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(54) Title: HUMAN ANTIBODIES TO BONE MORPHOGENETIC PROTEIN 6

(57) Abstract: The present invention provides antibodies that bind to BMP6, and methods of use. According to certain embodiments of the invention, the antibodies are fully human antibodies that bind to BMP6. The antibodies of the invention are useful for inhibiting binding of BMP6 to the hemojuvelin receptor, thereby down-regulating transcription and expression of hepcidin, thus providing a means of preventing or treating an iron-deficiency anemia or an iron-deficiency related disorder. In some embodiments, the antibodies of the present invention are used in treating at least one symptom or complication of an iron-deficiency anemia or an iron-deficiency related disorder.

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HUMAN ANTIBODIES TO BONE MORPHOGENETIC PROTEIN 6

PRIORITY DATA

[001] This application claims priority to U.S. Provisional Application Serial No. 62/860,597, filed June 12, 2019, entitled "HUMAN ANTIBODIES TO BONE MORPHOGENETIC PROTEIN 6", which is hereby incorporated by reference in its entirety.

REFERENCE TO A SEQUENCE LISTING

[002] This application incorporates by reference the Sequence Listing submitted in computer readable form as file 0431_31PCT_ST25, created on June 5, 2019, and containing 43.2 kilobytes.

FIELD OF THE INVENTION

[003] The present invention is related to human antibodies and antigen-binding fragments thereof that specifically bind to bone morphogenetic protein 6 (BMP6), and to therapeutic and diagnostic methods of using such antibodies and fragments.

BACKGROUND

[004] BMP6 is a member of bone morphogenetic proteins, or BMPs, in the TGF-β superfamily. BMP6 has been shown to be involved in a variety of physiological process including in particular regulating iron levels.

[005] The uptake of nutritional iron involves reduction of Fe^{3+} in the intestinal lumen by ferric reductases and the subsequent transport of Fe^{2+} across the apical membrane of enterocytes. The ferroportin-mediated efflux of Fe^{2+} from enterocytes into the plasma is critical for systemic iron homoeostasis. This process is negatively regulated by hepcidin, a liver-derived peptide hormone that binds to ferroportin and promotes its phosphorylation, internalization and lysosomal degradation.

[006] The expression of hepcidin is controlled transcriptionally. Iron-dependent induction of hepcidin requires BMP (bone morphogenetic protein) signaling. Iron triggers the expression of BMP6 in the liver sinusoidal endothelial cells, for binding to a BMP receptor on the surface of hepatocytes. BMP6 signaling leads to phosphorylation of SMAD1/5/8 and translocation along with SMAD4 to the nucleus, where it promotes hepcidin transcription upon binding to proximal and distal sites on its promoter.

[007] Hemojuvelin (HJV, HFE2) is a co-receptor for BMP6 which enhances Bmp6 signaling in liver to induce Hepcidin expression. Mutations in hemojuvelin (HJV) have been found to lead to low hepcidin levels and excess iron accumulation (in the liver).

[008] Antibodies which block BMP6 binding to hemojuvelin or its type II Bmp receptors provide a promising treatment for conditions associated with low plasma iron levels because reduction of such binding reduces plasma hepcidin levels which in turn promotes ferroportin-mediated efflux of Fe^{2+} from enterocytes into the plasma.

SUMMARY OF THE INVENTION

[009] The invention provides fully human monoclonal antibodies (mAbs) and antigen-binding fragments thereof that bind specifically to bone morphogenetic protein 6 (BMP6). Such antibodies may be useful to treat conditions associated with low plasma iron levels. The antibodies may act to reduce transcription of hepcidin, in turn promoting ferroportin-mediated efflux of Fe^{2+} from enterocytes into the plasma. Such antibodies may prevent, halt the progression of, or lessen the severity of conditions associated with low plasma iron levels or ameliorate at least one symptom associated with such conditions, including but not limited to the group consisting of extreme fatigue, weakness, pale skin, chest pain, fast heartbeat, heart palpitations, shortness of breath, headache, dizziness, lightheadedness, cold hands, cold feet, inflammation of the tongue and restless legs. In some cases, the antibodies may be used to prevent or treat a condition or indication associated with low plasma iron levels such as iron-deficiency anemia or iron-deficiency related disorders. Such antibodies may be used alone or in conjunction with a second agent useful for treating iron-deficiency anemia or iron-deficiency related disorders. In certain embodiments, the antibodies specific for BMP6 may be given therapeutically in conjunction with a second agent to prevent, halt the progression of, or lessen the severity of conditions associated with low plasma iron levels or ameliorate at least one symptom associated with such conditions. In certain embodiments, the antibodies may be used prophylactically as stand-alone therapy to protect patients who are at risk for developing an iron-deficiency anemia or an iron-deficiency related disorder. For example, certain patient populations may be at risk for developing an iron-deficiency anemia or an iron-deficiency related disorder, including elderly patients or patients who have experienced a loss of blood. Any of such patient populations may benefit from treatment with the antibodies of the invention, when given alone or in conjunction with a second agent.

[010] The antibodies of the present invention may be used to treat iron-deficiency anemia or an iron-deficiency related disorder in a patient. The antibodies can be full-length (for example, an IgG1 or IgG4 antibody) or may comprise only an antigen-binding portion (for example, a Fab, $F(ab')_2$ or scFv fragment), and may be modified to affect functionality, *e.g.,* to eliminate residual effector functions (Reddy *et al.,* (2000), J. Immunol. 164:1925-1933) or increase mAb half-life (Zalevsky *et al.*, (2010), Nature Biotechnology 28:157-159). The present invention includes any antibody or antigen-binding fragment thereof which comprises any of the V_H regions specified herein linked to a heavy chain constant region (e.g., human constant region) such as gamma (e.g., gamma-1,

gamma-2, gamma-3 or gamma-4), delta, alpha, mu or epsilon and/or any V_L region specified herein linked to a light chain constant region (e.g., human constant region) such as lambda or kappa.

[011] Accordingly, in a first aspect, the invention provides an isolated fully human monoclonal antibody or antigen-binding fragment thereof that specifically binds to BMP6.

[012] In one embodiment, the isolated human antibody or antigen-binding fragment thereof binds to BMP6 with a K_D equal to or less than 10^{-7} M as measured by surface plasmon resonance.

[013] In some embodiments, the isolated antibody or antigen-binding fragment thereof exhibits one or more properties selected from the group consisting of: (a) binds to human BMP6 at 37°C with a binding dissociation equilibrium constant (K_D) of less than about 2nM as measured by surface plasmon resonance; (b) binds to human BMP6 at 37°C with a dissociative half-life (t½) of greater than about 130 minutes as measured by surface plasmon resonance; (c) binds to human BMP6 at 25°C with a K_D of less than about 1nM as measured by surface plasmon resonance; (d) binds to human BMP6 at 25°C with a t½ of greater than about 180 minutes as measured by surface plasmon resonance; (e) binds to a mouse BMP6 at 37°C with a binding dissociation equilibrium constant (K_D) of less than about 10nM as measured by surface plasmon resonance; (f) binds to a mouse BMP6 at 37°C with a dissociative half-life (t½) of greater than about 70 minutes as measured by surface plasmon resonance; (g) binds to a mouse BMP6 at 25°C with a K_D of less than about 4nM as measured by surface plasmon resonance; and (h) binds to a mouse BMP6 at 25°C with a t½ of greater than about 80 minutes as measured by surface plasmon resonance.

[014] In some cases, the isolated human antibody or antigen-binding fragment thereof which binds to BMP6 comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences selected from the group consisting of SEQ ID NOs: 1 and 3; and/or three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences selected from the group consisting of SEQ ID NOs: 2 and 4. Methods and techniques for identifying CDRs within HCVR and LCVR amino acid sequences are well known in the art and can be used to identify CDRs within the specified heavy chain variable region(s) (HCVR) and/or light chain variable region(s) (LCVR) amino acid sequences disclosed herein. Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.*, the Kabat definition, the Chothia definition, and the AbM definition. In general terms, the Kabat definition is based on sequence variability, the Chothia definition is based on the location of the structural loop regions, and the AbM definition is a compromise between the Kabat and Chothia approaches. *See, e.g.*, Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991); Al-Lazikani *et al.*, (1997), *J. Mol. Biol. 273*:927-948; and Martin *et al.*, (1989), *Proc. Natl. Acad. Sci. USA 86*:9268-9272. Public databases are also available for identifying CDR sequences within an antibody.

[015] In some embodiments, the isolated human antibody or antigen-binding fragment thereof, which binds to BMP6, comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 3.

[016] In some embodiments, the isolated human antibody or antigen-binding fragment thereof, which binds to BMP6, comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4.

[017] In some cases, the isolated human antibody or antigen-binding fragment thereof, which binds to BMP6, comprises (a) a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 3; and (b) a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4.

[018] In one embodiment, the isolated human antibody or antigen-binding fragment thereof, which binds to BMP6, comprises:

(a) a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 11;

(b) a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6 and 12;

(c) a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 7 and 13;

(d) a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 14;

(e) a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 9 and 15; and

(f) a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10 and 16.

[019] In various embodiments, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds to BMP6, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iii) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs 5 and 11, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 14, or a substantially similar sequence thereof having at

least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6 and 12, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 7 and 13, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 14, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 9 and 15, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NOs: 10 and 16, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and/or (v) binds to BMP6 with a K_D equal to or less than 10^{-7} M as measured by surface plasmon resonance.

[020] In another aspect, the invention provides an isolated antibody or antigen-binding fragment thereof that competes for binding to BMP6 with a reference antibody or antigen-binding fragment comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 3; and the CDRs of a light chain variable region (LCVR) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4.

[021] In another aspect, the invention provides an isolated antibody or antigen-binding fragment thereof that binds the same epitope on BMP6 as a reference antibody or antigen-binding fragment comprising the CDRs of a heavy chain variable region (HCVR) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 3; and the CDRs of a light chain variable region (LCVR) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4.

[022] In some embodiments, the invention provides an isolated human antibody or antigen-binding fragment thereof that binds BMP6, wherein the antibody or fragment thereof comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 1/3 and 2/4.

[023] In another aspect, the invention provides nucleic acid molecules encoding anti-BMP6 antibodies or fragments thereof. Recombinant expression vectors carrying the nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are methods of producing the antibodies by culturing the host cells under conditions permitting production of the antibodies, and recovering the antibodies produced.

[024] In some embodiments, the invention provides an antibody or fragment thereof comprising a HCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 17 and 19, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[025] In some embodiments, the antibody or fragment thereof further comprises a LCVR encoded by a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 18 and 20, or a substantially identical sequence having at least 90%, at least 95%, at least 98%, or at least 99% homology thereof.

[026] In some cases, the invention provides an antibody or antigen-binding fragment of an antibody comprising a HCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs: 23 and 29, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs: 26 and 32, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[027] In some embodiments, the invention provides an antibody or fragment thereof further comprising a HCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs: 21 and 27, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs: 22 and 28, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs: 24 and 30, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain encoded by a nucleotide sequence selected from the group consisting of SEQ ID NOs: 25 and 31, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity.

[028] In some embodiments, the antibody or antigen-binding fragment thereof that binds to BMP6, as described herein, may be linked to a detectable label such as a radionuclide label or an MRI-detectable label.

[029] In another aspect, the invention provides a pharmaceutical composition comprising an isolated fully human monoclonal antibody or antigen-binding fragment thereof that binds to BMP6, as described above or herein, and a pharmaceutically acceptable carrier or diluent.

[030] In some embodiments, the pharmaceutical composition comprises a fully human monoclonal antibody that binds to BMP6 having any one or more of the characteristics described above or herein. In one embodiment, the antibody binds to BMP6 with a K_D equal to or less than 10^{-}

^{7}M. In various embodiments, the composition comprises an antibody that binds to BMP6 and has a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 1/3 and 2/4. The present invention also provides an isolated human antibody or antigen-binding fragment, wherein the antibody or antigen-binding fragment comprises (i) a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 34, and a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 33; (ii) a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 36, and a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 35;

[031] In some cases, the invention features a composition, which is a combination of an antibody or antigen-binding fragment of an antibody of the invention, and a second therapeutic agent. The second therapeutic agent may be a small molecule drug, a protein/polypeptide, an antibody, a nucleic acid molecule, such as an anti-sense oligonucleotide, or a siRNA. The second therapeutic agent may be synthetic or naturally derived. The second therapeutic agent may be any agent that is advantageously combined with the antibody or fragment thereof of the invention.

[032] In certain embodiments, the second therapeutic agent may be an agent that helps to counteract or reduce any possible side effect(s) associated with the antibody or antigen-binding fragment of an antibody of the invention, if such side effect(s) should occur.

[033] It will also be appreciated that the antibodies and pharmaceutically acceptable compositions of the present invention can be employed in combination therapies, that is, the antibodies and pharmaceutically acceptable compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an antibody may be administered concurrently with another agent used to treat the same disorder), or they may achieve different effects (*e.g.,* control of any adverse effects). As used herein, additional therapeutic agents that are normally administered to treat or prevent a particular disease, or condition, are appropriate for the disease, or condition, being treated. When multiple therapeutics are co-administered, dosages may be adjusted accordingly, as is recognized in the pertinent art.

[034] In another aspect, the invention provides a method for preventing, treating or managing an iron-deficiency anemia or an iron-deficiency related disorder. In certain embodiments, the invention provides a method for treating a patient suffering from an iron-deficiency anemia or an iron-deficiency related disorder, the method comprising administering to the patient an effective amount of an antibody or an antigen-binding fragment thereof that binds to BMP6; or a pharmaceutical composition comprising an effective amount of an antibody or an antigen-binding fragment thereof

that binds to BMP6, such that the an iron-deficiency anemia or an iron-deficiency related disorder is either prevented, or lessened in severity and/or duration, or at least one symptom or complication associated with the condition or disease is prevented, or ameliorated, or that the frequency and/or duration of, or the severity of the iron-deficiency anemia or an iron-deficiency related disorder is reduced.

[035] In some embodiments of the method, the pharmaceutical composition comprising the antibodies of the invention is administered to the patient in combination with a second therapeutic agent.

[036] In embodiments of the invention, the antibody or antigen-binding fragment thereof or the pharmaceutical composition comprising the antibody is administered subcutaneously, intravenously, intradermally, orally or intramuscularly.

[037] In related embodiments, the invention includes the use of an isolated anti-BMP6 antibody or antigen binding portion of an antibody of the invention in the manufacture of a medicament for the prevention or treatment of a disease or disorder related to or caused by an iron-deficiency anemia or an iron-deficiency related disorder. The invention also includes use of an isolated anti-BMP6 antibody or antigen binding portion thereof for preventing or treating a disease or disorder related to or caused by an iron-deficiency anemia or an iron-deficiency related disorder. In one embodiment, the invention includes the use of an isolated anti-BMP6 antibody or antigen-binding fragment thereof in the manufacture of a medicament for the treatment of an iron-deficiency anemia or an iron-deficiency related disorder. In some cases, the invention includes the use of an anti-BMP6 antibody or antigen-binding fragment thereof as discussed above or herein for treating a patient suffering from or at risk of developing an iron-deficiency anemia or an iron-deficiency related disorder.

[038] Other embodiments will become apparent from a review of the ensuing detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[039] Figure 1 shows mice receiving a BMP6 antibody of the invention, H4H17855P, had increased serum iron compared to mice receiving an isotype control antibody at 10mg/kg. There is a dose dependent effect of the BMP6 antibody, which demonstrated increased serum iron at 20mg/kg as compared to 10mg/kg.

[040] Figure 2 shows mice receiving a BMP6 antibody of the invention, H4H17855P, had decreased serum hepcidin compared to mice receiving an isotype control antibody at 10mg/kg. There is a dose dependent effect of the BMP6 antibody, which demonstrated decreased hepcidin at 20mg/kg as compared to 10mg/kg.

[041] Figure 3 shows that mice receiving a BMP6 antibody of the invention, H4H17871P, had increased serum iron compared to mice receiving an isotype control antibody.

[042] Figure 4 shows that mice receiving a BMP6 antibody of the invention, H4H17871P, had decreased serum hepcidin compared to mice receiving an isotype control antibody.

DETAILED DESCRIPTION

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

[044] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference in their entirety.

Definitions

[045] The terms “BMP6” and “Bone Morphogenetic Protein 6” refer, interchangeably, to a 57 kDa monomeric protein. BMP6 is a member of the transforming growth factor beta (TGF-beta) superfamily of regulatory molecules. The amino acid sequence of human BMP6 is shown in SEQ ID NO: 40. The amino acid sequence of mouse BMP6 is shown in SEQ ID NO: 42. Unless otherwise noted, reference to BMP6 refers to the human form.

[046] The term "antibody", as used herein, is intended to refer to immunoglobulin molecules comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds (*i.e.*, "full antibody molecules"), as well as multimers thereof (*e.g.* IgM) or antigen-binding fragments thereof. Each heavy chain is comprised of a heavy chain variable region (“HCVR” or “V_H”) and a heavy chain constant region (comprised of domains C_H1, C_H2 and C_H3). Each light chain is comprised of a light chain variable region (“LCVR or “V_L”) and a light chain constant region (C_L). The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. In certain embodiments of the invention, the FRs of the antibody (or antigen binding fragment thereof) may be identical to the human germline sequences, or may be naturally or artificially modified. An amino acid consensus sequence may be defined based on a side-by-side analysis of two or more CDRs.

[047] Substitution of one or more CDR residues or omission of one or more CDRs is also possible. Antibodies have been described in the scientific literature in which one or two CDRs can be dispensed with for binding. Padlan *et al.* (FASEB J. 1995, 9:133-139) analyzed the contact regions between antibodies and their antigens, based on published crystal structures, and concluded that only about one fifth to one third of CDR residues actually contact the antigen. Padlan also found many antibodies in which one or two CDRs had no amino acids in contact with an antigen (see also, Vajdos *et al.* 2002 J Mol Biol 320:415-428).

[048] CDR residues not contacting antigen can be identified based on previous studies by molecular modeling and/or empirically. If a CDR or residue(s) thereof is omitted, it is usually substituted with an amino acid occupying the corresponding position in another human antibody sequence or a consensus of such sequences. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically. Empirical substitutions can be conservative or non-conservative substitutions.

[049] The fully human BMP6 monoclonal antibodies disclosed herein may comprise one or more amino acid substitutions, insertions and/or deletions in the framework and/or CDR regions of the heavy and light chain variable domains as compared to the corresponding germline sequences. Such mutations can be readily ascertained by comparing the amino acid sequences disclosed herein to germline sequences available from, for example, public antibody sequence databases. The present invention includes antibodies, and antigen-binding fragments thereof, which are derived from any of the amino acid sequences disclosed herein, wherein one or more amino acids within one or more framework and/or CDR regions are mutated to the corresponding residue(s) of the germline sequence from which the antibody was derived, or to the corresponding residue(s) of another human germline sequence, or to a conservative amino acid substitution of the corresponding germline residue(s) (such sequence changes are referred to herein collectively as "germline mutations"). A person of ordinary skill in the art, starting with the heavy and light chain variable region sequences disclosed herein, can easily produce numerous antibodies and antigen-binding fragments that comprise one or more individual germline mutations or combinations thereof. In certain embodiments, all of the framework and/or CDR residues within the V_H and/or V_L domains are mutated back to the residues found in the original germline sequence from which the antibody was derived. In other embodiments, only certain residues are mutated back to the original germline sequence, *e.g.*, only the mutated residues found within the first 8 amino acids of FR1 or within the last 8 amino acids of FR4, or only the mutated residues found within CDR1, CDR2 or CDR3. In other embodiments, one or more of the framework and/or CDR residue(s) are mutated to the corresponding residue(s) of a different germline sequence (*i.e.*, a germline sequence that is different from the germline sequence from which the antibody was originally derived). Furthermore, the antibodies of the present invention may contain any combination of two or more germline

mutations within the framework and/or CDR regions, *e.g.*, wherein certain individual residues are mutated to the corresponding residue of a particular germline sequence while certain other residues that differ from the original germline sequence are maintained or are mutated to the corresponding residue of a different germline sequence. Once obtained, antibodies and antigen-binding fragments that contain one or more germline mutations can be easily tested for one or more desired property such as, improved binding specificity, increased binding affinity, improved or enhanced antagonistic or agonistic biological properties (as the case may be), reduced immunogenicity, etc. Antibodies and antigen-binding fragments obtained in this general manner are encompassed within the present invention.

[050] The present invention also includes fully human anti-BMP6 monoclonal antibodies comprising variants of any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein having one or more conservative substitutions. For example, the present invention includes anti-BMP6 antibodies having HCVR, LCVR, and/or CDR amino acid sequences with, *e.g.*, 10 or fewer, 8 or fewer, 6 or fewer, 4 or fewer, etc. conservative amino acid substitutions relative to any of the HCVR, LCVR, and/or CDR amino acid sequences disclosed herein.

[051] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human mAbs of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.,* mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species (e.g., mouse), have been grafted onto human FR sequences.

[052] The term "specifically binds," or “binds specifically to”, or the like, means that an antibody or antigen-binding fragment thereof forms a complex with an antigen that is relatively stable under physiologic conditions. Specific binding can be characterized by an equilibrium dissociation constant of at least about $1x10^{-6}$ M or less (*e.g.*, a smaller K_D denotes a tighter binding). Methods for determining whether two molecules specifically bind are well known in the art and include, for example, equilibrium dialysis, surface plasmon resonance, and the like. As described herein, antibodies that bind specifically to BMP6 have been identified by surface plasmon resonance, *e.g.*, BIACORE™. Moreover, multi-specific antibodies that bind to one domain in BMP6 and one or more additional antigens or a bi-specific that binds to two different regions of BMP6 are nonetheless considered antibodies that “specifically bind”, as used herein.

[053] The term “high affinity” antibody refers to those mAbs having a binding affinity to BMP6, expressed as K_D, of at least 10^{-7} M; preferably 10^{-8} M; more preferably 10^{-9}M, even more preferably

10^{-10} M, even more preferably 10^{-11} M, as measured by surface plasmon resonance, e.g., BIACORE™ or solution-affinity ELISA.

[054] By the term “slow off rate”, “Koff” or “kd” is meant to describe an antibody that dissociates from BMP6 with a rate constant of 1 x 10^{-3} s^{-1} or less, preferably 1 x 10^{-4} s^{-1} or less, as determined by surface plasmon resonance, *e.g.*, BIACORE™.

[055] The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment”, as used herein, refers to one or more fragments of an antibody that retain the ability to bind to BMP6.

[056] In specific embodiments, antibody or antibody fragments of the invention may be conjugated to a therapeutic moiety (“immunoconjugate”), such as an antibiotic, a second anti-BMP6 antibody, or an antibody to a cytokine such as IL-1, IL-6, or TGF-β, or any other therapeutic moiety useful for treating an iron-deficiency anemia or an iron-deficiency related disorder.

[057] An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies (Abs) having different antigenic specificities (*e.g.,* an isolated antibody that specifically binds BMP6, or a fragment thereof, is substantially free of Abs that specifically bind antigens other than BMP6).

[058] The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biomolecular interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIACORE™ system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, N.J.).

[059] The term "K_D ", as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction.

[060] The term “epitope” refers to an antigenic determinant that interacts with a specific antigen-binding site in the variable region of an antibody molecule known as a paratope. A single antigen may have more than one epitope. Thus, different antibodies may bind to different areas on an antigen and may have different biological effects. The term “epitope” also refers to a site on an antigen to which B and/or T cells respond. It also refers to a region of an antigen that is bound by an antibody. Epitopes may be defined as structural or functional. Functional epitopes are generally a subset of the structural epitopes and have those residues that directly contribute to the affinity of the interaction. Epitopes may also be conformational, that is, composed of non-linear amino acids. In certain embodiments, epitopes may include determinants that are chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl groups, or sulfonyl groups, and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics.

[061] The term "substantial identity" or "substantially identical," when referring to a nucleic acid or fragment thereof, indicates that, when optimally aligned with appropriate nucleotide insertions or deletions with another nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 90%, and more preferably at least about 95%, 96%, 97%, 98% or 99% of the nucleotide bases, as measured by any well-known algorithm of sequence identity, such as FASTA, BLAST or GAP, as discussed below. A nucleic acid molecule having substantial identity to a reference nucleic acid molecule may, in certain instances, encode a polypeptide having the same or substantially similar amino acid sequence as the polypeptide encoded by the reference nucleic acid molecule.

[062] As applied to polypeptides, the term "substantial similarity" or “substantially similar” means that two peptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 90% sequence identity, even more preferably at least 95%, 98% or 99% sequence identity. Preferably, residue positions, which are not identical, differ by conservative amino acid substitutions. A "conservative amino acid substitution" is one in which an amino acid residue is substituted by another amino acid residue having a side chain (R group) with similar chemical properties (e.g., charge or hydrophobicity). In general, a conservative amino acid substitution will not substantially change the functional properties of a protein. In cases where two or more amino acid sequences differ from each other by conservative substitutions, the percent or degree of similarity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. See, *e.g.*, Pearson (1994) Methods Mol. Biol. 24: 307-331, which is herein incorporated by reference. Examples of groups of amino acids that have side chains with similar chemical properties include 1) aliphatic side chains: glycine, alanine, valine, leucine and isoleucine; 2) aliphatic-hydroxyl side chains: serine and threonine; 3) amide-containing side chains: asparagine and glutamine; 4) aromatic side chains: phenylalanine, tyrosine, and tryptophan; 5) basic side chains: lysine, arginine, and histidine; 6) acidic side chains: aspartate and glutamate, and 7) sulfur-containing side chains: cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, glutamate-aspartate, and asparagine-glutamine. Alternatively, a conservative replacement is any change having a positive value in the PAM250 log-likelihood matrix disclosed in Gonnet *et al.* (1992) Science 256: 1443 45, herein incorporated by reference. A "moderately conservative" replacement is any change having a nonnegative value in the PAM250 log-likelihood matrix.

[063] Sequence similarity for polypeptides is typically measured using sequence analysis software. Protein analysis software matches similar sequences using measures of similarity assigned to various substitutions, deletions and other modifications, including conservative amino acid substitutions. For instance, GCG software contains programs such as GAP and BESTFIT which

can be used with default parameters to determine sequence homology or sequence identity between closely related polypeptides, such as homologous polypeptides from different species of organisms or between a wild type protein and a mutein thereof. See, *e.g.,* GCG Version 6.1. Polypeptide sequences also can be compared using FASTA with default or recommended parameters; a program in GCG Version 6.1. FASTA (*e.g.,* FASTA2 and FASTA3) provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson (2000) *supra*). Another preferred algorithm when comparing a sequence of the invention to a database containing a large number of sequences from different organisms is the computer program BLAST, especially BLASTP or TBLASTN, using default parameters. See, *e.g.,* Altschul *et al.* (1990) J. Mol. Biol. 215: 403-410 and (1997) Nucleic Acids Res. 25: 3389-3402, each of which is herein incorporated by reference.

[064] In specific embodiments, the antibody or antibody fragment for use in the method of the invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for epitopes of more than one target polypeptide.

[065] By the phrase "therapeutically effective amount" is meant an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) The Art, Science and Technology of Pharmaceutical Compounding).

General Description

[066] Bone morphogenetic protein 6 (BMP6) is a 57 kDa protein which has been shown to be involved in a variety of physiological processes including regulating iron levels. BMP6 signaling leads to phosphorylation of SMAD1/5/8 and translocation of SMAD4 to the nucleus, where it promotes hepcidin (a negative regulator of serum iron) transcription upon binding to proximal and distal sites on its promoter. Hemojuvelin (HJV, HFE2) is a co-receptor for BMP6 which, enhances signaling. Reduced binding of BMP6 to HJV reduces hepcidin transcription, thereby promoting serum iron levels.

[067] The antibodies described herein demonstrate specific binding to BMP6 and in some embodiments, may be useful for treating patients suffering from iron-deficiency anemia or an iron-deficiency related disorder. They may be used alone or as adjunct therapy with other therapeutic moieties or modalities known in the art for treating iron-deficiency anemia or an iron-deficiency related disorder, such as, but not limited to, iron supplementation through iron supplements, dietary changes to promote serum iron and/or intravenous delivery of iron, blood transfusion, and iron promoting medications. They may be used in conjunction with additional antibodies specific for antigens other than BMP6 or may combined with other types of treatments.

[068] In some embodiments, the antibodies described herein may be useful in preventing, treating or managing an iron-deficiency anemia or an iron-deficiency related disorder.

[069] In certain embodiments, the antibodies of the invention are obtained from mice immunized with a primary immunogen, such as a native, full length human BMP6 (SEQ ID NO: 40) or BMP6 fragments, followed by immunization with a secondary immunogen, or with an immunogenically active fragment of BMP6.

[070] The immunogen may be an immunogenic fragment of BMP6 or DNA encoding the fragment thereof. The immunogen may be BMP6 coupled to a histidine tag and/or to a fragment of Fc region of an antibody.

[071] The amino acid sequence of full length human BMP6 is shown as SEQ ID NO: 40. The full length amino acid sequence of mouse BMP6is shown as SEQ ID NO: 42.

[072] In certain embodiments, antibodies that bind specifically to BMP6 may be prepared using fragments of the above-noted regions, or peptides that extend beyond the designated regions by about 5 to about 20 amino acid residues from either, or both, the N or C terminal ends of the regions described herein. In certain embodiments, any combination of the above-noted regions or fragments thereof may be used in the preparation of BMP6 -specific antibodies. In certain embodiments, any one or more of the above-noted regions of BMP6, or fragments thereof may be used for preparing monospecific, bispecific, or multi-specific antibodies.

Antigen-Binding Fragments of Antibodies

[073] Unless specifically indicated otherwise, the term "antibody," as used herein, shall be understood to encompass antibody molecules comprising two immunoglobulin heavy chains and two immunoglobulin light chains (*i.e.*, "full antibody molecules") as well as antigen-binding fragments thereof. The terms "antigen-binding portion" of an antibody, "antigen-binding fragment" of an antibody, and the like, as used herein, include any naturally occurring, enzymatically obtainable, synthetic, or genetically engineered polypeptide or glycoprotein that specifically binds an antigen to form a complex. The terms "antigen-binding fragment" of an antibody, or "antibody fragment", as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to BMP6. An antibody fragment may include a Fab fragment, a $F(ab')_2$ fragment, a Fv fragment, a dAb fragment, a fragment containing a CDR, or an isolated CDR. Antigen-binding fragments of an antibody may be derived, *e.g.*, from full antibody molecules using any suitable standard techniques such as proteolytic digestion or recombinant genetic engineering techniques involving the manipulation and expression of DNA encoding antibody variable and (optionally) constant domains. Such DNA is known and/or is readily available from, *e.g.*, commercial sources, DNA libraries (including, *e.g.*, phage-antibody libraries), or can be synthesized. The DNA may be sequenced and manipulated chemically or by using molecular biology techniques, for example, to

arrange one or more variable and/or constant domains into a suitable configuration, or to introduce codons, create cysteine residues, modify, add or delete amino acids, etc.

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

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

[076] In certain embodiments, an antigen-binding fragment of an antibody may contain at least one variable domain covalently linked to at least one constant domain. Non-limiting, exemplary configurations of variable and constant domains that may be found within an antigen-binding fragment of an antibody of the present invention include: (i) V_H -C_H1; (ii) V_H -C_H2; (iii) V_H -C_H3; (iv) V_H -C_H1-C_H2; (v) V_H -C_H1-C_H2-C_H3; (vi) V_H -C_H2-C_H3; (vii) V_H -C_L; (viii) V_L -C_H1; (ix) V_L -C_H2; (x) V_L -C_H3; (xi) V_L -C_H1-C_H2; (xii) V_L -C_H1-C_H2-C_H3; (xiii) V_L -C_H2-C_H3; and (xiv) V_L -C_L. In any configuration of variable and constant domains, including any of the exemplary configurations listed above, the variable and constant domains may be either directly linked to one another or may be linked by a full or partial hinge or linker region. A hinge region may consist of at least 2 (*e.g.*, 5, 10, 15, 20, 40, 60 or more) amino acids, which result in a flexible or semi-flexible linkage between adjacent variable and/or constant domains in a single polypeptide molecule. Moreover, an antigen-binding fragment of an antibody of the present invention may comprise a homo-dimer or hetero-dimer (or other multimer) of any of the variable and constant domain configurations listed above in non-covalent association with one another and/or with one or more monomeric V_H or V_L domain (*e.g.*, by disulfide bond(s)).

[077] As with full antibody molecules, antigen-binding fragments may be mono-specific or multi-specific (*e.g.*, bi-specific). A multi-specific antigen-binding fragment of an antibody will typically

comprise at least two different variable domains, wherein each variable domain is capable of specifically binding to a separate antigen or to a different epitope on the same antigen. Any multi-specific antibody format, including the exemplary bi-specific antibody formats disclosed herein, may be adapted for use in the context of an antigen-binding fragment of an antibody of the present invention using routine techniques available in the art.

[078] The present invention includes anti-BMP6 antibodies and antigen-binding fragments having immunoglobulin chains that include the amino acid sequences set forth herein as well as variants having cellular and/or *in vitro* post-translational modifications. For example, the present invention includes antibodies and antigen-binding fragments thereof that specifically bind to BMP6 comprising heavy and/or light chain amino acid sequences set forth herein (e.g., CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and/or CDR-L3) as well as antibodies and fragments wherein one or more amino acid residues is glycosylated, one or more Asn residues is deamidated, one or more residues (e.g., Met, Trp and/or His) is oxidized, the N-terminal Gln is pyroglutamate (pyroE) and/or the C-terminal Lysine is missing.

[079] The present invention includes recombinant methods for making anti-BMP6 antibodies or antigen-binding fragments thereof of the present invention, or an immunoglobulin chain thereof, comprising (i) introducing one or more polynucleotides encoding a light and/or a heavy immunoglobulin chain of said antibody or antigen-binding fragment (e.g., a heavy chain or V_H thereof or immunoglobulin comprising the HCDR1, HCDR2 and HCDR3 thereof and/or a light chain or V_L thereof or immunoglobulin comprising the LCDR1, LCDR2 and LCDR3 thereof), for example, wherein the polynucleotide is in a vector and/or is operably linked to a promoter; (ii) culturing the host cell (e.g., Chinese hamster ovary (CHO) cell or *Pichia* cell or *Pichia pastoris* cell) under condition favorable to expression of the polynucleotide(s) and, (iii) optionally, isolating the antibody or fragment or chain from the host cell and/or medium in which the host cell is grown. When making an antibody or antigen-binding fragment comprising more than one immunoglobulin chain, e.g., an antibody that comprises two heavy immunoglobulin chains and two light immunoglobulin chains, co-expression of the chains in a single host cell leads to association of the chains, e.g., in the cell or on the cell surface or outside the cell if such chains are secreted, so as to form the antibody or antigen-binding fragment molecule. The methods include those wherein only a heavy immunoglobulin chain or only a light immunoglobulin chain (e.g., any of those discussed herein including mature fragments and/or variable domains thereof) is expressed. Such chains are useful, for example, as intermediates in the expression of an antibody or antigen-binding fragment that includes such a chain. The present invention includes the products of such expression methods (e.g., antibodies, antigen-binding fragments, V_Hs, or V_Ls).

Preparation of Human Antibodies

[080] Methods for generating human antibodies in transgenic mice are known in the art. Any such known methods can be used in the context of the present invention to make human antibodies that specifically bind to BMP6.

[081] Using VELOCIMMUNE® technology (see, for example, US 6,596,541, Regeneron Pharmaceuticals, VELOCIMMUNE®) or any other known method for generating monoclonal antibodies, high affinity chimeric antibodies to BMP6 are initially isolated having a human variable region and a mouse constant region. The VELOCIMMUNE® technology involves generation of a transgenic mouse having a genome comprising human heavy and light chain variable regions operably linked to endogenous mouse constant region loci such that the mouse produces an antibody comprising a human variable region and a mouse constant region in response to antigenic stimulation. The DNA encoding the variable regions of the heavy and light chains of the antibody are isolated and operably linked to DNA encoding the human heavy and light chain constant regions. The DNA is then expressed in a cell capable of expressing the fully human antibody.

[082] Generally, a VELOCIMMUNE® mouse is challenged with the antigen of interest, and lymphatic cells (such as B-cells) are recovered from the mice that express antibodies. The lymphatic cells may be fused with a myeloma cell line to prepare immortal hybridoma cell lines, and such hybridoma cell lines are screened and selected to identify hybridoma cell lines that produce antibodies specific to the antigen of interest. DNA encoding the variable regions of the heavy chain and light chain may be isolated and linked to desirable isotypic constant regions of the heavy chain and light chain. Such an antibody protein may be produced in a cell, such as a CHO cell. Alternatively, DNA encoding the antigen-specific chimeric antibodies or the variable domains of the light and heavy chains may be isolated directly from antigen-specific lymphocytes.

[083] Initially, high-affinity chimeric antibodies are isolated having a human variable region and a mouse constant region. The antibodies are characterized and selected for desirable characteristics, including affinity, selectivity, epitope, etc. The mouse constant regions are replaced with a desired human constant region to generate the fully human antibody of the invention, for example wild type or modified IgG1 or IgG4. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

[084] In general, the antibodies of the instant invention possess very high affinities, typically possessing K_D of from about 10^{-12} through about 10^{-7} M, when measured by binding to antigen either immobilized on solid phase or in solution phase. The mouse constant regions are replaced with desired human constant regions to generate the fully human antibodies of the invention. While the constant region selected may vary according to specific use, high affinity antigen-binding and target specificity characteristics reside in the variable region.

Bioequivalents

[085] The anti-BMP6 antibodies and antibody fragments of the present invention encompass proteins having amino acid sequences that vary from those of the described antibodies, but that retain the ability to bind BMP6. Such variant antibodies and antibody fragments comprise one or more additions, deletions, or substitutions of amino acids when compared to parent sequence, but exhibit biological activity that is essentially equivalent to that of the described antibodies. Likewise, the antibody-encoding DNA sequences of the present invention encompass sequences that comprise one or more additions, deletions, or substitutions of nucleotides when compared to the disclosed sequence, but that encode an antibody or antibody fragment that is essentially bioequivalent to an antibody or antibody fragment of the invention.

[086] Two antigen-binding proteins, or antibodies, are considered bioequivalent if, for example, they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose under similar experimental conditions, either single dose or multiple doses. Some antibodies will be considered equivalents or pharmaceutical alternatives if they are equivalent in the extent of their absorption but not in their rate of absorption and yet may be considered bioequivalent because such differences in the rate of absorption are intentional and are reflected in the labeling, are not essential to the attainment of effective body drug concentrations on, *e.g.*, chronic use, and are considered medically insignificant for the particular drug product studied.

[087] In one embodiment, two antigen-binding proteins are bioequivalent if there are no clinically meaningful differences in their safety, purity, and potency.

[088] In one embodiment, two antigen-binding proteins are bioequivalent if a patient can be switched one or more times between the reference product and the biological product without an expected increase in the risk of adverse effects, including a clinically significant change in immunogenicity, or diminished effectiveness, as compared to continued therapy without such switching.

[089] In one embodiment, two antigen-binding proteins are bioequivalent if they both act by a common mechanism or mechanisms of action for the condition or conditions of use, to the extent that such mechanisms are known.

[090] Bioequivalence may be demonstrated by *in vivo* and/or *in vitro* methods. Bioequivalence measures include, *e.g.*, (a) an *in vivo* test in humans or other mammals, in which the concentration of the antibody or its metabolites is measured in blood, plasma, serum, or other biological fluid as a function of time; (b) an *in vitro* test that has been correlated with and is reasonably predictive of human *in vivo* bioavailability data; (c) an *in vivo* test in humans or other mammals in which the appropriate acute pharmacological effect of the antibody (or its target) is measured as a function of

time; and (d) in a well-controlled clinical trial that establishes safety, efficacy, or bioavailability or bioequivalence of an antibody.

[091] Bioequivalent variants of the antibodies of the invention may be constructed by, for example, making various substitutions of residues or sequences or deleting terminal or internal residues or sequences not needed for biological activity. For example, cysteine residues not essential for biological activity can be deleted or replaced with other amino acids to prevent formation of unnecessary or incorrect intramolecular disulfide bridges upon renaturation. In other contexts, bioequivalent antibodies may include antibody variants comprising amino acid changes, which modify the glycosylation characteristics of the antibodies, *e.g.*, mutations that eliminate or remove glycosylation.

Anti-BMP6 Antibodies Comprising Fc Variants

[092] According to certain embodiments of the present invention, anti-BMP6 antibodies are provided comprising an Fc domain comprising one or more mutations which enhance or diminish antibody binding to the FcRn receptor, *e.g.*, at acidic pH as compared to neutral pH. For example, the present invention includes anti-BMP6 antibodies comprising a mutation in the C_H2 or a C_H3 region of the Fc domain, wherein the mutation(s) increases the affinity of the Fc domain to FcRn in an acidic environment (*e.g.,* in an endosome where pH ranges from about 5.5 to about 6.0). Such mutations may result in an increase in serum half-life of the antibody when administered to an animal. Non-limiting examples of such Fc modifications include, *e.g.*, a modification at position 250 (e.g., E or Q); 250 and 428 (e.g., L or F); 252 (e.g., L/Y/F/W or T), 254 (e.g., S or T), and 256 (e.g., S/R/Q/E/D or T); or a modification at position 428 and/or 433 (e.g., H/L/R/S/P/Q or K) and/or 434 (*e.g.,* A, W, H, F or Y [N434A, N434W, N434H, N434F or N434Y]); or a modification at position 250 and/or 428; or a modification at position 307 or 308 (*e.g.,* 308F, V308F), and 434. In one embodiment, the modification comprises a 428L (*e.g.,* M428L) and 434S (*e.g.,* N434S) modification; a 428L, 259I (*e.g.,* V259I), and 308F (*e.g.,* V308F) modification; a 433K (*e.g.,* H433K) and a 434 (*e.g.,* 434Y) modification; a 252, 254, and 256 (*e.g.,* 252Y, 254T, and 256E) modification; a 250Q and 428L modification (*e.g.,* T250Q and M428L); and a 307 and/or 308 modification(s) (*e.g.,* 308F and/or 308P). In yet another embodiment, the modification comprises a 265A (*e.g.*, D265A) and/or a 297A (*e.g.*, N297A) modification.

[093] For example, the present invention includes anti-BMP6 antibodies comprising an Fc domain comprising one or more pairs or groups of mutations selected from the group consisting of: 250Q and 248L (*e.g.*, T250Q and M248L); 252Y, 254T and 256E (*e.g.*, M252Y, S254T and T256E); 428L and 434S (*e.g.*, M428L and N434S); 257I and 311I (*e.g.*, P257I and Q311I); 257I and 434H (*e.g.*, P257I and N434H); 376V and 434H (*e.g.*, D376V and N434H); 307A, 380A and 434A (*e.g.*, T307A, E380A and N434A); and 433K and 434F (*e.g.*, H433K and N434F). All possible combinations of

the foregoing Fc domain mutations, and other mutations within the antibody variable domains disclosed herein, are contemplated within the scope of the present invention.

[094] The present invention also includes anti-BMP6 antibodies comprising a chimeric heavy chain constant (C_H) region, wherein the chimeric C_H region comprises segments derived from the C_H regions of more than one immunoglobulin isotype. For example, the antibodies of the invention may comprise a chimeric C_H region comprising part or all of a C_H2 domain derived from a human IgG1, human IgG2 or human IgG4 molecule, combined with part or all of a C_H3 domain derived from a human IgG1, human IgG2 or human IgG4 molecule. According to certain embodiments, the antibodies of the invention comprise a chimeric C_H region having a chimeric hinge region. For example, a chimeric hinge may comprise an "upper hinge" amino acid sequence (amino acid residues from positions 216 to 227 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence (amino acid residues from positions 228 to 236 according to EU numbering) derived from a human IgG1, a human IgG2 or a human IgG4 hinge region. According to certain embodiments, the chimeric hinge region comprises amino acid residues derived from a human IgG1 or a human IgG4 upper hinge and amino acid residues derived from a human IgG2 lower hinge. An antibody comprising a chimeric C_H region as described herein may, in certain embodiments, exhibit modified Fc effector functions without adversely affecting the therapeutic or pharmacokinetic properties of the antibody. (*See*, *e.g.*, U.S. Provisional Appl. No. 61/759,578, filed February 1, 2013, the disclosure of which is hereby incorporated by reference in its entirety).

Biological Characteristics of the Antibodies

[095] In general, the antibodies of the present invention may function by binding to BMP6. In some embodiments, the antibodies of the present invention may bind to another antigen (cross-reactive antibodies).

[096] In certain embodiments, the antibodies of the present invention may be bi-specific antibodies. The bi-specific antibodies of the invention may bind one epitope in one domain and may also bind one epitope in a second domain of BMP6. In certain embodiments, the bi-specific antibodies of the invention may bind two different epitopes in the same domain.

[097] In one embodiment, the invention provides a fully human monoclonal antibody or antigen-binding fragment thereof that binds to BMP6, wherein the antibody or fragment thereof exhibits one or more of the following characteristics: (i) comprises a HCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 3, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (ii) comprises a LCVR having an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and 4, or a substantially similar sequence thereof having at least 90%, at least 95%, at least

98% or at least 99% sequence identity; (iii) comprises a HCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 7 and 13, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR3 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 10 and 16, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; (iv) comprises a HCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and 11, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a HCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 6 and 12, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; a LCDR1 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 8 and 14, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and a LCDR2 domain having an amino acid sequence selected from the group consisting of SEQ ID NO: 9 and 15, or a substantially similar sequence thereof having at least 90%, at least 95%, at least 98% or at least 99% sequence identity; and (v) binds to BMP6 with a K_D equal to or less than 10^{-7}.

[098] Certain anti-BMP6 antibodies of the present invention are able to bind to and neutralize the activity of BMP6, as determined by *in vitro* or *in vivo* assays. The ability of the antibodies of the invention to bind to and neutralize the activity of BMP6 may be measured using any standard method known to those skilled in the art, including binding assays, or activity assays, as described herein.

[099] The peptides may be modified to include addition or substitution of certain residues for tagging or for purposes of conjugation to carrier molecules, such as, KLH. For example, a cysteine may be added at either the N-terminal or C-terminal end of a peptide, or a linker sequence may be added to prepare the peptide for conjugation to, for example, KLH for immunization.

[0100] The antibodies specific for BMP6 may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface. In one embodiment, the label may be a radionuclide, a fluorescent dye or a MRI-detectable label. In certain embodiments, such labeled antibodies may be used in diagnostic assays including imaging assays.

Epitope Mapping and Related Technologies

[0101] The present invention includes anti-BMP6 antibodies which interact with one or more amino acids found within one or more regions of BMP6. The epitope to which the antibodies bind may consist of a single contiguous sequence of 3 or more (*e.g.*, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more) amino acids located within any of the aforementioned regions of the BMP6 molecule (*e.g.* a linear epitope in a domain). Alternatively, the epitope may consist of a plurality of non-contiguous amino acids (or amino acid sequences) located within either or both of the aforementioned regions of the BMP6 molecule (*e.g.* a conformational epitope).

[0102] Various techniques known to persons of ordinary skill in the art can be used to determine whether an antibody "interacts with one or more amino acids" within a polypeptide or protein. Exemplary techniques include, for example, routine cross-blocking assays, such as that described in Antibodies, Harlow and Lane (Cold Spring Harbor Press, Cold Spring Harbor, NY). Other methods include alanine scanning mutational analysis, peptide blot analysis (Reineke (2004) Methods Mol Biol 248:443-63), peptide cleavage analysis, crystallographic studies and NMR analysis. In addition, methods such as epitope excision, epitope extraction and chemical modification of antigens can be employed (Tomer (2000) Protein Science 9: 487-496). Another method that can be used to identify the amino acids within a polypeptide with which an antibody interacts is hydrogen/deuterium exchange detected by mass spectrometry. In general terms, the hydrogen/deuterium exchange method involves deuterium-labeling the protein of interest, followed by binding the antibody to the deuterium-labeled protein. Next, the protein/antibody complex is transferred to water and exchangeable protons within amino acids that are protected by the antibody complex undergo deuterium-to-hydrogen back-exchange at a slower rate than exchangeable protons within amino acids that are not part of the interface. As a result, amino acids that form part of the protein/antibody interface may retain deuterium and therefore exhibit relatively higher mass compared to amino acids not included in the interface. After dissociation of the antibody, the target protein is subjected to protease cleavage and mass spectrometry analysis, thereby revealing the peptides containing the deuterium-labeled residues that contain specific amino acids with which the antibody interacts. *See, e.g.*, Ehring (1999) *Analytical Biochemistry* 267(2):252-259; Engen and Smith (2001) *Anal. Chem. 73*: 256A-265A.

[0103] The term "epitope" refers to a site on an antigen to which B and/or T cells respond. B-cell epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation.

[0104] Modification-Assisted Profiling (MAP), also known as Antigen Structure-based Antibody Profiling (ASAP) is a method that categorizes large numbers of monoclonal antibodies (mAbs) directed against the same antigen according to the similarities of the binding profile of each antibody to chemically or enzymatically modified antigen surfaces (see US 2004/0101920, herein specifically incorporated by reference in its entirety). Each category may reflect a unique epitope either distinctly different from or partially overlapping with epitope represented by another category. This technology allows rapid filtering of genetically identical antibodies, such that characterization can be focused on genetically distinct antibodies. When applied to hybridoma screening, MAP may facilitate identification of rare hybridoma clones that produce mAbs having the desired characteristics. MAP may be used to sort the antibodies of the invention into groups of antibodies binding different epitopes.

[0105] In certain embodiments, the anti-BMP6 antibodies or antigen-binding fragments thereof bind an epitope within any one or more of the regions exemplified in human BMP6, as exemplified in SEQ ID NO: 40, or to a fragment thereof.

[0106] The present invention includes human anti-BMP6 antibodies that bind to the same epitope, or a portion of the epitope, as any of the specific exemplary antibodies described herein, or an antibody having the CDR sequences of any of the exemplary antibodies described herein. Likewise, the present invention also includes anti-BMP6 antibodies that compete for binding to BMP6 or a BMP6 fragment with any of the specific exemplary antibodies described herein, or an antibody having the CDR sequences of any of the exemplary antibodies described herein.

[0107] One can easily determine whether an antibody binds to the same epitope as, or competes for binding with, a reference anti-BMP6 antibody by using routine methods known in the art. For example, to determine if a test antibody binds to the same epitope as a reference anti-BMP6 antibody of the invention, the reference antibody is allowed to bind to a BMP6 protein or peptide under saturating conditions. Next, the ability of a test antibody to bind to the BMP6 molecule is assessed. If the test antibody is able to bind to BMP6 following saturation binding with the reference anti-BMP6 antibody, it can be concluded that the test antibody binds to a different epitope than the reference anti-BMP6 antibody. On the other hand, if the test antibody is not able to bind to the BMP6 protein following saturation binding with the reference anti-BMP6 antibody, then the test antibody may bind to the same epitope as the epitope bound by the reference anti-BMP6 antibody of the invention.

[0108] To determine if an antibody competes for binding with a reference anti-BMP6 antibody, the above-described binding methodology is performed in two orientations: In a first orientation, the reference antibody is allowed to bind to a BMP6 protein under saturating conditions followed by assessment of binding of the test antibody to the BMP6 molecule. In a second orientation, the test

antibody is allowed to bind to a BMP6 molecule under saturating conditions followed by assessment of binding of the reference antibody to the BMP6 molecule. If, in both orientations, only the first (saturating) antibody is capable of binding to the BMP6 molecule, then it is concluded that the test antibody and the reference antibody compete for binding to BMP6. As will be appreciated by a person of ordinary skill in the art, an antibody that competes for binding with a reference antibody may not necessarily bind to the identical epitope as the reference antibody, but may sterically block binding of the reference antibody by binding an overlapping or adjacent epitope.

[0109] Two antibodies bind to the same or overlapping epitope if each competitively inhibits (blocks) binding of the other to the antigen. That is, a 1-, 5-, 10-, 20- or 100-fold excess of one antibody inhibits binding of the other by at least 50% but preferably 75%, 90% or even 99% as measured in a competitive binding assay (see, *e.g.,* Junghans *et al.*, Cancer Res. 1990 50:1495-1502). Alternatively, two antibodies have the same epitope if essentially all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0110] Additional routine experimentation (*e.g.*, peptide mutation and binding analyses) can then be carried out to confirm whether the observed lack of binding of the test antibody is in fact due to binding to the same epitope as the reference antibody or if steric blocking (or another phenomenon) is responsible for the lack of observed binding. Experiments of this sort can be performed using ELISA, RIA, surface plasmon resonance, flow cytometry or any other quantitative or qualitative antibody-binding assay available in the art.

Immunoconjugates

[0111] The invention encompasses a human anti-BMP6 monoclonal antibody conjugated to a therapeutic moiety ("immunoconjugate"), such as an agent that is capable of reducing the severity of an iron-deficiency anemia or an iron-deficiency related disorder, or to ameliorate at least one symptom associated with an iron-deficiency anemia or an iron-deficiency related disorder. As used herein, the term "immunoconjugate" refers to an antibody that is chemically or biologically linked to a radioactive agent, a cytokine, an interferon, a target or reporter moiety, an enzyme, a toxin, or a therapeutic agent. The antibody may be linked to the radioactive agent, cytokine, interferon, target or reporter moiety, enzyme, toxin, or therapeutic agent at any location along the molecule so long as it is able to bind its target. An example of immunoconjugate is an antibody drug conjugate. In some embodiments, the agent may be a second different antibody to BMP6, or to a cytokine such as IL-1, IL-6, or a chemokine such as TGF-β. The type of therapeutic moiety that may be conjugated to the anti-BMP6 antibody will take into account the condition to be treated and the

desired therapeutic effect to be achieved. Examples of suitable agents for forming immunoconjugates are known in the art; see for example, WO 05/103081. The preparation of immunoconjugates and immunotoxins is generally well known in the art (see, e.g., U.S. Pat. No. 4340535). Immunoconjugates are described in detail, for example, in US 7250492, US 7420040 and US 7411046, each of which is incorporated herein in their entirety.

Multi-specific Antibodies

[0112] The antibodies of the present invention may be mono-specific, bi-specific, or multi-specific. Multi-specific antibodies may be specific for different epitopes of one target polypeptide or may contain antigen-binding domains specific for more than one target polypeptide. See, *e.g.,* Tutt et al., 1991, J. Immunol. 147:60-69; Kufer et al., 2004, Trends Biotechnol. 22:238-244.The antibodies of the present invention can be linked to or co-expressed with another functional molecule, *e.g.*, another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (*e.g.,* by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multi-specific antibody with a second binding specificity. For example, the present invention includes bi-specific antibodies wherein one arm of an immunoglobulin is specific for the N-terminal region of BMP6, or a fragment thereof, and the other arm of the immunoglobulin is specific for the C-terminal region of BMP6, or a second therapeutic target, or is conjugated to a therapeutic moiety. An exemplary bi-specific antibody format that can be used in the context of the present invention involves the use of a first immunoglobulin (Ig) C_{H3} domain and a second Ig C_{H3} domain, wherein the first and second Ig C_{H3} domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the bi-specific antibody to BMP6 as compared to a bi-specific antibody lacking the amino acid difference. In one embodiment, the first Ig C_{H3} domain binds BMP6 and the second Ig C_{H3} domain contains a mutation that reduces or abolishes BMP6 binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second C_{H3} may further comprise a Y96F modification (by IMGT; Y436F by EU). Further modifications that may be found within the second C_{H3} include: D16E, L18M, N44S, K52N, V57M, and V82I (by IMGT; D356E, L358M, N384S, K392N, V397M, and V422I by EU) in the case of IgG1 antibodies; N44S, K52N, and V82I (IMGT; N384S, K392N, and V422I by EU) in the case of IgG2 antibodies; and Q15R, N44S, K52N, V57M, R69K, E79Q, and V82I (by IMGT; Q355R, N384S, K392N, V397M, R409K, E419Q, and V422I by EU) in the case of IgG4 antibodies. Variations on the bi-specific antibody format described above are contemplated within the scope of the present invention.

[0113] Other exemplary bispecific formats that can be used in the context of the present invention include, without limitation, *e.g.*, scFv-based or diabody bispecific formats, IgG-scFv fusions, dual

variable domain (DVD)-Ig, Quadroma, knobs-into-holes, common light chain (*e.g.*, common light chain with knobs-into-holes, etc.), CrossMab, CrossFab, (SEED)body, leucine zipper, Duobody, IgG1/IgG2, dual acting Fab (DAF)-IgG, and Mab^2 bispecific formats (*see*, *e.g.*, Klein *et al.* 2012, mAbs 4:6, 1-11, and references cited therein, for a review of the foregoing formats). Bispecific antibodies can also be constructed using peptide/nucleic acid conjugation, *e.g.*, wherein unnatural amino acids with orthogonal chemical reactivity are used to generate site-specific antibody-oligonucleotide conjugates which then self-assemble into multimeric complexes with defined composition, valency and geometry. (*See*, *e.g.*, Kazane *et al.*, *J. Am. Chem. Soc.* [*Epub*: *Dec. 4, 2012*]).

Therapeutic Administration and Formulations

[0114] The invention provides therapeutic compositions comprising the anti-BMP6 antibodies or antigen-binding fragments thereof as discussed herein. The therapeutic compositions in accordance with the invention can be administered with suitable carriers, excipients, and other agents that are incorporated into formulations to provide improved transfer, delivery, tolerance, and the like. A multitude of appropriate formulations can be found in the formulary known to all pharmaceutical chemists: Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA. These formulations include, for example, powders, pastes, ointments, jellies, waxes, oils, lipids, lipid (cationic or anionic) containing vesicles (such as LIPOFECTIN®), DNA conjugates, anhydrous absorption pastes, oil-in-water and water-in-oil emulsions, emulsions carbowax (polyethylene glycols of various molecular weights), semi-solid gels, and semi-solid mixtures containing carbowax. See also Powell *et al.* "Compendium of excipients for parenteral formulations" PDA (1998) J Pharm Sci Technol 52:238-311.

[0115] The dose of antibody may vary depending upon the age and the size of a subject to be administered, target disease, conditions, route of administration, and the like. When the antibody of the present invention is used for preventing or treating an iron-deficiency anemia or an iron-deficiency related disorder, it is advantageous to intravenously administer the antibody of the present invention normally at a single dose of about 0.1 to about 100 mg/kg body weight, more preferably about 5 to about 100, about 10 to about 90, or about 20 to about 70 mg/kg body weight. Depending on the severity of the condition, the frequency and the duration of the treatment can be adjusted. In certain embodiments, the antibody or antigen-binding fragment thereof of the invention can be administered as an initial dose of at least about 0.1 mg to about 800 mg, about 1 to about 500 mg, about 5 to about 300 mg, or about 10 to about 200 mg, to about 100 mg, or to about 50 mg. In certain embodiments, the initial dose may be followed by administration of a second or a plurality of subsequent doses of the antibody or antigen-binding fragment thereof in an amount that can be approximately the same or less than that of the initial dose, wherein the subsequent doses

are separated by at least 1 day to 3 days; at least one week, at least 2 weeks; at least 3 weeks; at least 4 weeks; at least 5 weeks; at least 6 weeks; at least 7 weeks; at least 8 weeks; at least 9 weeks; at least 10 weeks; at least 12 weeks; or at least 14 weeks.

[0116] Various delivery systems are known and can be used to administer the pharmaceutical composition of the invention, *e.g.,* encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the mutant viruses, receptor mediated endocytosis (see, *e.g.*, Wu et al. (1987) J. Biol. Chem. 262:4429-4432). Methods of introduction include, but are not limited to, intradermal, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. The composition may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.,* oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. The pharmaceutical composition can be also delivered in a vesicle, in particular a liposome (see, for example, Langer (1990) Science 249:1527-1533).

[0117] The use of nanoparticles to deliver the antibodies of the present invention is also contemplated herein. Antibody-conjugated nanoparticles may be used both for therapeutic and diagnostic applications. Antibody-conjugated nanoparticles and methods of preparation and use are described in detail by Arruebo, M., et al. 2009 ("Antibody-conjugated nanoparticles for biomedical applications" in J. Nanomat. Volume 2009, Article ID 439389, 24 pages, doi: 10.1155/2009/439389), incorporated herein by reference. Nanoparticles for drug delivery have also been described in, for example, US 8277812, US 8258256, US 8257740, US 8246995, US 8236330, each incorporated herein in its entirety.

[0118] In certain situations, the pharmaceutical composition can be delivered in a controlled release system. In one embodiment, a pump may be used. In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity of the composition's target, thus requiring only a fraction of the systemic dose.

[0119] The injectable preparations may include dosage forms for intravenous, subcutaneous, intracutaneous and intramuscular injections, drip infusions, etc. These injectable preparations may be prepared by methods publicly known. For example, the injectable preparations may be prepared, *e.g.*, by dissolving, suspending or emulsifying the antibody or its salt described above in a sterile aqueous medium or an oily medium conventionally used for injections. As the aqueous medium for injections, there are, for example, physiological saline, an isotonic solution containing glucose and other auxiliary agents, etc., which may be used in combination with an appropriate solubilizing agent such as an alcohol (*e.g.*, ethanol), a polyalcohol (e.g., propylene glycol, polyethylene glycol), a nonionic surfactant [e.g., polysorbate 80, HCO-50 (polyoxyethylene (50 mol) adduct of hydrogenated castor oil)], etc. As the oily medium, there are employed, *e.g.*, sesame oil,

soybean oil, etc., which may be used in combination with a solubilizing agent such as benzyl benzoate, benzyl alcohol, etc. The injection thus prepared is preferably filled in an appropriate ampoule.

[0120] A pharmaceutical composition of the present invention can be delivered subcutaneously or intravenously with a standard needle and syringe. In addition, with respect to subcutaneous delivery, a pen delivery device readily has applications in delivering a pharmaceutical composition of the present invention. Such a pen delivery device can be reusable or disposable. A reusable pen delivery device generally utilizes a replaceable cartridge that contains a pharmaceutical composition. Once all of the pharmaceutical composition within the cartridge has been administered and the cartridge is empty, the empty cartridge can readily be discarded and replaced with a new cartridge that contains the pharmaceutical composition. The pen delivery device can then be reused. In a disposable pen delivery device, there is no replaceable cartridge. Rather, the disposable pen delivery device comes prefilled with the pharmaceutical composition held in a reservoir within the device. Once the reservoir is emptied of the pharmaceutical composition, the entire device is discarded.

[0121] Numerous reusable pen and autoinjector delivery devices have applications in the subcutaneous delivery of a pharmaceutical composition of the present invention. Examples include, but certainly are not limited to AUTOPEN® (Owen Mumford, Inc., Woodstock, UK), DISETRONIC™ pen (Disetronic Medical Systems, Burghdorf, Switzerland), HUMALOG MIX 75/25™ pen, HUMALOG® pen, HUMALIN 70/30™ pen (Eli Lilly and Co., Indianapolis, IN), NOVOPEN® I, II and III (Novo Nordisk, Copenhagen, Denmark), NOVOPEN JUNIOR™ (Novo Nordisk, Copenhagen, Denmark), BD pen (Becton Dickinson, Franklin Lakes, NJ), OPTIPEN®, OPTIPEN® PRO, OPTIPEN® STARLET, and OPTICLIK™ (Sanofi-aventis, Frankfurt, Germany), to name only a few. Examples of disposable pen delivery devices having applications in subcutaneous delivery of a pharmaceutical composition of the present invention include, but certainly are not limited to the SOLOSTAR® pen (Sanofi-aventis), the FLEXPEN® (Novo Nordisk), and the KWIKPEN® (HUMALOG®), the SURECLICK®Autoinjector (, the PENLET (Haselmeier, Stuttgart, Germany), the EPIPEN® (Mylan®and the HUMIRA ® Pen (Abbott Labs, Abbott Park, IL), to name only a few.

[0122] Advantageously, the pharmaceutical compositions for oral or parenteral use described above are prepared into dosage forms in a unit dose suited to fit a dose of the active ingredients. Such dosage forms in a unit dose include, for example, tablets, pills, capsules, injections (ampoules), suppositories, etc. The amount of the aforesaid antibody contained is generally about 5 to about 500 mg per dosage form in a unit dose; especially in the form of injection, it is preferred that the aforesaid antibody is contained in about 5 to about 100 mg and in about 10 to about 250 mg for the other dosage forms. The present invention includes an injection device (e.g., a pre-filled

syringe or pre-filled autoinjector) or a vial (e.g., a glass or plastic vial) comprising an antibody or antigen-binding fragment of the present invention or pharmaceutical composition thereof which includes a pharmaceutically acceptable carrier.

Therapeutic Uses of the Antibodies

[0123] In certain embodiments of the invention, the present antibodies are useful for treating an iron-deficiency anemia or an iron-deficiency related disorder, or at least one symptom associated with an iron-deficiency anemia or an iron-deficiency related disorder. The antibodies of the invention are also contemplated for prophylactic use in patients at risk for developing an iron-deficiency anemia or an iron-deficiency related disorder. These patients include the elderly, or patients immunocompromised due to illness or treatment with immunosuppressive therapeutics. It is contemplated that the antibodies of the invention may be used alone, or in conjunction with a second agent, or third agent for treating an iron-deficiency anemia or an iron-deficiency related disorder, or for alleviating at least one symptom or complication associated with an iron-deficiency anemia or an iron-deficiency related disorder. The second or third agents may be delivered concurrently with the antibodies of the invention, or they may be administered separately, either before or after the antibodies of the invention. A patient that may receive an antibody or antigen-binding fragment of the invention or a pharmaceutical composition thereof includes, for example, an animal such as a mammal such as a human (e.g., an elderly human, for example, 65 years of age or older), rabbit, mouse, rat, cow, pig, dog, primate, horse or sheep.

[0124] In a further embodiment of the invention the present antibodies are used for the preparation of a pharmaceutical composition for treating patients suffering from an iron-deficiency anemia or an iron-deficiency related disorder.

Combination Therapies

[0125] Combination therapies may include an anti-BMP6 antibody of the invention and any additional therapeutic agent(s) that may be advantageously combined with an antibody of the invention, or with a biologically active fragment of an antibody of the invention.

[0126] The antibodies may be used in conjunction with other therapies, such as moieties or modalities known in the art for treating iron-deficiency anemia or an iron-deficiency related disorder, such as, but not limited to, iron supplementation through iron supplements, dietary changes to promote serum iron and/or intravenous delivery of iron, blood transfusion, and iron promoting medications.

[0127] The additional therapeutically active component(s) may be administered prior to, concurrent with, or after the administration of the anti-BMP6 antibody of the present invention. For purposes of

the present disclosure, such administration regimens are considered the administration of an anti-BMP6 antibody "in combination with" one or more additional therapeutically active component(s).

Diagnostic Uses of the Antibodies

[0128] The anti-BMP6 antibodies of the present invention may also be used to detect and/or measure BMP6 in a sample, *e.g.*, for diagnostic purposes. Exemplary diagnostic assays for BMP6 may comprise, *e.g.*, contacting a sample, obtained from a patient, with an anti-BMP6 antibody of the invention, wherein the anti-BMP6 antibody is labeled with a detectable label or reporter molecule or used as a capture ligand to selectively isolate BMP6 from patient samples. Alternatively, an unlabeled anti-BMP6 antibody can be used in diagnostic applications in combination with a secondary antibody which is itself detectably labeled. The detectable label or reporter molecule can be a radioisotope, such as ^{3}H, ^{14}C, ^{32}P, ^{35}S, or ^{125}I; a fluorescent or chemiluminescent moiety such as fluorescein isothiocyanate, or rhodamine; or an enzyme such as alkaline phosphatase, β-galactosidase, horseradish peroxidase, or luciferase. Specific exemplary assays that can be used to detect or measure BMP6 in a sample include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence-activated cell sorting (FACS).

[0129] Samples that can be used in BMP6 diagnostic assays according to the present invention include any tissue or fluid sample obtainable from a patient, which contains detectable quantities of either BMP6, or fragments thereof, under normal or pathological conditions. Generally, levels of BMP6 in a particular sample obtained from a healthy patient (*e.g.*, a patient not afflicted with an iron-deficiency anemia or an iron-deficiency related disorder) will be measured to initially establish a baseline, or standard, level of BMP6. This baseline level of BMP6 can then be compared against the levels of BMP6 measured in samples obtained from individuals suspected of having an iron-deficiency anemia or an iron-deficiency related disorder related condition, or symptoms associated with such condition.

[0130] The antibodies specific for BMP6 may contain no additional labels or moieties, or they may contain an N-terminal or C-terminal label or moiety. In one embodiment, the label or moiety is biotin. In a binding assay, the location of a label (if any) may determine the orientation of the peptide relative to the surface upon which the peptide is bound. For example, if a surface is coated with avidin, a peptide containing an N-terminal biotin will be oriented such that the C-terminal portion of the peptide will be distal to the surface. In some embodiments, the label may be detectable label such as a radionuclide, a fluorescent dye or a MRI-detectable label. Detectable labels may be linked to the antibodies wherein such antibodies may be used in imaging assays.

EXAMPLES

[0131] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the methods and compositions of the invention, and are not intended to limit the scope of what the inventors regard as their invention. Unless indicated otherwise, parts are parts by weight, molecular weight is average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric.

Example 1. Generation of Human Antibodies to BMP6

[0132] Human antibodies to BMP6 were generated in a VELOCIMMUNE® mouse comprising DNA encoding human Immunoglobulin heavy and kappa light chain variable regions. The mice were immunized with a stabilized full-length BMP6 protein.

[0133] The antibody immune response was monitored by a BMP6-specific immunoassay. When a desired immune response was achieved, splenocytes were harvested and fused with mouse myeloma cells to preserve their viability and form hybridoma cell lines. The hybridoma cell lines were screened and selected to identify cell lines that produce BMP6-specific antibodies. The cell lines were used to obtain several anti-BMP6 chimeric antibodies (i.e., antibodies possessing human variable domains and mouse constant domains).

[0134] Exemplary antibodies generated as disclosed above were designated as H4H17855P and H4H17871P. The biological properties of the exemplary antibodies generated in accordance with the methods of this Example are further described in the Examples below.

Example 2. Heavy and Light Chain Variable Region Amino Acid Sequences

[0135] Table 1 sets forth the heavy and light chain variable region amino acid sequence pairs of selected antibodies specific for BMP6 and their corresponding antibody identifiers. Antibodies are typically referred to herein according to the following nomenclature: Fc prefix (e.g. “H4H”), followed by a numerical identifier (*e.g.,* “7855” as shown in Table 1), followed by a “P” suffix. Thus, according to this nomenclature, an antibody may be referred to as, *e.g.,* “H4H17855P”. The H4H prefix on the antibody designations used herein indicates the particular Fc region of the antibody. For example, an “H4H” antibody has a human IgG4 Fc.

[0136]

Table 1

	SEQ ID NOs:							
Antibody Designation	HCVR	HCDR1	HCDR2	HCDR3	LCVR	LCDR1	LCDR2	LCDR3
H4H17855P	1	5	6	7	2	8	9	10
H4H17871P	3	11	12	13	4	14	15	16

Example 3. Binding kinetics of human monoclonal antibodies to BMP6

Equilibrium dissociation constants (K_D values) for human and mouse BMP6 binding to purified anti-BMP6 monoclonal antibodies of this invention were determined using a real-time surface plasmon resonance (SPR) biosensor instrument, MASS-1. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, 1µg/mL Heparin, and 0.05% v/v Surfactant Tween-20, pH 7.4 (HBS-EHT) running buffer at 25°C and 37°C. The HCA sensor surface was first derivatized by amine coupling the monoclonal mouse anti-human Fc antibody (GE, # BR100839) and anti-BMP6 monoclonal antibodies were individually captured. Different concentrations of human BMP6 reagents (hBMP6; R&D Systems, Cat#507-BP; 60nM – 0.94nM; 4-fold serial dilution) or mouse BMP6 (mBMP6; R&D Systems, Cat#6325-BM; 60nM and 15nM) prepared in HBS-EHT running buffer were injected over the captured anti-BMP6 monoclonal antibody for 4 minutes at a flow rate of 30µL/minute, while the dissociation of BMP6 reagent bound to captured anti-BMP6 monoclonal antibody was monitored for 10 minutes in HBS-EHT running buffer. Kinetic association (k_a) and dissociation (k_d) rate constants were determined by fitting the real-time binding sensorgrams to a 1:1 binding model with mass transport limitation using Scrubber 2.0c software. Binding dissociation equilibrium constant (K_D) and dissociative half-life (t½) for different anti-BMP6 monoclonal antibodies were calculated from the kinetic rate constants as:

$$K_D\ (M) = \frac{kd}{ka}, \quad \text{and} \quad t½\ (min) = \frac{\ln(2)}{60 * kd}$$

Binding kinetic parameters for hBMP6 or mBMP6 binding to different anti-BMP6 monoclonal antibodies of this invention at 25°C and 37°C are shown in Tables 2 through 5.

As shown in Table 2, at 25°C, both antibodies of the invention bound human BMP6 with K_D values of 195pM and 355pM. As shown in Table 3, at 37°C, both antibodies of the invention bound human BMP6 with K_D values of 240pM and 1.34nM. As shown in Table 4, at 25°C, both antibodies of the invention bound mouse BMP6 with K_D values of 3.39nM and 3.55nM. As shown in Table 5, at 37°C, both antibodies of the invention bound mouse BMP6 with K_D values of 4.0nM and 6.46nM.

Table 2: Binding kinetics parameters of hBMP6 binding to anti-BMP6 monoclonal antibodies at 25°C.

mAb Captured	mAb Capture Level (RU)	60nM hBMP6 Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H17855P	282 ± 2.4	72	1.78E+05	6.30E-05	3.55E-10	183
H4H17871P	243 ± 1.8	52	2.83E+05	4.14E-05	1.95E-10	279
Isotype Control mAb	345 ± 1.8	2	NB	NB	NB	NB

NB; no binding was observed under the current experimental conditions.

Table 3: Binding kinetics parameters of hBMP6 binding to anti-BMP6 monoclonal antibodies at 37°C.

mAb Captured	mAb Capture Level (RU)	60nM hBMP6 Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H17855P	319 ± 9.3	83	6.11E+04	8.17E-05	1.34E-09	141
H4H17871P	286 ± 7.7	43	4.16E+04	1.00E-05 #	2.40E-10	1155
Isotype Control mAb	407 ± 3.4	2	NB	NB	NB	NB

NB; no binding was observed under the current experimental conditions.

under the current experimental conditions, no dissociation of hBMP6 was observed from the captured anti-BMP6 monoclonal antibody and k_d
value was manually fixed at 1.00E-05 when fitting the real time binding sensorgrams.

Table 4: Binding kinetics parameters of mBMP6 binding to anti-BMP6 monoclonal antibodies at 25°C.

mAb Captured	mAb Capture Level (RU)	60nM mBMP6 Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H17855P	274 ± 4.8	41	3.66E+04	1.30E-04	3.55E-09	89
H4H17871P	237 ± 2.8	25	3.74E+04	1.27E-04	3.39E-09	91
Isotype Control mAb	346 ± 1.1	3	NB	NB	NB	NB

NB: no binding was observed under the current experimental conditions.

Table 5: Binding kinetics parameters of mBMP6 binding to anti-BMP6 monoclonal antibodies at 37°C.

mAb Captured	mAb Capture Level (RU)	60nM mBMP6 Bound (RU)	k_a (1/Ms)	k_d (1/s)	K_D (M)	t½ (min)
H4H17855P	295 ± 3.7	46	2.38E+04	1.54E-04	6.46E-09	75
H4H17871P	288 ± 2.5	19	2.35E+04	9.43E-05	4.00E-09	123
Isotype Control mAb	408 ± 6.2	3	NB	NB	NB	NB

*NB indicates that no binding was observed under the current experimental conditions.

Example 4. Blocking of BMP6 receptor binding by anti-BMP6 monoclonal antibodies

Blocking of BMP6 from binding to its receptors, Hemojuvelin, ActR2A or ActR2B by anti-BMP6 monoclonal antibodies (mAbs) was determined using a real-time surface plasmon resonance (SPR) biosensor instrument, Biacore 3000. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, 1µg/mL Heparin, and 0.05% v/v Surfactant Tween-20, pH 7.4 (HBS-EHT) buffer at 25°C.

Approximately 10500, 5000 and 5000 RU of human Hemojuvelin expressed with a human Fc tag (hHJV-hFc; SEQ ID: 37), human ActR2A extracellular domain expressed with a C-terminal human Fc tag (hActR2A-hFc; SEQ ID: 38), human ActR2B extracellular domain expressed with a C-terminal human Fc tag (hActR2B-hFc; SEQ ID: 39), respectively were individually immobilized on different flow cells of CM4 sensor surface using the EDC/NHS surface chemistry; while the activated/deactivated surface was used as reference control surface. A concentration of 10nM human BMP-6 was pre-mixed with 400nM anti-BMP-6 mAbs for at least 2 hours before the start of the

experiment. The mixture of BMP6 and anti-BMP6 mAbs was injected over different immobilized sensor surfaces for 10 minutes at a flow rate of 5µL/min. The binding of 10nM BMP6 to immobilized surfaces was used to assess percent blocking while the non-specific binding of anti-BMP6 mAbs without BMP6 was also tested.

As shown in Table 6, the anti-BMP6 antibodies of the invention demonstrated partial blocking of BMP6 binding to hHJV-hFc. The anti-BMP6 antibodies of the invention demonstrated enhanced binding of BMP6 to the hActR2A-hFc and hActR2B-hFc surfaces.

Table 6: Blocking of BMP6 from binding to its receptors, Hemojuvelin, ActR2A or ActR2B by anti-BMP6 monoclonal antibodies.

		H4H17855P	**H4H17871P**	**Isotype Control mAb**
hHJV-hFc Surface	400nM mAb Binding (RU)	39	27	14
	400nM mAb + 10nM hBMP-6 Binding (RU)	266	111	299
	% Blocking	61	84	41
hActR2A-hFc Surface	400nM mAb Binding (RU)	34	30	17
	400nM mAb + 10nM hBMP-6 Binding (RU)	2490	659	246
	% Blocking	-400	-46	43
hActR2B-hFc Surface	400nM mAb Binding (RU)	37	28	15
	400nM mAb + 10nM hBMP-6 Binding (RU)	2486	711	187
	% Blocking	-563	-94	25

Example 5. Anti-BMP6 Antibodies Blocking BMP6 Binding to Hemojuvelin and ActivinR2A

The ability of anti-BMP6 monoclonal antibodies to block binding of human BMP6 to two natural binding partners, the co-receptor Hemojuvelin (HJV), and a type II binding receptor, Activin Receptor 2a (ActR2a), was measured using two competition sandwich ELISAs.

The human BMP6 protein used in the experiments was purchased from R&D systems (hBMP6; Cat# #507-BP/CF) and was biotinylated for detection purposes (biot-hBMP6) and the molecular weight of 30kDa was used for calculations as BMP6 protein is naturally a dimer. The HJV protein used in the experiments was comprised of a portion of the human HJV extracellular

domain (aa Gln36-Ser399) expressed with a linker sequence and the Fc portion of the human IgG1 at the C-terminus (hHJV-hFc; SEQ ID: 37) The Activin R2a protein was purchased from R&D Systems (hActR2a-hFc; R&D systems, Cat#340-RC2). An isotype antibody control, was included, along with a commercially available goat-anti-hBMP6 positive blocking control antibody (R&D Systems, Cat#AF507).

Experiments were carried out using the following procedure. Receptors were coated in Hank's Balanced Salt Solution (HBSS) separately at a concentration of 5 µg/mL for hHJV-hFc and 2.5 µg/mL for hActR2a-hFc on a 96-well microtiter plate overnight at 4°C. Nonspecific binding sites were subsequently blocked using a 1.0% (w/v) solution of BSA in HBSS. In other microtiter plates, a constant amount of 2.5nM of biot-BMP6 (for HJV capture) or 1.5nM biot-BMP6 (for ActR2a capture) protein was titrated with anti-BMP6 antibodies or isotype control antibodies ranging from 5.1pM to 300nM in serial dilution in HBSS with 1.0% BSA and 3.33µg/mL heparin. These antibody-protein complexes, after a one-hour incubation, were transferred to the microtiter plate coated with hHJV-hFc or hActR2a-hFc. After two hours of incubation at room temperature, the wells were washed, and plate-bound biot-BMP6 was detected with neutravidin conjugated with horseradish peroxidase (HRP) (Thermo Scientific, Cat#31030). The plates were then developed using TMB substrate solution (BD Biosciences, Cat #555214) according to manufacturer's recommendation and absorbance at 450nm was measured on a Victor X5 plate reader.

Data analysis was performed using a sigmoidal dose-response model within Prism™ software (GraphPad). The calculated IC_{50} value, defined as the concentration of antibody required to reduce 50% of biot-BMP6 binding to hHJV-hFc or hActR2a-hFc, was used as an indicator of blocking potency. Percent blockade at indicated concentration of the antibody tested was calculated as an indicator of the ability of the antibodies to block the binding of 2.5nM or 1.5nM of biot-BMP6 to hHJV-hFc or hActR2a-hFc on the plate, respectively. In the calculation, binding signal of the sample of the constant biot-BMP6 without the presence of the antibody for each assay was referenced as 100% binding or 0% blocking; and the baseline signal of the sample of buffer only without the presence of biot-BMP6 was referenced as 0% binding or 100% blocking.

The blockade of each antibody at the maximum concentration of 300nM antibody was calculated and compared. In addition, for the blocking of BMP6 binding to ActR2a, the blockade of each antibody at 11.1nM was calculated and reported to reflect the enhanced binding signal of biot-BMP6 in the presence of some antibodies in this assay.

The blocking results of the two assays are summarized in Table 7. The two antibodies of the invention blocked >90% of 2.5nM biot-BMP6 protein binding to hHJV-hFc with 300nM of antibody. The blocking potency (IC_{50} value) of H4H17855P was calculated at 0.522nM, which was below the lower limit of quantitation for the assay of 1.25nM. The IC_{50} value of H4H17871P blocking BMP6 protein binding to hHJV-hFc was 1.61nM. The potency of the positive control anti-BMP6 commercial Ab was 2.32nM with 93% blocking at 300nM antibody. The Comparator Ab blocked with an IC_{50} value of 2.99nM with maximal blocking of 88%. The isotype control antibody blocked <30% at concentrations up to 300nM antibody, as expected.

The two test antibodies, H4H17855P and H4H17871P, enhanced biot-BMP6 protein binding to hActR2a-hFc 29.8% and 15.5%, respectively at 11.1nM, while blocking 19.5% and 37.3% at 300nM antibody. The positive control anti-BMP6 commercial Ab blocked 92.8% with an IC_{50} of 11.8nM. The Comparator Ab blocked >30% at 300nM, but did not have a sigmoidal curve so no IC_{50} value was reported. The isotype control antibody blocked <30%, as expected.

Table 7: Effect of anti-BMP6 antibodies in Blocking ELISA

Antibody	Blocking of 2.5nM Biot-hBMP6 Binding to HJV-hFc IC50 (M)	300nM Ab Blocking of 2.5nM Biot-hBMP6 Binding to HJV-hFc (%)	Blocking of 1.5nM Biot- hBMP6 Binding to ActR2a-hFc IC50 (M)	300nM Ab Blocking of 1.5nM Biot-hBMP6 Binding to ActR2a-hFc (%)	11.1nM Ab Blocking of 1.5nM Biot-hBMP6 Binding to ActR2a-hFc (%)
H4H17855P	5.22E-10*	97.0	Enhancer	19.5	-29.8
H4H17871P	1.61E-09	94.9	Enhancer/Blocker	37.3	-15.5
Controls					
Comparator Ab	2.99E-09	88.2	IC	47.7	-1.6
ant-BMP6 commercial Ab	2.32E-09	93.0	1.18E-08	92.8	53.7
Isotype control Ab	NB	26.3	NB	19.9	25.7

IC indicates inconclusive IC_{50} value due to non-sigmoidal blocking curve
NB indicated non-blocking (<30%)
* indicates value below the lower limit of quantitation of 1.25E-09M for the BMP6 blocking HJV assay

Example 6. Binding cross-reactivity of anti-BMP6 monoclonal antibodies.

Binding cross-reactivity of anti-BMP6 monoclonal antibodies to BMP6 family members, (Human: BMP6, BMP5, BMP7, BMP8A, BMP9, BMP10, BMP12, BMP14, BMP3b, Activin A, and GDF3 and mouse: BMP6 and GDF6) was determined using a real-time Bio-Layer Interferometry (BLI) biosensor

using Octet HTX instrument. All binding studies were performed in 10mM HEPES, 150mM NaCl, 3mM EDTA, 1mg/mL BSA, 50µg/mL Heparin, and 0.05% v/v Surfactant Tween-20, pH 7.4 (HBS-EBHT) buffer at 25°C with the plate shaking at a speed of 1000 rpm. To assess binding cross-reactivity, Protein A coated Octet biosensor (Pall ForteBio Corp., # 18-5010) was first dipped in wells containing 20 µg/mL of anti-BMP6 monoclonal antibodies for 4 minutes followed by submerging in wells containing 100 nM of different BMP6 family members for 4 minutes. The biosensors were washed in HBS-EBHT buffer in between every step of the experiment. At the end of each cycle, the Protein A biosensor was regenerated using three alternate 5 second dips in 10mM Glycine pH 2.0 and 10 second dip HBS-EBHT buffer. The real-time binding response was monitored during the course of the experiment and the binding response at the end of every step was recorded and tabulated as shown in Tables 8A and 8B.

Table 8A: Binding cross-reactivity of anti-BMP6 monoclonal antibodies.

mAb Captured	mAb Capture Level (nm)	Human BMP6	Mouse BMP6	Human BMP7	Human BMP8A	Human BMP9	Human BMP10
H4H17855P	4.76 ± 0.19	0.29	0.09	0.05	-0.02	0.00	-0.01
H4H17871P	4.31 ± 0.14	0.21	0.09	0.00	0.00	0.01	0.01
Isotype control mAb	4.12 ± 0.06	-0.05	-0.06	-0.05	-0.05	-0.03	-0.04

Table 8B: Binding cross-reactivity of anti-BMP6 monoclonal antibodies.

mAb Captured	Human BMP12 /GDF7	Human BMP14 /GDF5	Human BMP5	Human Activin A	Human GDF3	Mouse GDF6	Human BMP3b
H4H17855P	0.03	-0.02	0.01	-0.02	0.03	-0.02	0.27
H4H17871P	0.01	-0.01	-0.01	-0.01	0.03	-0.02	0.19
Isotype control mAb	-0.04	-0.05	-0.06	-0.06	-0.01	-0.06	0.12

Example 7. Testing for antibody inhibition of BMP6 activation in bioassay with Hep3B/BRE-luc cells (human BMP6) or W-20-17/BRE-luc cells (mouse BMP6)

Cell lines were engineered to stably express a luciferase reporter [BMP-responsive element (BRE(2X)-luciferase-IRES-GFP)], and sorted for high expression of GFP to detect the regulation of BMP6 signaling. To test human BMP6 (hBMP6), Hep3B2.1-7 cells (referred to hereafter as Hep3B cells), a human hepatocellular carcinoma cell line was used and, to test mouse BMP6 (mBMP6), W-20-17 cells, a mouse bone marrow stromal cell line previously shown to be responsive to BMP2 (Thies et al. 1992), was used. The resulting reporter cell lines are referred to as Hep3B/BRE-luc and W-20-17/BRE-luc cells. Hep3B/BRE-luc cells were maintained in a media comprised of MEM, 10% FBS, Penicillin/Streptomycin, L-Glutamine, NEAA and Sodium Pyruvate (this media is referred

to Hep3B Media) and W-20-17/BRE-luc cells were maintained in a media comprised of 10% FBS, DMEM, Penicillin/Streptomycin/L-Glutamine, and 200ug/ml G418 (this media is referred to W-20-17 Media).

For the hBMP6 bioassay, Hep3B/BRE-luc cells were seeded onto 96-well assay plates in Hep3B media at 10,000 cells/well and incubated at 37°C in 5% CO_2 overnight. The next day, the Hep3B media was removed from the Hep3B cells and replaced with media comprised of MEM, 1% FBS, Penicillin/Streptomycin, L-Glutamine, NEAA and Sodium Pyruvate, and then incubated at 37°C in 5% CO_2 for an additional 6 hours before BMP6 and antibodies diluted in the assay media (media comprised of MEM, 0.1% BSA, Penicillin/Streptomycin + L-Glutamine) were added to the cells. For the mBMP6 bioassay, W-20-17/BRE-luc cells were seeded onto 96-well assay plates in W-20-17 media at 10,000 cells/well and incubated at 37°C in 5% CO_2 overnight. BMP6 and antibodies were added to the cells the following day in assay media comprised of DMEM, 0.1% BSA and Penicillin/Streptomycin/L-Glutamine.

For BMP6 activation, human BMP6 (hBMP6; R&D systems, Cat#507-BP/CF) or mouse BMP6 (mBMP6; R&D systems, Cat#6325-BM/CF) was serially diluted 1:3 from 300nM to 0.005nM, and added to cells including a no BMP6 control for dose responses. For antibody inhibition of BMP6, antibodies were serially diluted at 1:3 from either 1000nM to 0.02nM or 100nM to 0.002nM and mixed with either 1nM of hBMP6 or 5nM of mBMP6. A no antibody control was included in all antibody dose responses. These antibody/BMP6 mixtures were then incubated at 25°C for 30 minutes and added to the cells. Cells were incubated at 37°C and 5% CO_2 overnight for Hep3B/BRE-luc cells or 5.5 hours for W-20-17/BRE-luc cells. At the conclusion of these incubations, cells were incubated at 25°C for 15 minutes, followed by addition of OneGlo™ reagent (Promega E6130) to measure the amount of luciferase present in cells. Plates were read for luminescence by a Victor™X instrument (Perkin Elmer) 4 minutes after the addition of OneGlo™ with the results being analyzed using nonlinear regression (4-parameter logistics) with Prism6 software (GraphPad) to obtain EC_{50} and IC_{50} values. Inhibition of antibodies was calculated such that 0 – 100% inhibition is the range of inhibition of either 1 – 1nM hBMP6 or 0 – 5nM mBMP6 without inhibitor.

As shown in Table 9, both of the anti-BMP6 antibodies of the invention demonstrated complete inhibition of 1nM hBMP6 mediated activation of Hep3B/BRE-luc cells. The IC_{50} values of the hBMP6 inhibition ranged from 0.25nM to 1.6nM. Comparator Ab demonstrated complete inhibition of 1nM hBMP6 with an IC_{50} value of 0.53nM. A dose response of hBMP6 activated Hep3B/BRE-luc cells with EC_{50} values of 0.94nM and 0.33nM.

As shown in Table 9, both of the anti-BMP6 antibodies of the invention demonstrated complete inhibition of 5nM mBMP6 mediated activation of W-20-17/BRE-luc cells. The IC_{50} values of the mBMP6 inhibition ranged from 1.9nM to 11nM. Comparator Ab demonstrated complete inhibition of 5nM mBMP6 with an IC_{50} of 1.9nM. A dose response of mBMP6 activated W-20-17/BRE-luc cells with EC_{50} values of 1.2nM and 1.3nM.

An isotype control antibody demonstrated no inhibition of either human BMP6 or mouse BMP6.

Table 9: Anti-BMP6 antibody inhibition of BMP6 activation in cell-based assays

BMP6	**Human**		**Mouse**	
Cell Line	**Hep3B/BRE-luc**		**W-20-17/BRE-luc**	
EC_{50} [M] of BMP6	**9.4E-10**	**3.3E-10**	**1.2E-09**	**1.3E-09**
Constant concentration of BMP6	**1nM**		**5nM**	
Antibody	IC_{50} [M]	IC_{50} [M]	IC_{50} [M]	IC_{50} [M]
H4H17871P	1.6E-09	Not Tested	1.1E-08	Not Tested
H4H17855P	2.5E-10	Not Tested	1.9E-09	Not Tested
Comparator Ab	Not Tested	5.3E-10	Not Tested	1.9E-09
Isotype control	No Inhibition	No Inhibition	No Inhibition	No Inhibition

Example 8. Mouse experiment *in vivo*, serum hepcidin and iron levels after Bmp6 antibody treatment (H4H17855P)

To determine the efficacy of a BMP6 antibody of the invention in increasing serum and decreasing serum Hepcidin, an in vivo experiment was performed in mice homozygous for the expression of human BMP6 and HJV in place of mouse BMP6 and HJV. For the study, 5 or 6 mice per group received 2 doses of antibody on day 1 and 3 at either 10 or 20 mg/kg. Mice were sacrificed on day 5 and serum was taken to measure hepcidin and iron levels.

As shown in Table 10 and Figures 1 and 2, mice receiving a BMP6 antibody of the invention, H4H17855P, had increased serum iron and decreased serum hepcidin compared to mice receiving an isotype control antibody at 10mg/kg. There is a dose dependent effect of the BMP6 antibody, which demonstrated increased serum iron and decreased Hepcidin at 20mg/kg as compared to 10mg/kg

Table 10: Effect of a BMP6 antibody on serum iron and Hepcidin *in vivo*

Antibody	dose	n	Serum iron (mean)	Serum iron (standard deviation)	Serum hepcidin (mean)	Serum hepcidin (standard deviation)
Isotype control	10mg/kg	6	208.3	105	847.3	147.7
H4H17855P	10mg/kg	5	306.1	161.5	648.5	141.9
H4H17855P	20mg/kg	6	425.5	118.9	398.7	109.1
Comparator Ab	10mg/kg	6	385.0	228.5	171.7	20.57

Example 9. Mouse experiment *in vivo*, serum hepcidin and iron levels after Bmp6 antibody treatment (H4H17871P)

To determine the efficacy of a BMP6 antibody of the invention in increasing serum and decreasing serum Hepcidin, an in vivo experiment was performed in mice homozygous for the expression of human BMP6 and HJV in place of mouse BMP6 and HJV. For the study, 7 mice per group received one s.c. dose of 5mg/kg of antibody at day 1. Mice were sacrificed at day 5 and serum was taken to measure hepcidin and iron levels.

Serum iron levels were measured using the QuantiChrom Iron Assay Kit (BioAssay Systems DIFE-250). Serum Hepcidin was measured using the Hepcidin Murine-Compete ELISA kit (Intrinsic Lifesciences HMC-001)

As shown in Table 11 and Figures 3 and 4, mice receiving a BMP6 antibody of the invention, H4H17871P, had increased serum iron and decreased serum hepcidin compared to mice receiving an isotype control antibody.

Table 11: Effect of a BMP6 antibody on serum iron and Hepcidin in vivo

Antibody	Dose	n	Serum iron (mean)	Serum iron (standard deviation)	Serum hepcidin (mean)	Serum hepcidin (standard deviation)
Isotype control	5mg/kg	7	196.7	42.1	244.4	80.75
H4H17871P	5mg/kg	7	290.6	125.6	142.3	73.24
Comparator Ab	5mg/kg	7	400.4	93.52	76.37	57.7

What is claimed is:

1. An isolated human monoclonal antibody or antigen-binding fragment thereof that binds specifically to human bone morphogenetic protein 6 (BMP6), wherein the antibody or antigen-binding fragment exhibits one or more properties selected from the group consisting of:

(a) binds to human BMP6 at 37°C with a binding dissociation equilibrium constant (K_D) of less than about 2nM as measured by surface plasmon resonance;

(b) binds to human BMP6 at 37°C with a dissociative half-life (t½) of greater than about 130 minutes as measured by surface plasmon resonance;

(c) binds to human BMP6 at 25°C with a K_D of less than about 0.4nM as measured by surface plasmon resonance;

(d) binds to human BMP6 at 25°C with a t½ of greater than about 180 minutes as measured by surface plasmon resonance; and

(e) blocks interaction between human BMP6 and human hemojuvelin (HJV).

2. An isolated human antibody or antigen-binding fragment thereof that specifically binds to human BMP6, wherein the antibody or antigen-binding fragment comprises three heavy chain complementarity determining regions (CDRs) (HCDR1, HCDR2 and HCDR3) contained within any one of the heavy chain variable region (HCVR) sequences selected from the group consisting of SEQ ID NOs: 1 and 3; and three light chain CDRs (LCDR1, LCDR2 and LCDR3) contained within any one of the light chain variable region (LCVR) sequences selected from the group consisting of SEQ ID NOs: 2 and 4.

3. The isolated human antibody or antigen-binding fragment thereof of any one of claims 1-2, wherein the antibody or antigen-binding fragment comprises a HCVR comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 3.

4. The isolated human antibody or antigen-binding fragment thereof of any one of claims 1-3, wherein the antibody or antigen-binding fragment comprises a LCVR comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4.

5. The isolated human antibody or antigen-binding fragment thereof of any one of claims 1-4, wherein the antibody or antigen-binding fragment comprises: (a) a HCVR comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 3; and (b) a LCVR comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and 4.

6. The isolated human antibody or antigen-binding fragment thereof of any one of claims 1-5, wherein the antibody or antigen-binding fragment comprises:

(a) a HCDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5 and 11;

(b) a HCDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 6 and 12;

(c) a HCDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 7 and 13;

(d) a LCDR1 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 14;

(e) a LCDR2 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 9 and 15; and/or

(f) a LCDR3 domain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 10 and 16.

7. The isolated human antibody or antigen-binding fragment of any one of claims 1-6, wherein the antibody or antigen-binding fragment comprises a HCVR/LCVR amino acid sequence pair selected from the group consisting of SEQ ID NOs: 1/3 and 2/4.

8. The isolated human antibody or antigen-binding fragment of any one of claims 1-7, wherein the antibody or antigen-binding fragment comprises

(i) a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 34, and

a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 33; and/or

(ii) a light chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 36, and

a heavy chain immunoglobulin comprising the amino acid sequence set forth in SEQ ID NO: 35.

9. The antigen-binding fragment of any one of claims 1-8, wherein the antigen-binding fragment is a Fab fragment, a $F(ab')_2$ fragment, a Fd fragment, a Fv fragment, a single-chain Fv (scFv) molecule, or a dAb fragment.

10. An isolated antibody or antigen-binding fragment thereof that binds the same epitope on human BMP6 as a reference antibody comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 3; and the CDRs of a light chain variable region (LCVR) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4.

11. An isolated antibody or antigen-binding fragment thereof that competes for binding to human BMP6 with a reference antibody comprising the complementarity determining regions (CDRs) of a heavy chain variable region (HCVR) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 and 3, and a light chain variable region (LCVR) comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 2 and 4.

12. A method for making an antibody or antigen-binding fragment of any one of claims 1-11 comprising:

(i) introducing one or more polynucleotides encoding a light immunoglobulin chain of said antibody or fragment and a heavy immunoglobulin chain of said antibody or fragment into a host cell;

(ii) culturing the host cell in a growth medium under condition favorable to expression of the polynucleotide(s); and

(iii) optionally, isolating the antibody or fragment from the host cell and/or medium in which the host cell is grown.

13. An antibody or antigen-binding fragment which is the product of the method of claim 12.

14. An injection device or vessel comprising an antibody or antigen-binding fragment of any one of claims 1-11 and 13.

15. A pharmaceutical composition comprising an isolated human antibody or antigen-binding fragment thereof that binds to human BMP6 according to any one of claims 1-11 and 13, and a pharmaceutically acceptable carrier or diluent; and, optionally, one or more additional therapeutic agents.

16. The pharmaceutical composition of claim 15 comprising said additional therapeutic agent which is an iron supplement.

17. A method for preventing or treating an iron-deficiency anemia or an iron-deficiency related disorder in a patient in need thereof, comprising administering an effective amount of an antibody or an antigen-binding fragment thereof according to any one of claims 1-11 and 13; or a pharmaceutical composition according to any one of claims 15-16 to the patient.

18. The method of claim 17, wherein the antibody or antigen-binding fragment thereof is administered subcutaneously, intravenously, intradermally, orally, or intramuscularly.

19. The method of any one of claims 17-18, wherein the iron-deficiency anemia or an iron-deficiency related disorder produces a condition selected from the group consisting of extreme fatigue, weakness, pale skin, chest pain, fast heartbeat, heart palpitations, shortness of breath, headache, dizziness, lightheadedness, cold hands, cold feet, inflammation of the tongue and restless legs;

and administration of the antibody or antigen-binding fragment treats the condition or reduces the severity of one or more symptoms of the condition.

20. An antibody or antigen-binding fragment thereof of any one of claims 1-11 and 13 for use in treating a patient with an iron-deficiency anemia or an iron-deficiency related disorder.

21. A composition comprising one or more antibodies or antigen-binding fragments thereof of any one of claims 1-11 and 13 for use in treating an iron-deficiency anemia or an iron-deficiency related disorder.

22. Use of the isolated antibody or antigen-binding fragment thereof of any one of claims 1-11 and 13 in the manufacture of a medicament for treating a patient with an iron-deficiency anemia or an iron-deficiency related disorder.

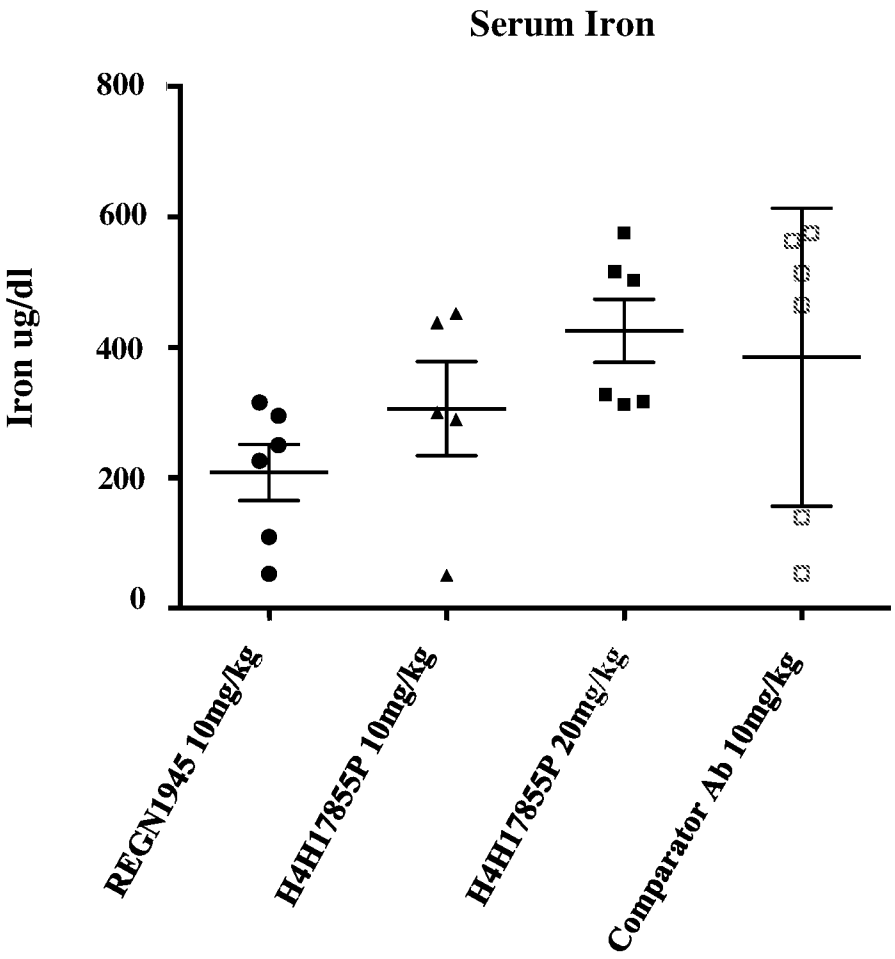
Serum Iron
Iron ug/dl
800
600
400
200
0
REGN1945 10mg/kg
H4H17855P 10mg/kg
H4H17855P 20mg/kg
Comparator Ab 10mg/kg

FIG. 1

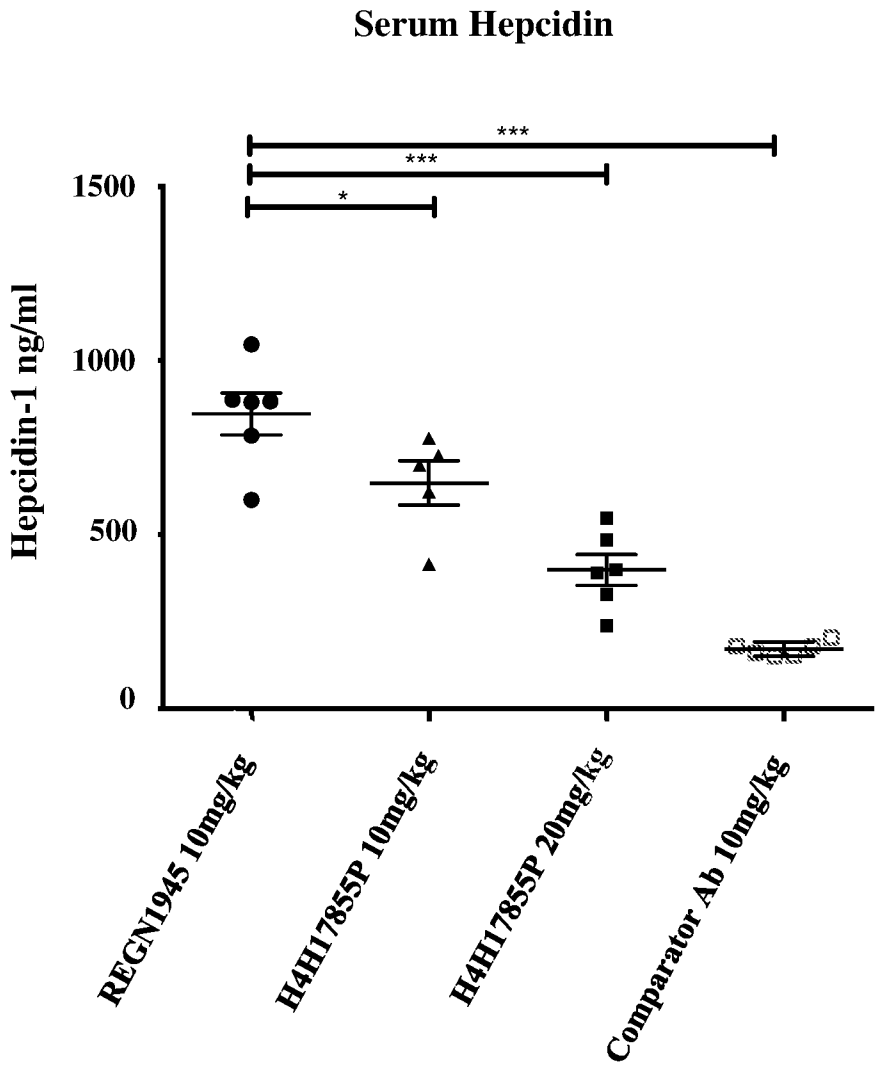
Serum Hepcidin
***
***
*
1500
1000
500
0
Hepcidin-1 ng/ml
REGN1945 10mg/kg
H4H17855P 10mg/kg
H4H17855P 20mg/kg
Comparator Ab 10mg/kg

FIG. 2

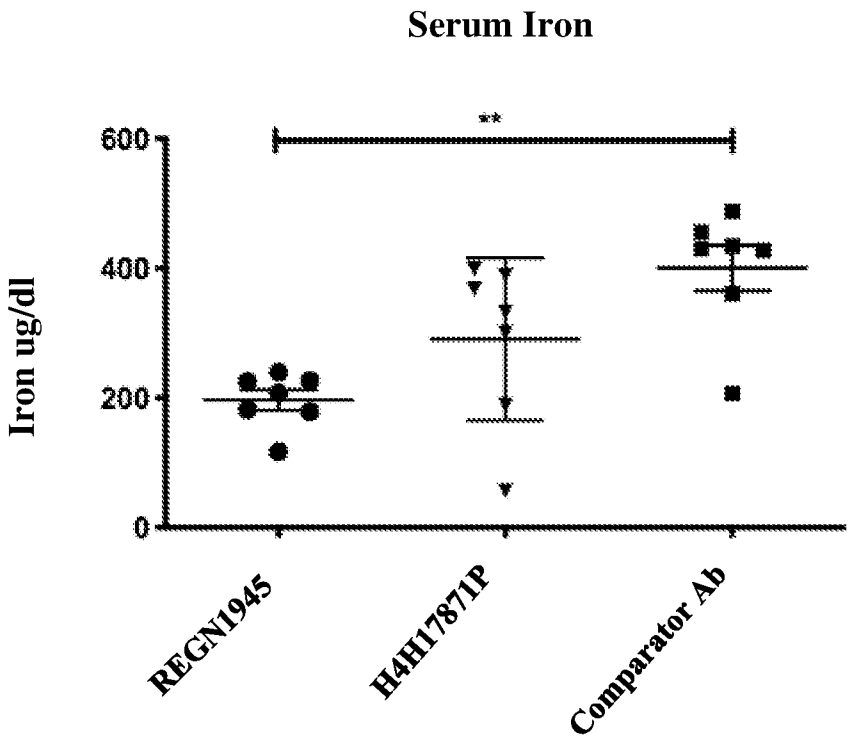
Serum Iron
**
600
400
200
0
Iron ug/dl
REGN1945
H4H17871P
Comparator Ab

FIG. 3

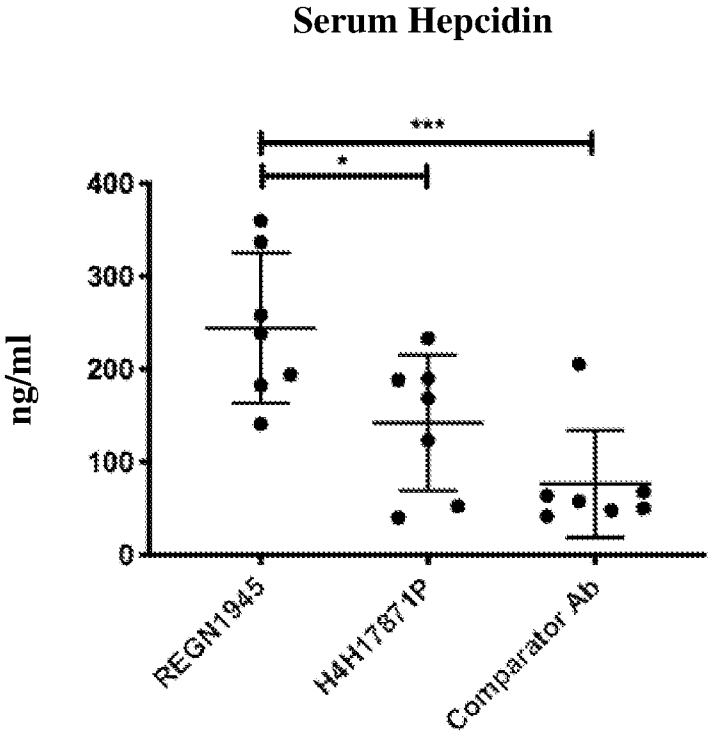
Serum Hepcidin
***
*
400
300
200
100
0
ng/ml
REGN1945
H4H17871P
Comparator Ab

FIG. 4

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/22 A61P7/06 A61K39/395
ADD.

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)
A61P A61K C07K

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)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2016/098079 A2 (NOVARTIS AG [CH]; CONG FENG [US] ET AL.) 23 June 2016 (2016-06-23) examples, in particular paragraph [0591], tables 7, 8 -----	1-22
X	AU 2016 211 890 A1 (GEN HOSPITAL CORP) 25 August 2016 (2016-08-25) whole document, in particular paragraphs [0021], [0032], [0113]; embodiments 1-45; examples, claims ----- -/--	1-22

[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
16 September 2020	23/11/2020

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bernhardt, Wiebke

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Volker Germaschewski: "Generation and development of KY1070, a fully human anti-BMP6 antibody for treatment of Anemia of Chronic Disease", Programme of the 8th Congress of the International BioIron Society , 5 May 2019 (2019-05-05), pages 1-27, XP055730961, Retrieved from the Internet: URL:https://www.embl.de/training/events/2019/BIR19-01/BIR19-01_Programme-V4.pdf [retrieved on 2020-09-15] page 11	1-22
X	Heinz Zoller ET AL: "Iron in Cancer, Infection, Kidney and Liver diseases: Innocent bystander or therapeutic target?", , 5 May 2019 (2019-05-05), pages 1-67, XP055730962, Retrieved from the Internet: URL:https://bioironforum.org/wp-content/uploads/2018/10/7.-Iron-in-cancer-infection-kideny-and-liver-diseases.Theurl-Zoller.pdf [retrieved on 2020-09-15] slides 65-66	1-22
Y	US 8 795 665 B2 (LILLY CO ELI [US]) 5 August 2014 (2014-08-05) paragraphs [0006] - [0012], [0036], table 2; examples, claims	1-22
Y	STEVEN S: "HUMAN ANTIBODY DISCOVERY VELOCIMMUNE- A NOVEL PLATFORM", ASIA FOCUS, CHRISTIAN CONFERENCE OF ASIA, THAILAND, no. 8, 1 January 2008 (2008-01-01), pages 72-74, XP003035153, ISSN: 0044-9164 abstract, figures	1-22
Y	EP 1 360 287 A1 (REGENERON PHARMA [US]) 12 November 2003 (2003-11-12) example 3, claims	1-22

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International application No
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GERSHONI JONATHAN M ET AL: "Epitope mapping - The first step in developing epitope-based vaccines", BIODRUGS, ADIS INTERNATIONAL LTD, NZ, vol. 21, no. 3, 1 January 2007 (2007-01-01), pages 145-156, XP009103541, ISSN: 1173-8804, DOI: 10.2165/00063030-200721030-00002 pages 148-149	10
Y	MATHIEU DONDELINGER ET AL: "Understanding the Significance and Implications of Antibody Numbering and Antigen-Binding Surface/Residue Definition", FRONTIERS IN IMMUNOLOGY, vol. 9, 16 October 2018 (2018-10-16), pages 1-15, XP055572450, DOI: 10.3389/fimmu.2018.02278 whole document, in particular abstract, figures 1-8	2,10,11
A	RUDIKOFF S ET AL: "Single amino acid substitution altering antigen-binding specificity", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES (PNAS), NATIONAL ACADEMY OF SCIENCES, US, vol. 79, no. 6, 1 March 1982 (1982-03-01), pages 1979-1983, XP002683593, ISSN: 0027-8424, DOI: 10.1073/PNAS.79.6.1979 abstract	1-22
X,P	VERENA PETZER ET AL: "A fully human anti-BMP6 antibody reduces the need for erythropoietin in rodent models of the anemia of chronic disease", BLOOD, vol. 136, no. 9, 21 May 2020 (2020-05-21), pages 1080-1090, XP055730960, US ISSN: 0006-4971, DOI: 10.1182/blood.2019004653 whole document, in particular abstract, figures 1-3	1-22

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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:

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 additional sheet

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 an 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.:

1-22(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-22(partially)

   Human antibody binding to human BMP-6 comprising the HCDRs of Seq ID No. 1 and the LCDRs of Seq ID No. 2; antibody binding to the same epitope of said antibody; antibody competing with said antibody; method for making said antibody; injection device or vessel comprising said antibody; pharmaceutical composition comprising said antibody; medical use of said antibody

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2. claims: 1-22(partially)

   Human antibody binding to human BMP-6 comprising the HCDRs of Seq ID No. 3 and the LCDRs of Seq ID No. 4; antibody binding to the same epitope of said antibody; antibody competing with said antibody; method for making said antibody; injection device or vessel comprising said antibody; pharmaceutical composition comprising said antibody; medical use of said antibody

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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