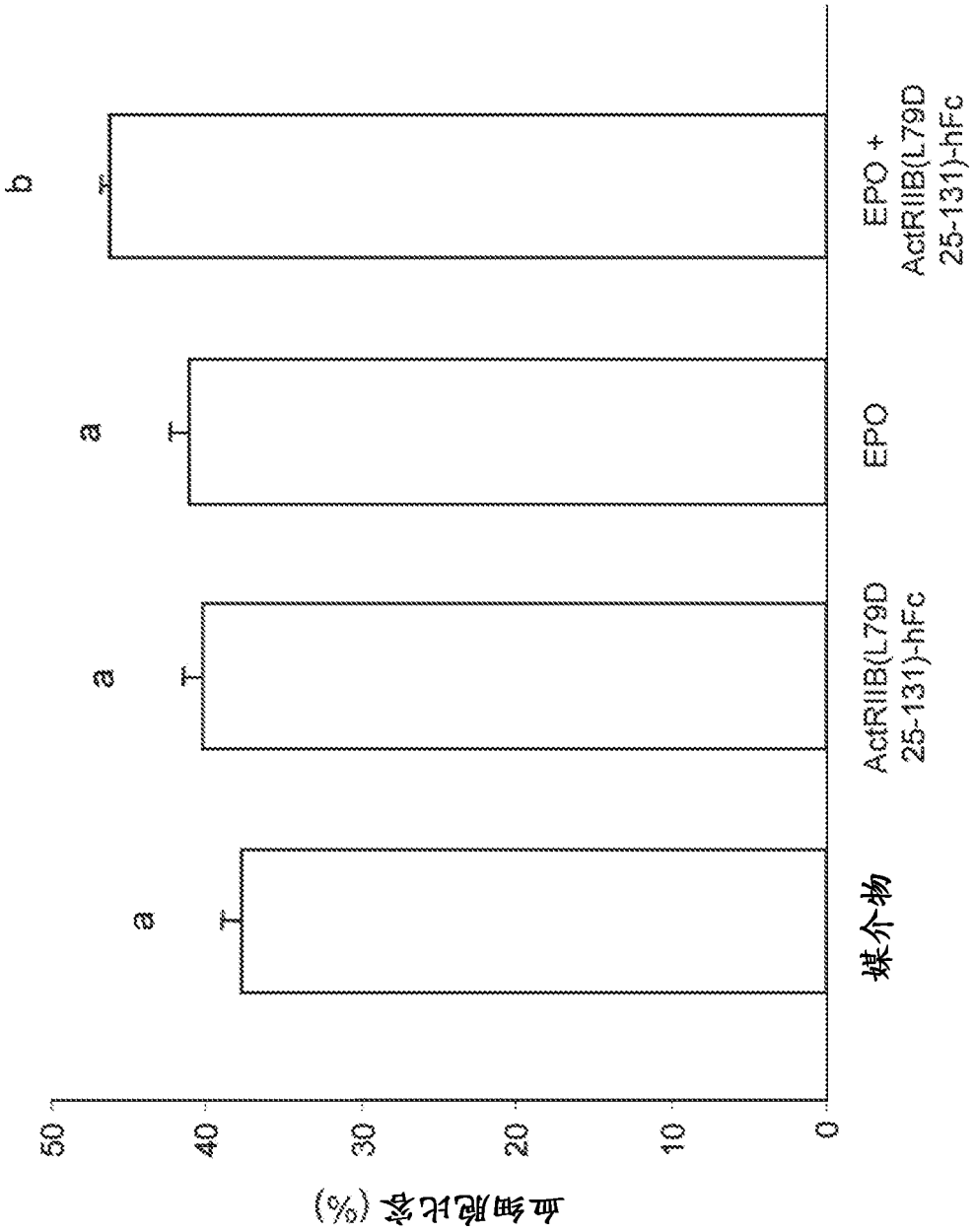

网织红细胞比率 (%)
0
10
20
30
40
50
a
a
a
b
媒介物
ActRIIB(L79D 25-131)-hFc
EPO
EPO + ActRIIB(L79D 25-131)-hFc

图 28

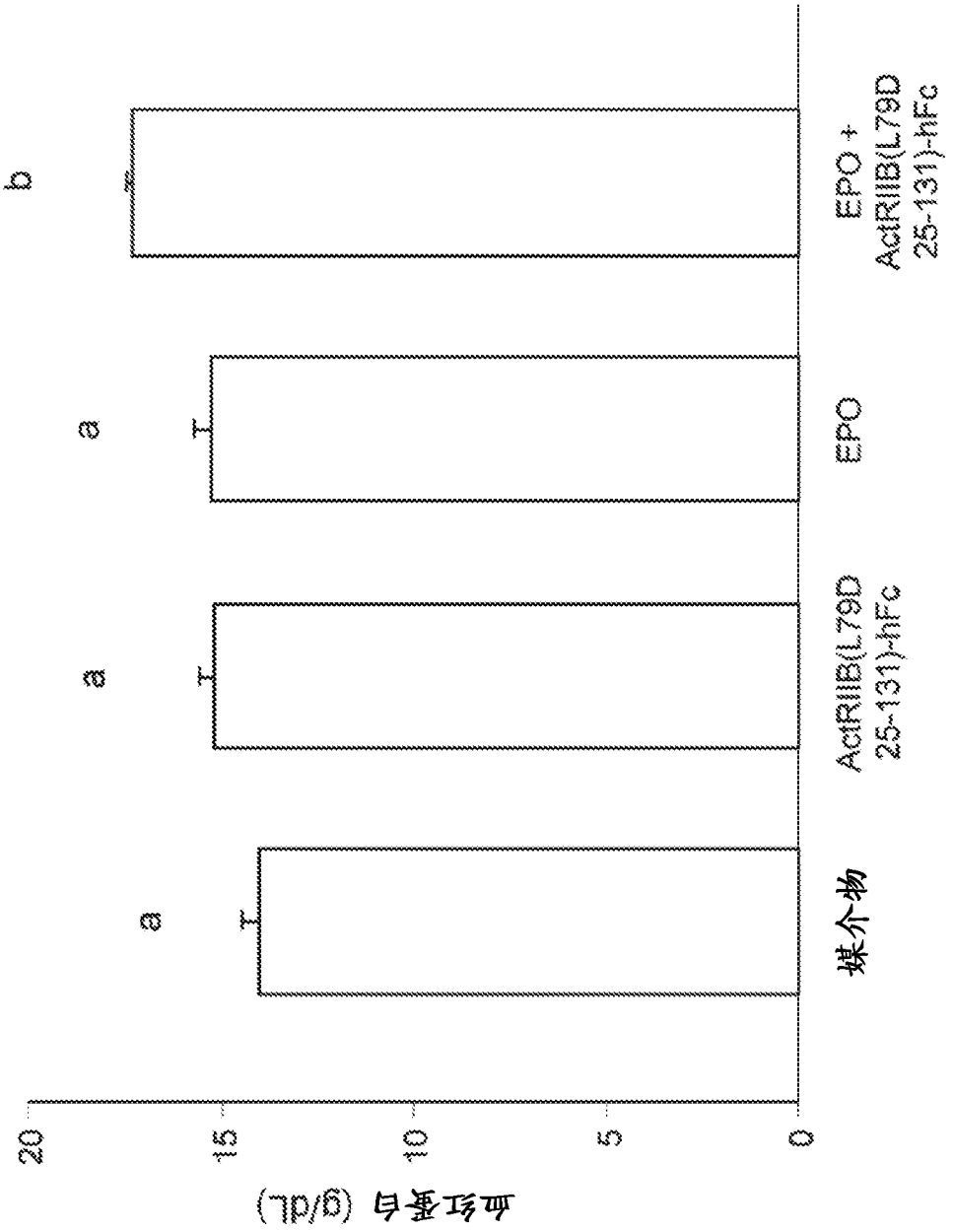

血红蛋白 (g/dL)
0
5
10
15
20
媒介物
ActRIIB(L79D 25-131)-hFc
EPO
EPO + ActRIIB(L79D 25-131)-hFc
a
a
a
b

图 29

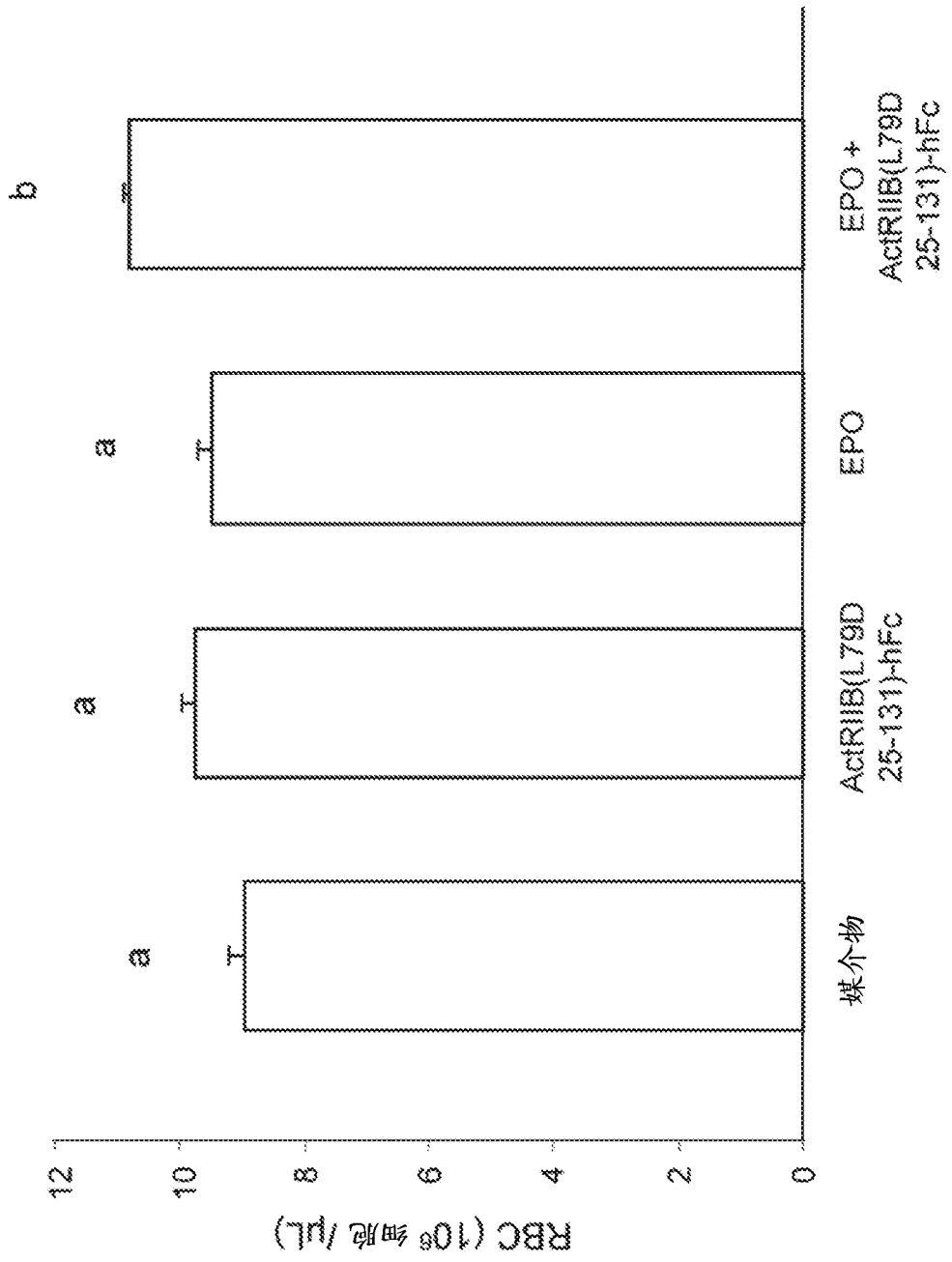

RBC (10⁶ 细胞 /μL)
0
2
4
6
8
10
12
媒介物
ActRIIB(L79D 25-131)-hFc
EPO
EPO + ActRIIB(L79D 25-131)-hFc
a
a
a
b

图 30

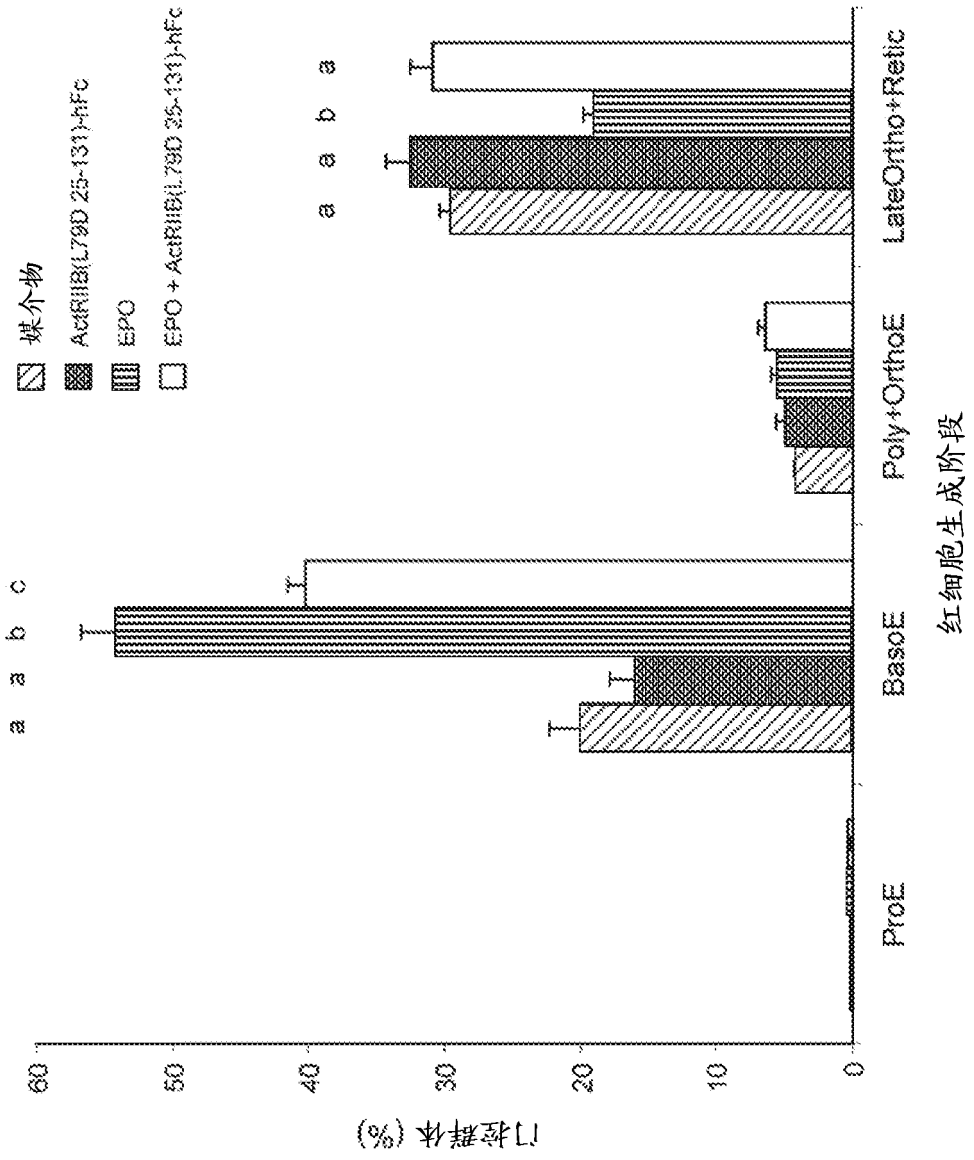

媒介物
ActRIIB(L79D 25-131)-hFc
EPO
EPO + ActRIIB(L79D 25-131)-hFc
a a b c
a a b a
a a b a
门控群体 (%)
0
10
20
30
40
50
60
ProE
BasoE
Poly+OrthoE
LateOrtho+Retic
红细胞生成阶段

图 31

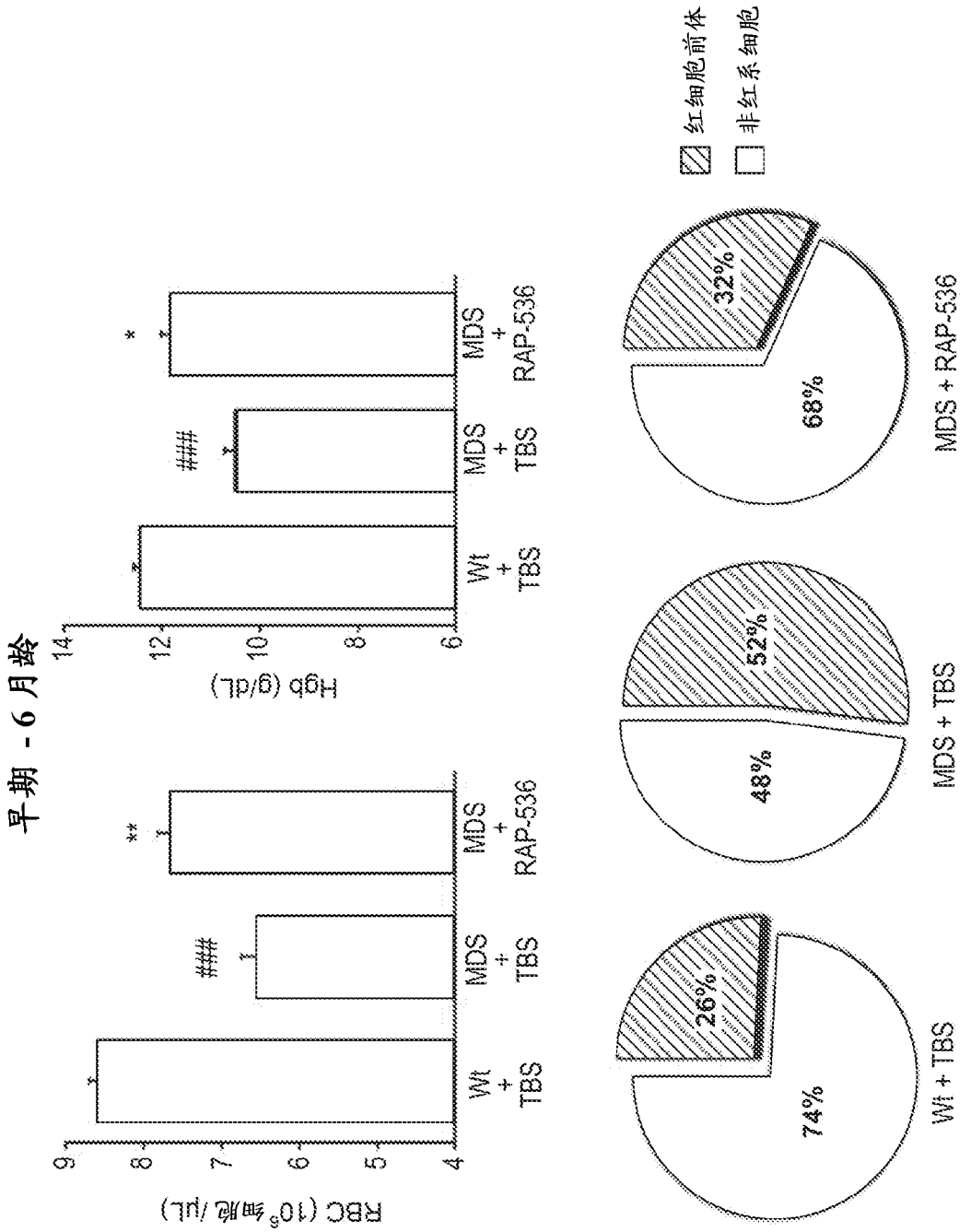

早期 - 6 月龄
RBC (10⁶细胞/μL)
Hgb (g/dL)
Wt + TBS
MDS + TBS
MDS + RAP-536
红细胞前体
非红系细胞
26%
74%
52%
48%
32%
68%

图 32A

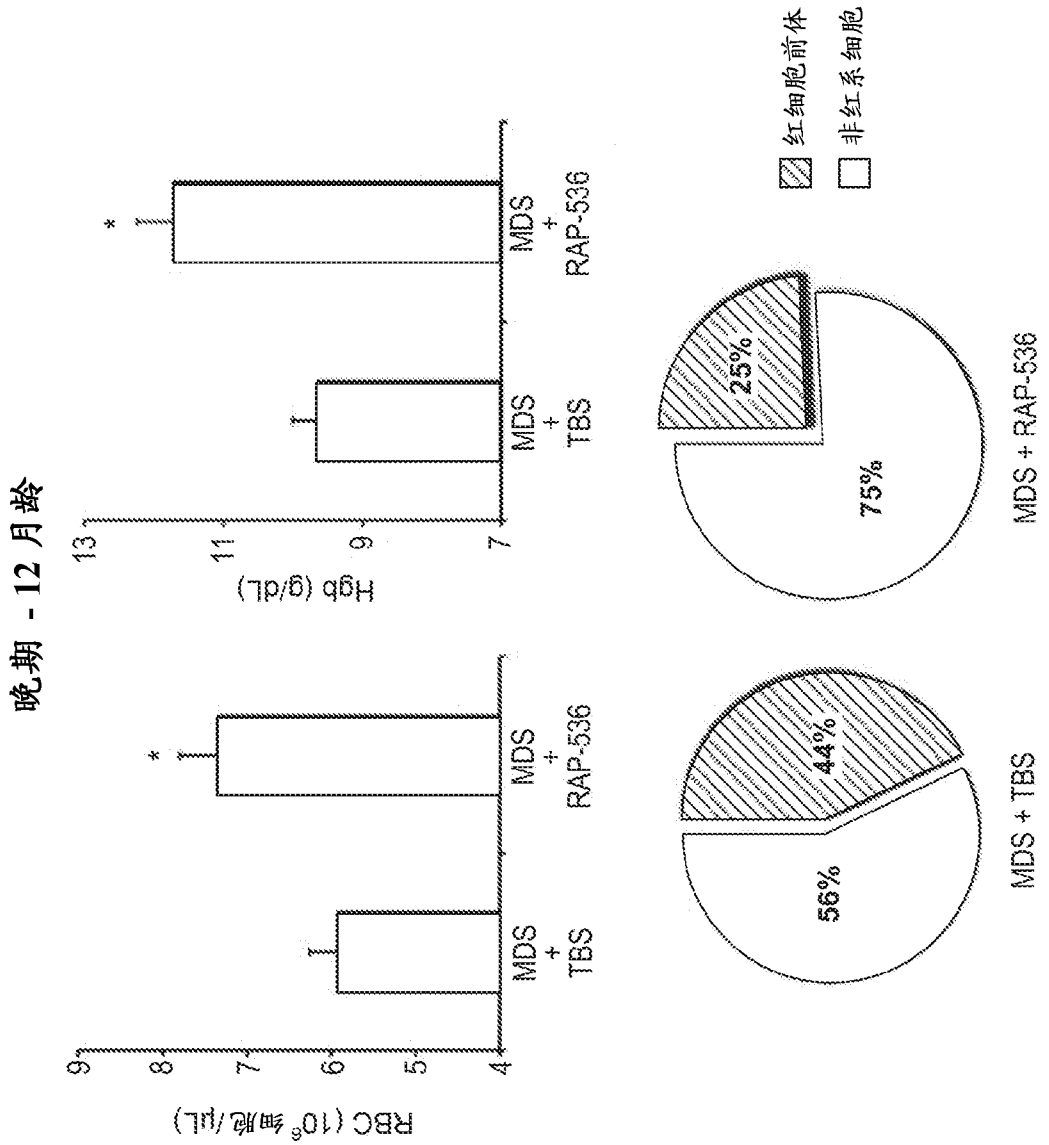

晚期 - 12 月龄
RBC (10⁶ 细胞/μL)
Hgb (g/dL)
MDS + TBS
MDS + RAP-536
红细胞前体
非红系细胞
44%
56%
25%
75%

图 32B