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(54) Title: TREATMENT OF IRON DISORDERS

(57) Abstract: Treatment of a disease of iron metabolism including the step of administering a peptide effective for inhibiting the binding of hepcidin to $\alpha 2$ - macroglobulin, to an individual requiring said treatment.



WO 2018/107241 A1

Treatment of iron disorders

Field of the invention

The invention relates to diseases of iron metabolism including anemia, especially to anemia of chronic disease, to hepcidin, and to the synthesis and design of peptides contemplated for therapeutic use as competitive inhibitors.

Background of the invention

Reference to any prior art in the specification is not an acknowledgment or suggestion that this prior art forms part of the common general knowledge in any jurisdiction or that this prior art could reasonably be expected to be understood, regarded as relevant, and/or combined with other pieces of prior art by a skilled person in the art.

Iron is an essential transition metal for life [1,2]. In most organisms, a highly coordinated system controls the regulation and cellular and systemic metabolism of iron [1,2].

Hepcidin, the 'hormone of iron metabolism' appears to be a major regulator of systemic iron homeostasis [3].

The dysregulation of hepcidin is etiologically involved in many diseases of iron metabolism. Pathologically low hepcidin induces iron overload (e.g., in hereditary hemochromatosis types 1-3), while pathologically high hepcidin typically induces iron-limited anemia [1], with the anemia of chronic disease being of particular concern [4].

Hepcidin is believed to exert its effects, principally through binding to the iron release transporter, ferroportin [3]. Ferroportin acts to release cellular iron into the circulation and results in the absorption of iron from the gut. By binding to ferroportin, hepcidin down-regulates ferroportin levels by causing its cellular internalisation and degradation [7]. This decrease in ferroportin prevents (1) iron being released from cellular stores (e.g., the liver) into the blood; and (2) iron uptake from the gut into the blood [7]. Hence, pathologically high hepcidin levels, as occurs in the anemia of chronic disease, leads to a debilitating anemia (as ferroportin expression is reduced) [3].

Considering its role in cellular iron release, ferroportin has been suggested as a target for the development of inhibitors for treatment of diseases of iron metabolism, whereby it has been hypothesised that inhibition of ferroportin I – hepcidin binding might enable treatment of these diseases [5].

5 In one study, the hepcidin pharmacophore at the ferroportin binding site was established [5]. However, the hepcidin analogs utilized in that study to define the pharmacophore were observed not to inhibit hepcidin binding to ferroportin in a ferroportin-GFP assay when hepcidin and each analog were co-incubated [5]. The results strongly suggested that these peptide analogs would not be useful for treatment
10 of diseases of iron metabolism. This is because they do not have sufficient affinity to inhibit hepcidin- mediated ferroportin internalisation and degradation [5].

Hepcidin has also been shown to bind to other molecules including α -2 macroglobulin [6], and the latter has been proposed as a target for screening for inhibitors for treatment of diseases of iron metabolism by increasing urinary excretion of
15 hepcidin arising from competitive inhibition of hepcidin binding with α -2 macroglobulin [8].

WO2010/065815, WO2013/086143, WO2014/071015 and WO2016/109363 (all by The Regents of University of California) and WO2014/145561 and WO2015/200916 (by Protagonist Therapeutics Inc) each propose peptides having hepcidin activity,
20 especially activity for binding to ferroportin to cause degradation or internalisation of same, for treatment of iron related disorders.

There remains a need to treat disease of iron metabolism, particularly those diseases arising from or associated with excessive hepcidin production or activity, and for pharmaceutical compositions for same.

25 There remains a need to treat anemia, in particular the anemia of chronic disease and for pharmaceutical compositions for same.

Summary of the invention

The invention seeks to address one or more of the above mentioned needs or limitations and in one embodiment provides peptides and methods of use of same for

antagonising the binding of hepcidin to $\alpha 2$ macroglobulin, or for increasing excretion of hepcidin from the body, or for increasing ferroportin -mediated export of Fe ions from a cell, or for improving red blood cell production, or for treating a disease of iron metabolism, especially a disease associated with excessive hepcidin production,
5 expression or activity, and in particular, for treating anemia of chronic disease.

The peptides utilised in the methods of the invention for treatment of diseases associated with excessive hepcidin production or activity inhibit the binding of hepcidin to $\alpha 2$ macroglobulin, and otherwise do not have hepcidin activity in the presence of natural or endogenous hepcidin. That is, they do not bind to ferroportin to cause
10 internalisation or degradation of ferroportin that would otherwise prevent ferroportin – mediated export of Fe ions from inside a cell in the presence of natural or endogenous hepcidin because they do not inhibit hepcidin from binding to ferroportin the presence of natural or endogenous hepcidin. Thus, in one embodiment, there is provided a method of treating a disease associated with excessive hepcidin production or activity including
15 providing an individual in need of said treatment with a peptide that: (i) inhibits the binding of hepcidin to $\alpha 2$ macroglobulin; and (ii) that does not have hepcidin activity, for example, does not prevent ferroportin from export of Fe ions from a cell, in the presence of natural or endogenous hepcidin.

In another embodiment, there is provided a method of treating anemia of chronic
20 disease including providing an individual in need of said treatment with a peptide that: (i) inhibits the binding of hepcidin to $\alpha 2$ macroglobulin; and (ii) that does not inhibit the binding of hepcidin to ferroportin. In these embodiments, the peptide has an amino acid sequence with at least 90% or greater amino acid homology to the sequence shown in SEQ ID No:1, provided that at position 4, the residue is not Phe, and at position 6, the
25 residue is not Ile:

SEQ ID No: 1 AspThrHisPheProlleCysIlePhe

In another embodiment, there is provided a method of treating a disease associated with excessive hepcidin production or activity, preferably for treating anemia of chronic disease, including providing in an individual in need of said treatment a
30 peptide that inhibits the binding of hepcidin to $\alpha 2$ macroglobulin, wherein the peptide does not have hepcidin activity, or does not inhibit the binding of hepcidin to ferroportin,

and wherein the peptide includes or consists of an amino acid sequence according to Formula 1A:

(SEQ ID No: 2) $X_1X_2X_3X_4X_5X_6$ CysIlePhe

5 or Formula 1B:

(SEQ ID No:3) PhelleCys $X_6X_5X_4X_3X_2X_1$

wherein

X_1 is Asp, Ala, Gly, Ser or Thr

10 X_2 is Thr, Ala or Gly

X_3 is His, Asp or Glu

X_4 is Phe, Leu, Nle, Lys, Ile or Val

X_5 is Pro, Ala or Gly

X_6 is Ile, Lys, Arg, or His

15

In the above described embodiments where the peptide includes an amino acid sequence according to Formula 1A, the peptide may consist of about 25 amino acids. For example, the peptide may have the sequence:

(SEQ ID No: 4)

20 $X_1X_2X_3X_4X_5X_6$ CysIlePheCysCysGlyCysCysBBXXCysGlyXCysCysBThr

wherein:

X_1 to X_6 are as described above;

B is a positively charged residue, for example His, Asn, Arg or Lys

X is any amino acid residue.

25 Preferably the peptide may have the sequence:

(SEQ ID No:5)

$X_1X_2X_3X_4X_5X_6$ CysIlePheCysCysGlyCysCysHisArgSerLysCysGlyMetCysCysLysThr

wherein X_1 to X_6 are as described above

The peptide may be linear or cyclic.

In some embodiments the peptides of the invention bind to $\alpha 2$ macroglobulin with a binding affinity sufficient to inhibit the binding of hepcidin to $\alpha 2$ macroglobulin..

Generally, the peptides of the invention are designed not to provide hepcidin hormonal activity in the presence of natural or endogenous hepcidin. As such, they do not generally prevent ferroportin from exporting Fe ions from a cell, for example by causing the degradation or internalisation of ferroportin that is otherwise seen when hepcidin binds to ferroportin. In this context, the peptides of the invention may be referred to as “null hepcidin analogues”.

In one embodiment, a null hepcidin analog may mimic a hepcidin function or activity (such as $\alpha 2$ macroglobulin-binding) although as mentioned above, a null hepcidin analog does not prevent ferroportin from export Fe ions from a cell in the presence of natural or endogenous hepcidin.

In one embodiment, there is provided a method of treating anemia of chronic disease comprising providing a peptide which does not bind to ferroportin in the presence of natural or endogenous hepcidin, wherein the peptide is selected from the group consisting of D1A (SEQ ID No: 6), T2A (SEQ ID No: 7), H3D (SEQ ID No: 8), F4Nle (SEQ ID No: 9), F4K (SEQ ID No: 10), P5A (SEQ ID No: 11), I6K (SEQ ID No: 12), D1A/H3D (SEQ ID No: 13), D1A/F4K (SEQ ID No: 14), I6D (SEQ ID No: 15), C7G (SEQ ID No: 16) or cyclic Hep9 or CHH1 as described herein.

Generally, a natural or endogenous hepcidin has the amino acid sequence shown in SEQ ID No: 17.

In some embodiments, the present invention provides compositions and medicaments which comprise at least one peptide, which may be isolated, synthesized and/or purified, comprising, consisting essentially, or consisting of Formula 1A or 1B as set forth herein.

In some embodiments, the present invention provides method of manufacturing medicaments for the treatment of diseases of iron metabolism, particularly disease that arise from or are associated with excessive hepcidin production, expression or activity, which comprise at least one peptide, which may be isolated and/or purified, comprising, consisting essentially or consisting of Formula 1A or 1B as set forth herein.

Also, provided are methods of treating a disease of iron metabolism in a subject, such as a mammalian subject, preferably a human subject, which comprises administering at least one peptide, which may be isolated and/or purified, comprising, consisting essentially or consisting of Formula 1A or 1B, as set forth herein, or a
5 composition comprising said at least one peptide to the subject. In some embodiments, the peptide is administered in a therapeutically effective amount. In some embodiments, the therapeutically effective amount is an effective daily dose administered as a single daily dose or as divided daily doses. The peptides of the present invention can also be administered at a variety of doses.

10 In some embodiments the dose is given as a weekly dose, e.g. from 1-10,000 $\mu\text{g/kg/dose}$. In some embodiments, the daily dose is about 1-1,000, preferably about 10-500 $\mu\text{g/kg/day}$. Dosages can vary according to the type of formulation of peptidyl drug administered as well as the route of administration. One skilled in the art can adjust the dosage by changing the route of administration or formulation, so that the
15 dosage administered would result in a similar pharmacokinetic or biological profile as would result from the preferred dosage ranges described herein. In some embodiments, the composition to be administered is formulated for oral, pulmonary or mucosal administration.

Some embodiments include any dosage with any route of administration which
20 results in an effective pharmacokinetic and pharmacodynamic profile that decreases serum hepcidin levels and increases serum iron levels. Some preferred doses include those that result in a desired reduction in serum hepcidin. Administration of the peptidyl or protein formulations of the present invention includes both direct administration, including self-administration, and indirect administration, including the act of prescribing
25 a drug. For example, a physician who instructs a patient to self-administer a drug and/or provides a patient with a prescription for a drug is considered to be administering the drug to the patient.

In some embodiments, the present invention provides methods of binding an $\alpha 2$ macroglobulin, which comprises contacting the $\alpha 2$ macroglobulin with at least one
30 peptide or composition as disclosed herein.

In some embodiments, the present invention provides kits comprising at least one peptide or composition as disclosed herein packaged together with a reagent, a device, instructional material, or a combination thereof.

5 In some embodiments, the present invention provides complexes which comprise at least one peptide as disclosed herein bound to an $\alpha 2$ macroglobulin, preferably a human $\alpha 2$ macroglobulin, or an antibody, such as an antibody which specifically binds a peptide as disclosed herein, or a combination thereof.

In some embodiments, the present invention provides the use of at least one peptide, which may be isolated and/or purified, comprising, consisting essentially or
10 consisting of Formula 1A or 1B as set forth herein or a composition comprising, consisting essentially of, or consisting of said at least one peptide for the manufacture of a medicament for treating a disease of iron metabolism and/or increasing the amount of extracellular iron in a subject in need thereof, wherein the medicament is prepared to be administered at an effective daily dose, as a single daily dose, or as divided daily doses.
15 In some embodiments, the dose is about 1-1,000, preferably about 10-500 $\mu\text{g/kg/day}$. In some embodiments, the medicament is formulated for subcutaneous injection or oral, pulmonary or mucosal administration.

Both the foregoing general description and the following detailed description are exemplary and explanatory only and are intended to provide further explanation of the
20 invention as claimed. The accompanying drawings are included to provide a further understanding of the invention and are incorporated in and constitute part of this specification, illustrate several embodiments of the invention, and together with the description serve to explain the principles of the invention.

Brief description of the drawings

25 Figure 1. Sequestration and transport of hepcidin in the circulation by native and activated $\alpha 2$ -macroglobulin ($\alpha 2\text{M}$). (1) Hepcidin binds to native and activated $\alpha 2\text{M}$. (2) Hepcidin-binding to high Mr (725 kDa) $\alpha 2\text{M}$ or its activated form largely prevents urinary excretion of low Mr hepcidin (2.8 kDa). (3) Hepcidin binding to native and activated $\alpha 2\text{M}$ is labile and in the presence of its target, ferroportin (Fpn1), hepcidin can dissociate

from $\alpha 2M$. Release of hepcidin and binding to Fpn1 prevents cellular iron release leading to increased iron storage.

Figure 2. Null hepcidin analogues compete for the hepcidin bound to α -2 macroglobulin and induce its urinary excretion. This is an innovative therapy for the anaemia of chronic disease, where there is inappropriate and excessive hepcidin production induced by a chronic disease (e.g., cancer, etc.). This excessive hepcidin prevents iron release from tissue stores, e.g., the liver and also decreases iron uptake from enterocytes in the gut. Iron is required for red blood cell production and a lack of iron release from tissues leads to a debilitating anaemia which exacerbates the underlying disease.

Figure 3. Null hepcidin analogue competition assay in vitro. The peptides D1A and I6K effectively compete with hepcidin for binding to $\alpha 2$ -macroglobulin ($\alpha 2M$). Hence, this indicates that these peptides can displace hepcidin from $\alpha 2M$ more effectively, leading to the urinary excretion of low molecular weight hepcidin. This is beneficial in the anaemia of chronic disease where there is excessive quantities of hepcidin produced. Densitometry is mean $\pm$ SD (3 expts).

Figure 4. I6K given i.v increases serum Fe & attenuates hepcidin activity. In vivo study in mice showing that I6K at 100-fold excess to hepcidin significantly ($p < 0.001$) increases serum Fe and totally prevents hepcidin's ability to decrease serum Fe levels. The increase in serum Fe above the control is probably due to I6K competing with hepcidin on $\alpha 2M$ (Figs. 2 & 3). Result is mean $\pm$ SEM ($n=5\sim 6$).

Figure 5. I6K given i.v. dose-dependently increases serum Fe *in vivo* in mice. I6K dose-dependently (at 1-, 10- and 100-fold excess of hepcidin) increases serum Fe and was well tolerated. Thus, these new data in vivo show the great potential of our analogues as they increase serum Fe to fight the anaemia of chronic disease which affects millions. Result is mean $\pm$ SEM ($n=5\sim 6$).

Figure 6 Hepcidin and its analogues, structure and activity correlations.

Detailed description of the embodiments

A particularly important finding of the invention is that certain peptides that had previously been considered not useful for treatment of diseases of iron metabolism, because they do not competitively inhibit binding of hepcidin with ferroportin and
5 therefore, do not have hepcidin activity for degradation of ferroportin in the presence of natural or endogenous hepcidin, have been found to inhibit the binding of α -2 macroglobulin to hepcidin.

This inhibition is important because the binding of α -2 macroglobulin to hepcidin enables hepcidin to function as a hormone and increases the half-life of hepcidin in the
10 body. That is, in the absence of hepcidin binding to high molecular weight α -2 macroglobulin protein (725 kDa), the low molecular weight hepcidin hormone (2kDa) is excreted by the kidney into the urine.

In light of this finding the inventors have realised that these peptides could be used to ostensibly elute hepcidin from α 2 macroglobulin, thereby enabling excretion of
15 hepcidin via the kidney. The result is to reduce the amount of hepcidin in individuals having pathologically excessive hepcidin production, expression or activity.

The present invention provides peptides which are useful in the study and treatment of diseases of iron metabolism, and in particular, diseases associated with, or arising from, excessive hepcidin activity or production. Such a disease may be one
20 where aberrant iron metabolism directly causes the disease, or where iron blood levels are dysregulated causing disease, or where iron dysregulation is a consequence of another disease, or where diseases can be treated by modulating iron levels, and the like.

In one embodiment the disease is an anemia including anemia of chronic
25 disease, anemia of inflammation, anemia of infection, hypochromic microcytic anemia, iron- deficiency anemia, iron-refractory iron deficiency anemia, anemia of chronic kidney disease, iron deficiency of obesity, congenital dyserythropoietic anemia, or other anemias.

In another embodiment, the disease may be a condition, for example a benign or
30 malignant tumor that overproduces hepcidin, or induces its over-production, conditions

with hepcidin excess, Friedreich ataxia, gracile syndrome, Hallervorden-Spatz disease, Wilson's disease, pulmonary hemosiderosis, hepatocellular carcinoma, cancer, hepatitis, cirrhosis of liver, pica, chronic renal failure, insulin resistance, diabetes, atherosclerosis, neurodegenerative disorders, multiple sclerosis, Parkinson's disease,
5 Huntington's disease, and Alzheimer's disease.

In a particularly preferred embodiment of the present invention, the disease of iron metabolism is anemia of chronic disease. Anemia of chronic disease is a condition well known in the art, affecting more than 80% of hospitalised patients suffering from infections, malignancy and inflammation (NEJM 2005;352:1011). This disorder is
10 associated with excessive levels of hepcidin, as a response to chronic diseases. Excessive hepcidin promotes liver iron storage, leading to inadequate iron release for red blood cell production, causing a severe anemia.

The peptides of the present invention may be made using methods known in the art including chemical synthesis (solid-phase, solution phase, or a combination of both),
15 biosynthesis, or *in vitro* synthesis using recombinant DNA methods,. See e.g. Kelly & Winkler (1990) Genetic Engineering Principles and Methods, vol. 12, J. K. Setlow ed., Plenum Press, NY, pp. 1-19; Merrifield (1964) J Amer Chem Soc 85:2149; Houghten (1985) PNAS USA 82:5131-5135; and Stewart & Young (1984) Solid Phase Peptide Synthesis, 2ed. Pierce, Rockford, IL, which are herein incorporated by reference. The
20 peptides of the present invention may be purified using protein purification techniques known in the art such as reverse phase high- performance liquid chromatography (HPLC), ion-exchange or immunoaffinity chromatography, precipitation, filtration, size exclusion, or electrophoresis. See Olsnes, S. and A. Pihl (1973) Biochem. 12(16):3121-3126; and Scopes (1982) Protein Purification, Springer- Verlag, NY, which are herein
25 incorporated by reference.

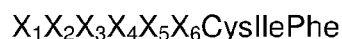
Alternatively, the peptides of the present invention may be made by recombinant DNA techniques known in the art. Thus, polynucleotides that encode the polypeptides of the present invention are contemplated herein. In preferred embodiments, the polynucleotides are isolated. As used herein "isolated polynucleotides" refers to
30 polynucleotides that are in an environment different from that in which the polynucleotide naturally occurs.

In some embodiments, the peptides of the present invention are substantially purified. As used herein, a "substantially purified" compound refers to a compound that is at least about 60% free, preferably about 75% free, and most preferably about 90% free from other macromolecular components with which the compound may otherwise be associated, or a compound that is at least about 60% free, preferably about 75% free, and most preferably about 90% free from other peptide components as measured by HPLC with detection at 214 nm.

The peptides of the present invention bind $\alpha 2$ macroglobulin, preferably human $\alpha 2$ macroglobulin. Preferred peptides of the present invention specifically bind human $\alpha 2$ macroglobulin. As used herein, "specifically binds" refers to a specific binding agent's preferential interaction with a given ligand over other agents in a sample. For example, a specific binding agent that specifically binds a given ligand, binds the given ligand, under suitable conditions, in an amount or a degree that is observable over that of any nonspecific interaction with other components in the sample.

Suitable conditions are those that allow interaction between a given specific binding agent and a given ligand. These conditions include pH, temperature, concentration, solvent, time of incubation, and the like, and may differ among given specific binding agent and ligand pairs, but may be readily determined by those skilled in the art.

According to the invention there is provided a method for treating a disease of iron metabolism, especially anemia of chronic disease including the step of administering a peptide effective for inhibiting the binding of hepcidin to $\alpha 2$ -macroglobulin, to an individual requiring said treatment, wherein the peptide has an amino acid sequence according to Formula 1:



wherein

X_1 is Asp, Ala, Gly, Ser or Thr

X_2 is Thr, Ala or Gly

X_3 is His, Asp or Glu

X_4 is Phe, Leu, Nle, Lys, Ile or Val

X_5 is Pro, Ala or Gly

X₆ is Ile, Lys, Arg, or His

wherein the peptide does not have hepcidin activity in the presence of endogenous or natural hepcidin.

Typically the peptide has a binding affinity similar to that of hepcidin for α -2
5 macroglobulin in a competitive binding assay described herein.

In one embodiment, X₁ is Ala, Gly, Ser or Thr, and X₂ is Thr, X₃ is His, X₄ is Phe, X₅ is Pro, and X₆ is Ile. Preferably, X₁ is Ala.

In another embodiment, X₁ is Asp, X₂ is Ala or Gly, X₃ is His, X₄ is Phe, X₅ is Pro, and X₆ is Ile. Preferably X₂ is Ala.

10 In a further embodiment, X₁ is Asp, X₂ is Thr, X₃ is Asp or Glu, X₄ is Phe, X₅ is Pro, and X₆ is Ile. Preferably X₃ is Asp.

In yet a further embodiment, X₁ is Asp, X₂ is Thr, X₃ is His, X₄ is Leu, Ile or Val, X₅ is Pro, and X₆ is Ile. Preferably X₄ is Nle or Leu.

In still a further embodiment, X₁ is Asp, X₂ is Thr, X₃ is His, X₄ is Phe, X₅ is Ala or
15 Gly, and X₆ is Ile. Preferably X₅ is Ala.

In still a further embodiment, X₁ is Asp, X₂ is Thr, X₃ is His, X₄ is Phe, X₅ is Pro, and X₆ is Lys, Arg or His. Preferably X₆ is Lys.

In still a further embodiment, wherein X₁ is Ala, Gly, Ser or Thr, and X₂ is Thr, X₃ is Asp or Glu, X₄ is Phe, X₅ is Pro, and X₆ is Ile. Preferably X₁ is Ala and X₃ is Asp.

20 In still a further embodiment, X₁ is Ala, Gly, Ser or Thr, and X₂ is Thr, X₃ is His, X₄ is Lys, Arg or His, X₅ is Pro, and X₆ is Ile. Preferably X₁ is Ala and X₄ is Lys.

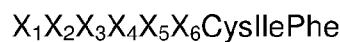
The peptide may be linear or cyclic, preferably linear.

In one embodiment, the peptide may contain an alkyl group at the C-terminal residue.

A peptide as described above may be provided for use in the method of the invention in the form of a pharmaceutical composition including the peptide and an excipient, diluent or carrier.

In further embodiments there are provided peptides and uses of same for treatment of iron disorders, particularly disorders such as anemia of chronic disease. These peptides do not have hepcidin activity in the presence of natural or endogenous hepcidin. These peptides inhibit the binding of hepcidin to $\alpha 2$ -macroglobulin. In one embodiment, there is provided a peptide for inhibiting the binding of hepcidin to $\alpha 2$ -macroglobulin, wherein the peptide has an amino acid sequence according to Formula

1:



wherein

X_1 is Ala, Gly, Ser or Thr, and X_2 is Thr, X_3 is Asp or Glu, X_4 is Phe, X_5 is Pro, and X_6 is Ile. Preferably X_1 is Ala and X_3 is Asp.

In another embodiment, the peptide has an amino acid sequence according to Formula 1C:



wherein

X_1 is Ala, Gly, Ser or Thr, and X_2 is Thr, X_3 is Asp or Glu, X_4 is Phe, X_5 is Pro, and X_6 is Ile. Preferably X_1 is Ala and X_3 is Asp.

B is a positively charged residue, for example His, Asn, Arg or Lys

X is any amino acid residue.

Preferably the peptide of Formula 1C may have the sequence:



where X_1 is Ala and X_3 is Asp

In another embodiment there is provided a peptide for inhibiting the binding of hepcidin to $\alpha 2$ -macroglobulin, wherein the peptide has an amino acid sequence according to Formula 1:



wherein

X₁ is Ala, Gly, Ser or Thr, and X₂ is Thr, X₃ is His, X₄ is Lys, Arg or His, X₅ is Pro, and X₆ is Ile. Preferably X₁ is Ala and X₄ is Lys.

In another embodiment, the peptide has an amino acid sequence according to Formula 1C:

5 X₁X₂X₃X₄X₅X₆CysIlePheCysCysGlyCysCysBBXXCysGlyXCysCysBThr

wherein

X₁ is Ala, Gly, Ser or Thr, and X₂ is Thr, X₃ is His, X₄ is Lys, Arg or His, X₅ is Pro, and X₆ is Ile. Preferably X₁ is Ala and X₄ is Lys.

B is a positively charged residue, for example His, Asn, Arg or Lys

10 X is any amino acid residue.

Preferably the peptide of Formula 1C may have the sequence:

X₁X₂X₃X₄X₅X₆CysIlePheCysCysGlyCysCysHisArgSerLysCysGlyMetCysCysLysThr

where X₁ is Ala and X₄ is Lys.

15 In another embodiment there is provided a peptide having an amino acid sequence according to Formula 1C:

X₁X₂X₃X₄X₅X₆CysIlePheCysCysGlyCysCysBBXXCysGlyXCysCysBThr

wherein:

X₁ is Asp, Ala, Gly, Ser or Thr

X₂ is Thr, Ala or Gly

20 X₃ is His, Asp or Glu

X₄ is Phe, Leu, Nle, Lys, Ile or Val

X₅ is Pro, Ala or Gly

X₆ is Ile, Lys, Arg, or His

B is a positively charged residue, for example His, Asn, Arg or Lys

25 X is any amino acid residue.

wherein the peptide does not have hepcidin activity in the presence of natural or endogenous hepcidin.

Typically the peptide has a binding affinity similar to that of hepcidin for α -2 macroglobulin in a competitive binding assay described herein.

Preferably the peptide of Formula 1C may have the sequence:

$X_1X_2X_3X_4X_5X_6$ CysIlePheCysCysGlyCysCysHisArgSerLysCysGlyMetCysCysLysThr

In one embodiment of Formula 1C, X_1 is Ala, Gly, Ser or Thr, and X_2 is Thr, X_3 is His, X_4 is Phe, X_5 is Pro, and X_6 is Ile. Preferably, X_1 is Ala.

5 In another embodiment of Formula 1C, X_1 is Asp, X_2 is Ala or Gly, X_3 is His, X_4 is Phe, X_5 is Pro, and X_6 is Ile. Preferably X_2 is Ala.

In a further embodiment of Formula 1C, X_1 is Asp, X_2 is Thr, X_3 is Asp or Glu, X_4 is Phe, X_5 is Pro, and X_6 is Ile. Preferably X_3 is Asp.

10 In yet a further embodiment of Formula 1C, X_1 is Asp, X_2 is Thr, X_3 is His, X_4 is Leu, Ile or Val, X_5 is Pro, and X_6 is Ile. Preferably X_4 is Nle or Leu.

In still a further embodiment of Formula 1C, X_1 is Asp, X_2 is Thr, X_3 is His, X_4 is Phe, X_5 is Ala or Gly, and X_6 is Ile. Preferably X_5 is Ala.

In still a further embodiment of Formula 1C, X_1 is Asp, X_2 is Thr, X_3 is His, X_4 is Phe, X_5 is Pro, and X_6 is Lys, Arg or His. Preferably X_6 is Lys.

15 In still a further embodiment of Formula 1C, wherein X_1 is Ala, Gly, Ser or Thr, and X_2 is Thr, X_3 is Asp or Glu, X_4 is Phe, X_5 is Pro, and X_6 is Ile. Preferably X_1 is Ala and X_3 is Asp.

20 In still a further embodiment of Formula 1C, X_1 is Ala, Gly, Ser or Thr, and X_2 is Thr, X_3 is His, X_4 is Lys, Arg or His, X_5 is Pro, and X_6 is Ile. Preferably X_1 is Ala and X_4 is Lys.

In certain embodiments, the present invention includes pharmaceutical compositions comprising one or more null hepcidin analogues of the present invention and a pharmaceutically acceptable carrier, diluent or excipient. A pharmaceutically acceptable carrier, diluent or excipient refers to a non-toxic solid, semi-solid or liquid
25 filler, diluent, encapsulating material or formulation auxiliary of any type. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the

like. It may also be desirable to include isotonic agents such as sugars, sodium chloride, and the like.

The term "pharmaceutically acceptable carrier" includes any of the standard pharmaceutical carriers. Pharmaceutically acceptable carriers for therapeutic use are
5 well known in the pharmaceutical art and are described, for example, in "Remington's Pharmaceutical Sciences", 17th edition, Alfonso R. Gennaro (Ed.), Mark Publishing Company, Easton, PA, USA, 1985. For example, sterile saline and phosphate-buffered saline at slightly acidic or physiological pH may be used.

Suitable pH-buffering agents may, e.g., be phosphate, citrate, acetate,
10 tris(hydroxymethyl)aminomethane (TRIS), N-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), ammonium bicarbonate, diethanolamine, histidine, arginine, lysine or acetate (e.g. as sodium acetate), or mixtures thereof. The term further encompasses any carrier agents listed in the US Pharmacopeia for use in animals, including humans.

15 It is to be understood that the inclusion of a null hepcidin analogue of the invention (i.e., one or more null hepcidin analogue peptide monomers of the invention or one or more null hepcidin analogue peptide dimers of the present invention) in a pharmaceutical composition also encompasses inclusion of a pharmaceutically acceptable salt or solvate of a null hepcidin analogue of the invention. In particular
20 embodiments, the pharmaceutical compositions further comprise one or more pharmaceutically acceptable carrier, excipient, or vehicle.

In certain embodiments, the invention provides a pharmaceutical composition comprising a null hepcidin analogue, or a pharmaceutically acceptable salt or solvate thereof, for treating a variety of conditions, diseases, or disorders as disclosed herein or
25 elsewhere (see, e.g., Methods of Treatment, herein).

In particular embodiments, the invention provides a pharmaceutical composition comprising a null hepcidin analogue peptide monomer, or a pharmaceutically acceptable salt or solvate thereof, for treating a variety of conditions, diseases, or disorders as disclosed herein elsewhere (see, e.g., Methods of Treatment, herein).

In particular embodiments, the invention provides a pharmaceutical composition comprising a null hepcidin analogue peptide dimer, or a pharmaceutically acceptable salt or solvate thereof, for treating a variety of conditions, diseases, or disorders as disclosed herein elsewhere (see, e.g., Methods of Treatment, herein).

5 The null hepcidin analogues of the present invention may be formulated as pharmaceutical compositions which are suited for administration with or without storage, and which typically comprise a therapeutically effective amount of at least one null hepcidin analogue of the invention, together with a pharmaceutically acceptable carrier, excipient or vehicle.

10 In some embodiments, the null hepcidin analogue pharmaceutical compositions of the invention are in unit dosage form. In such forms, the composition is divided into unit doses containing appropriate quantities of the active component or components. The unit dosage form may be presented as a packaged preparation, the package containing discrete quantities of the preparation, for example, packaged tablets,
15 capsules or powders in vials or ampoules. The unit dosage form may also be, e.g., a capsule, cachet or tablet in itself, or it may be an appropriate number of any of these packaged forms. A unit dosage form may also be provided in single-dose injectable form, for example in the form of a pen device containing a liquid-phase (typically aqueous) composition. Compositions may be formulated for any suitable route and
20 means of administration, e.g., any one of the routes and means of administration disclosed herein.

In particular embodiments, the null hepcidin analogue, or the pharmaceutical composition comprising a null hepcidin analogue, is suspended in a sustained-release matrix. A sustained-release matrix, as used herein, is a matrix made of materials,
25 usually polymers, which are degradable by enzymatic or acid-base hydrolysis or by dissolution. Once inserted into the body, the matrix is acted upon by enzymes and body fluids. A sustained-release matrix desirably is chosen from biocompatible materials such as liposomes, polylactides (polylactic acid), polyglycolide (polymer of glycolic acid), polylactide co-glycolide (copolymers of lactic acid and glycolic acid) polyanhydrides,
30 poly(ortho)esters, polypeptides, hyaluronic acid, collagen, chondroitin sulfate, carboxylic acids, fatty acids, phospholipids, polysaccharides, nucleic acids, polyamino acids,

amino acids such as phenylalanine, tyrosine, isoleucine, polynucleotides, polyvinyl propylene, polyvinylpyrrolidone and silicone. One embodiment of a biodegradable matrix is a matrix of one of either polylactide, polyglycolide, or polylactide co-glycolide (co-polymers of lactic acid and glycolic acid).

5 In certain embodiments, the compositions are administered enterally or parenterally. In particular embodiments, the compositions are administered orally, intracisternally, intravaginally, intraperitoneally, intrarectally, topically (as by powders, ointments, drops, suppository, or transdermal patch, including delivery intravitreally, intranasally, and via inhalation) or buccally. The term "parenteral" as used herein refers
10 to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous, intradermal and intraarticular injection and infusion. Accordingly, in certain embodiments, the compositions are formulated for delivery by any of these routes of administration.

 In certain embodiments, pharmaceutical compositions for parenteral injection
15 comprise pharmaceutically acceptable sterile aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders, for reconstitution into sterile injectable solutions or dispersions just prior to use. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents or vehicles include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), carboxymethylcellulose
20 and suitable mixtures thereof, beta-cyclodextrin, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate. Proper fluidity may be maintained, for example, by the use of coating materials such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants. These compositions may also contain adjuvants such as preservative, wetting agents,
25 emulsifying agents, and dispersing agents. Prolonged absorption of an injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption, such as aluminum monostearate and gelatin.

 Injectable depot forms include those made by forming microencapsule matrices of the null hepcidin analogue in one or more biodegradable polymers such as
30 polylactide-polyglycolide, poly(orthoesters), poly(anhydrides), and (poly)glycols, such as PEG. Depending upon the ratio of peptide to polymer and the nature of the particular

polymer employed, the rate of release of the null hepcidin analogue can be controlled. Depot injectable formulations are also prepared by entrapping the null hepcidin analogue in liposomes or microemulsions compatible with body tissues.

5 The injectable formulations may be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium just prior to use.

10 Topical administration includes administration to the skin or mucosa, including surfaces of the lung and eye. Compositions for topical lung administration, including those for inhalation and intranasal, may involve solutions and suspensions in aqueous and nonaqueous formulations and can be prepared as a dry powder which may be pressurized or non-pressurized. In non-pressurized powder compositions, the active ingredient may be finely divided form may be used in admixture with a larger-sized pharmaceutically acceptable inert carrier comprising particles having a size, for
15 example, of up to 100 micrometers in diameter. Suitable inert carriers include sugars such as lactose.

Alternatively, the composition may be pressurized and contain a compressed gas, such as nitrogen or a liquefied gas propellant. The liquefied propellant medium and indeed the total composition may be such that the active ingredient does not dissolve
20 therein to any substantial extent. The pressurized composition may also contain a surface active agent, such as a liquid or solid non-ionic surface active agent or may be a solid anionic surface active agent. It is preferred to use the solid anionic surface active agent in the form of a sodium salt.

A further form of topical administration is to the eye. A null hepcidin analogue of
25 the invention may be delivered in a pharmaceutically acceptable ophthalmic vehicle, such that the null hepcidin analogue is maintained in contact with the ocular surface for a sufficient time period to allow the null hepcidin analogue to penetrate the corneal and internal regions of the eye, as for example the anterior chamber, posterior chamber, vitreous body, aqueous humor, vitreous humor, cornea, iris/ciliary, lens, choroid/retina
30 and sclera. The pharmaceutically acceptable ophthalmic vehicle may, for example, be an ointment, vegetable oil or an encapsulating material. Alternatively, the null hepcidin

analogues of the invention may be injected directly into the vitreous and aqueous humour.

Compositions for rectal or vaginal administration include suppositories which may be prepared by mixing the null hepcidin analogues of this invention with suitable non-
5 irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax, which are solid at room temperature but liquid at body temperature and, therefore, melt in the rectum or vaginal cavity and release the active compound.

Null hepcidin analogues of the present invention may also be administered in liposomes or other lipid-based carriers. As is known in the art, liposomes are generally
10 derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolizable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a null hepcidin analogue of the present invention, stabilizers, preservatives,
15 excipients, and the like. In certain embodiments, the lipids comprise phospholipids, including the phosphatidyl cholines (lecithins) and serines, both natural and synthetic. Methods to form liposomes are known in the art.

Pharmaceutical compositions to be used in the invention suitable for parenteral administration may comprise sterile aqueous solutions and/or suspensions of the
20 peptide inhibitors made isotonic with the blood of the recipient, generally using sodium chloride, glycerin, glucose, mannitol, sorbitol, and the like.

In some aspects, the invention provides a pharmaceutical composition for oral delivery. Compositions and null hepcidin analogues of the instant invention may be prepared for oral administration according to any of the methods, techniques, and/or
25 delivery vehicles described herein. Further, one having skill in the art will appreciate that the null hepcidin analogues of the instant invention may be modified or integrated into a system or delivery vehicle that is not disclosed herein, yet is well known in the art and compatible for use in oral delivery of peptides.

In certain embodiments, formulations for oral administration may comprise
30 adjuvants (e.g. resorcinols and/or nonionic surfactants such as polyoxyethylene oleyl

ether and n-hexadecylpolyethylene ether) to artificially increase the permeability of the intestinal walls, and/or enzymatic inhibitors (e.g. pancreatic trypsin inhibitors, diisopropylfluorophosphate (DFF) or trasylol) to inhibit enzymatic degradation. In certain embodiments, the null hepcidin analogue of a solid-type dosage form for oral administration can be mixed with at least one additive, such as sucrose, lactose, cellulose, mannitol, trehalose, raffinose, maltitol, dextran, starches, agar, alginates, chitins, chitosans, pectins, gum tragacanth, gum arabic, gelatin, collagen, casein, albumin, synthetic or semisynthetic polymer, or glyceride. These dosage forms can also contain other type(s) of additives, e.g., inactive diluting agent, lubricant such as magnesium stearate, paraben, preserving agent such as sorbic acid, ascorbic acid, alpha-tocopherol, antioxidants such as cysteine, disintegrators, binders, thickeners, buffering agents, pH adjusting agents, sweetening agents, flavoring agents or perfuming agents.

In particular embodiments, oral dosage forms or unit doses compatible for use with the null hepcidin analogues of the present invention may include a mixture of null hepcidin analogue and nondrug components or excipients, as well as other non-reusable materials that may be considered either as an ingredient or packaging. Oral compositions may include at least one of a liquid, a solid, and a semi-solid dosage forms. In some embodiments, an oral dosage form is provided comprising an effective amount of null hepcidin analogue, wherein the dosage form comprises at least one of a pill, a tablet, a capsule, a gel, a paste, a drink, a syrup, ointment, and suppository. In some instances, an oral dosage form is provided that is designed and configured to achieve delayed release of the null hepcidin analogue in the subject's small intestine and/or colon.

In one embodiment, an oral pharmaceutical composition comprising a null hepcidin analogue of the present invention comprises an enteric coating that is designed to delay release of the null hepcidin analogue in the small intestine. In at least some embodiments, a pharmaceutical composition is provided which comprises a null hepcidin analogue of the present invention and a protease inhibitor, such as aprotinin, in a delayed release pharmaceutical formulation. In some instances, pharmaceutical compositions of the instant invention comprise an enteric coat that is soluble in gastric juice at a pH of about 5.0 or higher. In at least one embodiment, a pharmaceutical

composition is provided comprising an enteric coating comprising a polymer having dissociable carboxylic groups, such as derivatives of cellulose, including hydroxypropylmethyl cellulose phthalate, cellulose acetate phthalate and cellulose acetate trimellitate and similar derivatives of cellulose and other carbohydrate polymers.

5 In one embodiment, a pharmaceutical composition comprising a null hepcidin analogue of the present invention is provided in an enteric coating, the enteric coating being designed to protect and release the pharmaceutical composition in a controlled manner within the subject's lower gastrointestinal system, and to avoid systemic side effects. In addition to enteric coatings, the null hepcidin analogues of the instant
10 invention may be encapsulated, coated, engaged or otherwise associated within any compatible oral drug delivery system or component. For example, in some embodiments a null hepcidin analogue of the present invention is provided in a lipid carrier system comprising at least one of polymeric hydrogels, nanoparticles, microspheres, micelles, and other lipid systems.

15 To overcome peptide degradation in the small intestine, some embodiments of the present invention comprise a hydrogel polymer carrier system in which a null hepcidin analogue of the present invention is contained, whereby the hydrogel polymer protects the null hepcidin analogue from proteolysis in the small intestine and/or colon. The null hepcidin analogues of the present invention may further be formulated for
20 compatible use with a carrier system that is designed to increase the dissolution kinetics and enhance intestinal absorption of the peptide. These methods include the use of liposomes, micelles and nanoparticles to increase GI tract permeation of peptides.

Various bioresponsive systems may also be combined with one or more null hepcidin analogue of the present invention to provide a pharmaceutical agent for oral
25 delivery. In some embodiments, a null hepcidin analogue of the instant invention is used in combination with a bioresponsive system, such as hydrogels and mucoadhesive polymers with hydrogen bonding groups (e.g., PEG, poly(methacrylic) acid [PMAA], cellulose, Eudragit®, chitosan and alginate) to provide a therapeutic agent for oral administration. Other embodiments include a method for optimizing or prolonging drug
30 residence time for a null hepcidin analogue disclosed herein, wherein the surface of the null hepcidin analogue surface is modified to comprise mucoadhesive properties

through hydrogen bonds, polymers with linked mucins or/and hydrophobic interactions. These modified peptide molecules may demonstrate increase drug residence time within the subject, in accordance with a desired feature of the invention. Moreover, targeted mucoadhesive systems may specifically bind to receptors at the enterocytes
5 and M-cell surfaces, thereby further increasing the uptake of particles containing the null hepcidin analogue.

Other embodiments comprise a method for oral delivery of a null hepcidin analogue of the present invention, wherein the null hepcidin analogue is provided to a subject in combination with permeation enhancers that promote the transport of the
10 peptides across the intestinal mucosa by increasing paracellular or transcellular permeation. For example, in one embodiment, a permeation enhancer is combined with a null hepcidin analogue, wherein the permeation enhancer comprises at least one of a long-chain fatty acid, a bile salt, an amphiphilic surfactant, and a chelating agent. In one
15 embodiment, a permeation enhancer comprising sodium N-[hydroxybenzoyl)amino] caprylate is used to form a weak noncovalent association with the null hepcidin analogue of the instant invention, wherein the permeation enhancer favors membrane transport and further dissociation once reaching the blood circulation.

In another embodiment, a null hepcidin analogue of the present invention is conjugated to oligoarginine, thereby increasing cellular penetration of the peptide into
20 various cell types. Further, in at least one embodiment, a noncovalent bond is provided between a peptide inhibitor of the present invention and a permeation enhancer selected from the group consisting of a cyclodextrin (CD) and a dendrimers, wherein the permeation enhancer reduces peptide aggregation and increasing stability and solubility for the null hepcidin analogue molecule.

Other embodiments of the invention provide a method for treating a subject with a null hepcidin analogue of the present invention having an increased half-life. In one
25 aspect, the present invention provides a null hepcidin analogue having a half-life of at least several hours to one day *in vitro* or *in vivo* (e.g., when administered to a human subject) sufficient for daily (q.d.) or twice daily (b.i.d.) dosing of a therapeutically
30 effective amount. In another embodiment, the null hepcidin analogue has a half-life of three days or longer sufficient for weekly (q.w.) dosing of a therapeutically effective

amount. Further, in another embodiment, the null hepcidin analogue has a half-life of eight days or longer sufficient for bi-weekly (b.i.w.) or monthly dosing of a therapeutically effective amount. In another embodiment, the null hepcidin analogue is derivatized or modified such that it has a longer half-life as compared to the
5 underivatized or unmodified null hepcidin analogue.

In another embodiment, the null hepcidin analogue contains one or more chemical modifications to increase serum half-life. When used in at least one of the treatments or delivery systems described herein, a null hepcidin analogue of the present invention may be employed in pure form or, where such forms exist, in pharmaceutically
10 acceptable salt form. The total daily usage of the null hepcidin analogues and compositions of the present invention can be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including: a) the disorder being treated and the severity of the disorder; b) activity of the specific compound
15 employed; c) the specific composition employed, the age, body weight, general health, sex and diet of the patient; d) the time of administration, route of administration, and rate of excretion of the specific null hepcidin analogue employed; e) the duration of the treatment; f) drugs used in combination or coincidental with the specific null hepcidin analogue employed, and like factors well known in the medical arts.

20 In particular embodiments, the total daily dose of the null hepcidin analogues of the invention to be administered to a human or other mammal host in single or divided doses may be in amounts, for example, from 0.0001 to 300 mg/kg body weight daily or 1 to 300 mg/kg body weight daily. In certain embodiments, a dosage of a null hepcidin analogue of the present invention is in the range from about 0.0001 to about 100 mg/kg
25 body weight per day, such as from about 0.0005 to about 50 mg/kg body weight per day, such as from about 0.001 to about 10 mg/kg body weight per day, e.g. from about 0.01 to about 1 mg/kg body weight per day, administered in one or more doses, such as from one to three doses.

In various embodiments, a null hepcidin analogue of the invention may be
30 administered continuously (e.g. by intravenous administration or another continuous drug administration method), or may be administered to a subject at intervals, typically

at regular time intervals, depending on the desired dosage and the pharmaceutical composition selected by the skilled practitioner for the particular subject. Regular administration dosing intervals include, e.g., once daily, twice daily, once every two, three, four, five or six days, once or twice weekly, once or twice monthly, and the like.

5 Such regular null hepcidin analogue administration regimens of the invention may, in certain circumstances such as, e.g., during chronic long-term administration, be advantageously interrupted for a period of time so that the medicated subject reduces the level of or stops taking the medication, often referred to as taking a "drug holiday." Drug holidays are useful for, e.g., maintaining or regaining sensitivity to a drug
10 especially during long-term chronic treatment, or to reduce unwanted side-effects of long-term chronic treatment of the subject with the drug. The timing of a drug holiday depends on the timing of the regular dosing regimen and the purpose for taking the drug holiday (e.g., to regain drug sensitivity and/or to reduce unwanted side effects of continuous, long- term administration). In some embodiments, the drug holiday may be
15 a reduction in the dosage of the drug (e.g. to below the therapeutically effective amount for a certain interval of time). In other embodiments, administration of the drug is stopped for a certain interval of time before administration is started again using the same or a different dosing regimen (e.g. at a lower or higher dose and/or frequency of administration). A drug holiday of the invention may thus be selected from a wide range
20 of time-periods and dosage regimens. An exemplary drug holiday is two or more days, one or more weeks, or one or more months, up to about 24 months of drug holiday. So, for example, a regular daily dosing regimen with a peptide, a peptide analogue, or a dimer of the invention may, for example, be interrupted by a drug holiday of a week, or two weeks, or four weeks, after which time the preceding, regular dosage regimen (e.g.
25 a daily or a weekly dosing regimen) is resumed. A variety of other drug holiday regimens are envisioned to be useful for administering the null hepcidin analogues of the invention.

Thus, the null hepcidin analogues may be delivered via an administration regime which comprises two or more administration phases separated by respective drug
30 holiday phases.

During each administration phase, the null hepcidin analogue is administered to the recipient subject in a therapeutically effective amount according to a pre-determined administration pattern. The administration pattern may comprise continuous administration of the drug to the recipient subject over the duration of the administration phase. Alternatively, the administration pattern may comprise administration of a plurality of doses of the null hepcidin analogue to the recipient subject, wherein said doses are spaced by dosing intervals.

A dosing pattern may comprise at least two doses per administration phase, at least five doses per administration phase, at least 10 doses per administration phase, at least 20 doses per administration phase, at least 30 doses per administration phase, or more.

Said dosing intervals may be regular dosing intervals, which may be as set out above, including once daily, twice daily, once every two, three, four, five or six days, once or twice weekly, once or twice monthly, or a regular and even less frequent dosing interval, depending on the particular dosage formulation, bioavailability, and pharmacokinetic profile of the null hepcidin analogue of the present invention.

An administration phase may have a duration of at least two days, at least a week, at least 2 weeks, at least 4 weeks, at least a month, at least 2 months, at least 3 months, at least 6 months, or more.

Where an administration pattern comprises a plurality of doses, the duration of the following drug holiday phase is longer than the dosing interval used in that administration pattern. Where the dosing interval is irregular, the duration of the drug holiday phase may be greater than the mean interval between doses over the course of the administration phase. Alternatively the duration of the drug holiday may be longer than the longest interval between consecutive doses during the administration phase.

The duration of the drug holiday phase may be at least twice that of the relevant dosing interval (or mean thereof), at least 3 times, at least 4 times, at least 5 times, at least 10 times, or at least 20 times that of the relevant dosing interval or mean thereof.

Within these constraints, a drug holiday phase may have a duration of at least two days, at least a week, at least 2 weeks, at least 4 weeks, at least a month, at least 2

months, at least 3 months, at least 6 months, or more, depending on the administration pattern during the previous administration phase.

An administration regime comprises at least 2 administration phases. Consecutive administration phases are separated by respective drug holiday phases.

5 Thus the administration regime may comprise at least 3, at least 4, at least 5, at least 10, at least 15, at least 20, at least 25, or at least 30 administration phases, or more, each separated by respective drug holiday phases.

Consecutive administration phases may utilise the same administration pattern, although this may not always be desirable or necessary. However, if other drugs or
10 active agents are administered in combination with a null hepcidin analogue of the invention, then typically the same combination of drugs or active agents is given in consecutive administration phases. In certain embodiments, the recipient subject is human.

It will be understood that the invention disclosed and defined in this specification
15 extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

Examples

20 Example 1 - Screening assays for determining appropriate α 2- macroglobulin binders.

For competition assay experiment, biotinylated hepcidin (BioHepcidin) was incubated at a 4:1 molar ratio of BioHepcidin: α 2M/ α 2M-MA in the presence of unlabeled hepcidin or its analogues. In these studies, 27.6 pmol of α 2M or α 2M-MA were incubated with 110.4 pmol BioHepcidin in the presence of hepcidin or its analogue at 25, 50, 100 or 200
25 molar excess of BioHepcidin, for 1 h at 37 °C. Any unbound BioHepcidin was removed from the α 2M•BioHepcidin/ α 2M-MA•BioHepcidin complexes using Sephadex G-25 columns. Samples are then applied dropwise to a nitrocellulose membrane before biotin-streptavidin-HRP detection *via* chemiluminescence (Chemiluminescence Detection Module, Thermo Scientific).

Example 2 - Screening assays for determining peptides that do not have other hepcidin activities

Assay to determine the activity of hepcidin analogues were performed in two stages. First, cells were pre-treated with culture media alone (DMEM + 10% fetal calf serum; control), or this medium containing DFO or FAC for 24 h/37°C (primary incubation). This medium was then removed and the cells rinsed with serum-free media. In the second stage, cells were incubated with serum-free DMEM or this medium containing DFO (100 µM) or FAC (250 µg/mL) in the presence or absence of either: hepcidin and hepcidin analogues (0.7 µM), for 6 h/37°C (secondary incubation). At the end of the secondary incubation, cells were lysed for assessment of Fpn1 expression by Western analysis. The activity of hepcidin analogues are measured in terms of Fpn1 expression relative to cell treated with hepcidin.

Example 3 - Screening assays for determining peptides that do not degrade/cause internalisation of Ferroportin.

To measure serum iron levels in vivo, 100 µL 0.9% saline (Control) or this vehicle containing: Hepcidin (0.9 nmole), or hepcidin analogues at 0.9 nmole (1x), 9 nmole (10x) or 90 nmole (100x) in the presence or absence of Hepcidin (0.9 nmole) were injected into the tail vein of mice. After 2 h, blood (600 µL) was collected by cardiac puncture from anesthetized mice and placed in Capiject® Micro Collection tubes and serum isolated. Serum iron was measured using a Konelab Clinical Chemistry Analyzer (Thermo Scientific, Waltham, MA).

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CLAIMS

1. A method for treating a disease of iron metabolism including the step of administering a peptide effective for inhibiting the binding of hepcidin to $\alpha 2$ -macroglobulin, to an individual requiring said treatment, wherein the peptide has an amino acid sequence according to Formula 1:
- $X_1X_2X_3X_4X_5X_6\text{CysIlePhe}$
- wherein
- X_1 is Asp, Ala, Gly, Ser or Thr
- X_2 is Thr, Ala or Gly
- X_3 is His, Asp or Glu
- X_4 is Phe, Leu, Lys, Nle, Ile or Val
- X_5 is Pro, Ala or Gly
- X_6 is Ile, Lys, Arg, or His
2. The method of claim 1 wherein X_1 is Ala, Gly, Ser or Thr, and X_2 is Thr, X_3 is His, X_4 is Phe, X_5 is Pro, and X_6 is Ile.
3. The method of claim 2 wherein X_1 is Ala.
4. The method of claim 1 wherein X_1 is Asp, X_2 is Ala or Gly, X_3 is His, X_4 is Phe, X_5 is Pro, and X_6 is Ile.
5. The method of claim 4 wherein X_2 is Ala.
6. The method of claim 1 wherein X_1 is Asp, X_2 is Thr, X_3 is Asp or Glu, X_4 is Phe, X_5 is Pro, and X_6 is Ile.
7. The method of claim 6 wherein X_3 is Asp.
8. The method of claim 1 wherein X_1 is Asp, X_2 is Thr, X_3 is His, X_4 is Leu, Ile or Val, X_5 is Pro, and X_6 is Ile.
9. The method of claim 8 wherein X_4 is Nle or Leu.
10. The method of claim 1 wherein X_1 is Asp, X_2 is Thr, X_3 is His, X_4 is Phe, X_5 is Ala or Gly, and X_6 is Ile.

11. The method of claim 10 wherein X₅ is Ala.
12. The method of claim 1 wherein X₁ is Asp, X₂ is Thr, X₃ is His, X₄ is Phe, X₅ is Pro, and X₆ is Lys, Arg or His.
13. The method of claim 12 wherein X₆ is Lys.
- 5 14. The method of claim 1 wherein X₁ is Ala, Gly, Ser or Thr, and X₂ is Thr, X₃ is Asp or Glu, X₄ is Phe, X₅ is Pro, and X₆ is Ile.
15. The method of claim 14 wherein X₁ is Ala and X₃ is Asp.
16. The method of claim 1 wherein X₁ is Ala, Gly, Ser or Thr, and X₂ is Thr, X₃ is His, X₄ is Lys, Arg or His, X₅ is Pro, and X₆ is Ile.
- 10 17. The method of claim 16 wherein X₁ is Ala and X₄ is Lys.
18. The method of any one of the preceding claims wherein the peptide is linear or cyclic.
19. The method of any one of the preceding claims wherein the peptide contains an alkyl group at the C-terminal residue.
- 15 20. The method of any one of the preceding claims wherein the peptide is provided in the form of a pharmaceutical composition including the peptide and an excipient, diluent or carrier.
21. The method of any one of the preceding claims wherein the disease is anemia of inflammation.
- 20 22. The method of any one of the preceding claims wherein the peptide is provided in an amount of about 10-500mg/kg individual.
23. The method of any one of the preceding claims wherein the peptide is provided daily.
24. The method of any one of the preceding claims wherein the peptide is
25 administered IV.

25. The method of any one of the preceding claims wherein the peptide is provided once to the individual in the form of a sustained release formulation.

26. A peptide for inhibiting the binding of hepcidin to $\alpha 2$ - macroglobulin, wherein the peptide has an amino acid sequence according to Formula 1:

5 $X_1X_2X_3X_4X_5X_6\text{CysIlePhe}$

wherein

X_1 is Ala, Gly, Ser or Thr, and X_2 is Thr, X_3 is Asp or Glu, X_4 is Phe, X_5 is Pro, and X_6 is Ile.

27. The peptide of claim 26 wherein X_1 is Ala and X_3 is Asp.

10 28. A peptide for inhibiting the binding of hepcidin to $\alpha 2$ - macroglobulin, wherein the peptide has an amino acid sequence according to Formula 1:

$X_1X_2X_3X_4X_5X_6\text{CysIlePhe}$

wherein

15 X_1 is Ala, Gly, Ser or Thr, and X_2 is Thr, X_3 is His, X_4 is Lys, Arg or His, X_5 is Pro, and X_6 is Ile.

29. The peptide of claim 28 wherein X_1 is Ala and X_4 is Lys.

30. A peptide for inhibiting the binding of hepcidin to $\alpha 2$ - macroglobulin, wherein the peptide has an amino acid sequence:

$\text{AspThrHisPheProlleCysIlePhe}$

20 wherein the peptide is a cyclic peptide, or wherein the peptide contains an alkyl group at the C-terminal residue.

31. The peptide according to any one of claims 26 to 29 wherein the peptide contains about 25 amino acids.

25 32. The peptide according to any one of claims 26 or 29 wherein the peptide has the sequence

$X_1X_2X_3X_4X_5X_6\text{CysIlePheCysCysGlyCysCysHisArgSerLysCysGlyMetCysCysLysThr}$

33. A peptide having an amino acid sequence according to Formula 1C:

$X_1X_2X_3X_4X_5X_6\text{CysIlePheCysCysGlyCysCysBBXXCysGlyXCysCysBThr}$

wherein:

X₁ is Asp, Ala, Gly, Ser or Thr

X₂ is Thr, Ala or Gly

X₃ is His, Asp or Glu

5 X₄ is Phe, Leu, Nle, Lys, Ile or Val

X₅ is Pro, Ala or Gly

X₆ is Ile, Lys, Arg, or His

B is a positively charged residue, for example His, Asn, Arg or Lys

X is any amino acid residue.

- 10 34. The peptide of claim 33 wherein X₁ is Ala, Gly, Ser or Thr, and X₂ is Thr, X₃ is His, X₄ is Phe, X₅ is Pro, and X₆ is Ile, preferably, X₁ is Ala.
35. The peptide of claim 33 wherein X₁ is Asp, X₂ is Ala or Gly, X₃ is His, X₄ is Phe, X₅ is Pro, and X₆ is Ile, preferably X₂ is Ala.
- 15 36. The peptide of claim 33 wherein X₁ is Asp, X₂ is Thr, X₃ is Asp or Glu, X₄ is Phe, X₅ is Pro, and X₆ is Ile, preferably X₃ is Asp.
37. The peptide of claim 33 wherein X₁ is Asp, X₂ is Thr, X₃ is His, X₄ is Leu, Ile or Val or Nle, X₅ is Pro, and X₆ is Ile, preferably X₄ is Leu or Nle.
38. The peptide of claim 33 wherein X₁ is Asp, X₂ is Thr, X₃ is His, X₄ is Phe, X₅ is Ala or Gly, and X₆ is Ile, preferably X₅ is Ala.
- 20 39. The peptide of claim 33 wherein X₁ is Asp, X₂ is Thr, X₃ is His, X₄ is Phe, X₅ is Pro, and X₆ is Lys, Arg or His, preferably X₆ is Lys.
40. A pharmaceutical composition including a peptide of any one of claims 26 to 39.

Figure 1

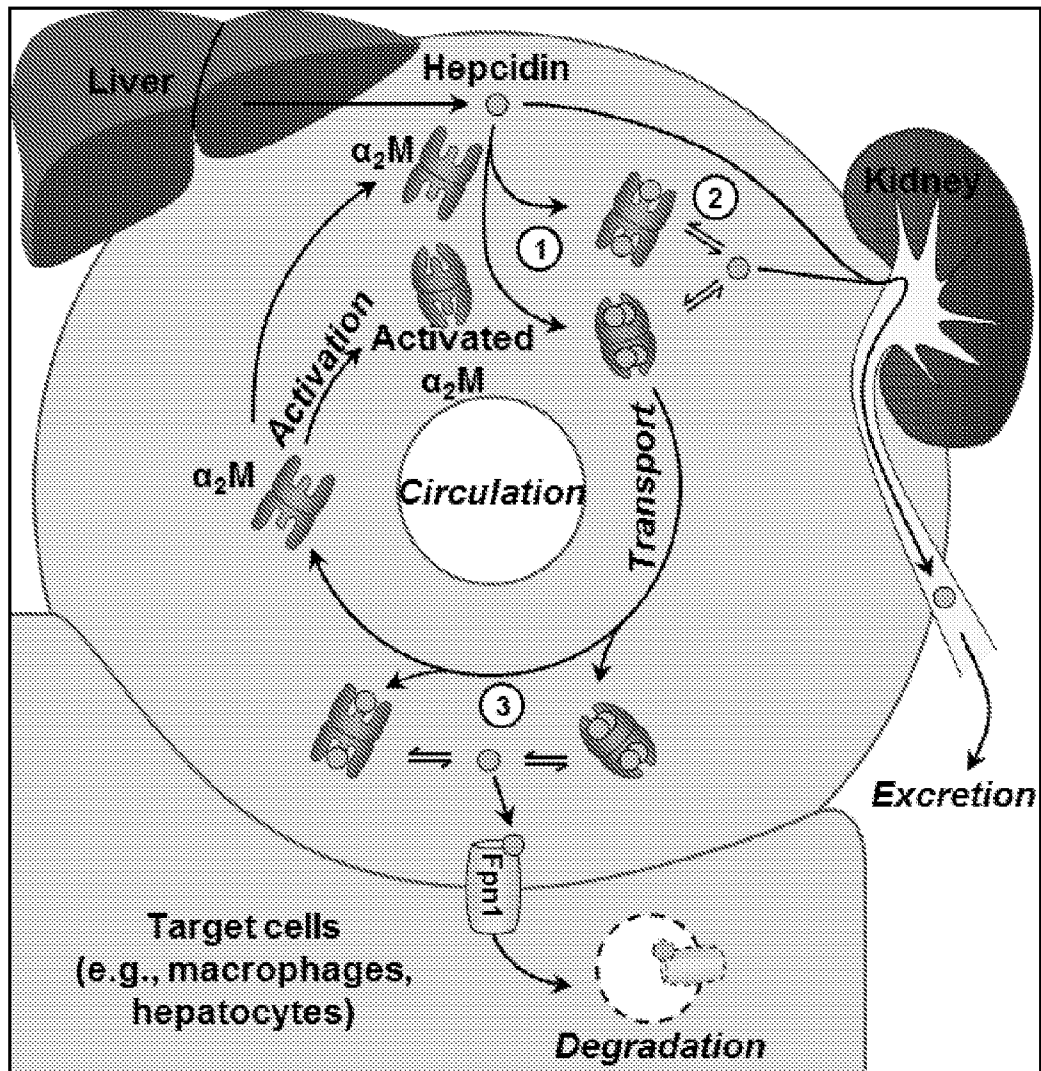


Figure 2

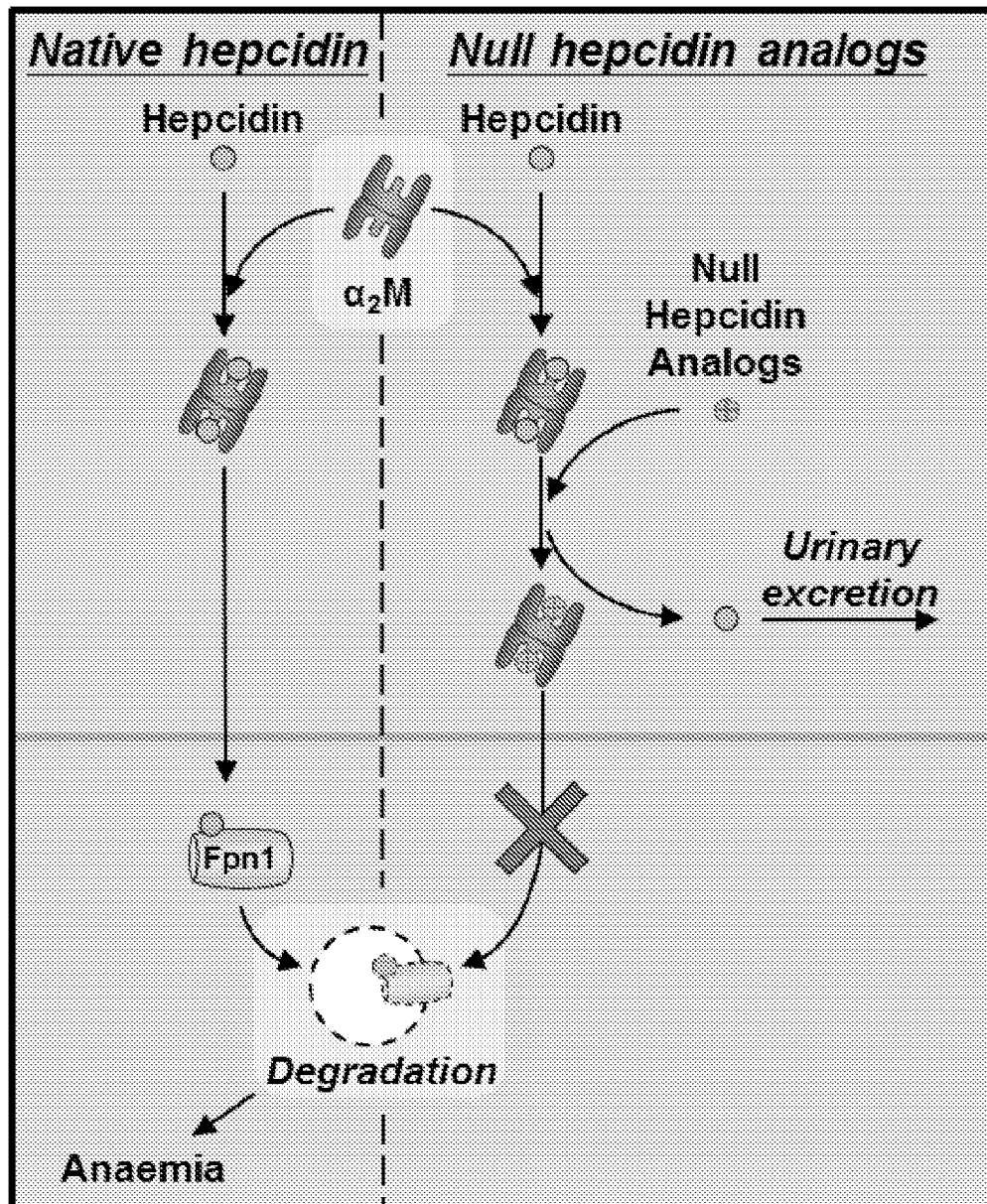


Figure 3

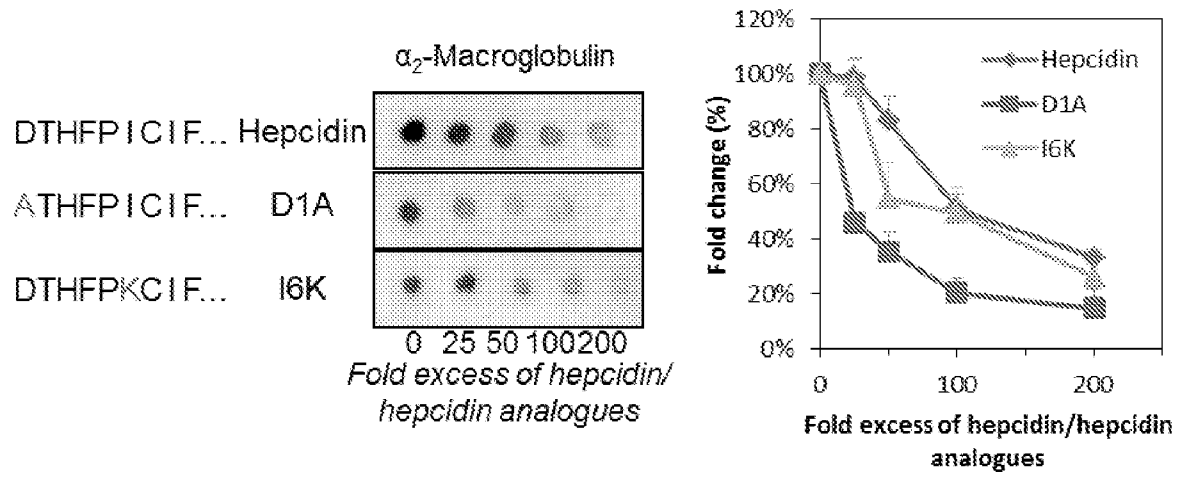


Figure 4

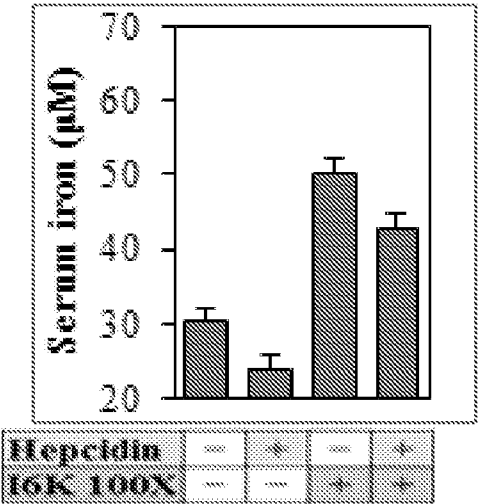


Figure 5

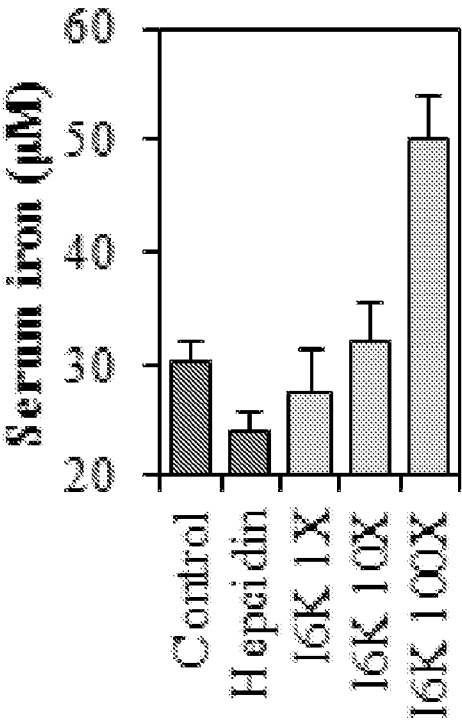
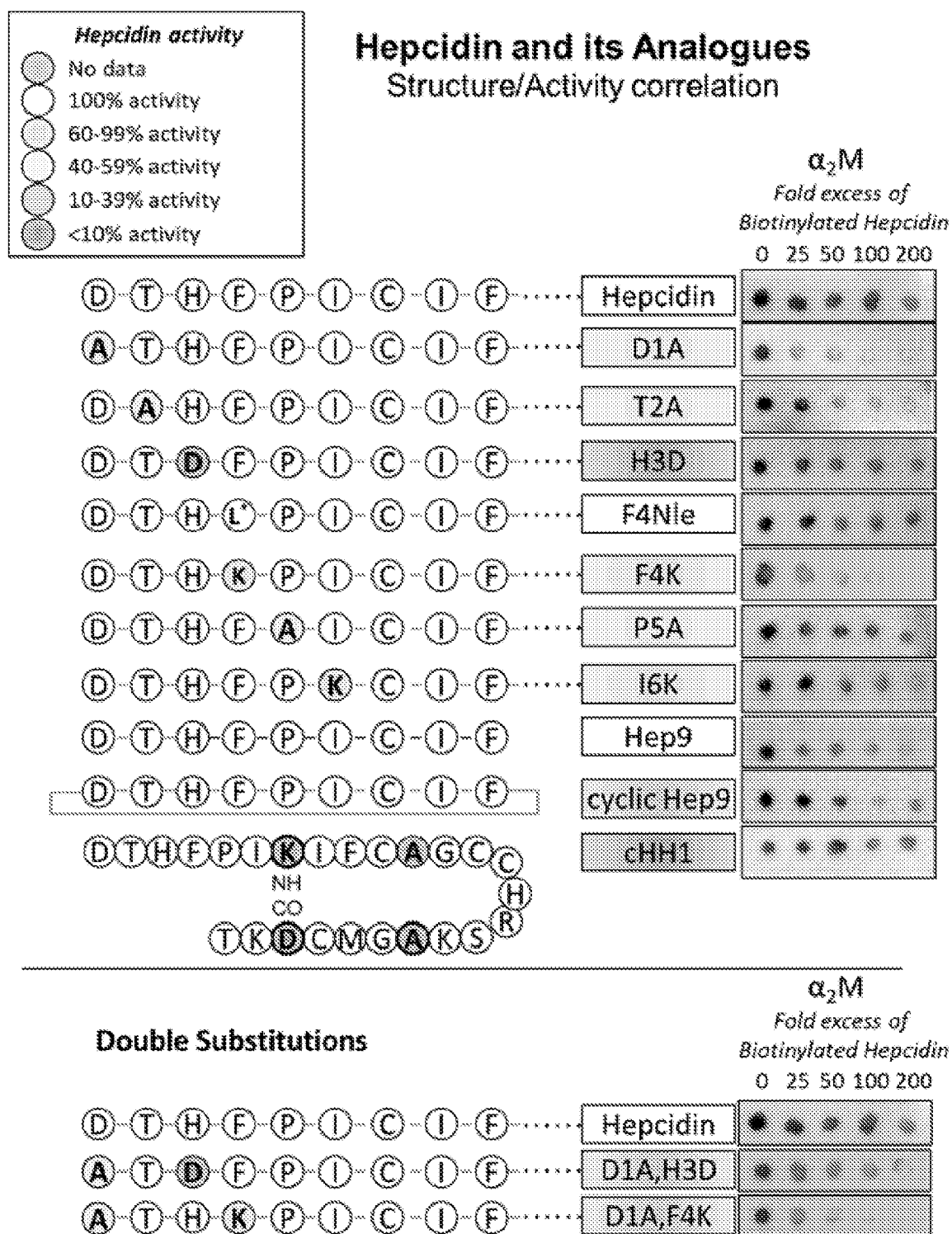


Figure 6



INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU2017/051399

A. CLASSIFICATION OF SUBJECT MATTER

A61K 38/16 (2006.01) A61K 38/08 (2006.01) A61K 38/17 (2006.01) C07K 7/04 (2006.01) C07K 7/06 (2006.01)
C07K 7/64 (2006.01) C07K 14/00 (2006.01) C07K 14/47 (2006.01) A61P 7/06 (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases: EPOQUE: PATENW; STN: Registry, HCAPLUS, BIOSIS, EMBASE, MEDLINE; Public: ESPACENET, PubMed, Google Patents.
 Keywords: Mini-hepcidin, null hepcidin, hep9, hep25, hepcidin, leap 1, HAMP, HEPC, HFE2B, PLTR, analogue, fragment, alpha 2
 macroglobulin, and like terms.

Applicant and Inventor name searches were completed using Internal IPA Databases PAMS NOSE and INTESS and using public databases
 ESPACENET and PubMed.

Various claimed peptide sequences were searched using STN (Registry) and GENOMEQUEST, including DTHFPICIF (SEQ ID NO: 1). See
 GENOMEQUEST and STN portions of the SIS for specific sequences.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		
Date of the actual completion of the international search 19 February 2018		Date of mailing of the international search report 19 February 2018	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaaustralia.gov.au		Authorised officer Holly Thomas AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61262256157	

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
the subject matter listed in Rule 39 on which, under Article 17(2)(a)(i), an international search is not required to be carried out, including
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

See Supplemental Box for Details

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:
 - a. (means)
☐ on paper
☒ in electronic form
 - b. (time)
☐ in the international application as filed
☒ together with the international application in electronic form
☐ subsequently to this Authority for the purposes of search
2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:
Hepcidin sequences for wildtype Hep9 and Hep25, as well as claimed variants, were searched taking into account restrictions based on Article 6 (see ISO Box VIII; Item 1).

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2017/051399
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/086143 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 13 June 2013 Table 1; [13]-[14]; [18], [19]-[20]; [51]; [71]; [75]; [84]; [122]-[123]; [125]; Figure 1 and Figure 2A.	1-11 and 14-40
X	WO 2010/065815 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 10 June 2010 Table 1; [50]; [92]-[93]; Figures 1 and 2A.	1-11 and 14-40
X	CLARK, R. J. et al. "Understanding the Structure/Activity Relationships of the Iron Regulatory Peptide Hepcidin" Chem Biol. 2011, Vol. 18, No. 3, Pages 336-343. Abstract; 'Peptide synthesis' starting Page 2; 'Bioactivity of the hepcidin analogues' starting Page 4; Figure 4.	1-11 and 14-40
X	PREZA, G. C. et al. "Minihepcidins are rationally designed small peptides that mimic hepcidin activity in mice and may be useful for the treatment of iron overload", The Journal of Clinical Investigation, 2011, Vol. 121, No. 12, Pages 4880-4888 & Supplementary Material retrieved online https://www.jci.org/articles/view/57693#sd . Abstract; Page 4881, Col. 2, Para. 2; Page 4883, Col. 1, Para. 3; Page 4887, Col. 1, Para. 7 – Col. 2, Para. 4; Supplemental Table 2.	1-11 and 14-40
X	CHUA, K. et al. "Small cyclic agonists of iron regulatory hormone hepcidin", Bioorg Med Chem Lett. 2015, Vol. 25, No. 21, Pages 4961-9. Abstract; Page 2, Para. 1 and 3; Page 5, Para. 3; Figure 1.	1-11 and 14-40
A	PESLOVA, G. et al. "Hepcidin, the hormone of iron metabolism, is bound specifically to α -2-macroglobulin in blood", Blood, 2009, Vol. 113, No. 24, Pages 6225-6236. Whole document.	1-40
P,X	WO 2017/117411 A1 (PROTAGONIST THERAPEUTICS, INC.) 06 July 2017 Abstract; Page 6, Para. 2-3; Page 9, Para. 3; Page 63, Para. 2; Example 2; Table 9.	1, 18, 20-21, 23-25, 33 and 40
P,X	WO 2017/120419 A1 (LA JOLLA PHARMACEUTIAL COMPANY) 13 July 2017 Abstract; Page 3, Para. 3; Page 14, Line 25; Page 15, Lines 19-27; Page 32, Lines 12-15; Page 34, Lines 16-30.	1, 18, 20-21, 23-24, 33-35, 37 and 39-40
Form PCT/ISA/210 (fifth sheet) (July 2009)		

Supplemental Box**Continuation of: Box III**

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

Invention 1: Claims 1, 18-25, 33 and 40 (in part). These claims relate to peptides and uses thereof having Formula 1, or Formula 1c, wherein: X1 is Asp, X2 is Thr, X3 is His, X4 is Phe, X5 is Pro and X6 is Ile (i.e. unmodified hepcidin; Hep9 and Hep25).

Invention 2: Claims 1, 18-25, 33 and 40 (in part) and claims 2, 3, and 34 (in full). These claims relate to peptides and uses thereof as described in Invention 1, wherein X1 is substituted with Ala (i.e. 9 amino acid and 25 amino acid peptides of modified hepcidin designated D1A).

Invention 3: Claims 1, 18-25, 33 and 40 (in part) and claims 4-5 and 35 (in full). These claims relate to peptides and uses thereof as described in Invention 1, wherein X2 is substituted with Ala (i.e. 9 amino acid and 25 amino acid peptides of modified hepcidin designated T2A).

Invention 4: Claims 1, 18-25, 33 and 40 (in part) and claims 6-7 and 36 (in full). These claims relate to peptides and uses thereof as described in Invention 1, wherein X3 is substituted with Asp (i.e. 9 amino acid and 25 amino acid peptides of modified hepcidin designated H3D).

Invention 5: Claims 1, 18-25, 33 and 40 (in part) and claims 8-9 and 37 (in full). These claims relate to peptides and uses thereof as described in Invention 1, wherein X4 is substituted with Nle or Leu (i.e. 9 amino acid and 25 amino acid peptides of modified hepcidin designated F4Nle).

Invention 6: Claims 1, 18-25, 33 and 40 (in part) and claims 10-11 and 38 (in full). These claims relate to peptides and uses thereof as described in Invention 1, wherein X5 is substituted with Ala (i.e. 9 amino acid and 25 amino acid peptides of modified hepcidin designated P5A).

Invention 7: Claims 1, 18-25, 33 and 40 (in part) and claims 12-13 and 39 (in full). These claims relate to peptides and uses thereof as described in Invention 1, wherein X6 is substituted with Lys (i.e. 9 amino acid and 25 amino acid peptides of modified hepcidin designated I6K).

Invention 8: Claims 1, 18-25, 33 and 40 (in part), claims 14-15 and 26-27 (in full) and claims 31-32 (in part). These claims relate to peptides and uses thereof as described in Invention 1, wherein X1 is substituted with Ala and X3 is substituted with Asp (i.e. 9 amino acid and 25 amino acid peptides of modified hepcidin with double mutations designated D1A/H3D).

Invention 9: Claims 1, 18-25, 33 and 40 (in part), claims 16-17 and 28-29 (in full) and claims 31-32 (in part). These claims relate to peptides and uses thereof as described in Invention 1, wherein X1 is substituted with Ala and X4 is substituted with Lys (i.e. 9 amino acid and 25 amino acid peptides of modified hepcidin with double mutations designated D1A/F4K).

Invention 10: Claims 1, 18-25, 33 and 40 (in part) and claim 30 (in full). These claims relate to peptides and uses thereof having the claimed formula (wt Hep9) that are cyclic or comprise an alkyl group at the C-terminal residue.

Invention 11+: Claims 1, 18-25, 33 and 40 (in part). Peptides and uses thereof as described in Invention 1, wherein each substitution or combination of substitutions of X1-X6 from the sequences of Invention 1, apart from those already given above for Inventions 2-10, represent a separate invention.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. The only feature common to all of the claimed inventions is that the peptides are either 9mer or 25mer wildtype hepcidin peptides, or are based on such peptides

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PCT/AU2017/051399**Supplemental Box**

(E.g. comprise single or double mutations based on wildtype hepcidin). However it is considered that this feature is generic in this particular art. See for example D1: WO2013/086143.

Therefore this common feature cannot be a special technical feature. Hence there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied a posteriori.

Despite the above issue, no restrictions have been placed on the search or examination due to the lack of unity present; however, it should be noted that restrictions have been placed on the search and examination of the claims due to Article 6 issues (see ISO Box VIII Item 1 for more information).

INTERNATIONAL SEARCH REPORT		International application No.	
Information on patent family members		PCT/AU2017/051399	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
WO 2013/086143 A1	13 June 2013	WO 2013086143 A1	13 Jun 2013
		AU 2009322260 A1	07 Jul 2011
		AU 2009322260 B2	12 Sep 2013
		AU 2012327226 A1	27 Jun 2013
		AU 2012327226 B2	14 Apr 2016
		CA 2855122 A1	13 Jun 2013
		CN 102245626 A	16 Nov 2011
		CN 104011066 A	27 Aug 2014
		EP 2373679 A2	12 Oct 2011
		EP 2373679 B1	08 Mar 2017
		EP 2788370 A1	15 Oct 2014
		EP 3208279 A1	23 Aug 2017
		HK 1199892 A1	24 Jul 2015
		JP 2015504859 A	16 Feb 2015
		US 2012040894 A1	16 Feb 2012
		US 8435941 B2	07 May 2013
		US 2014336110 A1	13 Nov 2014
		US 9328140 B2	03 May 2016
		US 2013203662 A1	08 Aug 2013
		US 2016200765 A1	14 Jul 2016
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		AU 2009322260 A1	07 Jul 2011
		AU 2009322260 B2	12 Sep 2013
		AU 2012327226 A1	27 Jun 2013
		AU 2012327226 B2	14 Apr 2016
		CA 2855122 A1	13 Jun 2013
		CN 102245626 A	16 Nov 2011
		CN 104011066 A	27 Aug 2014
		EP 2373679 A2	12 Oct 2011
		EP 2373679 B1	08 Mar 2017
		EP 2788370 A1	15 Oct 2014
		EP 3208279 A1	23 Aug 2017
		HK 1199892 A1	24 Jul 2015
		JP 2015504859 A	16 Feb 2015

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

Form PCT/ISA/210 (Family Annex)(July 2009)

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.
Form PCT/ISA/210 (Family Annex)(July 2009)

INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/AU2017/051399	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
		US 2012040894 A1	16 Feb 2012
		US 8435941 B2	07 May 2013
		US 2014336110 A1	13 Nov 2014
		US 9328140 B2	03 May 2016
		US 2013203662 A1	08 Aug 2013
		US 2016200765 A1	14 Jul 2016
		WO 2013086143 A1	13 Jun 2013
WO 2017/117411 A1	06 July 2017	WO 2017117411 A1	06 Jul 2017
WO 2017/120419 A1	13 July 2017	WO 2017120419 A1	13 Jul 2017
		US 2017246256 A1	31 Aug 2017
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			