

[0012] US Patent, US9315545, describe additional novel hepcidin analogs and their use to treat diseases of iron metabolism, beta thalassemia, hemochromatosis, iron-loading anemias, alcoholic liver disease, or chronic hepatitis C.

[0013] Polycythemia vera (PV) is a chronic, progressive trilineage clonal disorder signified by increased myeloid, erythroid, and megakaryocytic cell proliferation/accumulation and is characterized by the World Health Organization (WHO) as a myeloproliferative neoplasm (Arber et al., 2016,127(20):2391-405). Diagnosis is defined by two criteria; the first being increased red blood cell mass, bone marrow biopsy showing trilineage hypercellularity and presence of JAK2V617F or JAK2 exon 12 mutations and the second criteria incorporates polycythemia, bone marrow biopsy confirmation and subnormal serum erythropoietin levels (Arber et al., 2016,127(20):2391-405).

[0014] An estimated 148,000 people in the United States are living with PV with a median age at diagnosis of 61 years (Stein et al., J Clin Oncol. 2015 Nov 20;33(33):3953-60). Polycythemia symptoms, related to blood hyperviscosity include fatigue, bone pain, headaches, lightheadedness, visual disturbances, atypical chest pain, pruritus, erythromelalgia, and paresthesia (Tefferi et al., Blood Cancer J. 2018, 8(1):3). Clinical features include splenomegaly, thrombotic and bleeding complications, and risk of leukemic transformation.

[0015] As PV is a disease characterized by increased erythropoiesis, it has been shown in animal models that high doses of hepcidin mimetics can ameliorate this disease by diminishing erythropoiesis (Casu et al., Blood. 2016;128(2):265-276). In PV mice that express the orthologous JAK2 mutation causing human PV, administration of minihepcidin significantly reduces splenomegaly and normalizes hematocrit. These studies indicate that drug-like minihepcidins have a potential as future therapeutics for untransfused β -thalassemia and PV (Casu et al., Blood. 2016;128(2):265-276).

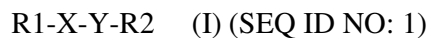
[0016] There is clearly a need for new therapeutic agents and methods for treating and preventing PV, including PV in high-risk patients or those who cannot tolerate phlebotomy. The present invention addresses such needs of treating PV.

BRIEF SUMMARY

[0017] In a specific example, the present disclosure provides methods of treating polycythemia vera in a subject in need thereof comprising administering to the subject an effective amount of a pharmaceutical composition of a hepcidin analogue.

[0018] In a more specific example, the present disclosure provides methods of treating polycythemia vera in a subject in need thereof, comprising administering to the subject an effective amount of a pharmaceutical composition comprising a hepcidin analogue and a pharmaceutically acceptable carrier, diluent or excipient.

[0019] In one example, the hepcidin analogue comprises a peptide comprising Formula (I):



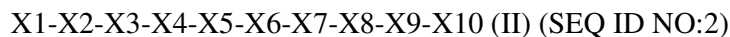
or a pharmaceutically acceptable salt or solvate thereof,

wherein

R1 is hydrogen, a C1-C6 alkyl, a C6-C12 aryl, a C1-C20 alkanoyl or pGlu;

R2 is NH₂ or OH;

X is a peptide sequence having the formula II



wherein

X1 is Asp, Ala, Ida, pGlu, bhAsp, Leu, D-Asp or absent;

X2 is Thr, Ala, or D-Thr;

X3 is His, Lys, or D-His;

X4 is Phe, Ala, Dpa or D-Phe;

X5 is Pro, Gly, Arg, Lys, Ala, D-Pro or bhPro;

X6 is Ile, Cys, Arg, Lys, D-Ile or D-Cys;

X7 is Cys, Ile, Leu, Val, Phe, D-Ile or D-Cys;

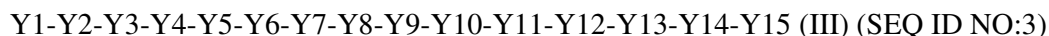
X8 is Ile, Arg, Phe, Gln, Lys, Glu, Val, Leu or D-Ile;

X9 is Phe or bhPhe; and

X10 is Lys, Phe or absent;

wherein if Y is absent, X7 is Ile; and

Y is a peptide sequence having the formula III



wherein

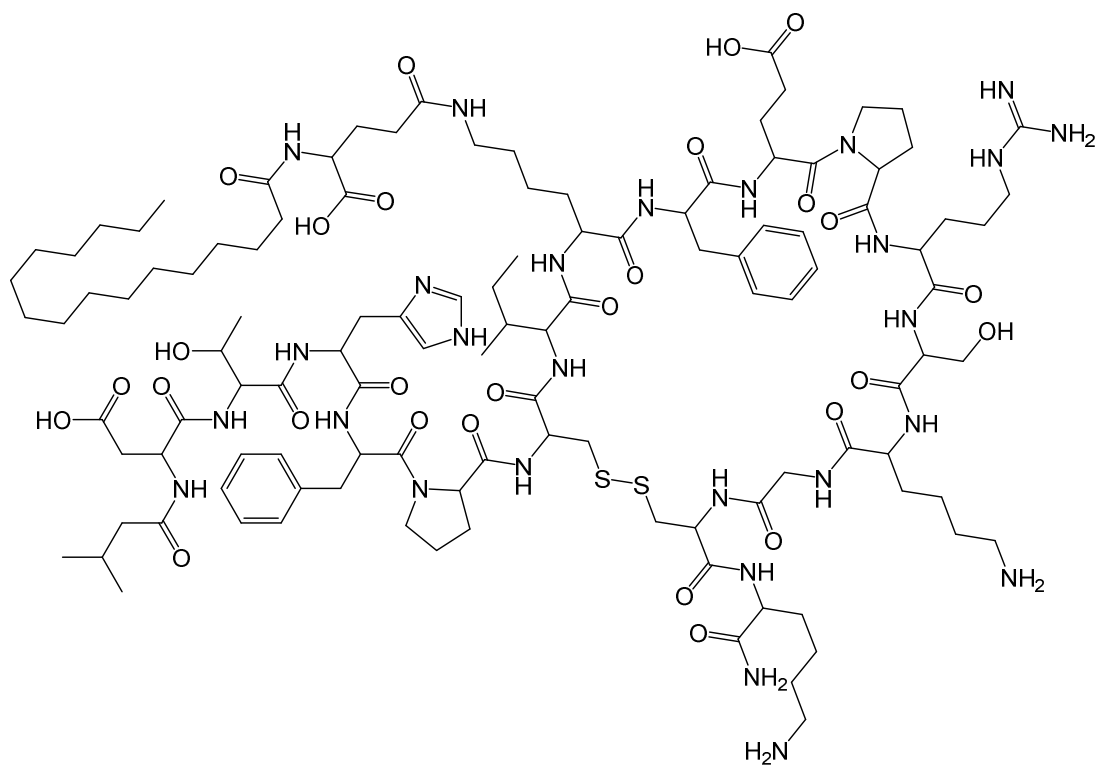
Y1 is Gly, Cys, Ala, Phe, Pro, Glu, Lys, D-Pro, Val, Ser or absent;

Y2 is Pro, Ala, Cys, Gly or absent;

Y3 is Arg, Lys, Pro, Gly, His, Ala, Trp or absent;
Y4 is Ser, Arg, Gly, Trp, Ala, His, Tyr or absent;
Y5 is Lys, Met, Arg, Ala or absent;
Y6 is Gly, Ser, Lys, Ile, Ala, Pro, Val or absent;
Y7 is Trp, Lys, Gly, Ala, Ile, Val or absent;
Y8 is Val, Thr, Gly, Cys, Met, Tyr, Ala, Glu, Lys, Asp, Arg or absent;
Y9 is Cys, Tyr or absent;
Y10 is Met, Lys, Arg, Tyr or absent;
Y11 is Arg, Met, Cys, Lys or absent;
Y12 is Arg, Lys, Ala or absent;
Y13 is Arg, Cys, Lys, Val or absent;
Y14 is Arg, Lys, Pro, Cys, Thr or absent; and
Y15 is Thr, Arg or absent;

wherein said peptide of formula I is optionally PEGylated on R1, X, or Y, and wherein a side chain of an amino acid of the peptide is optionally conjugated to a lipophilic substituent or polymeric moiety. In a related example, formula II is shown as above, but X3 is D-Lys.

[0019a] In one aspect, disclosed herein is a use of a hepcidin analogue or a pharmaceutically acceptable salt or solvate thereof in the manufacture of a medicament for treating polycythemia vera in a human subject in need thereof, wherein the hepcidin analogue is:



Isovaleric acid-DTHFPCI(K(isoGlu-Palm))FEPRSKGCK-NH₂ (SEQ ID NO:45);
wherein the amino acids are L-amino acids; and
wherein the hepcidin analogue or pharmaceutically acceptable salt or solvate thereof is to be administered via subcutaneous injection; the subject is to be administered from 20 mg to 100 mg of the hepcidin analogue or pharmaceutically acceptable salt or solvate thereof; and the hepcidin analogue or pharmaceutically acceptable salt or solvate thereof is to be provided to the subject about twice a week, about once a week, or about once every two week.

[0020] In one example, R1 is hydrogen, isovaleric acid, isobutyric acid or acetyl.

[0021] In particular examples of any of the hepcidin analogues or dimers of the present disclosure, the half-life extension moiety is selected from C12 (Lauric acid), C14 (Myristic acid), C16 (Palmitic acid), C18 (Stearic acid), C20, C12 diacid, C14 diacid, C16 diacid, C18 diacid, C20 diacid, biotin, and isovaleric acid. In certain examples, the half-life extension moiety is attached to a linker moiety that is attached to the peptide. In certain examples, the half-life extension moiety increases the molecular weight of the hepcidin analogue by about 50 D to about 2 KD. In various examples, the half-life extension moiety increases serum half-life, enhances solubility, and/or improves bioavailability of the hepcidin analogue.

[0022] In certain examples, a peptide analogue or dimer of the present disclosure comprises an isovaleric acid moiety conjugated to an N-terminal Asp residue.

[0023] In certain examples, a peptide analogue of the present disclosure comprises an amidated C-terminal residue.

[0024] In certain examples, a hepcidin analogue or dimer of the present disclosure comprises the sequence: Asp-Thr-His-Phe-Pro-Cys-Ile-Lys-Phe-Glu-Pro-Arg-Ser-Lys-Gly-Cys-Lys (SEQ ID NO:19), or comprises a sequence having at least 80%, at least 90%, or at least 94% identity to this sequence.

[0025] In certain examples, a hepcidin analogue or dimer of the present disclosure comprises the sequence: Asp-Thr-His-Phe-Pro-Cys-Ile-Lys-Phe-Pro-Arg-Ser-Lys-Gly-Cys-Lys (SEQ ID NO: 19), or comprises a sequence having at least 80%, at least 90%, or at least 94% identity to this sequence.

[0026] In a related example, the present disclosure includes a polynucleotide that encodes a peptide of a hepcidin analogue or dimer (or monomer subunit of a dimer) of the present disclosure.

[0027] In a further related example, the present disclosure includes a vector comprising a polynucleotide of the disclosure.

[0028] In another example, the present disclosure includes a pharmaceutical composition, comprising a hepcidin analogue, dimer, polynucleotide, or vector of the present disclosure, and a pharmaceutically acceptable carrier, excipient or vehicle.

[0029] In another example, the present disclosure provides a method of binding a ferroportin or inducing ferroportin internalization and degradation, comprising contacting the ferroportin with at least one hepcidin analogue, dimer or composition of the present disclosure.

[0030] In another example, the present disclosure provides methods to treat Polycythemia vera.

[0031] In a further example, the present disclosure includes a method for treating Polycythemia vera in a subject in need thereof comprising providing to the subject an effective amount of a hepcidin analogue or pharmaceutical composition of the present disclosure. In certain examples, the hepcidin analogue or pharmaceutical composition is provided to the subject by an oral, intravenous, peritoneal, intradermal, subcutaneous, intramuscular, intrathecal, inhalation, vaporization, nebulization, sublingual, buccal, parenteral, rectal, vaginal, or topical route of administration. In certain examples, the hepcidin analogue or pharmaceutical composition is provided to the subject by an oral or subcutaneous route of administration. In certain examples, the hepcidin analogue or pharmaceutical composition is provided to the subject at most or about twice daily, at most or about once daily, at most or about once every two days, at most or about once a week, or at most or about once a month.

[0032] In particular examples, the hepcidin analogue is provided to the subject at a dosage of about 10 mg to about 100 mg, about 10 mg to about 80 mg, or about 10 mg to about 50 mg. In a more particular example, the hepcidin analogue is provided to the subject at a dosage of about 20 mg to about 40 mg. In particular examples, the hepcidin analogue is provided to the subject at a dosage of about 10 mg, about 15 mg, about 20 mg, about 25 mg, about 30 mg, about 40 mg, about 50 mg, about 60 mg, about 70 mg, or about 80 mg. In particular examples, the hepcidin analogue is provided to the subject about once a week. In another

particular example, the hepcidin analogue is provided to the subject about twice a week, e.g., subcutaneously.

[0033] In certain examples, any of the disclosed methods further comprises determining the subject's hematocrit level at one or more time points following administration of the hepcidin analogue, and maintaining or adjusting the amount of the hepcidin analogue or pharmaceutically acceptable salt thereof administered to the subject, wherein the amount is increased if the subject's determined hematocrit is greater than 44% or 45%, wherein the amount is decreased if the subject's determined hematocrit is less than either 37.5% or 40%, and maintaining the amount if the subject's determined hematocrit is between 37.5% and 45%, between 37.5% and 44%, between 40% and 45%, or between 40% and 44%.

[0034] In another example, the present disclosure provides a device comprising pharmaceutical composition of the present disclosure, for delivery of a hepcidin analogue or dimer of the disclosure to a subject, optionally orally or subcutaneously.

[0035] In yet another example, the present disclosure includes a kit comprising a pharmaceutical composition of the disclosure, packaged with a reagent, a device, or an instructional material, or a combination thereof.

[0036] In particular examples of any of the methods disclosed, the Polycythemia vera is phlebotomy-requiring Polycythemia vera, or phlebotomy-requiring Polycythemia vera in a low risk patient.

[0037] In particular examples of any of the methods disclosed, the subject is a low risk Polycythemia vera patient, a high risk Polycythemia vera patient, a symptomatic phlebotomy-requiring Polycythemia vera patient, a high risk patient with phlebotomy-requiring Polycythemia vera or a low risk patient with phlebotomy-requiring Polycythemia vera.

[0038] In particular examples of any of the methods disclosed, the subject is diagnosed with Polycythemia vera and has received at least three phlebotomies to goal hematocrit $\leq 45\%$ in the 24 weeks prior to administration of the hepcidin analog or peptide to the subject.

[0039] In certain examples of any of the methods disclosed, the subject is administered from about 5 mg to about 200 mg of the hepcidin analog or the peptide, e.g., about 10 mg to about 100 mg, about 20 mg to about 100 mg, about 20 mg, about 40 mg, about 80 mg, about 100 mg, or about 120 mg.

[0040] In certain examples of any of the methods disclosed, the pharmaceutical composition is administered via subcutaneous injection.

[0041] In certain examples of any of the methods disclosed, the pharmaceutical composition is administered about weekly over a period of time.

[0042] In certain examples of any of the methods disclosed, the amount of the hepcidin analog or peptide administered is increased over a period of time.

[0043] In certain examples of any of the methods disclosed, the subject is a mammal, e.g., a human.

[0044] In certain examples of any of the methods disclosed, the method results in a decrease in the subject's hematocrit level to $\leq 45\%$, a decrease in the subject's hematocrit of at least 3%, and/or an increase in the subject's serum ferritin. In some examples, the subject remains phlebotomy free during a course of treatment, e.g., treatment about once a week for a period of time.

BRIEF DESCRIPTION OF THE FIGURES

[0045] Figures 1A and 1B describe the data obtained from the Cobas Iron2 Analysis experiment for Compound A and Compound B. Figure 1A is a graph showing serum iron levels and serum concentration of Compound A at the indicated times following treatment with Compound A. Figure 1B is a graph showing serum iron levels and serum concentration of Compound B at the indicated times following treatment with Compound B.

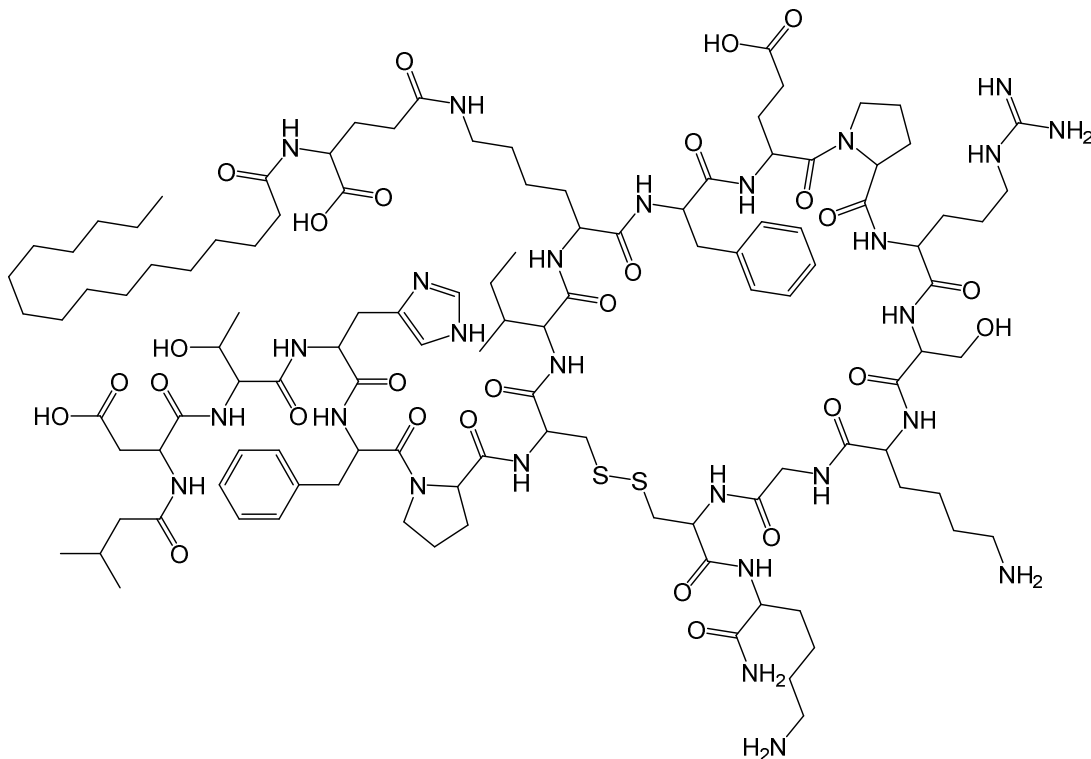
[0046] Figure 2 shows temporal profile of hematocrit and RBC indices in male Cynomolgus monkeys following subcutaneous administration of vehicle ($\circ$) or Compound A at doses of 1 ($\bullet$), 3 ($\square$), and 10 ($\blacksquare$) mg/kg/dose once weekly for 4 administrations with a 28-day recovery period. Compound A induced changes in secondary hematologic indices (mean corpuscular hemoglobin concentration, MCHC, and mean corpuscular hemoglobin, MCH) indicative of iron-restricted erythropoiesis. Each point represents mean $\pm$ SD of up to 6 animals in the Main treatment (all groups) and up to 2 animals in the Recovery phase (vehicle and 10 mg/kg Compound A). Arrows indicate when Compound A was administered.

[0047] Figure 3 demonstrates that Compound A induces significant changes in hematocrit (Hct) and secondary hematologic indices. Alterations in Hct, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) were assessed after subcutaneous administration of vehicle ($\circ$) or Compound A at doses of 0.6 ($\bullet$), 2 ($\square$), and 6 ($\blacksquare$) mg/kg once weekly for 13 consecutive weeks followed by a 35-day recovery period. Arrows represent administration days starting on Day 1. Each point

CLAIMS

What is claimed is:

1. Use of a hepcidin analogue or a pharmaceutically acceptable salt or solvate thereof in the manufacture of a medicament for treating polycythemia vera in a human subject in need thereof, wherein the hepcidin analogue is:



Isovaleric acid-DTHFPCI(K(isoGlu-Palm))FEPRSKGCK-NH2 (SEQ ID NO:45);

wherein the amino acids are L-amino acids; and

wherein the hepcidin analogue or pharmaceutically acceptable salt or solvate thereof is to be administered via subcutaneous injection; the subject is to be administered from 20 mg to 100 mg of the hepcidin analogue or pharmaceutically acceptable salt or solvate thereof; and the hepcidin analogue or pharmaceutically acceptable salt or solvate thereof is to be provided to the subject about twice a week, about once a week, or about once every two weeks.

2. The use of claim 1, wherein the use results in a decrease in the subject's hematocrit level to <45% and an increase in the subject's serum ferritin level.

3. The use of claim 1 or claim 2, wherein the hepcidin analogue is to be administered to the subject in a pharmaceutical composition comprising one or more pharmaceutically acceptable carriers, excipients, or diluents.

4. The use of any one of claims 1-3, wherein:

(a) the hepcidin analogue is to be provided to the subject at a dosage of 20 mg to 50 mg, 20 mg to 40 mg, about 80 mg, about 70 mg, about 60 mg, about 50 mg, about 40 mg, about 30 mg, about 25 mg, or about 20 mg;

(b) the peptide or the pharmaceutical composition is to be provided to the subject at a dosage of about 20 mg, about 25 mg, about 30 mg, about 35 mg, or about 40 mg;

(c) the peptide or the pharmaceutical composition is to be provided to the subject at a dosage of about 20 mg, about 25 mg, about 30 mg, or about 40 mg about once a week.

(d) the peptide or the pharmaceutical composition is to be provided to the subject at a dosage of about 20 mg, about 25 mg, about 30 mg, or about 40 mg about twice a week;

(e) the hepcidin analogue or pharmaceutically acceptable salt thereof is to be provided to the subject at a dosage of about 20 mg;

(f) the hepcidin analogue or pharmaceutically acceptable salt thereof is to be provided to the subject at a dosage of about 25 mg;

(g) the hepcidin analogue or pharmaceutically acceptable salt thereof is to be provided to the subject at a dosage of about 30 mg;

(h) the hepcidin analogue or pharmaceutically acceptable salt thereof is to be provided to the subject at a dosage of about 40 mg;

(i) the hepcidin analogue or pharmaceutically acceptable salt thereof is to be provided to the subject at a dosage of about 50 mg;

(j) the hepcidin analogue or pharmaceutically acceptable salt thereof is to be provided to the subject at a dosage of about 60 mg;

(k) the hepcidin analogue or pharmaceutically acceptable salt thereof is to be provided to the subject at a dosage of about 70 mg; or

(l) the hepcidin analogue or pharmaceutically acceptable salt thereof is to be provided to the subject at a dosage of about 80 mg.

5. The use according to any one of claims 1-4, wherein the polycythemia vera is phlebotomy-requiring polycythemia vera, optionally wherein the subject is a symptomatic phlebotomy-requiring polycythemia vera patient.
6. The use according to any one of claims 1-5, wherein the subject is diagnosed with polycythemia vera and has received at least three phlebotomies to goal hematocrit $\leq 45\%$ in the 24 weeks prior to administration of the pharmaceutical composition to the subject.
7. The use according to any one of claims 1-6, wherein:
- (a) the amount of the hepcidin analogue or pharmaceutically acceptable salt thereof to be administered is increased over a period of time; or
 - (b) the use further comprises determining the subject's hematocrit at one or more time points following administration of the hepcidin analogue or pharmaceutically acceptable salt thereof, and maintaining or adjusting the amount of the hepcidin analogue or pharmaceutically acceptable salt thereof to be administered to the subject, wherein the amount is increased if the subject's determined hematocrit is greater than 45%, wherein the amount is decreased if the subject's determined hematocrit is less than either 37.5% or 40%, and maintaining the amount if the subject's determined hematocrit is between 37.5% and 45% or between 40% and 44%.
8. The use according to any one of claims 1-7, wherein the subject is to be treated by cytoreductive therapy, optionally hydroxyurea.