

METHODS FOR TREATING HEPCIDIN-MEDIATED DISORDERS

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority benefit to U.S. Provisional Application No. 62/199,434, filed July 31, 2015, and U.S. Provisional Application No. 62/268,788, filed December 17, 2015, each of which is incorporated by reference in its entirety.

2. SEQUENCE LISTING

[0001.1] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 12, 2016, is named 34392PCT_CRF-sequencelisting.txt and is 52,278 bytes in size.

3. BACKGROUND

[0002] The peptide hormone, hepcidin, plays a central role in systemic iron homeostasis. Hentze *et al.*, *Cell* 142:24-38 (2010). Hepcidin expression is known to be influenced by the product of the *TMPRSS6* gene, matriptase-2, a type II transmembrane serine protease. Common variants in the *TMPRSS6* gene have been shown to correlate with iron status, Benyamin *et al.*, *Nature Genetics* 41(11):1173-1175 (2009), with the rs855791 SNP (2321G→A; A736V) having been shown to correlate with naturally occurring variations in hepcidin expression and blood hemoglobin levels.

[0003] Hepcidin expression has also been implicated in human iron disorders, Pietrangelo, *J. Hepatology* 54:173-181 (2011), and in anemias of chronic disease (ACD) (also known as anemia of inflammation (AI)). ACD is prevalent in patients with chronic infection, autoimmune disease, cancer, and chronic kidney disease (CKD). Sun *et al.*, *Am. J. Hematol.* 87(4):392-400 (2012).

[0004] There is a need in the art for methods of treating hepcidin-mediated disorders.

4. SUMMARY

[0005] We have demonstrated that reducing IL-6 signaling provides clinical benefit in patients with a hepcidin-mediated disorder, including anemia of chronic disease and hepcidin-mediated cellular toxicities, but only in those patients having at least one copy of the *TMPRSS6* rs855791 major allele, with greatest effect in patients with elevated levels of IL-6.

[0006] Accordingly, in a first aspect, methods of treating a hepcidin-mediated disorder are provided. The methods comprise administering a therapeutically effective amount of an IL-6 antagonist to a patient with a hepcidin-mediated disorder who has been determined to have at least one copy of the major allele at the *TMPRSS6* rs855791 SNP. In a first series of embodiments, the patient has previously been determined to have at least one copy of the *TMPRSS6* rs855791 major allele. In another series of embodiments, the method further comprises the earlier step of determining that the patient has at least one copy of the *TMPRSS6* rs855791 major allele. Typically, the patient has elevated pre-treatment serum

[0030] In some embodiments, the hepcidin-mediated disorder is Castleman's Disease.

[0031] In another aspect, methods are provided for improving treatment of a hepcidin-mediated disorder. The method comprises discontinuing administration of an IL-6 antagonist to a patient with a hepcidin-mediated disorder, wherein the patient has been determined to be homozygous for the *TMPRSS6* rs855791 minor allele.

[0032] In another aspect, methods are provided for improving treatment of a hepcidin-mediated disorder by discontinuing therapy that is ineffective, thereby reducing side effects and reducing cost without loss of treatment efficacy. The methods comprise discontinuing administration of an IL-6 antagonist to a patient with a hepcidin-mediated disorder, wherein the patient has been determined to be homozygous for the *TMPRSS6* rs855791 minor allele. In one series of embodiments, the patient has previously been determined to be homozygous for the *TMPRSS6* rs855791 minor allele. In another series of embodiments, the method further comprises the earlier step of determining that the patient is homozygous for the *TMPRSS6* rs855791 minor allele. In typical embodiments, the patient has elevated pre-treatment serum levels of IL-6. In various embodiments, the patient has elevated pre-treatment serum levels of CRP. In various embodiments, the patient has a hepcidin-mediated disorder selected from those described in Section 6.4.1 herein. In certain embodiments, the patient has anemia of chronic disease.

[0033] The data presented in Examples 2, 3 and 5 below demonstrate that IL-6 antagonists provide therapeutic benefit in subjects having elevated pre-treatment IL-6 levels and at least one copy of the *TMPRSS6* major allele, even in the absence of anemia. Accordingly, in another aspect, methods are provided for treating IL-6 mediated inflammatory disorders in patients without anemia of chronic inflammation. The methods comprise administering a therapeutically effective amount of an IL-6 antagonist to a subject, typically a human patient, who has an IL-6 mediated inflammatory disorder, wherein the patient does not have anemia, and wherein the subject has been determined to have at least one copy of the *TMPRSS6* rs855791 major allele. In a first series of embodiments, the subject has previously been determined to have at least one copy of the *TMPRSS6* rs855791 major allele. In another series of embodiments, the method further comprises the earlier step of determining that the subject has at least one copy of the *TMPRSS6* rs855791 major allele. Typically, the methods affirmatively exclude treatment of subjects who are homozygous for the *TMPRSS6* rs855791 minor allele. Typically, the patient has elevated pre-treatment serum levels of IL-6.

[0034] In particular embodiments of any of the treatment methods, the patient has elevated pre-treatment serum levels of IL-6. In certain embodiments, the patient has a pre-treatment

[0049] In a variety of embodiments, the IL-6 antagonist is a JAK inhibitor. In particular embodiments, the JAK inhibitor is selected from the group consisting of tofacitinib (Xeljanz), decernotinib, ruxolitinib, upadacitinib, baricitinib, filgotinib, lestaurtinib, pacritinib, peficitinib, INCB-039110, ABT-494, INCB-047986 and AC-410.

[0050] In various embodiments, the IL-6 antagonist is a STAT3 inhibitor.

[0051] In some embodiments in which the IL-6 antagonist is an antibody or antigen-binding fragment or derivative, the IL-6 antagonist is administered parenterally. In particular embodiments, the IL-6 antagonist is administered subcutaneously.

[0052] In some embodiments in which the IL-6 antagonist is a JAK inhibitor or a STAT3 inhibitor, wherein the IL-6 antagonist is administered orally.

5. BRIEF DESCRIPTION OF THE DRAWINGS

[0053] FIGS. 1A and 1B provide boxplots showing that increased amounts of erythropoietin (“EPO”) were required for treatment in chronic kidney disease patients (CKD stage 5 dialysis subjects) who had elevated levels of serum IL-6 and at least one copy of the major allele at a known SNP in the *TMPRSS6* gene, rs855791 (G or C at nucleotide position 2321, encoding a *TMPRSS6* polypeptide comprising an alanine at amino acid position 736; 736A), but not in chronic kidney disease patients who had elevated levels of IL-6 and were homozygous for the rs855791 *TMPRSS6* minor allele (T or A at nucleotide position 2321, encoding a *TMPRSS6* polypeptide having a valine at position 736; 736V). Data from patients homozygous for the minor allele (A/A) are shown in FIG. 1A; data from patients having at least one copy of the major allele (homozygous G/G and heterozygous G/A) were pooled and are shown in FIG. 1B. Each of the two patient populations was further stratified into groups based on tertiles of serum IL-6 level: “Low” tertile (IL-6 < 5 pg/ml); “Middle” tertile (IL-6 = 5-15 pg/ml); “Highest” tertile (IL-6 > 15pg/ml). Boxplots with error bars are overlaid over raw data. Each boxplot represents a patient group based on both IL-6 level and genotype. Details are provided in Example 1.

[0054] FIGS. 2A and 2B provide survival curves that demonstrate that the *TMPRSS6* rs855791 major allele confers a higher all-cause mortality in response to elevated IL-6 levels in chronic kidney disease stage 5 dialysis subjects. FIG. 2A shows data from patients homozygous for the minor allele (A/A). FIG. 2B shows data from patients having at least one copy of the major allele (homozygous G/G and heterozygous G/A). Each group was separated into tertiles of serum IL-6 level using the IL-6 levels used in FIG. 1. Details are provided in Example 1.

[0062] FIGS. 10A – 10C show that anti-IL-6 therapy had an anti-cardiorenal syndrome effect equivalent to standard of care therapy, as measured by levels of fibrosis in heart tissue from rodent groups treated with anti-IL-6 (“IL-6 Ab”), standard of care (perindopril or “Peri”), and a control (“IgG”). Figure 10A is a micrograph showing a histological section of heart tissue stained with picrosirius-red. Two regions of the tissue were analyzed: a “Normal” region and a “Fibrosis Margin” region. An example “Normal” region is indicated by the delineated portion of the tissue slice. The inset in the micrograph shows a magnified view of the “Normal” region, showing that small portions of the “Normal” region has fibrotic tissue. The “Fibrosis Margin” region is a region of tissue in the “Normal” region peripheral to the fibrotic tissue. Figure 10B is a plot showing percentages of the area of the “Normal” region indicated as fibrotic tissue (i.e., stained/dark regions) in tissue samples from all groups. Figure 10C is a plot showing percentages of the area of the “Fibrosis Margin” region indicated as fibrotic tissue in tissue samples from all groups. Results are expressed as mean +/-SEM. Details are provided in Example 4.

[0063] FIGS. 11A and 11B show data from an *in vivo* model in which myocardial infarction was induced in mice genotypically analogous to human beings homozygous for the *TMPRSS6* rs855791 major allele. The control group received no therapy. The experimental group were treated with an anti-murine-IL-6 antibody. FIG. 11A shows that treatment with anti-IL-6 provide statistically significant improvement in ejection fraction. FIG. 11B shows that treatment with anti-IL-6 provides statistically significant improvement in contractility, measured as cardiac left ventricular fractional shortening. The data demonstrate that anti-IL-6 therapy given immediately after myocardial infarction improves functional recovery of the left ventricle in rodents that mimic human patients having the *TMPRSS6* rs855791 major allele. Details are provided in Example 5.

[0064] The figures depict various embodiments of the present invention for purposes of illustration only. One skilled in the art will readily recognize from the following discussion that alternative embodiments of the structures and methods illustrated herein may be employed without departing from the principles of the invention described herein.

6. DETAILED DESCRIPTION

6.1. Overview of experimental results

[0065] The peptide hormone, hepcidin, plays a central role in systemic iron homeostasis. Hentze *et al.*, *Cell* 142:24-38 (2010). Hepcidin expression is known to be influenced by the product of the *TMPRSS6* gene, matriptase-2, a type II transmembrane serine protease. Common variants in the *TMPRSS6* gene have been shown to correlate with iron status,

infarction improves functional recovery of the left ventricle in rodents that are genotypically analogous to human patients having the *TMPRSS6* rs855791 major allele.

[0085] Collectively, the experimental data demonstrate that therapeutic interventions that reduce IL-6 signaling will provide clinical benefit in patients with a hepcidin-mediated disorder, such as anemia or a hepcidin-mediated cellular toxicity, but only in those who have at least one copy of the *TMPRSS6* rs855791 major allele, with greatest effect in patients with elevated levels of IL-6.

[0086] Accordingly, as further described below, in a first aspect methods of treating a hepcidin-mediated disorder are provided. The methods comprise administering a therapeutically effective amount of an IL-6 antagonist to a patient with a hepcidin-mediated disorder who has been determined to have at least one copy of the major allele at the *TMPRSS6* rs855791 SNP. In a second aspect, methods are provided for improving treatment of hepcidin-mediated disorders, the method comprising discontinuing administration of an IL-6 antagonist to a patient with a hepcidin-mediated disorder, wherein the patient has been determined to be homozygous for the *TMPRSS6* rs855791 minor allele. Treatment is improved by discontinuing therapy that is ineffective, thereby reducing side effects and reducing cost without loss of treatment efficacy. In a further aspect, methods are provided for treating IL-6 mediated inflammatory disorders in patients without anemia of chronic inflammation, the methods comprising administering a therapeutically effective amount of an IL-6 antagonist to a patient who has an IL-6 mediated inflammatory disorder, does not have anemia, and the subject has been determined to have at least one copy of the *TMPRSS6* rs855791 major allele.

6.2. Definitions

[0087] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art to which this invention belongs. As used herein, the following terms have the meanings ascribed to them below.

[0088] By “**hepcidin**” is meant a polypeptide having at least about 85% or greater amino acid identity to the amino acid sequence provided at NCBI Accession No. NP_066998 (“hepcidin preprotein”), or biologically active fragment thereof. Exemplary hepcidin biological activities include binding and reducing the levels of the iron export channel ferroportin, inhibiting iron transport, inhibiting intestinal iron absorption, and inhibiting iron release from macrophages and the liver. An exemplary hepcidin preprotein amino acid sequence is provided below:

```
1  MALSSQIWAA CLLLLLLLAS LTSGSVFPQQ TGQLAELQPQ DRAGARASWM PMFQRRRRRD
61  THFPICIFCC GCCHRSKCGM CCKT (SEQ ID NO:1)
```

With reference to the sequence above, hepcidin exists in various forms, including as a preprohormone (amino acids 25-84), prohormone (amino acids 25-84), and mature forms termed hepcidin-25 (amino acids 60-84), hepcidin-22 (amino acids 63-84), and hepcidin-20 (amino acids 65-84).

[0089] A “**hepcidin-mediated disorder**” is any disorder in which hepcidin expression contributes to the etiology of the disorder or any of its symptoms. The contribution of hepcidin to the etiology may be known, may be suspected, or may be inferred from an observation that administration of an IL-6 antagonist provides greater therapeutic benefit in patients with the disorder who have at least one copy of the *TMPRSS6* rs855791 SNP major allele as compared to patients with the disorder who are homozygous for the *TMPRSS6* rs855791 SNP minor allele. Hepcidin-mediated disorders are further described below in Section 6.4.1.

[0090] By “**transmembrane protease serine 6 (*TMPRSS6*) polypeptide**” is meant a polypeptide or fragment thereof having at least about 85% or greater amino acid identity to the amino acid sequence provided at NCBI Accession No. NP_001275929 and having serine proteinase activity. The *TMPRSS6* polypeptide, also known as Matriptase-2 (MT2), cleaves hemojuvelin and inhibits bone morphogenetic protein signaling. An exemplary *TMPRSS6* amino acid sequence having an alanine at position 736 (736A) is provided below:

```

1  MPVAEAPQVA GGQGDGGDGE EAEPEGMFKA CEDSKRKARG YLRLVPLFVL LALLVLASAG
61  VLLWYFLGYK AEVMVSQVYS GSLRVLNRHF SQDLTRRESS AFRSETAKAQ KMLKELITST
121 RLGTYYNSSS VVSFGEGLPT CFFWFILQIP EHRRLMLSPE VVQALLVEEL LSTVNSSAAV
181 PYRAEYEDVP EGLVILEASV KDIAALNSTL GCYRYSYVGQ GQVLRLKGPD HLAASSCLWHL
241 QGPKDLMLKL RLEWTLAECR DRLAMYDVAG PLEKRLITSV YGCSRQEPVV EVLASGAIMA
301 VVWKKGLHSY YDPFVLSVQP VVFQACEVNL TLDNRLDSQG VLSTPYFPSY YSPQTHCSWH
361 LTVPSLDYGL ALWFDAYALR RQKYDLPCTQ GQWTIQNRRL CGLRILQPYA ERIPVVATAG
421 ITINFTSQIS LTGPGVRVHY GLYNQSDPCP GEFLCSVNGL CVPACDGVKD CPNGLDERNC
481 VCRATFQCKE DSTCISLPKV CDGQPDCLNG SDEEQCEQEV PCGTFTFQCE DRSCVKKPNP
541 QCDGRPDGRD GSDEEHDCDG LQGPSSRIVG GAVSSEGEWP WQASLQVRGR HICGGALIAD
601 RWVITAACHF QEDSMASVTL WTVFLGKVVQ NSRWPGEVSF KVSRLLLHPY HEEDSHDYDV
661 ALLQLDHPVV RSAAVRPVCL PARSHFFEPG LHCWITGWGA LREGALRADA VALFYGWRNQ
721 GSETCCCPIS NALQKADVQL IPQDLCSEVY RYQVTPRMLC AGYRKGKKDA CQGDSSGGLPV
781 CKALSGRWFL AGLVSWGLGC GRPNYFGVYT RITGVISWIQ QVVT (SEQ ID NO:2)

```

An exemplary *TMPRSS6* amino acid sequence having a valine at position 736 (736V) is provided below:

```

1  MPVAEAPQVA GGQGDGGDGE EAEPEGMFKA CEDSKRKARG YLRLVPLFVL LALLVLASAG

```

```

181 SCTVDYSTVY FVNIEVWVEA ENALGKVTSD HINFDVPYKV KPNPPHNLSV INSEELSSIL
241 KLTWTNPSIK SVIILKYNIQ YRTKDASTWS QIPPEDTAST RSSFTVQDLK PFTEYVFRIR
301 CMKEDGKGYW SDWSEEASGI TYEDRPSKAP SFWYKIDPSH TQGYRTVQLV WKTLPPEAN
361 GKILDYEVTL TRWKSHLQNY TVNATKLTVN LTNDRYLATL TVRNLVGKSD AAVLTIPACD
421 FQATHPVMDL KAFFKDNMLW VEWTTPRESV KKYILEWCVL SDKAPCITDW QQEDGTVHRT
481 YLRGNLAESK CYLITVTPVY ADGPGSPESI KAYLKQAPPS KGPTVVRTKKV GKNEAVLEWD
541 QLPVDVQNGF IRNYTIFYRT IIGNETAVNV DSSHTEYTLS SLTSDTLYMV RMAAYTDEGG
601 KDGPEFTFTT PKFAQGEIEA IVVPVCLAFL LTLLGLVLC FNKRDLIKKH IWPNVDPDSK
661 SHIAQWSPHT PPRHNFNSKD QMYSDGNFTD VSVVEIEAND KKPFPEDLKS LDLFKKEKIN
721 TEGHSSGIGG SSCMSSSRPS ISSSDENESS QNTSSTVQYS TVVHSGYRHQ VPSVQVFSRS
781 ESTQPLLDSE ERPEDLQLVD HVDGGDGILP RQQYFKQNCS QHESSPDISH FERSKQVSSV
841 NEEDFVRLKQ QISDHISQSC GSGQMCMFQE VSAADAFGPG TEGQVERFET VGMEAATDEG
901 MPKSYLPQTV RQGGYMPQ (SEQ ID NO:9)

```

[0103] By “**IL-6 antagonist**” is meant an agent that is capable of decreasing the biological activity of IL-6. IL-6 antagonists include agents that decrease the level of IL-6 polypeptide in serum, including agents that decrease the expression of an IL-6 polypeptide or nucleic acid; agents that decrease the ability of IL-6 to bind to the IL-6R; agents that decrease the expression of the IL-6R; and agents that decrease signal transduction by the IL-6R receptor when bound by IL-6. In preferred embodiments, the IL-6 antagonist decreases IL-6 biological activity by at least about 10%, 20%, 30%, 50%, 70%, 80%, 90%, 95%, or even 100%. As further described in Section 6.9 below, IL-6 antagonists include IL-6 binding polypeptides, such as anti-IL-6 antibodies and antigen binding fragments or derivatives thereof; IL-6R binding polypeptides, such as anti-IL-6R antibodies and antigen binding fragments or derivatives thereof; and synthetic chemical molecules, such as JAK1 and JAK3 inhibitors.

[0104] By “**IL-6 antibody**” or “**anti-IL-6 antibody**” is meant an antibody that specifically binds IL-6. Anti-IL-6 antibodies include monoclonal and polyclonal antibodies that are specific for IL-6, and antigen-binding fragments or derivatives thereof. IL-6 antibodies are described in greater detail in Section 6.9.1 below.

[0105] By “**IL-6 mediated inflammatory disorder**” is meant any disorder in which IL-6 is known or suspected to contribute to the etiology of the disease or any of its symptoms.

[0106] By “**erythropoietin (EPO)**” is meant a polypeptide or fragment thereof having at least about 85% or greater amino acid identity to the amino acid sequence provided at NCBI Accession No. NP_000790 and having EPO biological activity. Exemplary EPO biological activities include binding to the erythropoietin receptor and resultant proliferation and

[0113] **“Pre-treatment”** means prior to the first administration of an IL-6 antagonist according to the methods described herein. Pre-treatment does not exclude, and often includes, the prior administration of treatments other than an IL-6 antagonist.

[0114] In this disclosure, **“comprises,” “comprising,” “containing,” “having,” “includes,” “including,”** and linguistic variants thereof have the meaning ascribed to them in U.S. Patent law, permitting the presence of additional components beyond those explicitly recited.

[0115] By **“biological sample”** is meant any tissue, cell, fluid, or other material derived from an organism (e.g., human subject). In certain embodiments, the biological sample is serum or blood.

[0116] By **“angiotensin converting enzyme (ACE) inhibitor”** is meant an agent that inhibits an angiotensin converting enzyme’s biological function of converting angiotensin I to angiotensin II. ACE inhibitors include, without limitation, quinapril, perindopril, ramipril, captopril, benazepril, trandolapril, fosinopril, lisinopril, moexipril, and enalapril. In various embodiments, the ACE inhibitor is perindopril.

6.3. Other interpretational conventions

[0117] Unless otherwise specified, antibody constant region residue numbering is according to the EU index as in Kabat.

[0118] Ranges provided herein are understood to be shorthand for all of the values within the range, inclusive of the recited endpoints. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50.

[0119] Unless specifically stated or apparent from context, as used herein the term “or” is understood to be inclusive. Unless specifically stated or apparent from context, as used herein, the terms “a”, “an”, and “the” are understood to be singular or plural.

[0120] Unless specifically stated or otherwise apparent from context, as used herein the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

6.4. Methods Of Treating Hepcidin-Mediated Disorders

[0121] In a first aspect, methods of treating a hepcidin-mediated disorder are provided.

[0122] The methods comprise administering a therapeutically effective amount of an IL-6 antagonist to a subject, typically a human patient, who has a hepcidin-mediated disorder,

wherein the subject has been determined to have at least one copy of the *TMPRSS6* rs855791 major allele. In a first series of embodiments, the subject has previously been determined to have at least one copy of the *TMPRSS6* rs855791 major allele. In another series of embodiments, the method further comprises the earlier step of determining that the subject has at least one copy of the *TMPRSS6* rs855791 major allele. Typically, the methods affirmatively exclude treatment of subjects who are homozygous for the *TMPRSS6* rs855791 minor allele. Typically, the patient has elevated pre-treatment serum levels of IL-6.

6.4.1. Hepcidin-Mediated Disorders

6.4.1.1. Anemia of Chronic Disease / Chronic Inflammation

[0123] In various embodiments, the hepcidin-mediated disorder treated by the methods described herein is an anemia of chronic disease, also known as anemia of chronic inflammation.

[0124] In various embodiments, the patient is male and has a pre-treatment hemoglobin (Hb) content of less than 14 g/dl. In some embodiments, the male patient has a pre-treatment Hb level of 13.0 – 13.9 g/dl, 12.0 – 12.9 g/dl, 11.0 – 11.9 g/dl, 10.0 – 10.9 g/dl, or less than 10 g/dl. In various embodiments, the patient is female and has a pre-treatment Hb content of less than 12 g/dl. In some embodiments, the female patient has a pre-treatment Hb level of 11.0 – 11.9 g/dl, 10.0 – 10.9 g/dl, 9.0 – 9.9 g/dl, 8.0 – 8.9 g/dl, or less than 8 g/dl. In some of these embodiments, prior the patient has been treated with an ESA. In some embodiments, the patient has been treated with iron supplementation. In some embodiments, the patient has been treated with transfusion of blood or packed red blood cells.

[0125] In various embodiments, the patient is male and has a pre-treatment hematocrit of less than 40%. In some embodiments, the male patient has a pre-treatment hematocrit less than 39%, less than 38%, less than 37%, less than 36%, or less than 35%. In certain embodiments, the male patient has a pre-treatment hematocrit of 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31% or 30%. In various embodiments, the patient is female, and has a pre-treatment hematocrit of less than 36%. In some embodiments, the female patient has a pre-treatment hematocrit of less than 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, or 26%. In certain embodiments, the female patient has a pre-treatment hematocrit of 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, or 26%. In some of these embodiments, the patient has been treated with an ESA. In some embodiments, the patient has been treated with iron supplementation. In some embodiments, the patient has been treated with transfusion of blood or packed red blood cells.

[0131] In some embodiments, the IL-6 antagonist is administered at a dose, on a schedule, and for a period sufficient to allow at least a 10% reduction in the patient's dose of ESA as compared to pre-treatment ESA dose. In certain embodiments, the IL-6 antagonist is administered at a dose, on a schedule, and for a period sufficient to allow at least a 20%, 30%, 40%, or 50% reduction in the patient's dose of ESA as compared to pre-treatment ESA dose. In particular embodiments, the IL-6 antagonist is administered at a dose, on a schedule, and for a period sufficient to allow at least 60%, or even at least 75% reduction in patient's dose of ESA as compared to pre-treatment ESA dose.

[0132] In some embodiments, the IL-6 antagonist is administered at a dose, on a schedule, and for a period sufficient to reverse functional iron deficiency.

6.4.1.1.1. Chronic Kidney Disease

[0133] In various embodiments, the chronic disease is chronic kidney disease (CKD).

[0134] In some embodiments, the patient has KDOQI stage 1 chronic kidney disease. In certain embodiments, the patient has KDOQI stage 2 chronic kidney disease, KDOQI stage 3 chronic kidney disease, KDOQI stage 4 chronic kidney disease, or KDOQI stage 5 chronic kidney disease.

[0135] In some embodiments, the patient has cardiorenal syndrome (CRS). In certain embodiments, the patient has CRS Type 4.

[0136] In some embodiments, the patient has been treated with dialysis.

[0137] In some embodiments, the IL-6 antagonist is administered at a dose, on a schedule, and for a period sufficient to reduce cardiovascular (CV) mortality as compared to age-matched and disease-matched historical cohorts.

6.4.1.1.2. Chronic Inflammatory Diseases

[0138] In various embodiments, the chronic disease is a chronic inflammatory disease.

[0139] In some embodiments, the chronic inflammatory disease is rheumatoid arthritis (RA).

[0140] In specific embodiments, the patient has a pre-treatment DAS28 score of greater than 5.1. In some embodiments, the patient has a pre-treatment DAS28 score of 3.2 to 5.1. In some embodiments, the patient has a pre-treatment DAS28 score of less than 2.6. In various embodiments, the patient's pre-treatment RA is severely active. In some embodiments, the patient's pre-treatment RA is moderately active.

[0141] In certain embodiments, the patient has been treated with methotrexate. In some embodiments, methotrexate is discontinued when treatment with an IL-6 antagonist is initiated. In some embodiments, treatment with methotrexate is continued when treatment with an IL-6 antagonist is initiated.

[0142] In certain embodiments, the patient has been treated with an anti-TNF α agent. In particular embodiments, the anti-TNF α agent is selected from etanercept, adalimumab, infliximab, certolizumab, and golimumab. In particular embodiments, the anti-TNF α agent is discontinued when treatment with an IL-6 antagonist is initiated.

[0143] In certain embodiments, the patient has been treated with an IL-1 receptor antagonist. In specific embodiments, the IL-1 receptor antagonist is anakinra. In particular embodiments, the IL-1 receptor antagonist is discontinued when treatment with an IL-6 antagonist is initiated.

[0144] In certain embodiments, the patient has been treated with abatacept. In particular embodiments, abatacept is discontinued when treatment with an IL-6 antagonist is initiated.

[0145] In certain embodiments, the patient has been treated with an IL-6 antagonist, and the method further comprises continuing to administer an IL-6 antagonist only to those patients newly determined to have at least one copy of the *TMPRSS6* rs855791 major allele. In specific embodiments, the IL-6 antagonist is tocilizumab. In specific embodiments, the IL-6 antagonist is tofacitinib.

[0146] In various embodiments, the chronic inflammatory disease is selected from the group consisting of juvenile idiopathic arthritis, ankylosing spondylitis, plaque psoriasis, psoriatic arthritis, inflammatory bowel disease, Crohn's disease, and ulcerative colitis.

6.4.1.1.3. Cancer

[0147] In various embodiments, the chronic disease is cancer.

[0148] In some embodiments, the cancer is selected from the group consisting of: solid tumors, small cell lung cancer, non-small cell lung cancer, hematological cancer, multiple myeloma, leukemias, chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), lymphomas, and Hodgkin's lymphoma.

6.4.1.1.4. Chronic Infection

[0149] In various embodiments, the chronic disease is a chronic infection.

6.4.1.1.5. Congestive Heart Failure

[0150] In various embodiments, the chronic disease is congestive heart failure (CHF).

6.4.1.2. Iron-refractory Iron-deficiency Anemia (IRIDA)

[0151] In various embodiments, the hepcidin-mediated disorder is iron-refractory iron-deficiency anemia (IRIDA).

6.4.1.3. Anemia Associated With Heparin-Producing Hepatic Adenomas

[0152] In various embodiments, the hepcidin-mediated disorder is anemia associated with a hepcidin-producing hepatic adenoma.

6.4.1.4. Acute Coronary Syndrome

[0153] The data presented in Examples 2, 3, and 5 below demonstrate that IL-6 antagonists are effective in reducing risk of heart failure and death, and in increasing cardiac function and reducing fibrosis, after acute myocardial infarction. Accordingly, in various embodiments, the hepcidin-mediated disorder is acute coronary syndrome.

[0154] In certain embodiments, the patient has suffered a myocardial infarction within the 60 days prior to first administration of an IL-6 antagonist. In particular embodiments, the patient has suffered a myocardial infarction within the 30 days, 14 days, 7 days, 48 hours, or 24 hours prior to first administration of an IL-6 antagonist.

[0155] In some embodiments, the IL-6 antagonist is administered at a dose, on a schedule, and for a period sufficient to improve myocardial contractility as compared to pre-treatment levels. In certain embodiments, the IL-6 antagonist is administered at dose, on a schedule, and for a period sufficient to improve cardiac ejection fraction as compared to pre-treatment levels. In certain embodiments, the IL-6 antagonist is administered at dose, on a schedule, and for a period sufficient to reduce cardiac fibrosis as compared to pre-treatment levels.

6.4.1.5. Castleman's Disease

[0156] In various embodiments, the hepcidin-mediated disorder is Castleman's Disease.

6.5. Methods Of Improving Treatment of Heparin-Mediated Disorders

[0157] In another aspect, methods are provided for improving treatment of a hepcidin-mediated disorder by discontinuing therapy that is ineffective, thereby reducing side effects and reducing cost without loss of treatment efficacy. The methods comprise discontinuing administration of an IL-6 antagonist to a patient with a hepcidin-mediated disorder, wherein the patient has been determined to be homozygous for the *TMPRSS6* rs855791 minor allele. In one series of embodiments, the patient has previously been determined to be homozygous for the *TMPRSS6* rs855791 minor allele. In another series of embodiments, the method further comprises the earlier step of determining that the patient is homozygous for the *TMPRSS6* rs855791 minor allele. In typical embodiments, the patient has elevated pre-treatment serum levels of IL-6. In various embodiments, the patient has elevated pre-treatment serum levels of CRP.

[0158] In various embodiments, the patient has a hepcidin-mediated disorder selected from those described in Section 6.4.1 above. In certain embodiments, the patient has anemia of chronic disease.

6.6. Methods of Treating IL-6 Mediated Inflammatory Disorders

[0159] The data presented in Examples 2, 3 and 5 below demonstrate that IL-6 antagonists provide therapeutic benefit in subjects having elevated pre-treatment IL-6 levels and at least one copy of the *TMPRSS6* major allele, even in the absence of anemia. Accordingly, in another aspect, methods are provided for treating IL-6 mediated inflammatory disorders in patients without anemia of chronic inflammation.

[0160] The methods comprise administering a therapeutically effective amount of an IL-6 antagonist to a subject, typically a human patient, who has an IL-6 mediated inflammatory disorder, wherein the patient does not have anemia, and wherein the subject has been determined to have at least one copy of the *TMPRSS6* rs855791 major allele. In a first series of embodiments, the subject has previously been determined to have at least one copy of the *TMPRSS6* rs855791 major allele. In another series of embodiments, the method further comprises the earlier step of determining that the subject has at least one copy of the *TMPRSS6* rs855791 major allele. Typically, the methods affirmatively exclude treatment of subjects who are homozygous for the *TMPRSS6* rs855791 minor allele. Typically, the patient has elevated pre-treatment serum levels of IL-6.

[0161] In some embodiments, the IL-6 mediated disorder is rheumatoid arthritis (RA).

[0162] In specific embodiments, the patient has a pre-treatment DAS28 score of greater than 5.1. In some embodiments, the patient has a pre-treatment DAS28 score of 3.2 to 5.1. In some embodiments, the patient has a pre-treatment DAS28 score of less than 2.6. In various embodiments, the patient's pre-treatment RA is severely active. In some embodiments, the patient's pre-treatment RA is moderately active.

[0163] In certain embodiments, the patient has been treated with methotrexate. In some embodiments, methotrexate is discontinued when treatment with an IL-6 antagonist is initiated. In some embodiments, methotrexate is continued when treatment with an IL-6 antagonist is initiated.

[0164] In certain embodiments, the patient has been treated with an anti-TNF α agent. In particular embodiments, the anti-TNF α agent is selected from etanercept, adalimumab, infliximab, certolizumab, and golimumab. In particular embodiments, the anti-TNF α agent is discontinued when treatment with an IL-6 antagonist is initiated.

[0165] In certain embodiments, the patient has been treated with an IL-1 receptor antagonist. In specific embodiments, the IL-1 receptor antagonist is anakinra. In particular embodiments, the IL-1 receptor antagonist is discontinued when treatment with an IL-6 antagonist is initiated.

[0166] In certain embodiments, the patient has been treated with abatacept. In particular embodiments, abatacept is discontinued when treatment with an IL-6 antagonist is initiated.

[0167] In various embodiments, the IL-6 mediated disorder is selected from the group consisting of juvenile idiopathic arthritis, ankylosing spondylitis, plaque psoriasis, psoriatic arthritis, inflammatory bowel disease, Crohn's disease, and ulcerative colitis.

6.7. Pre-Treatment Serum IL-6 And CRP Levels

[0168] In typical embodiments of the methods described herein, the patient has elevated pre-treatment serum levels of IL-6.

[0169] In some embodiments, the patient has a pre-treatment serum IL-6 level of greater than 2.5 pg/ml. In various embodiments, the patient has a pre-treatment serum IL-6 level of greater than 5 pg/ml, greater than 7.5 pg/ml, greater than 10 pg/ml, greater than 12.5 pg/ml, or greater than 15 pg/ml.

[0170] In some embodiments, the IL-6 antagonist is administered at a dose, on a schedule, and for a period sufficient to reduce the patient's serum IL-6 levels below pre-treatment levels. In certain embodiments, the IL-6 antagonist is administered at a dose, on a schedule, and for a period sufficient to reduce the patient's serum IL-6 levels by at least 10%, 20%, 30%, 40%, or 50% as compared to pre-treatment levels.

[0171] In various embodiments, the patient has elevated pre-treatment levels of C-reactive protein (CRP). In some embodiments, the patient has a pre-treatment CRP level greater than 2 mg/ml, 2.5 mg/ml, 3 mg/ml, 3.5 mg/ml, 4 mg/ml, 4.5 mg/ml, or 5 mg/ml. In some embodiments, the patient has pre-treatment CRP levels greater than 7.5 mg/ml, 10 mg/ml, 12.5 mg/ml, or 15 mg/ml.

[0172] In some embodiments, the IL-6 antagonist is administered at a dose, on a schedule, and for a period sufficient to reduce the patient's CRP levels below pre-treatment levels. In certain embodiments, the IL-6 antagonist is administered at a dose, on a schedule, and for a period sufficient to reduce the patient's CRP levels by at least 10%, 20%, 30%, 40%, or 50% as compared to pre-treatment levels.

6.8. TMRSS6 rs855791 Genotyping

[0173] Methods described herein comprise administering a therapeutically effective amount of an IL-6 antagonist to a subject who has been determined to have at least one copy of the

TMPRSS6 rs855791 major allele. Preferably, both alleles corresponding to a gene of interest are identified, thus permitting identification and discrimination of patients who are homozygous for the *TMPRSS6* rs855791 major allele, heterozygous for the major and minor *TMPRSS6* rs855791 alleles, and homozygous for the *TMPRSS6* rs855791 minor allele.

[0174] The absence (major allele) or presence (minor allele) of SNP rs855791 (2321G→A) in the *TMPRSS6* gene is determined using standard techniques.

[0175] Typically, PCR is used to amplify a biological sample obtained from the patient.

[0176] In some embodiments, the absence or presence of polymorphism is detected concurrently with amplification using real-time PCR (RT-PCR). In certain embodiments, the RT-PCR assay employs 5' nuclease (TaqMan® probes), molecular beacons, and/or FRET hybridization probes. *Reviewed in Espy et al., Clin. Microbiol. Rev.* 2006 January; 19(1): 165-256, incorporated herein by reference in its entirety. In typical embodiments, a commercially available assay is used. In select embodiments, the commercially available assay is selected from the group consisting of TaqMan™ SNP Genotyping Assays (ThermoFisher); PCR SNP Genotyping Assay (Qiagen); Novallele Genotyping Assays (Canon); and SNP Type™ assays (formerly SNPtype) (Fluidigm).

[0177] In some embodiments, the absence or presence of polymorphism is detected following amplification using hybridization with a probe specific for SNP rs855791, restriction endonuclease digestion, nucleic acid sequencing, primer extension, microarray or gene chip analysis, mass spectrometry, and/or a DNase protection assay. In some embodiments, the allelic variants are called by sequencing. In certain embodiments, Sanger sequencing is used. In certain embodiments, one of a variety of next-generation sequencing techniques is used, including for example a sequencing technique selected from the group consisting of microarray sequencing, Solexa sequencing (Illumina), Ion Torrent (Life Technologies), SOLiD (Applied Biosystems), pyrosequencing, single-molecule real-time sequencing (Pacific Bio), nanopore sequencing and tunneling currents sequencing.

6.9. IL-6 Antagonists

[0178] The IL-6 antagonist used in the methods described herein is capable of decreasing the biological activity of IL-6.

6.9.1. Anti-IL-6 Antibodies

[0179] In various embodiments, the IL-6 antagonist is an anti-IL-6 antibody or antigen-binding fragment or derivative thereof.

[0180] In some embodiments, the IL-6 antagonist is a full-length anti-IL-6 monoclonal antibody. In particular embodiments, the full-length monoclonal antibody is an IgG

substitution with glutamic acid (“YTE”). See U.S. Pat. No. 7,083,784, incorporated herein by reference in its entirety. In certain extended half-life embodiments, the IgG constant domain comprises substitutions selected from T250Q/M428L (Hinton *et al.*, *J. Immunology* 176:346-356 (2006)); N434A (Yeung *et al.*, *J. Immunology* 182:7663-7671 (2009)); or T307A/E380A/N434A (Petkova *et al.*, *International Immunology*, 18: 1759-1769 (2006)).

[0187] In some embodiments, the elimination half-life of the anti-IL-6 antibody is increased by utilizing the FcRN-binding properties of human serum albumin. In certain embodiments, the antibody is conjugated to albumin (Smith *et al.*, *Bioconjug. Chem.*, 12: 750-756 (2001)). In some embodiments, the anti-IL-6 antibody is fused to bacterial albumin-binding domains (Stork *et al.*, *Prot. Eng. Design Science* 20: 569-76 (2007)). In some embodiments, the anti-IL-6 antibody is fused to an albumin-binding peptide (Nguyen *et al.*, *Prot Eng Design Sel* 19: 291-297 (2006)). In some embodiments, the anti-IL-antibody is bispecific, with one specificity being to IL-6, and one specificity being to human serum albumin (Ablynx, WO 2006/122825 (bispecific Nanobody)).

[0188] In some embodiments, the elimination half-life of the anti-IL-6 antibody is increased by PEGylation (Melmed *et al.*, *Nature Reviews Drug Discovery* 7: 641-642 (2008)); by HPMA copolymer conjugation (Lu *et al.*, *Nature Biotechnology* 17: 1101-1104 (1999)); by dextran conjugation (*Nuclear Medicine Communications*, 16: 362-369 (1995)); by conjugation with homo-amino-acid polymers (HAPs; HAPylation) (Schlapschy *et al.*, *Prot Eng Design Sel* 20: 273-284 (2007)); or by polysialylation (Constantinou *et al.*, *Bioconjug. Chem.* 20: 924-931 (2009)).

6.9.1.1. MED5117 and Derivatives

[0189] In certain embodiments, the anti-IL-6 antibody or antigen-binding portion thereof comprises all six CDRs of MEDI5117. In particular embodiments, the antibody or antigen-binding portion thereof comprises the MEDI5117 heavy chain V region and light chain V region. In specific embodiments, the antibody is the full-length MEDI5117 antibody. The MEDI5117 antibody is described in WO 2010/088444 and US 2012/0034212, the disclosures of which are incorporated herein by reference in their entireties. The MEDI5117 antibody has the following CDR and heavy and light chain sequences:

MEDI5117 VH CDR1

SNYMI (SEQ ID NO:12)

MEDI5117 VH CDR2

DLYYYAGDTYYADSVKG (SEQ ID NO:13)

MEDI5117 VH CDR3

the amino acid sequence of the VH and VL domain of MEDI5117. The percent sequence identity is determined using BLAST algorithms using default parameters.

[0194] In certain embodiments, the MED5117 derivative comprises an amino acid sequence in which the CDRs comprise an amino acid sequence that is at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the amino acid sequence of the respective CDRs of MEDI5117. The percent sequence identity is determined using BLAST algorithms using default parameters.

[0195] In certain embodiments, the V_H and/or V_L CDR derivatives comprise conservative amino acid substitutions at one or more predicted nonessential amino acid residues (i.e., amino acid residues which are not critical for the antibody to specifically bind to human IL-6).

6.9.1.2. Other Anti-IL-6 Antibodies

[0196] In various embodiments, the anti-IL-6 antibody comprises the six CDRs from an antibody selected from the group consisting of siltuximab, gerilimumab, sirukumab, clazakizumab, olokizumab, elsilimomab, VX30 (VOP-R003; Vaccinex), EB-007 (EBI-029; Eleven Bio), ARGX-109 (ArGEN-X), FM101 (Femta Pharmaceuticals, Lonza) and ALD518/BMS-945429 (Alder Biopharmaceuticals, Bristol-Myers Squibb). In certain embodiments, the anti-IL-6 antibody comprises the heavy chain V region and light chain V region from an antibody selected from the group consisting of siltuximab, gerilimumab, sirukumab, clazakizumab, olokizumab, VX30 (VOP-R003; Vaccinex), EB-007 (EBI-029; Eleven Bio), ARGX-109 (ArGEN-X), FM101 (Femta Pharmaceuticals, Lonza) and ALD518/BMS-945429 (Alder Biopharmaceuticals, Bristol-Myers Squibb). In particular embodiments, the anti-IL-6 antibody is an antibody selected from the group consisting of siltuximab, gerilimumab, sirukumab, clazakizumab, olokizumab, VX30 (VOP-R003; Vaccinex), EB-007 (EBI-029; Eleven Bio), ARGX-109 (ArGEN-X), FM101 (Femta Pharmaceuticals, Lonza) and ALD518/BMS-945429 (Alder Biopharmaceuticals, Bristol-Myers Squibb).

[0197] In some embodiments, the anti-IL-6 antibody comprises the six CDRs from an antibody selected from those described in US 2016/0168243, US 2016/0130340, US 2015/0337036, US 2015/0203574, US 2015/0140011, US 2015/0125468, US 2014/0302058, US 2014/0141013, US 2013/0280266, US 2013/0017575, US 2010/0215654, US 2008/0075726, US Pat. No. 5,856,135, US 2006/0240012,

US 2006/0257407, or U.S. Pat. No. 7291721, the disclosures of which are incorporated herein by reference in their entireties.

6.9.2. Anti-IL-6 Receptor Antibodies

[0198] In various embodiments, the IL-6 antagonist is an anti-IL-6 receptor antibody or antigen-binding fragment or derivative thereof.

[0199] In some embodiments, the IL-6 antagonist is a full-length anti-IL-6 receptor monoclonal antibody. In particular embodiments, the full-length monoclonal antibody is an IgG antibody. In certain embodiments, the full-length monoclonal antibody is an IgG1, IgG2, IgG3, or IgG4 antibody. In some embodiments, the IL-6 antagonist is a polyclonal composition comprising a plurality of species of full-length anti-IL-6 receptor antibodies, each of the plurality having unique CDRs. In some embodiments, the IL-6 antagonist is an antibody fragment selected from Fab and Fab' fragments. In some embodiments, the IL-6 antagonist is a scFv, a single domain antibody, including a camelid-derived VHH single domain Nanobody. In some embodiments, the antibody is bispecific or multispecific, with at least one of the antigen-binding portions having specificity for IL-6R.

[0200] In some embodiments, the antibody is fully human. In some embodiments, the antibody is humanized. In some embodiments, the antibody is chimeric and has non-human V regions and human C region domains. In some embodiments, the antibody is murine.

[0201] In typical embodiments, the anti-IL-6 receptor antibody has a K_D for binding human IL-6R of less than 100 nM. In some embodiments, the anti-IL-6R antibody has a K_D for binding human IL-6R of less than 75 nM, 50 nM, 25 nM, 20 nM, 15 nM, or 10 nM. In particular embodiments, the anti-IL-6 receptor antibody has a K_D for binding human IL-6R of less than 5 nM, 4 nM, 3 nM, or 2 nM. In selected embodiments, the anti-IL-6 receptor antibody has a K_D for binding human IL-6R of less than 1 nM, 750 pM, or 500 pM. In specific embodiments, the anti-IL-6 receptor antibody has a K_D for binding human IL-6R of no more than 500 pM, 400 pM, 300 pM, 200 pM, or 100 pM.

[0202] In typical embodiments, the anti-IL-6R reduces the biological activity of IL-6.

[0203] In typical embodiments, the anti-IL-6R antibody has an elimination half-life following intravenous administration of at least 7 days. In certain embodiments, the anti-IL-6R antibody has an elimination half-life of at least 14 days, at least 21 days, or at least 30 days.

[0204] In some embodiments, the anti-IL-6R antibody has a human IgG constant region with at least one amino acid substitution that extends serum half-life as compared to the unsubstituted human IgG constant domain.

[0210] In certain embodiments, the anti-IL-6R antibody or antigen-binding portion thereof comprises all six CDRs of VX30 (Vaccinex), ARGX-109 (arGEN-X), FM101 (Formatech), , SA237 (Roche), NI-1201 (NovImmune), or an antibody described in US 2012/0225060.

[0211] In certain embodiments, the anti-IL-6R antibody or antigen-binding portion thereof is a single domain antibody. In particular embodiments, the single domain antibody is a camelid VHH single domain antibody. In specific embodiments, the antibody is vobarilizumab (ALX-0061) (Ablynx NV).

6.9.3. Anti-IL-6:IL-6R Complex Antibodies

[0212] In various embodiments, the IL-6 antagonist is an antibody specific for the complex of IL-6 and IL-6R. In certain embodiments, the antibody has the six CDRs of an antibody selected from those described in US 2011/0002936, which is incorporated herein by reference in its entirety.

6.9.4. JAK and STAT Inhibitors

[0213] IL-6 is known to signal via the JAK-STAT pathway.

[0214] In various embodiments, the IL-6 antagonist is an inhibitor of the JAK signaling pathway. In some embodiments, the JAK inhibitor is a JAK1-specific inhibitor. In some embodiments, the JAK inhibitor is a JAK3-specific inhibitor. In some embodiments, the JAK inhibitor is a pan-JAK inhibitor.

[0215] In certain embodiments, the JAK inhibitor is selected from the group consisting of tofacitinib (Xeljanz), decernotinib, ruxolitinib, upadacitinib, baricitinib, filgotinib, lestaurtinib, pacritinib, peficitinib, INCB-039110, ABT-494, INCB-047986 and AC-410.

[0216] In various embodiments, the IL-6 antagonist is a STAT3 inhibitor. In a specific embodiment, the inhibitor is AZD9150 (AstraZeneca, Isis Pharmaceuticals), a STAT3 antisense molecule.

6.9.5. Additional IL-6 Antagonists

[0217] In various embodiments, the IL-6 antagonist is an antagonist peptide.

[0218] In certain embodiments, the IL-6 antagonist is C326 (an IL-6 inhibitor by Avidia, also known as AMG220), or FE301, a recombinant protein inhibitor of IL-6 (Ferring International Center S.A., Conaris Research Institute AG). In some embodiments, the anti-IL-6 antagonist comprises soluble gp130, FE301 (Conaris/Ferring).

6.10. Dosage regimens

6.10.1 Antibodies, antigen-binding fragments, peptides

[0219] In typical embodiments, antibody, antigen-binding fragments, and peptide IL-6 antagonists are administered parenterally.

In some embodiments, the antagonist is administered at a dose of 1.5 mg/kg, 2 mg/kg, 2.5 mg/kg, 3 mg/kg, 3.5 mg/kg, 4 mg/kg, 4.5 mg/kg, or 5 mg/kg.

[0227] In various intravenous embodiments, the IL-6 antagonist is administered once every 7 days, once every 14 days, once every 21 days, once every 28 days, or once a month. In various subcutaneous embodiments, the IL-6 antagonist is administered once every 14 days, once every 28 days, once a month, once every two months (every other month), or once every three months.

[0228] In certain preferred embodiments, the IL-6 antagonist is the MEDI5117 antibody. In various embodiments, MEDI5117 is administered in a flat dose of 1 – 30 mg IV once every week. In certain embodiments, the MEDI5117 antibody is administered in a flat dose of 1, 2, 3, 4, 5, 7.5, 10, 15, 20, 25, or 30 mg IV once every week. In some embodiments, the MEDI5117 antibody is administered in a flat dose of 25 – 250 mg s.c. once every month to once every three months. In particular embodiments, MEDI5117 is administered at a dose of 30 mg, 45 mg, 60 mg, 75 mg, 100 mg, 120 mg, 125 mg, 150 mg, 175 mg, 200 mg, 225 mg, 240 mg, or 250 mg s.c. once every month, once every two months, or once every 3 months.

[0229] In some embodiments, the IL-6 antagonist is tocilizumab. In various embodiments, tocilizumab is administered s.c. in a starting dose for patients ≥ 100 kg of 162 mg once every week. In some embodiments, tocilizumab is administered intravenously at a dose of 4 mg/kg once every 4 weeks followed by an increase to 8 mg/kg every 4 weeks based on clinical response.

6.10.2. JAK and STAT inhibitors

[0230] In typical embodiments, small molecule JAK inhibitors and STAT inhibitors are administered orally.

[0231] In various embodiments, the inhibitor is administered once or twice a day at an oral dose of 1 – 10 mg, 10 – 20 mg, 20 – 30 mg, 30 – 40 mg, or 40 – 50 mg. In some embodiments, the inhibitor is administered once or twice a day at a dose of 50 – 60 mg, 60 – 70 mg, 70 – 80 mg, 80 – 90 mg, or 90 – 100 mg. In some embodiments, the inhibitor is administered at a dose of 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 mg PO once or twice a day. In some embodiments, the inhibitor is administered at a dose of 75 mg PO QD or BID, 100 mg PO QD or BID.

[0232] In certain embodiments, the JAK inhibitor is tofacitinib, and is administered at a dose of 5 mg PO BID or 11 mg PO qDay,

[0233] In certain embodiments, the JAK inhibitor is decernotinib, and is administered at a dose of 25 mg, 50 mg, 100 mg, or 150 mg PO BID.

[0234] In certain embodiments, the inhibitor is ruxolitinib, and is administered at dose of 25 mg PO BID, 20 mg PO BID, 15 mg PO BID, 10 mg PO BID, or 5 mg PO BID.

6.11. Additional therapeutic agents

[0235] In various embodiments of the methods described herein, the method further comprises administration of a therapeutic agent additional to the IL-6 antagonist, wherein the second therapeutic agent is also capable of reducing hepcidin expression.

[0236] In some embodiments, the second therapeutic agent is a BMP antagonist. In certain embodiments, the BMP antagonist is an anti-BMP6 antibody. In particular embodiments, the anti-BMP6 antibody has the six CDRs of an antibody described in US 2016/0176956, or US 2016/0159896, the disclosures of which are incorporated herein by reference in its entirety.

[0237] In certain embodiments, the second therapeutic agent is a hemojuvelin antagonist. In particular embodiments, the hemojuvelin antagonist is an anti-hemojuvelin antibody. In specific embodiments, the anti-hemojuvelin antibody has the six CDRs of the antibodies disclosed in Kovac *et al.*, *Haematologica* (2016) doi:10.3324/haematol.2015.140772 [ePub ahead of print].

[0238] In certain embodiments, the second therapeutic agent is a hepcidin antagonist. In particular embodiments, the hepcidin antagonist is an anti-hepcidin antibody. In specific embodiments, the antibody has the six CDRs from an antibody described in US 2016/0017032, the disclosure of which is incorporated herein by reference in its entirety.

6.12. Kits

[0239] In another aspect, kits are provided.

[0240] In typical embodiments, the kits provide reagents for determining, from a biological sample obtained from a patient, the patient's genotype at the location of the *TMPRSS6* SNP rs855791.

6.13. Additional Aspects And Embodiments

6.13.1. Methods Of Treating Inflammation In Chronic Kidney Disease Or Cardiovascular Disease

[0241] In other aspects and embodiments, compositions and methods are provided for characterizing and treating inflammation in chronic kidney disease or cardiovascular disease with an IL-6 antagonist, as well as methods for characterizing the responsiveness of a patient to treatment.

[0242] These aspects and embodiments are based, at least in part, on the discovery that inflammation in chronic kidney disease patients and cardiovascular disease patients having

DEQLKSGTASVVCLLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTLTL
SKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 19).

47. The method of item 42, wherein the anti-IL-6 antibody is MEDI5117.

48. The method of any one of items 1-47, wherein the subject is human.

6.13.2. Methods For Treating Cardiorenal Syndrome

[0234] In other aspects and embodiments, compositions and methods for treating cardiorenal syndrome are provided.

[0235] These aspects and embodiments are based, at least in part, on the discovery that anti-IL-6 treatment of heart injury in a rodent model of cardiorenal syndrome had an equivalent effect as standard of care treatment. As reported in more detail below, rodent models of cardiorenal syndrome were treated with anti-IL-6 or standard of care therapy (ACE inhibitor, perindopril) following a myocardial infarction. Following treatment, ejection fraction, force of cardiac contractility, and percentage of fibrotic tissue in heart tissue were measured. Levels of ejection fraction in both subject groups treated with anti-IL-6 and subject groups treated with standard of care therapy were increased compared to levels in a subject group treated with a control treatment. Cardiac contractility in both groups treated with anti-IL-6 and treated with standard of care therapy were increased compared to levels in a subject group treated with a control treatment. The amounts of fibrotic tissue in both groups treated with anti-IL-6 and treated with standard of care therapy were decreased compared to the amount in a subject group treated with a control treatment. Further, levels of ejection fraction and amount of fibrotic tissue were similar in the subject group treated with anti-IL-6 and the subject group treated with standard of care therapy. The results demonstrate that anti-IL-6 therapy had equivalent efficacy as standard of care therapy in treating cardiorenal syndrome in a rodent model.

[0236] These aspects and embodiments are further based, at least in part, on the discovery that patients identified as having cardiorenal syndrome following myocardial infarction and having elevated levels of IL-6 had particularly increased risk of cardiovascular death, including heart failure. Without being bound by theory, IL-6 may play a causal role in the development and/or progression of cardiorenal syndrome. Thus, patients having elevated levels of IL-6 following a myocardial infarction or patients having cardiorenal syndrome and elevated levels of IL-6 will likely benefit from IL-6 inhibition.

an IL-6 polynucleotide or polypeptide in a biological sample from the subject relative to a reference.

17. The method of item 15 or 16, wherein the subject has had a myocardial infarction.

18. The method of any one of items 1-10 or 15-17, wherein the IL-6 antagonist is an anti-IL-6 antibody.

19. The method of item 18, wherein the anti-IL-6 antibody is MEDI5117.

20. The method of any one of item 11-19, wherein the biological sample is a plasma sample.

21. The method of any one of items 1-20, wherein the subject is human.

6.14 Examples

[0297] The following examples are provided by way of illustration, not limitation.

6.14.1. Example 1: EPO dosage and overall survival in chronic kidney disease patients is correlated with serum IL-6 and CRP levels only in patients with at least one copy of the *TMPRSS6* SNP rs855791 major allele**

[0298] The peptide hormone, hepcidin, plays a central role in systemic iron homeostasis.

Hentze *et al.*, *Cell* 142:24-38 (2010). Hepcidin expression is known to be influenced by the product of the *TMPRSS6* gene, matriptase-2, a type II transmembrane serine protease.

Common variants in the *TMPRSS6* gene have been shown to correlate with iron status,

Benyamin *et al.*, *Nature Genetics* 41(11):1173-1175 (2009), and certain mutations in the

TMPRSS6 gene have been shown to cause iron-refractory iron deficiency anemia (IRIDA),

Finberg *et al.*, *Nature Genetics* 40(5):569-571 (2008). SNP rs855791 (2321G→A; A736V) is

a naturally occurring variation in the *TMPRSS6* gene that has been associated with naturally occurring variations in hepcidin expression and blood hemoglobin levels.

[0299] To determine whether the genotype at the *TMPRSS6* rs855791 SNP predicted extent of anemia in end stage renal disease, data previously collected in clinical studies of patients with chronic kidney disease were analyzed in conjunction with newly determined SNP

thereby increasing anemia. The increased mortality risk is a consequence of the dysregulated iron metabolism, the resulting anemia, and/or the increased dose of erythropoiesis stimulating agent, such as EPO. If these correlations reflect a causal relationship, they raise the possibility that reducing IL-6 levels or IL-6 signaling could reduce anemia, reduce required EPO dose, and increase survival in patients with chronic kidney disease, but only in those patients having at least one copy of the *TMPRSS6* rs855791 major allele, and with greatest effect in those patients having elevated serum levels of IL-6.

6.14.2. Example 2: Risk of death and risk of heart failure after acute myocardial infarction are correlated with IL-6 serum levels only in patients with at least one copy of the *TMPRSS6* SNP rs855791 major allele

[0310] To determine whether *TMPRSS6* rs855791 genotype affected IL-6 sensitivity in patients with acute rather than chronic disease, data previously collected in clinical studies of patients hospitalized for acute coronary syndrome were analyzed in conjunction with newly determined SNP genotyping.

Methods

[0311] Data were analyzed from subjects previously enrolled in a multi-center Study of Platelet Inhibition and Patient Outcomes (PLATO). Patients were eligible for enrollment in PLATO if they had been hospitalized for an acute coronary syndrome with an onset of symptoms during the previous 24 hours. Mortality and presence of heart failure was measured in these subjects beginning thirty days following a myocardial infarction.

Results

[0312] The mortality of subjects homozygous for the *TMPRSS6* rs855791 SNP minor allele (A) did not correlate with variations in IL-6 (FIG. 4A). However, one or two copies of the major allele (G) conferred a higher all-cause mortality in response to elevated IL-6 levels in subjects following myocardial infarction (FIG. 4B). Thus, *TMPRSS6* modulated IL-6 mediated risk of death following myocardial infarction.

[0313] The effect of *TMPRSS6* genotype on IL-6 mediated risk of heart failure was also measured in subjects enrolled in PLATO beginning thirty days post-myocardial infarction. Heart failure in subjects homozygous for the minor allele (A) did not correlate with variations in IL-6 (FIG. 5A). However, the G allele of *TMPRSS6* conferred a higher heart failure rate in response to elevated IL-6 levels in subjects following a myocardial infarction (FIG. 5B). Thus, *TMPRSS6* modulated IL-6 mediated risk of heart failure following myocardial infarction.

Discussion

[0314] These data demonstrate that the correlation between *TMPRSS6* genotype, IL-6 levels, and adverse clinical outcomes is not limited to patients with chronic kidney disease. Without intending to be bound by theory, in patients having at least one copy of the *TMPRSS6* major allele, increases in serum IL-6 drive may drive increased hepcidin expression, with consequent increased sequestration of iron in cardiomyocytes, followed by iron-mediated cellular toxicity. If these correlations reflect a causal relationship, they raise the possibility that reducing IL-6 levels or IL-6 signaling could reduce heart failure and mortality in patients with acute coronary syndrome, but only in those patients having at least one copy of the *TMPRSS6* rs855791 major allele, and with greatest effect in those patients with elevated serum levels of IL-6.

6.14.3. Example 3: *In vitro* studies on iPS-derived human cardiomyocytes confirm a causal relationship between *TMPRSS6* genotype and IL-6-mediated cytotoxicity

[0315] Although the correlations observed in Examples 1 and 2 imply that reducing IL-6 mediated signaling should provide clinical benefit in patients having at least one copy of the *TMPRSS6* rs855791 major allele, elevated levels of IL-6, and either anemia or a hepcidin-mediated cellular toxicity, the observed correlations fall short of proving a causal relationship. Accordingly, experiments were conducted in human induced pluripotent cell derived cardiac myocytes (iPS-CMs) transfected with variants of *TMPRSS6* to interrogate the effect of BMP and BMP plus IL-6 on hepcidin expression and cellular susceptibility to ischemic injury.

6.14.3.1. Methods

[0316] *Culturing of human iPS derived cardiomyocytes* – iCell cardiomyocytes (Cellular Dynamics International, CDI Inc.) were plated on 0.1% gelatin coated 6-well or 96-well cell culture plate with iCell Cardiomyocytes plating medium (CDI Inc.). Forty-eight hours after plating, plating medium was replaced with Maintenance Medium (CDI Inc.). Maintenance Medium was replaced every other day up to the day the experiment was performed.

[0317] *Simulated Ischemia/Reoxygenation Protocol* – The iPS cardiomyocytes were subjected to simulated ischemia (SI) for 90 min by replacing the cell medium with an “ischemia buffer” that contained 118 mm NaCl, 24 mm NaHCO₃, 1.0 mm NaH₂PO₄, 2.5 mm CaCl₂-2H₂O, 1.2 mm MgCl₂, 20 mm sodium lactate, 16 mm KCl, 10 mm 2-deoxyglucose (pH adjusted to 6.2) as reported previously (Das, A., Xi, L., and Kukreja, K. C. (2005) *J.*

[0321] *Real time PCR- Taqman assay* – Total RNA including small RNA was isolated using miRNeasy mini kit according to manufacturer’s protocol (QIAGEN Sciences, MD, USA). Concentration and purity of the isolated RNA was measured using Nanodrop ND-1000 spectrophotometer (Agilent technologies, CA, USA). Briefly 1 µg of total RNA was converted to cDNA with random hexamer using high capacity cDNA synthesis kit (Applied Biosystems, CA, USA). Reverse transcription reaction was carried out using the following PCR conditions: 25°C for 10 min; 37 °C for 120 min and 85°C for 5 min. Real-time PCR was performed using Taqman amplicon specific probes (Applied Biosystems, CA, USA) Hamp (CGGCTCTGCAGCCTTG) (SEQ ID NO:20) under the following PCR cycle condition: 95°C for 10 minutes; 95°C for 15 seconds and 60°C for 60 seconds. The expression of Hamp was normalized to GAPDH (CTTCCAGGAGCGAGATCCCGCTAA) (SEQ ID NO:21) housekeeping gene. The relative gene expression was analyzed using the 2^{-ΔΔCt} method.

[0322] *TMPRSS6 mutagenesis and transfection of iPS cells* – pCMV6-XL5 TMPRSS6 was purchased from Origene Technologies (Rockville, MD), catalog number SC306623 corresponding to GenBank accession number NM_153609. This clone contains a mutation resulting in an amino acid change, K253A. Site directed mutagenesis was performed to revert the amino acid at position 253 to the canonical lysine (K). Once the reversion was confirmed, site directed mutagenesis was performed to introduce the V736A mutation. All mutagenesis reactions were carried out using Agilent Technologies QuikChange II XL Site-Directed Mutagenesis Kit (Santa Clara, CA; catalog number 200521). All vectors were sequenced to confirm. The primer sequences used were: antisense (as) TMPRSS6 E253K GCATGAGGTCCTTGGGGCCCTGCAG (SEQ ID NO:22); sense (s) TMPRSS6 E253K CTGCAGGGCCCCAAGGACCTCATGC (SEQ ID NO:23); antisense (as) TMPRSS6 V736A CCTGGTAGCGATAGGCCTCGCTGCACAGG (SEQ ID NO:24); sense (s) TMPRSS6 V736A CCTGTGCAGCGAGGCCTATCGCTACCAGG (SEQ ID NO:25).

6.14.3.2. Results

[0323] Human iPS-CMs only minimally express matriptase-2 at baseline. Cells were transfected with a construct driving constitutive expression of matriptase-2 736A, encoded by the *TMPRSS6* rs855791SNP major allele, or matriptase-2 736V, encoded by the minor allele, mimicking homozygous major allele and homozygous minor allele cardiomyocytes, respectively.

[0324] Hepcidin expression is regulated by both the BMP6/SMAD and IL-6/STAT signaling pathways, with both BMP and IL-6 acting through their respective receptors to drive increased hepcidin expression. Casanovas *et al.*, *PLOS Comp. Biol.* 10(1):e1003421 (2014).

The major allele and minor allele iPS cardiomyocytes were treated *in vitro* with agonists of both signaling pathways — recombinant BMP2 and IL-6 — or with BMP2 alone to model clinical interventions in which IL-6 levels (or signaling) are reduced. Control iPS cells were not treated with either agonist. Cell mortality was measured under normal oxygen tension (normoxia), and also under conditions that simulate hypoxia followed by reoxygenation (reperfusion).

[0325] FIG. 6A shows the results when the cells were treated under normal oxygen levels. iPS cardiomyocytes expressing only the *TMPRSS6* rs855791 minor allele (“736V minor allele”) are not significantly affected (“n.s.”) by elimination of IL-6 signaling: cell mortality measured as percent Trypan Blue positive cells is insignificantly reduced when the cells are treated with BMP2 alone, as compared to treatment with BMP2+IL-6. In contrast, iPS cardiomyocytes expressing the *TMPRSS6* rs855791 major allele show statistically significantly lower cell death when IL-6 signaling is eliminated.

[0326] FIG. 6B shows the results when the cells were subjected to hypoxia followed by reoxygenation. As compared to normoxic conditions, hypoxia/reoxygenation is significantly toxic to the iPS cardiomyocytes, with about 40 percent of major and minor allele control cells killed, as compared to about 20% of the control cells under normoxic conditions (compare FIG. 6B to FIG. 6A). Against this increased background toxicity, minor allele iPS cardiomyocytes are not significantly affected by elimination of IL-6 signaling: cell mortality is insignificantly reduced when the cells are treated with BMP2 alone, as compared to treatment with BMP2+IL-6. In contrast, the iPS cardiomyocytes expressing the *TMPRSS6* rs855791 major allele show statistically significantly lower cell death when IL-6 signaling is eliminated.

6.14.3.3. Discussion

[0327] These data strengthen the inferences drawn from the post hoc analysis of clinical trial data in Example 1 and Example 2: reduction in IL-6 signaling can be effective to reduce IL-6 mediated toxicity in cardiomyocytes expressing the *TMPRSS6* rs855791 major allele, but not in cardiomyocytes expressing only the minor allele. Without intending to be bound by theory, the IL-6 driven increase in toxicity in the major allele iPS cardiomyocytes may result from IL-6 mediated increase in hepcidin expression, with consequent increased sequestration of iron in the cells, followed by iron-mediated cellular toxicity.

6.14.4. Example 4: Anti-IL-6 therapy is as effective as current standard of care in a model of cardiorenal syndrome in rats

example “Normal” region is indicated by the delineated portion of the tissue slice shown in the micrograph in FIG. 10A. The inset in the micrograph shows a magnified view of the “Normal” region, showing that small portions of the “Normal” region has fibrotic tissue. The “Fibrosis Margin” region is a region of tissue in the “Normal” region peripheral to the fibrotic tissue.

[0340] Plots in FIGS. 10B and 10C show that heart tissue from subjects in groups treated with anti-IL-6 or standard of care therapy had significantly decreased percentage area of fibrotic tissue compared to the isotype control group, both when measured in the “Normal” region (FIG. 10B) or in the “Fibrosis margin” region (FIG. 10C). In addition, the percentage areas of fibrotic tissue measured in the anti-IL-6 and standard of care therapy groups were similar (both in the “Normal” region and “Fibrosis margin” region), indicating that anti-IL-6 had an equivalent anti-fibrotic effect as the ACE inhibitor perindopril (standard of care therapy).

[0341] These data demonstrate that treatment with an anti-IL-6 agent is effective to reduce cardiac injury and restore function in an *in vivo* model of cardiorenal syndrome in animals that are genotypically analogous to human beings homozygous for the *TMPRSS6* rs855791 major allele.

6.14.5. Example 5: Anti-IL-6 therapy is effective in preserving cardiac function in a model of acute myocardial infarction in mice genotypically analogous to human *TMPRSS6* rs855791 major allele homozygotes

[0342] The data in Examples 2 and 3 suggested that reducing IL-6 levels or IL-6 signaling could reduce heart failure and mortality in patients with acute coronary syndrome, but only in those patients having at least one copy of the *TMPRSS6* rs855791 major allele, and with greatest effect in those patients with elevated serum levels of IL-6.

[0343] A rodent study was performed to determine the effect of anti-IL-6 therapy after acute myocardial infarction in mice genotypically analogous to human beings homozygous for the *TMPRSS6* rs855791 major allele.

[0344] FIGS. 11A and 11B show data from an *in vivo* model in which myocardial infarction was induced in mice genotypically analogous to human beings homozygous for the *TMPRSS6* rs855791 major allele. The control group received no therapy. The experimental group was treated with an anti-murine-IL-6 antibody. FIG. 11A shows that treatment with anti-IL-6 provides statistically significant improvement in ejection fraction. FIG. 11B shows that treatment with anti-IL-6 provides statistically significant improvement in contractility,

measured as cardiac fractional shortening. The data demonstrate that anti-IL-6 therapy given immediately after myocardial infarction improves functional recovery of the left ventricle in rodents that are genotypically analogous to human patients having the *TMPRSS6* rs855791 major allele.

7. INCORPORATION BY REFERENCE

[0345] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes.

8. EQUIVALENTS

[0346] While various specific embodiments have been illustrated and described, the above specification is not restrictive. It will be appreciated that various changes can be made without departing from the spirit and scope of the invention(s). Many variations will become apparent to those skilled in the art upon review of this specification.