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(71) Applicant *(for all designated States except US)*: **MODIGENE INC** [US/US]; 8000 Towers Crescent Drive, Suite 1300, Vienna, VA 22182 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **FARES, Fuad** [IL/IL]; P.o. Box 423, 25155 Hourfish Village (IL). **FIMA, Udi, Eyal** [IL/IL]; 30 Albek St., 84833 Beer-sheva (IL).

(74) Agent: **COHEN, Mark, S.**; Pearl Cohen Zedek Latzer, LLP, 1500 Broadway, 12th Floor, New York, NY 10036 (US).

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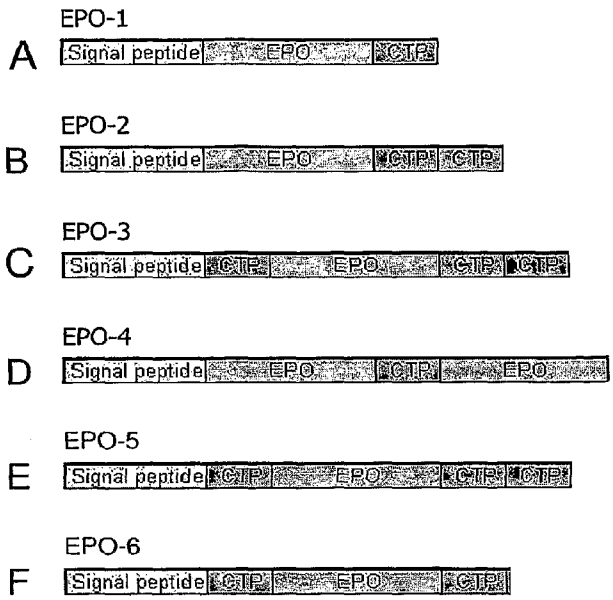
(54) **Title:** LONG-ACTING POLYPEPTIDES AND METHODS OF PRODUCING SAME



(57) **Abstract:** A polypeptide and polynucleotides encoding same comprising at least two carboxy-terminal peptide (CTP) sequences of chorionic gonadotrophin attached to a peptide-of-interest are disclosed. Pharmaceutical compositions comprising the polypeptide and polynucleotides of the invention and methods of using same are also disclosed.

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LONG-ACTING POLYPEPTIDES AND METHODS OF PRODUCING SAME

FIELD OF INVENTION

A polypeptide and polynucleotides encoding same comprising at least two carboxy-terminal peptides (CTP) of chorionic gonadotrophin attached to a peptide-of-interest are disclosed. Pharmaceutical compositions comprising the polypeptide and polynucleotides of the invention and methods of using same are also disclosed.

BACKGROUND OF THE INVENTION

Polypeptides are susceptible to denaturation or enzymatic degradation in the blood, liver or kidney. Accordingly, polypeptides typically have short circulatory half-lives of several hours. Because of their low stability, peptide drugs are usually delivered in a sustained frequency so as to maintain an effective plasma concentration of the active peptide. Moreover, since peptide drugs are usually administrated by infusion, frequent injection of peptide drugs cause considerable discomfort to a subject. Thus, there is a need for technologies that will prolong the half-lives of therapeutic polypeptides while maintaining a high pharmacological efficacy thereof. Such desirous peptide drugs should also meet the requirements of enhanced serum stability, high activity and a low probability of inducing an undesired immune response when injected into a subject.

Unfavorable pharmacokinetics, such as a short serum half-life, can prevent the pharmaceutical development of many otherwise promising drug candidates. Serum half-life is an empirical characteristic of a molecule, and must be determined experimentally for each new potential drug. For example, with lower molecular weight polypeptide drugs, physiological clearance mechanisms such as renal filtration can make the maintenance of therapeutic levels of a drug unfeasible because of cost or frequency of the required dosing regimen. Conversely, a long serum half-life is undesirable where a drug or its metabolites have toxic side effects.

SUMMARY OF THE INVENTION

In one embodiment, the present invention provides a polypeptide comprising at least two chorionic gonadotrophin carboxy terminal peptide (CTP) amino acid sequences attached to a polypeptide sequence-of-interest.

In another embodiment, the present invention provides a polypeptide comprising a first chorionic gonadotrophin CTP amino acid (AA) sequence attached to an amino terminus of a polypeptide sequence-of-interest and a second CTP amino acid sequence attached to a carboxy terminus of a polypeptide sequence of interest.

In another embodiment, the present invention provides a polypeptide comprising two chorionic gonadotrophin CTP sequences attached to a carboxy terminus of a polypeptide sequence-of-interest.

In another embodiment, the present invention provides a polypeptide comprising a first chorionic gonadotrophin CTP AA sequence attached to an amino terminus of polypeptide sequence-of-interest, and a second and third CTP AA sequences attached to a carboxy terminus of a polypeptide sequence of interest.

In another embodiment, the present invention provides a polypeptide comprising at least three chorionic gonadotrophin CTP AA sequences attached to a polypeptide sequence-of-interest.

In another embodiment, the present invention provides a polynucleotide comprising a sequence encoding a polypeptide, comprising at least two chorionic gonadotrophin CTP AA sequences attached to a polypeptide sequence-of-interest.

In another embodiment, the present invention provides a polynucleotide comprising a nucleotide sequence, encoding a first chorionic gonadotrophin CTP AA sequence attached to an amino terminus of polypeptide sequence-of-interest and a second CTP AA sequence attached to a carboxy terminus of a polypeptide sequence of interest.

In another embodiment, the present invention provides a polynucleotide comprising a sequence encoding two chorionic gonadotrophin CTP AA sequences attached to a carboxy terminus of polypeptide sequence-of-interest.

In another embodiment, the present invention provides a polynucleotide comprising a sequence encoding a first chorionic gonadotrophin CTP AA sequence attached to an amino terminus of polypeptide sequence-of-interest, and a second and third CTP AA sequences attached to a carboxy terminus of a polypeptide sequence of interest.

In another embodiment, the present invention provides a polynucleotide comprising a sequence encoding at least three chorionic gonadotrophin CTP AA sequences attached to a polypeptide sequence-of-interest.

In another embodiment, the present invention provides a method of treating a growth, weight-related or metabolic conditions in a subject, the method comprising the step of administering to a subject a therapeutically effective amount of CTP-hGH, thereby treating a subject having a growth or weight-related condition. (hGH is for growth disorders in general and Growth hormone deficiency related disorders in particularly. In our example, we demonstrated growth gain in hipophysectomized rats (which have no Growth hormone secretion) following injections of CTP-hGH.)

In another embodiment, the present invention provides a method of improving biological half life of a polypeptide sequence-of-interest, comprising the step of attaching at least two chorionic gonadotrophin CTP AA sequences to a polypeptide sequence-of-interest.

In another embodiment, the present invention provides a method of administering a polypeptide sequence-of-interest to a subject in need thereof, comprising the step of attaching at least two chorionic gonadotrophin CTP AA sequences to a polypeptide sequence-of-interest.

In another embodiment, the present invention provides a polypeptide comprising at least two chorionic gonadotrophin CTP AA sequences attached to an EPO peptide.

In another embodiment, the present invention provides a polypeptide comprising a first chorionic gonadotrophin CTP AA sequence attached to an amino terminus of EPO peptide and a second CTP AA sequence attached to a carboxy terminus of an EPO peptide.

In another embodiment, the present invention provides a polypeptide comprising two chorionic gonadotrophin CTP AA sequences attached to a carboxy terminus of EPO peptide.

In another embodiment, the present invention provides a polypeptide comprising a first chorionic gonadotrophin CTP AA sequence attached to an amino terminus of EPO peptide, and a second and third CTP AA sequences attached to a carboxy terminus of an EPO peptide.

In another embodiment, the present invention provides a polypeptide comprising at least three chorionic gonadotrophin CTP AA sequences attached to an EPO peptide.

In another embodiment, the present invention provides a polynucleotide comprising a nucleotide sequence, encoding at least two chorionic gonadotrophin CTP AA sequences attached to an EPO peptide.

In another embodiment, the present invention provides a polynucleotide comprising a nucleotide sequence, encoding a first chorionic gonadotrophin CTP AA sequence attached to an amino

terminus of EPO peptide and a second CTP AA sequence attached to a carboxy terminus of an EPO peptide.

In another embodiment, the present invention provides a polynucleotide comprising a nucleotide sequence, encoding two chorionic gonadotrophin CTP AA sequences attached to a carboxy terminus of polypeptide sequence-of-interest.

In another embodiment, the present invention provides a polynucleotide comprising a nucleotide sequence, encoding a first chorionic gonadotrophin CTP AA sequence attached to an amino terminus of polypeptide sequence-of-interest, and a second and third CTP AA sequences attached to a carboxy terminus of a polypeptide sequence of interest.

In another embodiment, the present invention provides a polynucleotide comprising a nucleotide sequence, encoding at least three chorionic gonadotrophin CTP AA sequences attached to a polypeptide sequence-of-interest.

In another embodiment, the present invention provides a method of treating or reducing the incidence associated with anaemia in a subject, comprising the step of administering to a subject a therapeutically effective amount of the EPO-CTP, thereby treating the subject having anemia.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1A-1F are diagrams illustrating six EPO-CTP constructs.

Figure 1A – is a diagram of the polypeptide of SEQ ID NO: 1

Figure 1B is a diagram of the polypeptide of SEQ ID NO: 2

Figure 1C is a diagram of the polypeptide of SEQ ID NO: 3

Figure 1D is a diagram of the polypeptide of SEQ ID NO: 4.

Figure 1E is a diagram of the polypeptide of SEQ ID NO: 5.

Figure 1F is a diagram of the polypeptide of SEQ ID NO: 6.

FIG. 2 is a photograph illustrating the expression of the EPO-CTP variants from transfected DG44 cells. Final test samples from transfected cells were prepared as described under "sample preparation" and run on SDS/PAGE. Proteins were detected by western blot.

FIG. 3 is a graph illustrating the in vivo bioactivity of recombinant hEPO derivatives and EPO-3 (SEQ ID NO: 3). ICR mice (n=7/group) received a single IV injection/week (15μg/kg) for three weeks of EPO-3, rhEPO-WT (SEQ ID NO: 16), Recormon (Commercial EPO) or Recormon (5μg/kg) 3 times a week. Control animals were injected IV with PBS. Blood samples were collected three times a week and haematocrit levels were detected. Each point represents the group average of haematocrit (%) ± SE.

FIG. 4 is a graph illustrating the in vivo bioactivity of recombinant hEPO derivatives and EPO-1 (SEQ ID NO: 1). ICR mice (n=7/group) received a single IV injection/week (15μg/kg) for three weeks of EPO-1, rhEPO-WT (SEQ ID NO: 16), Recormon or Recormon (5μg/kg) 3 times a week. Control animals were injected IV with PBS. Blood samples were collected three times a week and haematocrit levels were detected. Each point represents the group average of haematocrit (%) ± SE.

FIG. 5 is a graph illustrating the in vivo bioactivity of recombinant hEPO derivatives and EPO-2 (SEQ ID NO: 2). ICR mice (n=7/group) received a single IV injection/week (15μg/kg) for three weeks of EPO-2 (SEQ ID NO: 2), rhEPO-WT (SEQ ID NO: 16), Recormon or Recormon (5μg/kg) 3 times a week. Control animals were injected IV with PBS. Blood samples were collected three times a week and haematocrit levels were detected. Each point represents the group average of haematocrit (%) ± SE.

FIG. 6 is a time graph illustrating the change in reticulocyte level following a single bolus dose of EP0-0 (SEQ ID NO: 16), EPO-3 (SEQ ID NO: 3) and Aranesp.

FIG. 7 is a time graph illustrating the change in hemoglobin level (presented as change from baseline) following a single bolus dose of EP0-0 (SEQ ID NO: 16), EPO-3 (SEQ ID NO: 3) and Aranesp.

FIG. 8 is a time graph illustrating the change in hematocrit level following a single bolus dose of EP0-0 (SEQ ID NO: 16), EPO-3 (SEQ ID NO: 3) and Aranesp.

FIG. 9 is a graph illustrating the change in serum concentration of EPO -0 (SEQ ID NO: 16), EPO-3 (SEQ ID NO: 3) and Aranesp post i.v. injection.

FIG. 10 is a Western blot illustrating the molecular weight & identity of MOD-4020 (SEQ ID NO: 36), MOD-4021 (SEQ ID NO: 37), MOD-4022 (SEQ ID NO: 38), MOD-4023 (SEQ ID NO: 39) and MOD-4024 (SEQ ID NO: 40). PAGE SDS gel was blotted and stained using monoclonal anti-hGH antibodies. The photograph indicates that like commercial and wild type hGH, MOD-7020-4 variants are recognized by anti hGH antibodies.

FIG. 11 is a bar graph illustrating the weight gain of hypophysectomized rats following administration of the GH-CTP polypeptides of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

In one embodiment, the present invention describes long-acting polypeptides and methods of producing and using same. In one embodiment, long-acting polypeptides comprise carboxy terminal peptide (CTP) of human Chorionic Gonadotropin (hCG). In one embodiment, CTP acts as a protectant against degradation of proteins or peptides derived therefrom. In one embodiment, CTP extends circulatory half-lives of proteins or peptides derived therefrom. In some embodiments, CTP enhances the potency of proteins or peptides derived therefrom.

In another embodiment, "CTP peptide," "carboxy terminal peptide," and "CTP sequence" are used interchangeably herein. In another embodiment, the carboxy terminal peptide is a full-length CTP. In another embodiment, the carboxy terminal peptide is a truncated CTP. Each possibility represents a separate embodiment of the present invention.

In another embodiment, "EPO peptide" and "EPO sequence" are used interchangeably herein. In another embodiment, the EPO peptide is an EPO protein. In another embodiment, the carboxy terminal peptide is a truncated EPO protein. Each possibility represents a separate embodiment of the present invention.

In another embodiment, "signal sequence" and "signal peptide" are used interchangeably herein. In another embodiment, "sequence" when in reference to a polynucleotide can refer to a coding portion. Each possibility represents a separate embodiment of the present invention.

In another embodiment, "peptide of interest" and "polypeptide sequence-of-interest" are used interchangeably herein. In another embodiment, the peptide of interest is a full-length protein. In another embodiment, the peptide of interest is a protein fragment. Each possibility represents a separate embodiment of the present invention.

In one embodiment, a polypeptide comprising at least two carboxy-terminal peptide (CTP) sequences of chorionic gonadotrophin attached to a polypeptide sequence-of-interest, wherein a first CTP sequence of the at least two CTP sequences is attached to an amino terminus of the polypeptide sequence of interest and a second CTP sequence of the at least two CTP sequences is attached to the carboxy terminus of the polypeptide sequence of interest is provided. In another embodiment, the carboxy-terminal peptide (CTP) sequence is of human chorionic gonadotrophin.

In another embodiment, the carboxy-terminal peptide (CTP) is attached to the polypeptide sequence of interest via a linker. In another embodiment, the linker which connects the CTP sequence to the polypeptide sequence of interest is a covalent bond. In another embodiment, the linker which connects the CTP sequence to the polypeptide sequence of interest is a peptide bond. In another embodiment, the linker which connects the CTP sequence to the polypeptide sequence of interest is a substituted peptide bond.

The phrase "polypeptide sequence of interest" refers, in another embodiment, to any polypeptide sequence, such as one comprising a biological activity. In another embodiment, the peptide is glycosylated. In another embodiment, the peptide is non-glycosylated. Examples of polypeptides which benefit from an extension in their circulatory half-life include, but are not limited to erythropoietin (EPO), interferons, human growth hormone (hGH) and glucagon-like peptide-1(GLP-1).

In another embodiment, the carboxy terminal peptide (CTP) of human Chorionic Gonadotropin (hCG) is fused to a protein. In another embodiment, the carboxy terminal peptide (CTP) of human Chorionic Gonadotropin (hCG) is fused to a glycoprotein. In another embodiment, the carboxy terminal peptide (CTP) of human Chorionic Gonadotropin (hCG) is fused to a glycoprotein hormone. In another embodiment, the carboxy terminal peptide (CTP) of human Chorionic Gonadotropin (hCG) is fused to a peptide derived from a glycoprotein hormone. In some embodiments, glycoprotein hormones comprise EPO, FSH, or TSH and pepetides derived therefrom.

In some embodiments, a CTP sequences at both the amino terminal end of a polypeptide and at the carboxy terminal end of the polypeptide provide enhanced protection against degradation of a protein. In some embodiments, CTP sequences at both the amino terminal end of a polypeptide and at the carboxy terminal end of the polypeptide provide extended half-life of the attached protein.

In some embodiments, a CTP sequence at the amino terminal end of a polypeptide, a CTP sequence at the carboxy terminal end of the polypeptide, and at least one additional CTP sequence attached in tandem to the CTP sequence at the carboxy terminus provide enhanced protection against degradation of a protein. In some embodiments, a CTP sequence at the amino terminal end of a polypeptide, a CTP sequence at the carboxy terminal end of the polypeptide, and at least one additional CTP sequence attached in tandem to the CTP sequence at the carboxy terminus provide extended half-life of the attached protein. In some embodiments, a CTP sequence at the amino terminal end of a polypeptide, a CTP sequence at the carboxy terminal end of the polypeptide, and at least one additional CTP sequence attached in tandem to the CTP sequence at the carboxy terminus provide enhanced activity of the attached protein.

In some embodiments, a CTP sequence at the amino terminal end of a polypeptide, a CTP sequence at the carboxy terminal end of the polypeptide, and at least one additional CTP sequence attached in tandem to the CTP sequence at the amino terminus provide enhanced protection against degradation of the attached protein. In some embodiments, a CTP sequence at the amino terminal end of a polypeptide, a CTP sequence at the carboxy terminal end of the polypeptide, and at least one additional CTP sequence attached in tandem to the CTP sequence at the amino terminus provide extended half-life of the attached protein. In some embodiments, a CTP sequence at the amino terminal end of a polypeptide, a CTP sequence at the carboxy terminal end of the polypeptide, and at least one additional CTP sequence attached in tandem to the CTP sequence at the amino terminus provide enhanced activity the attached protein.

In another embodiment, the carboxy terminal peptide (CTP) peptide of the present invention comprises the amino acid (AA) sequence from AA 112 to position 145 of human chorionic gonadotrophin, as set forth in SEQ ID NO: 17. In another embodiment, the CTP sequence of the present invention comprises the AA sequence from AA 118 to position 145 of human chorionic gonadotropin, as set forth in SEQ ID NO: 18. In another embodiment, the CTP sequence also commences from any position between positions 112-118 and terminates at position 145 of human chorionic gonadotrophin. In some embodiments, the CTP sequence peptide is 28, 29, 30, 31, 32, 33 or 34 AAs long and commences at position 112, 113, 114, 115, 116, 117 or 118 of the CTP AA sequence.

In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 1-5 conservative AA substitutions as described in U.S. Pat. No. 5,712,122. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 1 conservative AA substitution. In another embodiment,

the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 2 conservative AA substitutions. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 3 conservative AA substitutions. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 4 conservative AA substitutions. In another embodiment, the CTP peptide is a variant of chorionic gonadotrophin CTP which differs from the native CTP by 5 conservative AA substitutions. In another embodiment, the CTP peptide AA sequence of the present invention is at least 70% homologous to the native CTP AA sequence or a peptide thereof. In another embodiment, the CTP peptide AA sequence of the present invention is at least 80% homologous to the native CTP AA sequence or a peptide thereof. In another embodiment, the CTP peptide AA sequence of the present invention is at least 90% homologous to the native CTP AA sequence or a peptide thereof. In another embodiment, the CTP peptide AA sequence of the present invention is at least 95% homologous to the native CTP AA sequence or a peptide thereof.

In another embodiment, the CTP peptide DNA sequence of the present invention is at least 70% homologous to the native CTP DNA sequence or a peptide thereof. In another embodiment, the CTP peptide DNA sequence of the present invention is at least 80% homologous to the native CTP DNA sequence or a peptide thereof. In another embodiment, the CTP peptide DNA sequence of the present invention is at least 90% homologous to the native CTP DNA sequence or a peptide thereof. In another embodiment, the CTP peptide DNA sequence of the present invention is at least 95% homologous to the native CTP DNA sequence or a peptide thereof.

In another embodiment, at least one of the chorionic gonadotrophin CTP AA sequences is truncated. In another embodiment, both of the chorionic gonadotrophin CTP AA sequences are truncated. In another embodiment, 2 of the chorionic gonadotrophin CTP AA sequences are truncated. In another embodiment, 2 or more of the chorionic gonadotrophin CTP AA sequences are truncated. In another embodiment, all of the chorionic gonadotrophin CTP AA sequences are truncated. In another embodiment, the truncated CTP comprises the first 10 AA of SEQ ID NO:43. In another embodiment, the truncated CTP comprises the first 11 AA of SEQ ID NO:43. In another embodiment, the truncated CTP comprises the first 12 AA of SEQ ID NO:43. In another embodiment, the truncated CTP comprises the first 13 AA of SEQ ID NO:43. In another embodiment, the truncated CTP comprises the first 14 AA of SEQ ID NO:43. In another embodiment, the truncated CTP comprises the first 15 AA of SEQ ID NO:43. In another embodiment, the truncated CTP comprises the first 16 AA of SEQ ID NO:43. In another

embodiment, the truncated CTP comprises the last 14 AA of SEQ ID NO:43. Each possibility represents a separate embodiment of the present invention.

In another embodiment, at least one of the chorionic gonadotrophin CTP AA sequences is glycosylated. In another embodiment, both of the chorionic gonadotrophin CTP AA sequences are glycosylated. In another embodiment, 2 of the chorionic gonadotrophin CTP AA sequences are glycosylated. In another embodiment, 2 or more of the chorionic gonadotrophin CTP AA sequences are glycosylated. In another embodiment, all of the chorionic gonadotrophin CTP AA sequences are glycosylated. In another embodiment, the CTP sequence of the present invention comprises at least one glycosylation site. In another embodiment, the CTP sequence of the present invention comprises 2 glycosylation sites. In another embodiment, the CTP sequence of the present invention comprises 3 glycosylation sites. In another embodiment, the CTP sequence of the present invention comprises 4 glycosylation sites.

In some embodiments, erythropoietin (EPO) is utilized according to the teachings of the present invention. In some embodiments, any EPO encoding AA sequence is an EPO sequence. In some embodiments, any EPO encoding nucleic acid sequence is an EPO sequence In some embodiments, the attachment of CTP sequence to both the amino and carboxy termini of the EPO protein results in increased potency at stimulating erythropoiesis (Figures 3-5) and (Table 6 of Example 4), as compared to recombinant EPO and other combinations of EPO and CTP. In some embodiments, an EPO attached to three CTP sequences does not impair binding to its receptor as evidenced in Table 4 of Example 3 which demonstrates that EPO attached to three CTP sequences is equally effective at stimulating proliferation of TF-1 cells as wild-type EPO. In some embodiments EPO-CTP polypeptides of the present invention are set forth in SEQ ID NO: 3 and SEQ ID NO: 6.

In one embodiment, "erythropoietin" refers to mammalian erythropoietin. In one embodiment, "erythropoietin" refers to human erythropoietin, such as set forth in GenBank Accession No. AAA52400.

In one embodiment, erythropoietin or EPO sequence of the present invention also refers to homologues. In one embodiment, the erythropoietin AA sequence of the present invention is at least 50% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, the erythropoietin AA sequence of the present invention is at least 60% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the

National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, the erythropoietin AA sequence of the present invention is at least 70% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, the erythropoietin AA sequence of the present invention is at least 80% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, the erythropoietin AA sequence of the present invention is at least 90% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, the erythropoietin AA sequence of the present invention is at least 95% homologous to an erythropoietin sequence set forth in GenBank Accession No. AAA52400 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters).

In another embodiment, the methods of the present invention provide an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus and at least one additional CTP AA peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 2 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 4 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an

EPO peptide set forth in SEQ ID NO: 5 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 6 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 16 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of anemia .

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 20 encoding an EPO peptide and one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of anemia. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 21 encoding an EPO peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of anemia .

In another embodiment, the methods of the present invention provide an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus and at least additional one CTP AA peptide on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 2 having

additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 4 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 5 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 6 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 16 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting anemia.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 20 encoding an EPO peptide and one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting anemia. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 21 encoding an EPO peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting anemia.

In another embodiment, the methods of the present invention provide an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of

tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus and at least additional one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 2 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 4 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 5 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 6 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 16 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide a nucleic acid set forth in

SEQ ID NO: 20 encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor-associated anemia. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 21 encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of tumor-associated anemia.

In another embodiment, the methods of the present invention provide an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus and at least additional one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 2 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 4 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 5 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 6 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 16 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of

the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 20 encoding an EPO peptide and one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting tumor-associated anemia. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 21 encoding an EPO peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting tumor-associated anemia.

In another embodiment, the methods of the present invention provide an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus and at least additional one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 2 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 4 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia. In another

embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 5 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 6 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 16 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 20 encoding an EPO peptide and one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of tumor hypoxia. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 21 encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of tumor hypoxia.

In another embodiment, the methods of the present invention provide an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO

peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus and at least additional one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 2 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 4 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 5 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 6 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 16 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-

terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 20 encoding an EPO peptide and one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 21 encoding an EPO peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of chronic infections such as HIV, inflammatory bowel disease, or septic episodes.

In another embodiment, the methods of the present invention provide an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus and at least additional one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 2 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 4 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 5 having additionally at least one CTP AA peptide on the N-

terminus and at least one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 6 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 16 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 20 encoding an EPO peptide and one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 21 encoding an EPO peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting chronic infections such as HIV, inflammatory bowel disease, or septic episodes.

In another embodiment, the methods of the present invention provide an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having

additionally at least one CTP AA peptide on the N-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus and at least additional one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 2 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 4 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 5 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 6 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 16 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of

fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 20 encoding an EPO peptide and one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 21 encoding an EPO peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for the treatment of fatigue syndrome following cancer chemotherapy.

In another embodiment, the methods of the present invention provide an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus and at least additional one CTP AA peptide on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 2 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 4 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 5 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 6 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 16 having additionally at least one CTP AA peptide on the N-

terminus and at least one CTP AA peptide on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving stem cell engraftment.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 20 encoding an EPO peptide and one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving stem cell engraftment. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 21 encoding an EPO peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving stem cell engraftment.

In another embodiment, the methods of the present invention provide an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 1 having additionally at least one CTP AA peptide on the N-terminus and at least additional one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 2 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide

an EPO peptide set forth in SEQ ID NO: 3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 4 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 5 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 6 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 16 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide an EPO peptide set forth in SEQ ID NO: 22 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome .

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an EPO peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 20 encoding an EPO peptide and one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome. In another embodiment, the methods of the present invention provide a nucleic acid set forth in SEQ ID NO: 21 encoding an EPO peptide

and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing the survival rate of a patient with aplastic anemia or myelodysplastic syndrome .

In some embodiments, homology according to the present invention also encompasses deletions, insertions, or substitution variants, including an AA substitution, thereof and biologically active polypeptide fragments thereof. In one embodiment the substitution variant comprises a glycine in position 104 of erythropoietin AA sequence is substituted by a serine (SEQ ID NO: 22).

In some embodiments, human growth hormone (hGH) is utilized according to the teachings of the present invention. In some embodiments, the attachment of CTP sequence to both the amino and carboxy termini of the hGH protein results in increased potency (Figures 11). In some embodiments, the attachment of CTP sequence to both the amino and carboxy termini of the hGH protein results in prolonged in-vivo activity. In one embodiment, CTP-hGH polypeptides of the present invention are set forth in SEQ ID NO: 39- 41.

In one embodiment, the phrase "human growth hormone" (hGH) refers to a polypeptide, such as set forth in Genbank Accession No. P01241 (SEQ ID NO: 47), exhibiting hGH activity (i.e. stimulation of growth).

In one embodiment, "human growth hormone" (hGH) refers to a polypeptide, such as set forth in Genbank Accession No. P01241, exhibiting hGH activity (i.e. stimulation of growth). In one embodiment, hGH of the present invention also refers to homologs. In one embodiment, hGH AA sequence of the present invention is at least 50% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH AA sequence of the present invention is at least 60% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH AA sequence of the present invention is at least 70% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH AA sequence of the present invention is at least 80% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH AA sequence of the present invention is at least 90% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, hGH AA

sequence of the present invention is at least 95% homologous to an hGH sequence set forth in GenBank Accession No. P01241 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters).

Exemplary CTP-hGH polypeptides of the present invention are set forth in SEQ ID NO: 39, SEQ ID NO: 40 and SEQ ID NO: 41.

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for stimulating muscle growth. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for stimulating muscle growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for stimulating muscle growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for stimulating muscle growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for stimulating muscle growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for stimulating muscle growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for stimulating muscle growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for stimulating muscle growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for stimulating muscle growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for stimulating muscle growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for stimulating muscle growth.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for stimulating muscle growth. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an

hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for stimulating muscle growth. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for stimulating muscle growth. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for stimulating muscle growth.

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for stimulating bone growth. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for stimulating bone growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for stimulating bone growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for stimulating bone growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for stimulating bone growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for stimulating bone growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for stimulating bone growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for stimulating bone growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for stimulating bone growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for stimulating bone growth. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for stimulating bone growth.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus

and at least one CTP AA peptide on the C-terminus for stimulating bone growth. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for stimulating bone growth. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for stimulating bone growth. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for stimulating bone growth .

In another embodiment, the methods of the present invention provide an hGh peptide of the present invention for maintaining muscle quality.

In another embodiment, the methods of the present invention provide an hGh of the present invention for maintaining bone quality.

In another embodiment, the methods of the present invention provide an hGH-CTP nucleic acid sequence of the present invention for maintaining bone quality.

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating a wasting disease. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for treating a wasting disease. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating a wasting disease. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating a wasting disease. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for treating a wasting disease. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for treating a wasting disease. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for treating a wasting disease. In another embodiment, the methods of the

present invention provide an hGH peptide set forth in SEQ ID NO: 40 for treating a wasting disease. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for treating a wasting disease. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for treating a wasting disease. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for treating a wasting disease.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating a wasting disease. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for treating a wasting disease. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for treating a wasting disease. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for treating a wasting disease.

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing cardiac function. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing cardiac function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing cardiac function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing cardiac function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for increasing cardiac function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for increasing

cardiac function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for increasing cardiac function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for increasing cardiac function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for increasing cardiac function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for increasing cardiac function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for increasing cardiac function.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing cardiac function. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing cardiac function. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing cardiac function. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing cardiac function.

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing lipolysis. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing lipolysis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing lipolysis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing lipolysis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for increasing lipolysis. In another embodiment, the methods of the present invention

provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for increasing lipolysis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for increasing lipolysis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for increasing lipolysis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for increasing lipolysis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for increasing lipolysis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for increasing lipolysis.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for increasing lipolysis. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing lipolysis. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing lipolysis. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for increasing lipolysis .

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving fluid balance. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving fluid balance. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving fluid balance. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving fluid balance. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for improving fluid balance. In another embodiment, the

methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for improving fluid balance. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for improving fluid balance. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for improving fluid balance. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for improving fluid balance. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for improving fluid balance. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for improving fluid balance.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving fluid balance. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving fluid balance. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving fluid balance. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving fluid balance.

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating osteoporosis. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for treating osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating osteoporosis. In another embodiment, the methods of the present invention provide an

hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for treating osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for treating osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for treating osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for treating osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for treating osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for treating osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for treating osteoporosis.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating osteoporosis. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for treating osteoporosis. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for treating osteoporosis. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for treating osteoporosis.

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide an

hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for inhibiting osteoporosis.

n another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting osteoporosis. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for inhibiting osteoporosis .

In another embodiment, the methods of the present invention provide an hGh peptide of the present invention for improving exercise capacity.

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving lung function. In another embodiment, the methods of the present invention provide an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving lung function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP

AA peptide on the C-terminus for improving lung function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving lung function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for improving lung function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for improving lung function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for improving lung function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for improving lung function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for improving lung function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for improving lung function. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for improving lung function.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving lung function. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving lung function. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving lung function. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving lung function.

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving immunity. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving immunity. In another embodiment,

the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving immunity. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving immunity. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for improving immunity. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for improving immunity. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for improving immunity. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for improving immunity. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for improving immunity. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for improving immunity. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for improving immunity.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for improving immunity. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving immunity. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving immunity. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for improving immunity.

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for regrowing vital organs. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for regrowing vital organs. In another embodiment,

the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for regrowing vital organs. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for regrowing vital organs. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for regrowing vital organs. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for regrowing vital organs. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for regrowing vital organs. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for regrowing vital organs. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for regrowing vital organs. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for regrowing vital organs. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for regrowing vital organs.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for regrowing vital organs. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for regrowing vital organs. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for regrowing vital organs. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for regrowing vital organs.

In another embodiment, the methods of the present invention provide an hGh peptide of the present invention for increasing sense of well-being .

In another embodiment, the methods of the present invention provide an hGh peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for restoring REM sleep. In another embodiment, the methods of the present invention provide an hGh peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for restoring REM sleep. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 23 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for restoring REM sleep. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 36 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for restoring REM sleep. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 37 having additionally at least one CTP AA peptide on the N-terminus for restoring REM sleep. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 38 having additionally at least one CTP AA peptide on the N-terminus for restoring REM sleep. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 39 for restoring REM sleep. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 40 for restoring REM sleep. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 41 for restoring REM sleep. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 42 having additionally at least one CTP AA peptide on the N-terminus for restoring REM sleep. In another embodiment, the methods of the present invention provide an hGH peptide set forth in SEQ ID NO: 44 for restoring REM sleep.

In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for restoring REM sleep. In another embodiment, the methods of the present invention provide a nucleic acid sequence encoding an hGH peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for restoring REM sleep. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 45 encoding an hGH peptide comprising one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for restoring REM sleep. In another embodiment, the methods of the present invention provide a nucleic acid of SEQ ID NO: 46 encoding an hGH peptide and one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for restoring REM sleep.

In some embodiments, homology according to the present invention also encompasses deletions, insertions, or substitution variants, including an AA substitution, thereof and biologically active polypeptide fragments thereof. In one embodiment the substitution variant is one, in which the glutamine in position 65 of hGH is substituted by a valine (SEQ ID NO: 23) [Gellerfors et al., J Pharm Biomed Anal 1989, 7:173-83].

In some embodiments, interferon is utilized according to the teachings of the present invention. In some embodiments, the attachment of CTP sequence to both the amino and carboxy termini of the interferon protein results in increased potency. In some embodiments, the attachment of CTP sequence to both the amino and carboxy termini of the interferon protein results in prolonged in-vivo activity.

In one embodiment, "interferon" refers to the mammalian interferon polypeptide Type I. In one embodiment, "interferon" refers to the mammalian interferon polypeptide Type II. In some embodiments, additional suitable interferon polypeptides as known to those of ordinary skill in the art are utilized. In some embodiments, the interferon is alpha-interferon. In some embodiments, the interferon is beta-interferon. In some embodiments, the interferon is gamma-interferon. In some embodiments, the interferon is omega-interferon. In some embodiments, the interferon is a subspecies interferon. In one embodiment, the subspecies interferon (IFN) is IFN-α2a. In one embodiment, the subspecies interferon (IFN) is IFN-α2b. In one embodiment, the subspecies interferon (IFN) is IFN-β1a. In one embodiment, the interferon (IFN) subspecies is IFN-β1b.

In one embodiment, interferon of the present invention exhibits interferon activity, such as antiviral or antiproliferative activity. In some embodiments, GenBank accession nos. of non-limiting examples of interferons are listed in Table 1 below.

In one embodiment, an interferon of the present invention also refers to homologs. In one embodiment, interferon AA sequence of the present invention is at least 50% homologous to interferon sequences listed in Table 1 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, interferon AA sequence of the present invention is at least 60% homologous interferon sequences listed in Table 1 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, interferon AA sequence of the present invention is at least 70% homologous interferon sequences listed in Table 1 as determined using BlastP software of the National Center of Biotechnology Information (NCBI)

using default parameters). In one embodiment, interferon AA sequence of the present invention is at least 80% homologous to interferon sequences listed in Table 1 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, interferon AA sequence of the present invention is at least 90% homologous to interferon sequences listed in Table 1 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, interferon AA sequence of the present invention is at least 95% homologous interferon sequences listed in Table 1 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In some embodiments, homology according to the present invention also encompasses deletions, insertions, or substitution variants, including an AA substitution, thereof and biologically active polypeptide fragments thereof. In one embodiment the cysteine in position 17 of interferon β is substituted by a Serine (SEQ ID NO: 24).

Table 1 below lists examples of interferons with their respective NCBI sequence numbers

Table 1

Interferon name	*NCBI sequence number*
interferon, α 1	NP_076918.1
interferon, α 10	NP_002162.1
interferon, α13	NP_008831.2
interferon, α14	NP_002163.1
interferon, α16	NP_002164.1
interferon, α17	NP_067091.1
interferon, α2	NP_000596.2
interferon, α21	NP_002166.1
interferon, α4	NP_066546.1
interferon, α5	NP_002160.1
interferon, α6	NP_066282.1
interferon, α7	NP_066401.2
interferon, α8	NP_002161.2

interferon, β 1	NP_002167.1
interferon, ε1	NP_795372.1
interferon, γ	NP_000610.2
interferon, ε	NP_064509.1
interferon, Ω1	NP_002168.1

In another embodiment, the methods of the present invention provide an interferon beta 1 peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating or inhibiting multiple sclerosis. In another embodiment, the methods of the present invention provide an interferon beta 1 peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for treating or inhibiting multiple sclerosis. In another embodiment, the methods of the present invention provide an interferon beta 1 peptide set forth in SEQ ID NO: 24 having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for treating or inhibiting multiple sclerosis. In another embodiment, the methods of the present invention provide an interferon beta 1 peptide set forth in SEQ ID NO: 24 having additionally on the N-terminus the signal peptide of SEQ ID NO: 26 and at least one CTP AA peptide on the N-terminus of SEQ ID NO: 26 and at least one CTP AA peptide on the C-terminus of SEQ ID NO: 24 for treating or inhibiting multiple sclerosis.

In some embodiments, glucagon-like peptide-1 is utilized according to the teachings of the present invention. In some embodiments, the attachment of CTP sequences to both the amino and carboxy termini of a "glucagon-like peptide-1" results in increased potency. In some embodiments, the attachment of CTP to both the amino and carboxy termini of a peptide results in prolonged in-vivo activity. In some embodiments, the attachment of CTP to both the amino and carboxy termini of the glucagon-like peptide-results in prolonged in-vivo activity.

In one embodiment, "glucagon-like peptide-1" (GLP-1) refers to a mammalian polypeptide. In one embodiment, "glucagon-like peptide-1" (GLP-1) refers to a human polypeptide. In some embodiments, GLP-1 is cleaved from the glucagon preproprotein (Genbank ID No. NP002045) that has the ability to bind to the GLP-1 receptor and initiate a signal transduction pathway, resulting in insulinotropic activity. In one embodiment, "insulinotropic activity" refers to the ability to stimulate insulin secretion in response to elevated glucose levels, thereby causing

glucose uptake by cells and decreased plasma glucose levels. In some embodiments, GLP-1 polypeptides include, but are not limited to those described in U.S. Pat. No. 5,118,666, which is incorporated by reference herein.

In one embodiment, "GLP-1" refers to a polypeptide, such as set forth in sequences set forth in SEQ ID NO: 25 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, a GLP-1 of the present invention also refers to a GLP-1 homologue. In one embodiment, GLP-1 AA sequence of the present invention is at least 50% homologous to GLP-1 sequences set forth in SEQ ID NO: 25 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, GLP-1 AA sequence of the present invention is at least 60% homologous to GLP-1 sequences set forth in SEQ ID NO: 25 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, GLP-1 AA sequence of the present invention is at least 70% homologous to GLP-1 sequences set forth in SEQ ID NO: 25 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, GLP-1 AA sequence of the present invention is at least 80% homologous to GLP-1 sequences set forth in SEQ ID NO: 25 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, GLP-1 AA sequence of the present invention is at least 90% homologous to GLP-1 sequences set forth in SEQ ID NO: 25 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters). In one embodiment, GLP-1 AA sequence of the present invention is at least 95% homologous to GLP-1 sequences set forth in SEQ ID NO: 25 as determined using BlastP software of the National Center of Biotechnology Information (NCBI) using default parameters).

In another embodiment, the methods of the present invention provide a GLP-1 peptide having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating or inhibiting type II diabetes. In another embodiment, the methods of the present invention provide a GLP-1 peptide having additionally one CTP AA peptide on the N-terminus and two CTP AA peptides on the C-terminus for treating or inhibiting type II diabetes. In another embodiment, the methods of the present invention provide a GLP-1 peptide set forth in SEQ ID NO: 25 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating or inhibiting type II diabetes.

In one embodiment, the homologue also refers to a deletion, insertion, or substitution variant, including an AA substitution, thereof and biologically active polypeptide fragments thereof.

In one embodiment the polypeptide sequence-of-interest is an EPO. In one embodiment the polypeptide sequence-of-interest is an interferon. In another embodiment the polypeptide sequence-of-interest is an hGH. In another embodiment the polypeptide sequence-of-interest is a GLP-1. In another embodiment the polypeptide sequence-of-interest is an insulin. In another embodiment the polypeptide sequence-of-interest is enkephalin. In another embodiment the polypeptide sequence-of-interest is an ACTH. In another embodiment the polypeptide sequence-of-interest is a glucagon. In another embodiment the polypeptide sequence-of-interest is an insulin-like growth factor. In another embodiment the polypeptide sequence-of-interest is an epidermal growth factor. In another embodiment the polypeptide sequence-of-interest is an acidic or basic fibroblast growth factor. In another embodiment the polypeptide sequence-of-interest is a platelet-derived growth factor. In another embodiment the polypeptide sequence-of-interest is a granulocyte-CSF. In another embodiment the polypeptide sequence-of-interest is a macrophage-CSF. In another embodiment the polypeptide sequence-of-interest is an IL-2. In another embodiment the polypeptide sequence-of-interest is an IL-3. In another embodiment the polypeptide sequence-of-interest is a tumor necrosis factor. In another embodiment the polypeptide sequence-of-interest is an LHRH. In another embodiment the polypeptide sequence-of-interest is an LHRH analog. In another embodiment the polypeptide sequence-of-interest is a somatostatin. In another embodiment the polypeptide sequence-of-interest is a growth hormone releasing factor. In another embodiment the polypeptide sequence-of-interest is an endorphin. In another embodiment the polypeptide sequence-of-interest is an alveolar surfactant protein. In another embodiment the polypeptide sequence-of-interest is a natriuretic factor. In another embodiment the polypeptide sequence-of-interest is an adhesin. In another embodiment the polypeptide sequence-of-interest is an angiostatin. In another embodiment the polypeptide sequence-of-interest is an endostatin. In another embodiment the polypeptide sequence-of-interest is a receptor peptide. In another embodiment the polypeptide sequence-of-interest is a receptor binding ligand. In another embodiment the polypeptide sequence-of-interest is an antibody In another embodiment the polypeptide sequence-of-interest is an antibody fragment. In another embodiment the polypeptide sequence-of-interest is a peptide or a protein including any modified form.

In another embodiment, the peptide of the invention comprises a peptide of interest having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus. In another embodiment, the peptide of interest having additionally at least one CTP

.AA peptide on the N-terminus and one CTP AA peptide on the C-terminus comprises a protein selected from the following list: insulin, Albutein/albumin, Activase altiplase/tPA, adenosine deaminase, immune globulin, glucocerebrosidase, Leukine-sargramostim/GM-CSF, G-CSF, Venoglobulin-S/IgG, Proleukin aldesleukin, DNase, factor VIII, Helixate, L-asparaginase, WinRho SDF Rh I, Retavase retaplase/tPA, Factor IX, FSH, globulin, fibrin, interleukin-11, becaplermin/PDGF, lepirudin/herudin, TNF, Thymoglobulin, factor VIIa, interferon alpha-2a, interferon alfa n-1, interferon alfa-N3, interferon beta-1b, interferon gamma-1b, Interleukin-2, HGH, or monoclonal antibodies.

In another embodiment, the methods of the present invention provide insulin having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of diabetes.

In another embodiment, the methods of the present invention provide albumin having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of hypovolemic shock, hemodialysis or cardiopulmonary bypass.

In another embodiment, the methods of the present invention provide Activase- altiplase/tPA having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of acute myocardial infarction, acute massive pulmonary embolism, or (change throughout) ischemic stroke.

In another embodiment, the methods of the present invention provide adenosine deaminase having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of severe combined immunodeficiency disease.

In another embodiment, the methods of the present invention provide immune globulin having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of transplant recipients.

In another embodiment, the methods of the present invention provide immune globulin is a CMV immune globulin. In another embodiment, the methods of the present invention provide glucocerebrosidase having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of Gaucher disease.

In another embodiment, the methods of the present invention provide Leukine-sargramostim/GM-CSF having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the Stimulation of hematopoietic progenitor cells.

In another embodiment, the methods of the present invention provide G-CSF having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of Neutropenia. In another embodiment, the methods of the present invention provide Venoglobulin-S/IgG having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of Immunodeficiency diseases.

In another embodiment, the methods of the present invention provide Proleukin-aldesleukin having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of renal carcinoma or metastatic melanoma.

In another embodiment, the methods of the present invention provide DNase having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of Cystic fibrosis.

In another embodiment, the methods of the present invention provide factor VIII having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of Hemophilia A.

In another embodiment, the methods of the present invention provide Helixate having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of Hemophilia A.

In another embodiment, the methods of the present invention provide L-asparaginase having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of acute lymphoblastic leukemia.

In another embodiment, the methods of the present invention provide WinRho SDF Rh IgG having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of of Rh isoimmunization and immune thrombocytopenic purpura.

In another embodiment, the methods of the present invention provide Retavase retaplase/tPA having additionally at least one CTP AA peptide on the N-terminus and one CTP AA peptide on the C-terminus for the treatment of acute myocardial infarction.

In another embodiment, the methods of the present invention provide Factor IX having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of Hemophilia B.

In another embodiment, the methods of the present invention provide Factor IX having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of Hemophilia B.

In another embodiment, the methods of the present invention provide FSH having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for stimulation of ovulation during assisted reproduction.

In another embodiment, the methods of the present invention provide globulin having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the prevention of respiratory syncytial virus disease.

In another embodiment, the methods of the present invention provide fibrin having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for wound management and hemostasis. In another embodiment, the methods of the present invention provide interleukin-11 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for chemotherapy-induced thrombocytopenia.

In another embodiment, the methods of the present invention provide becaplermin/PDGF having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of diabetic foot ulcers.

In another embodiment, the methods of the present invention provide lepirudin/herudin having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for anticoagulation in heparin-induced thrombocytopenia.

In another embodiment, the methods of the present invention provide soluble TNF having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of rheumatoid arthritis.

In another embodiment, the methods of the present invention provide Thymoglobulin having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of organ transplant rejection disease.

In another embodiment, the methods of the present invention provide factor VIIa having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of hemophilia.

In another embodiment, the methods of the present invention provide interferon alpha-2a having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of hairy cell leukemia and AIDS-related Kaposi's sarcoma.

In another embodiment, the methods of the present invention provide interferon alpha-2b having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of Hairy cell leukemia, genital warts, AIDS-related Kaposi's sarcoma, hepatitis C, hepatitis B, malignant melanoma, and follicular lymphoma.

In another embodiment, the methods of the present invention provide interferon alfa-N3 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of genital warts.

In another embodiment, the methods of the present invention provide interferon gamma-1b having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of chronic granulomatous disease.

In another embodiment, the methods of the present invention provide interferon alfa n-1 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of hepatitis C infection.

In another embodiment, the methods of the present invention provide Interleukin-2 having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of renal carcinoma and metastatic melanoma.

In another embodiment, the methods of the present invention provide interferon beta-1b having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of multiple sclerosis.

In another embodiment, the methods of the present invention provide hGH having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for the treatment of wasting disease, AIDS, cachexia, or hGH deficiency.

In another embodiment, the methods of the present invention provide an OKT3 monoclonal antibody having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for organ transplant.

In another embodiment, the methods of the present invention provide a Reo monoclonal antibody having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for prevention of complications from coronary intervention and angioplasty.

In another embodiment, the methods of the present invention provide a monoclonal antibody having additionally at least one CTP AA peptide on the N-terminus and at least one CTP AA peptide on the C-terminus for treating colorectal cancer, Non-Hodgkin's lymphoma, kidney transplant rejection, metastatic breast cancer, or the prevention of respiratory syncytial virus disease.

In some embodiments, the CTP sequences modification is advantageous in permitting lower dosages to be used.

In some embodiments, "polypeptide" as used herein encompasses native polypeptides (either degradation products, synthetically synthesized polypeptides or recombinant polypeptides) and peptidomimetics (typically, synthetically synthesized polypeptides), as well as peptoids and semipeptoids which are polypeptide analogs, which have, in some embodiments, modifications rendering the polypeptides even more stable while in a body or more capable of penetrating into cells.

In some embodiments, modifications include, but are not limited to N terminus modification, C terminus modification, polypeptide bond modification, including, but not limited to, CH2-NH, CH2-S, CH2-S=O, O=C-NH, CH2-O, CH2-CH2, S=C-NH, CH=CH or CF=CH, backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992), which is incorporated by reference as if fully set forth herein. Further details in this respect are provided hereinunder.

In some embodiments, polypeptide bonds (-CO-NH-) within the polypeptide are substituted. In some embodiments, the polypeptide bonds are substituted by N-methylated bonds (-N(CH3)-CO-). In some embodiments, the polypeptide bonds are substituted by ester bonds (-C(R)H-C-O-O-C(R)-N-). In some embodiments, the polypeptide bonds are substituted by ketomethylen

bonds (-CO-CH2-). In some embodiments, the polypeptide bonds are substituted by α-aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH2-NH-). In some embodiments, the polypeptide bonds are substituted by hydroxyethylene bonds (-CH(OH)-CH2-). In some embodiments, the polypeptide bonds are substituted by thioamide bonds (-CS-NH-). In some embodiments, the polypeptide bonds are substituted by olefinic double bonds (-CH=CH-). In some embodiments, the polypeptide bonds are substituted by retro amide bonds (-NH-CO-). In some embodiments, the polypeptide bonds are substituted by polypeptide derivatives (-N(R)-CH2-CO-), wherein R is the "normal" side chain, naturally presented on the carbon atom. In some embodiments, these modifications occur at any of the bonds along the polypeptide chain and even at several (2-3 bonds) at the same time.

In some embodiments, natural aromatic AA of the polypeptide such as Trp, Tyr and Phe, be substituted for synthetic non-natural acid such as Phenylglycine, TIC, naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr. In some embodiments, the polypeptides of the present invention include one or more modified AA or one or more non-AA monomers (e.g. fatty acid, complex carbohydrates etc).

In one embodiment, "AA" or "AA" is understood to include the 20 naturally occurring AA; those AA often modified post-translationally *in vivo*, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual AA including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. In one embodiment, "AA" includes both D- and L-AA.

In some embodiments, the polypeptides of the present invention are utilized in therapeutics which require the polypeptides to be in a soluble form. In some embodiments, the polypeptides of the present invention include one or more non-natural or natural polar AA, including but not limited to serine and threonine which are capable of increasing polypeptide solubility due to their hydroxyl-containing side chain.

In some embodiments, the polypeptides of the present invention are are utilized in a linear form, although it will be appreciated by one skilled in the art that in cases where cyclicization does not severely interfere with polypeptide characteristics, cyclic forms of the polypeptide can also be utilized.

In some embodiments, the polypeptides of present invention are biochemically synthesized such as by using standard solid phase techniques. In some embodiments, these biochemical methods include exclusive solid phase synthesis, partial solid phase synthesis, fragment condensation, or

classical solution synthesis. In some embodiments, these methods are used when the polypeptide is relatively short (about 5-15kDa) and/or when it cannot be produced by recombinant techniques (i.e., not encoded by a nucleic acid sequence) and therefore involves different chemistry.

In some embodiments, solid phase polypeptide synthesis procedures are well known to one skilled in the art and further described by John Morrow Stewart and Janis Dillaha Young, Solid Phase Polypeptide Syntheses (2nd Ed., Pierce Chemical Company, 1984). In some embodiments, synthetic polypeptides are purified by preparative high performance liquid chromatography [Creighton T. (1983) Proteins, structures and molecular principles. WH Freeman and Co. N.Y.] and the composition of which can be confirmed via AA sequencing by methods known to one skilled in the art.

In some embodiments, recombinant protein techniques are used to generate the polypeptides of the present invention. In some embodiments, recombinant protein techniques are used for generation of relatively long polypeptides (e.g., longer than 18-25 AAs). In some embodiments, recombinant protein techniques are used for the generation of large amounts of the polypeptide of the present invention. In some embodiments, recombinant techniques are described by Bitter et al., (1987) Methods in Enzymol. 153:516-544, Studier et al. (1990) Methods in Enzymol. 185:60-89, Brisson et al. (1984) Nature 310:511-514, Takamatsu et al. (1987) EMBO J. 6:307-311, Coruzzi et al. (1984) EMBO J. 3:1671-1680 and Brogli et al., (1984) Science 224:838-843, Gurley et al. (1986) Mol. Cell. Biol. 6:559-565 and Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463.

In one embodiment, a polypeptide of the present invention is synthesized using a polynucleotide encoding a polypeptide of the present invention. In some embodiments, the polynucleotide encoding a polypeptide of the present invention is ligated into an expression vector, comprising a transcriptional control of a cis-regulatory sequence (e.g., promoter sequence). In some embodiments, the cis-regulatory sequence is suitable for directing constitutive expression of the polypeptide of the present invention. In some embodiments, the cis-regulatory sequence is suitable for directing tissue specific expression of the polypeptide of the present invention. In some embodiments, the cis-regulatory sequence is suitable for directing inducible expression of the polypeptide of the present invention.

In some embodiments, polynucleotides which express the polypeptides of the present invention are as set forth in SEQ ID NOs: 20, 21, 44, 45 and 46.

In some embodiment, tissue-specific promoters suitable for use with the present invention include sequences which are functional in specific cell population, example include, but are not limited to promoters such as albumin that is liver specific [Pinkert et al., (1987) Genes Dev. 1:268-277], lymphoid specific promoters [Calame et al., (1988) Adv. Immunol. 43:235-275]; in particular promoters of T-cell receptors [Winoto et al., (1989) EMBO J. 8:729-733] and immunoglobulins; [Banerji et al. (1983) Cell 33729-740], neuron-specific promoters such as the neurofilament promoter [Byrne et al. (1989) Proc. Natl. Acad. Sci. USA 86:5473-5477], pancreas-specific promoters [Edlunch et al. (1985) Science 230:912-916] or mammary gland-specific promoters such as the milk whey promoter (U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Inducible promoters suitable for use with the present invention include for example the tetracycline-inducible promoter (Srour, M.A., et al., 2003. Thromb. Haemost. 90: 398-405).

In one embodiment, the phrase "a polynucleotide" refers to a single or double stranded nucleic acid sequence which be isolated and provided in the form of an RNA sequence, a complementary polynucleotide sequence (cDNA), a genomic polynucleotide sequence and/or a composite polynucleotide sequences (e.g., a combination of the above).

In one embodiment, "complementary polynucleotide sequence" refers to a sequence, which results from reverse transcription of messenger RNA using a reverse transcriptase or any other RNA dependent DNA polymerase. In one embodiment, the sequence can be subsequently amplified *in vivo* or *in vitro* using a DNA polymerase.

In one embodiment, "genomic polynucleotide sequence" refers to a sequence derived (isolated) from a chromosome and thus it represents a contiguous portion of a chromosome.

In one embodiment, "composite polynucleotide sequence" refers to a sequence, which is at least partially complementary and at least partially genomic. In one embodiment, a composite sequence can include some exonal sequences required to encode the polypeptide of the present invention, as well as some intronic sequences interposing therebetween. In one embodiment, the intronic sequences can be of any source, including of other genes, and typically will include conserved splicing signal sequences. In one embodiment, intronic sequences include cis acting expression regulatory elements.

In one embodiment, the polynucleotides of the present invention further comprise a signal sequence encoding a signal peptide for the secretion of the polypeptides of the present invention. In some embodiments, signal sequences include, but are not limited to the endogenous signal

sequence for EPO as set forth in SEQ ID NO: 19 or the endogenous signal sequence for IFN-□1 as set forth in SEQ ID NO: 26. In another embodiment, the signal sequence is N-terminal to the CTP sequence that is in turn N-terminal to the polypeptide sequence of interest; e.g. the sequence is (a) signal sequence- (b) CTP- (c) sequence-of-interest- (d) optionally 1 or more additional CTP sequences. In another embodiment, 1 or more CTP sequences is inserted between the signal sequence of a polypeptide sequence of interest and the polypeptide sequence of interest itself, thus interrupting the wild-type sequence of interest. Each possibility represents a separate embodiment of the present invention.

In one embodiment, following expression and secretion, the signal peptides are cleaved from the precursor proteins resulting in the mature proteins.

In some embodiments, polynucleotides of the present invention are prepared using PCR techniques as described in Example 1, or any other method or procedure known to one skilled in the art. In some embodiments, the procedure involves the ligation of two different DNA sequences (See, for example, "Current Protocols in Molecular Biology", eds. Ausubel et al., John Wiley & Sons, 1992).

In one embodiment, polynucleotides of the present invention are inserted into expression vectors (i.e., a nucleic acid construct) to enable expression of the recombinant polypeptide. In one embodiment, the expression vector of the present invention includes additional sequences which render this vector suitable for replication and integration in prokaryotes. In one embodiment, the expression vector of the present invention includes additional sequences which render this vector suitable for replication and integration in eukaryotes. In one embodiment, the expression vector of the present invention includes a shuttle vector which renders this vector suitable for replication and integration in both prokaryotes and eukaryotes. In some embodiments, cloning vectors comprise transcription and translation initiation sequences (e.g., promoters, enhances) and transcription and translation terminators (e.g., polyadenylation signals).

In one embodiment, a variety of prokaryotic or eukaryotic cells can be used as host-expression systems to express the polypeptides of the present invention. In some embodiments, these include, but are not limited to, microorganisms, such as bacteria transformed with a recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vector containing the polypeptide coding sequence; yeast transformed with recombinant yeast expression vectors containing the polypeptide coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with

recombinant plasmid expression vectors, such as Ti plasmid, containing the polypeptide coding sequence.

In some embodiments, non-bacterial expression systems are used (e.g. mammalian expression systems such as CHO cells) to express the polypeptide of the present invention. In one embodiment, the expression vector used to express polynucleotides of the present invention in mammalian cells is pCI-DHFR vector comprising a CMV promoter and a neomycin resistance gene. Construction of the pCI-dhfr vector is described, according to one embodiment, in Example 1.

In some embodiments, in bacterial systems of the present invention, a number of expression vectors can be advantageously selected depending upon the use intended for the polypeptide expressed. In one embodiment, large quantities of polypeptide are desired. In one embodiment, vectors that direct the expression of high levels of the protein product, possibly as a fusion with a hydrophobic signal sequence, which directs the expressed product into the periplasm of the bacteria or the culture medium where the protein product is readily purified are desired. In one embodiment, certain fusion protein engineered with a specific cleavage site to aid in recovery of the polypeptide. In one embodiment, vectors adaptable to such manipulation include, but are not limited to, the pET series of *E. coli* expression vectors [Studier *et al.*, Methods in Enzymol. 185:60-89 (1990)].

In one embodiment, yeast expression systems are used. In one embodiment, a number of vectors containing constitutive or inducible promoters can be used in yeast as disclosed in U.S. Pat. Application No: 5,932,447. In another embodiment, vectors which promote integration of foreign DNA sequences into the yeast chromosome are used.

In one embodiment, the expression vector of the present invention can further include additional polynucleotide sequences that allow, for example, the translation of several proteins from a single mRNA such as an internal ribosome entry site (IRES) and sequences for genomic integration of the promoter-chimeric polypeptide.

In some embodiments, mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Strategene, pTRES which is available from Clontech, and their derivatives.

In some embodiments, expression vectors containing regulatory elements from eukaryotic viruses such as retroviruses are used by the present invention. SV40 vectors include pSVT7 and pMT2. In some embodiments, vectors derived from bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include pHEBO, and p2O5. Other exemplary vectors include pMSG, pAV009/A^+, pMTO10/A^+, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

In some embodiments, recombinant viral vectors are useful for *in vivo* expression of the polypeptides of the present invention since they offer advantages such as lateral infection and targeting specificity. In one embodiment, lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. In one embodiment, the result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. In one embodiment, viral vectors are produced that are unable to spread laterally. In one embodiment, this characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

In one embodiment, various methods can be used to introduce the expression vector of the present invention into cells. Such methods are generally described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., Gene Targeting, CRC Press, Ann Arbor Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and Gilboa et at. [Biotechniques 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

In some embodiments, introduction of nucleic acid by viral infection offers several advantages over other methods such as lipofection and electroporation, since higher transfection efficiency can be obtained due to the infectious nature of viruses.

In one embodiment, it will be appreciated that the polypeptides of the present invention can also be expressed from a nucleic acid construct administered to the individual employing any suitable

mode of administration, described hereinabove (i.e., in-vivo gene therapy). In one embodiment, the nucleic acid construct is introduced into a suitable cell via an appropriate gene delivery vehicle/method (transfection, transduction, homologous recombination, etc.) and an expression system as needed and then the modified cells are expanded in culture and returned to the individual (i.e., ex-vivo gene therapy).

In one embodiment, in vivo gene therapy using EPO has been attempted in animal models such as rodents [Bohl et al., Blood. 2000; 95:2793-2798], primates [Gao et al., Blood, 2004, Volume 103, Number 9] and has proven successful in human clinical trials for patients with chronic renal failure [Lippin et al Blood 2005, 106, Number 7].

In one embodiment, plant expression vectors are used. In one embodiment, the expression of a polypeptide coding sequence is driven by a number of promoters. In some embodiments, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV [Brisson *et al.*, Nature 310:511-514 (1984)], or the coat protein promoter to TMV [Takamatsu *et al.*, EMBO J. 6:307-311 (1987)] are used. In another embodiment,, plant promoters are used such as, for example, the small subunit of RUBISCO [Coruzzi *et al.*, EMBO J. 3:1671-1680 (1984); and Brogli *et al.*, Science 224:838-843 (1984)] or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B [Gurley *et al.*, Mol. Cell. Biol. 6:559-565 (1986)]. In one embodiment, constructs are introduced into plant cells using Ti plasmid, Ri plasmid, plant viral vectors, direct DNA transformation, microinjection, electroporation and other techniques well known to the skilled artisan. See, for example, Weissbach & Weissbach [Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463 (1988)]. Other expression systems such as insects and mammalian host cell systems, which are well known in the art, can also be used by the present invention.

It will be appreciated that other than containing the necessary elements for the transcription and translation of the inserted coding sequence (encoding the polypeptide), the expression construct of the present invention can also include sequences engineered to optimize stability, production, purification, yield or activity of the expressed polypeptide.

Various methods, in some embodiments, can be used to introduce the expression vector of the present invention into the host cell system. In some embodiments, such methods are generally described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., Gene Targeting, CRC Press, Ann Arbor Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass.

(1988) and Gilboa et at. [Biotechniques 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

In some embodiments, transformed cells are cultured under effective conditions, which allow for the expression of high amounts of recombinant polypeptide. In some embodiments, effective culture conditions include, but are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. In one embodiment, an effective medium refers to any medium in which a cell is cultured to produce the recombinant polypeptide of the present invention. In some embodiments, a medium typically includes an aqueous solution having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. In some embodiments, cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes and petri plates. In some embodiments, culturing is carried out at a temperature, pH and oxygen content appropriate for a recombinant cell. In some embodiments, culturing conditions are within the expertise of one of ordinary skill in the art.

In some embodiments, depending on the vector and host system used for production, resultant polypeptides of the present invention either remain within the recombinant cell, secreted into the fermentation medium, secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or retained on the outer surface of a cell or viral membrane.

In one embodiment, following a predetermined time in culture, recovery of the recombinant polypeptide is effected.

In one embodiment, the phrase "recovering the recombinant polypeptide" used herein refers to collecting the whole fermentation medium containing the polypeptide and need not imply additional steps of separation or purification.

In one embodiment, polypeptides of the present invention are purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization.

In one embodiment, to facilitate recovery, the expressed coding sequence can be engineered to encode the polypeptide of the present invention and fused cleavable moiety. In one embodiment,

a fusion protein can be designed so that the polypeptide can be readily isolated by affinity chromatography; e.g., by immobilization on a column specific for the cleavable moiety. In one embodiment, a cleavage site is engineered between the polypeptide and the cleavable moiety and the polypeptide can be released from the chromatographic column by treatment with an appropriate enzyme or agent that specifically cleaves the fusion protein at this site [e.g., see Booth *et al.*, Immunol. Lett. 19:65-70 (1988); and Gardella *et al.*, J. Biol. Chem. 265:15854-15859 (1990)].

In one embodiment, the polypeptide of the present invention is retrieved in "substantially pure" form.

In one embodiment, the phrase "substantially pure" refers to a purity that allows for the effective use of the protein in the applications described herein.

In one embodiment, the polypeptide of the present invention can also be synthesized using *in vitro* expression systems. In one embodiment, *in vitro* synthesis methods are well known in the art and the components of the system are commercially available.

In one embodiment, production of CTP-EPO-CTP polypeptides using recombinant DNA technology is illustrated in Example 1.

In some embodiments, the recombinant polypeptides are synthesized and purified; their therapeutic efficacy can be assayed either in vivo or in vitro. In one embodiment, the binding activities of the recombinant EPO polypeptides of the present invention can be ascertained using various assays as described in Examples 2-6 and 8-9. In one embodiment, in vitro binding activity is ascertained by measuring the ability of the polypeptide to stimulate proliferation of TF-1 cells. In one embodiment, in vivo activity is deduced by analyzing heamatocrit levels (Figures 3-5) and/or as a percentage of reticulocytes.

In one embodiment, the EPO polypeptides of the present invention can be used to treat a subject, with a variety of erythropoietin-associated conditions. In some embodiments, a subject is a human subject.

In some embodiment, the phrase "erythropoietin-associated conditions" refers to any condition associated with below normal, abnormal, or inappropriate modulation of erythropoietin. In some embodiment, levels of erythropoietin associated with such conditions are determined by any measure accepted and utilized by those of skill in the art. In some embodiment, erythropoietin-associated conditions typically include anemic conditions.

In some embodiment, "anemic conditions" refers to any condition, disease, or disorder associated with anemia. In some embodiment, anemic conditions include, but are not limited to, aplastic anemia, autoimmune hemolytic anemia, bone marrow transplantation, Churg-Strauss syndrome, Diamond Blackfan anemia, Fanconi's anemia, Felty syndrome, graft versus host disease, hematopoietic stem cell transplantation, hemolytic uremic syndrome, myelodysplasic syndrome, nocturnal paroxysmal hemoglobinuria, osteomyelofibrosis, pancytopenia, pure red-cell aplasia, purpura Schoenlein-Henoch, sideroblastic anemia, refractory anemia with excess of blasts, rheumatoid arthritis, Shwachman syndrome, sickle cell disease, thalassemia major, thalassemia minor, thrombocytopenic purpura, etc.

In one embodiment, the present invention comprises CTP-hGH-CTP polypeptides. In one embodiment, recombinant DNA technology methods are used for the production of CTP-hGH-CTP polypeptides as illustrated in Example 7. In one embodiment, the therapeutic efficacy of the CTP-hGH-CTP polypeptides of the present invention is assayed either in vivo. In one embodiment, the therapeutic efficacy of the CTP-hGH-CTP polypeptides of the present invention is assayed either in vitro. In one embodiment, the binding activities of the recombinant hGH polypeptides of the present invention are measured using Nb2 (a prolactin-dependent rat lymphoma cell line (ECACC Cell Bank)) or a FCD-P1 murine cell line, previously transfected with human growth hormone receptor. In one embodiment, binding of hGH to these receptors induces cell proliferation which in one embodiment is measured by the levels of MTT cellular stain as a function of hGH activity. In one embodiment, in vivo activity is deduced by measuring weight gain over time in treated growth hormone deficient animals.

In some embodiment, human growth hormone polypeptides of the present invention can be used to treat a subject, with conditions related to growth and weight, such as a growth deficiency disorder, AIDS wasting, aging, impaired immune function of HIV-infected subjects, a catabolic illness, surgical recovery, a congestive cardiomyopathy, liver transplantation, liver regeneration

after hepatectomy, chronic renal failure, renal osteodystrophy, osteoporosis, achondroplasia/hypochondroplasia, skeletal dysplasia, a chronic inflammatory or nutritional disorder such as Crohn's disease, short bowel syndrome, juvenile chronic arthritis, cystic fibrosis, male infertility, X-linked hypophosphatemic rickets, Down's syndrome, Spina bifida, Noonan Syndrome, obesity, impaired muscle strength and fibromyalgia.

In some embodiments, interferon polypeptides of the present invention are used to treat a subject, with a variety of conditions such as hairy cell leukemia (HCL), Kaposi's sarcoma (KS), chronic myelogenous leukemia (CML), chronic hepatitis C (CHC), condylomata acuminata (CA), chronic hepatitis B, malignant melanoma, follicular non-Hodgkin's lymphoma, multiple sclerosis, chronic granulomatous disease, Mycobacterium avium complex (MAC), pulmonary fibrosis and osteoporosis.

In some embodiments, Glucagon-like peptide-1(GLP-1) polypeptides of the present invention are used to treat a subject with non-insulin dependent diabetes, obesity, stroke, myocardial infarction, stroke, stress-induced hyperglycemia, or irritable bowel syndrome.

In one embodiment, the polypeptides of the present invention can be provided to the individual *per se*. In one embodiment, the polypeptides of the present invention can be provided to the individual as part of a pharmaceutical composition where it is mixed with a pharmaceutically acceptable carrier.

In one embodiment, a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

In one embodiment, "active ingredient" refers to the polypeptide sequence of interest, which is accountable for the biological effect.

In some embodiments, any of the compositions of this invention will comprise at least two CTP sequences bound to a protein of interest, in any form. In one embodiment, the present invention provides combined preparations. In one embodiment, "a combined preparation" defines especially a "kit of parts" in the sense that the combination partners as defined above can be dosed independently or by use of different fixed combinations with distinguished amounts of the combination partners i.e., simultaneously, concurrently, separately or sequentially. In some embodiments, the parts of the kit of parts can then, e.g., be administered simultaneously or chronologically staggered, that is at

different time points and with equal or different time intervals for any part of the kit of parts. The ratio of the total amounts of the combination partners, in some embodiments, can be administered in the combined preparation. In one embodiment, the combined preparation can be varied, e.g., in order to cope with the needs of a patient subpopulation to be treated or the needs of the single patient which different needs can be due to a particular disease, severity of a disease, age, sex, or body weight as can be readily made by a person skilled in the art.

In one embodiment, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases. In one embodiment, one of the ingredients included in the pharmaceutically acceptable carrier can be for example polyethylene glycol (PEG), a biocompatible polymer with a wide range of solubility in both organic and aqueous media (Mutter et al. (1979).

In one embodiment, "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. In one embodiment, excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs are found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

In one embodiment, suitable routes of administration , for example, include oral, rectal, transmucosal, transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intravenous, inrtaperitoneal, intranasal, or intraocular injections.

In one embodiment, the preparation is administered in a local rather than systemic manner, for example, via injection of the preparation directly into a specific region of a patient's body.

Various embodiments of dosage ranges are contemplated by this invention. The dosage of the polypeptide of the present invention, in one embodiment, is in the range of 0.05-80 mg/day. In another embodiment, the dosage is in the range of 0.05-50 mg/day. In another embodiment, the dosage is in the range of 0.1-20 mg/day. In another embodiment, the dosage is in the range of 0.1-10 mg/day. In another embodiment, the dosage is in the range of 0.1-5 mg/day. In another embodiment,

the dosage is in the range of 0.5-5 mg/day. In another embodiment, the dosage is in the range of 0.5-50 mg/day. In another embodiment, the dosage is in the range of 5-80 mg/day. In another embodiment, the dosage is in the range of 35-65 mg/day. In another embodiment, the dosage is in the range of 35-65 mg/day. In another embodiment, the dosage is in the range of 20-60 mg/day. In another embodiment, the dosage is in the range of 40-60 mg/day. In another embodiment, the dosage is in a range of 45-60 mg/day. In another embodiment, the dosage is in the range of 40-60 mg/day. In another embodiment, the dosage is in a range of 60-120 mg/day. In another embodiment, the dosage is in the range of 120-240 mg/day. In another embodiment, the dosage is in the range of 40-60 mg/day. In another embodiment, the dosage is in a range of 240-400 mg/day. In another embodiment, the dosage is in a range of 45-60 mg/day. In another embodiment, the dosage is in the range of 15-25 mg/day. In another embodiment, the dosage is in the range of 5-10 mg/day. In another embodiment, the dosage is in the range of 55-65 mg/day.

In one embodiment, the dosage is 20 mg/day. In another embodiment, the dosage is 30 mg/day. In another embodiment, the dosage is 40 mg/day. In another embodiment, the dosage is 50 mg/day. In another embodiment, the dosage is 60 mg/day. In another embodiment, the dosage is 70 mg/day. In another embodiment, the dosage is 80 mg/day. In another embodiment, the dosage is 90 mg/day. In another embodiment, the dosage is 100 mg/day.

Oral administration, in one embodiment, comprises a unit dosage form comprising tablets, capsules, lozenges, chewable tablets, suspensions, emulsions and the like. Such unit dosage forms comprise a safe and effective amount of the desired compound, or compounds, each of which is in one embodiment, from about 0.7 or 3.5 mg to about 280 mg/70 kg, or in another embodiment, about 0.5 or 10 mg to about 210 mg/70 kg. The pharmaceutically-acceptable carriers suitable for the preparation of unit dosage forms for peroral administration are well-known in the art. In some embodiments, tablets typically comprise conventional pharmaceutically-compatible adjuvants as inert diluents, such as calcium carbonate, sodium carbonate, mannitol, lactose and cellulose; binders such as starch, gelatin and sucrose; disintegrants such as starch, alginic acid and croscarmelose; lubricants such as magnesium stearate, stearic acid and talc. In one embodiment, glidants such as silicon dioxide can be used to improve flow characteristics of the powder-mixture. In one embodiment, coloring agents, such as the FD&C dyes, can be added for appearance. Sweeteners and flavoring agents, such as aspartame, saccharin, menthol, peppermint, and fruit flavors, are useful adjuvants for chewable tablets. Capsules typically comprise one or more solid diluents disclosed above. In some embodiments, the selection of carrier components depends on secondary considerations like taste, cost, and shelf stability, which are not critical for the purposes of this invention, and can be readily made by a person skilled in the art.

In one embodiment, the oral dosage form comprises predefined release profile. In one embodiment, the oral dosage form of the present invention comprises an extended release tablets, capsules, lozenges or chewable tablets. In one embodiment, the oral dosage form of the present invention comprises a slow release tablets, capsules, lozenges or chewable tablets. In one embodiment, the oral dosage form of the present invention comprises an immediate release tablets, capsules, lozenges or chewable tablets. In one embodiment, the oral dosage form is formulated according to the desired release profile of the pharmaceutical active ingredient as known to one skilled in the art.

Peroral compositions, in some embodiments, comprise liquid solutions, emulsions, suspensions, and the like. In some embodiments, pharmaceutically-acceptable carriers suitable for preparation of such compositions are well known in the art. In some embodiments, liquid oral compositions comprise from about 0.012% to about 0.933% of the desired compound or compounds, or in another embodiment, from about 0.033% to about 0.7%.

In some embodiments, compositions for use in the methods of this invention comprise solutions or emulsions, which in some embodiments are aqueous solutions or emulsions comprising a safe and effective amount of the compounds of the present invention and optionally, other compounds, intended for topical intranasal administration. In some embodiments, h compositions comprise from about 0.01% to about 10.0% w/v of a subject compound, more preferably from about 0.1% to about 2.0, which is used for systemic delivery of the compounds by the intranasal route.

In another embodiment, the pharmaceutical compositions are administered by intravenous, intra-arterial, or intramuscular injection of a liquid preparation. In some embodiments, liquid formulations include solutions, suspensions, dispersions, emulsions, oils and the like. In one embodiment, the pharmaceutical compositions are administered intravenously, and are thus formulated in a form suitable for intravenous administration. In another embodiment, the pharmaceutical compositions are administered intra-arterially, and are thus formulated in a form suitable for intra-arterial administration. In another embodiment, the pharmaceutical compositions are administered intramuscularly, and are thus formulated in a form suitable for intramuscular administration.

Further, in another embodiment, the pharmaceutical compositions are administered topically to body surfaces, and are thus formulated in a form suitable for topical administration. Suitable topical formulations include gels, ointments, creams, lotions, drops and the like. For topical administration, the compounds of the present invention are combined with an additional appropriate therapeutic agent or agents, prepared and applied as solutions, suspensions, or emulsions in a physiologically acceptable diluent with or without a pharmaceutical carrier.

In one embodiment, pharmaceutical compositions of the present invention are manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

In one embodiment, pharmaceutical compositions for use in accordance with the present invention is formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. In one embodiment, formulation is dependent upon the route of administration chosen.

In one embodiment, injectables, of the invention are formulated in aqueous solutions. In one embodiment, injectables, of the invention are formulated in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. In some embodiments, for transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

In one embodiment, the preparations described herein are formulated for parenteral administration, e.g., by bolus injection or continuous infusion. In some embodiments, formulations for injection are presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. In some embodiments, compositions are suspensions, solutions or emulsions in oily or aqueous vehicles, and contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

The compositions also comprise, in some embodiments, preservatives, such as benzalkonium chloride and thimerosal and the like; chelating agents, such as edetate sodium and others; buffers such as phosphate, citrate and acetate; tonicity agents such as sodium chloride, potassium chloride, glycerin, mannitol and others; antioxidants such as ascorbic acid, acetylcystine, sodium metabisulfote and others; aromatic agents; viscosity adjustors, such as polymers, including cellulose and derivatives thereof; and polyvinyl alcohol and acid and bases to adjust the pH of these aqueous compositions as needed. The compositions also comprise, in some embodiments, local anesthetics or other actives. The compositions can be used as sprays, mists, drops, and the like.

In some embodiments, pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients , in some embodiments, are prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include, in some embodiments, fatty oils such as sesame oil, or synthetic fatty acid esters such as ethyl oleate, triglycerides or

liposomes. Aqueous injection suspensions contain, in some embodiments, substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. In another embodiment, the suspension also contains suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

In another embodiment, the active compound can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez- Berestein and Fidler (eds.), Liss, New York, pp. 353-365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid).

In another embodiment, the pharmaceutical composition delivered in a controlled release system is formulated for intravenous infusion, implantable osmotic pump, transdermal patch, liposomes, or other modes of administration. In one embodiment, a pump is used (see Langer, supra; Sefton, CRC Crit. Ref. *Biomed. Eng.* 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989). In another embodiment, polymeric materials can be used. In yet another embodiment, a controlled release system can be placed in proximity to the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984). Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990).

In some embodiments, the active ingredient is in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use. Compositions are formulated, in some embodiments, for atomization and inhalation administration. In another embodiment, compositions are contained in a container with attached atomizing means.

In one embodiment, the preparation of the present invention is formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

In some embodiments, pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. In some embodiments, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

In one embodiment, determination of a therapeutically effective amount is well within the capability of those skilled in the art.

The compositions also comprise preservatives, such as benzalkonium chloride and thimerosal and the like; chelating agents, such as edetate sodium and others; buffers such as phosphate, citrate and acetate; tonicity agents such as sodium chloride, potassium chloride, glycerin, mannitol and others; antioxidants such as ascorbic acid, acetylcystine, sodium metabisulfote and others; aromatic agents; viscosity adjustors, such as polymers, including cellulose and derivatives thereof; and polyvinyl alcohol and acid and bases to adjust the pH of these aqueous compositions as needed. The compositions also comprise local anesthetics or other actives. The compositions can be used as sprays, mists, drops, and the like.

Some examples of substances which can serve as pharmaceutically-acceptable carriers or components thereof are sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, and methyl cellulose; powdered tragacanth; malt; gelatin; talc; solid lubricants, such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and oil of theobroma; polyols such as propylene glycol, glycerine, sorbitol, mannitol, and polyethylene glycol; alginic acid; emulsifiers, such as the Tween™ brand emulsifiers; wetting agents, such sodium lauryl sulfate; coloring agents; flavoring agents; tableting agents, stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; and phosphate buffer solutions. The choice of a pharmaceutically-acceptable carrier to be used in conjunction with the compound is basically determined by the way the compound is to be administered. If the subject compound is to be injected, in one embodiment, the pharmaceutically-acceptable carrier is sterile, physiological saline, with a blood-compatible suspending agent, the pH of which has been adjusted to about 7.4.

In addition, the compositions further comprise binders (e.g. acacia, cornstarch, gelatin, carbomer, ethyl cellulose, guar gum, hydroxypropyl cellulose, hydroxypropyl methyl cellulose, povidone), disintegrating agents (e.g. cornstarch, potato starch, alginic acid, silicon dioxide, croscarmelose sodium, crospovidone, guar gum, sodium starch glycolate), buffers (e.g., Tris-HCl., acetate, phosphate) of various pH and ionic strength, additives such as albumin or gelatin to prevent absorption to surfaces, detergents (e.g., Tween 20, Tween 80, Pluronic F68, bile acid salts), protease inhibitors, surfactants (e.g. sodium lauryl sulfate), permeation enhancers, solubilizing agents (e.g., glycerol, polyethylene glycerol), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite, butylated hydroxyanisole), stabilizers (e.g. hydroxypropyl cellulose, hyroxypropylmethyl cellulose), viscosity

increasing agents(e.g. carbomer, colloidal silicon dioxide, ethyl cellulose, guar gum), sweeteners (e.g. aspartame, citric acid), preservatives (e.g., Thimerosal, benzyl alcohol, parabens), lubricants (e.g. stearic acid, magnesium stearate, polyethylene glycol, sodium lauryl sulfate), flow-aids (e.g. colloidal silicon dioxide), plasticizers (e.g. diethyl phthalate, triethyl citrate), emulsifiers (e.g. carbomer, hydroxypropyl cellulose, sodium lauryl sulfate), polymer coatings (e.g., poloxamers or poloxamines), coating and film forming agents (e.g. ethyl cellulose, acrylates, polymethacrylates) and/or adjuvants.

Typical components of carriers for syrups, elixirs, emulsions and suspensions include ethanol, glycerol, propylene glycol, polyethylene glycol, liquid sucrose, sorbitol and water. For a suspension, typical suspending agents include methyl cellulose, sodium carboxymethyl cellulose, cellulose (e.g. Avicel™, RC-591), tragacanth and sodium alginate; typical wetting agents include lecithin and polyethylene oxide sorbitan (e.g. polysorbate 80). Typical preservatives include methyl paraben and sodium benzoate. In another embodiment, peroral liquid compositions also contain one or more components such as sweeteners, flavoring agents and colorants disclosed above.

The compositions also include incorporation of the active material into or onto particulate preparations of polymeric compounds such as polylactic acid, polglycolic acid, hydrogels, etc, or onto liposomes, microemulsions, micelles, unilamellar or multilamellar vesicles, erythrocyte ghosts, or spheroplasts.) Such compositions will influence the physical state, solubility, stability, rate of *in vivo* release, and rate of *in vivo* clearance.

Also comprehended by the invention are particulate compositions coated with polymers (e.g. poloxamers or poloxamines) and the compound coupled to antibodies directed against tissue-specific receptors, ligands or antigens or coupled to ligands of tissue-specific receptors.

In some embodiments, compounds modified by the covalent attachment of water-soluble polymers such as polyethylene glycol, copolymers of polyethylene glycol and polypropylene glycol, carboxymethyl cellulose, dextran, polyvinyl alcohol, polyvinylpyrrolidone or polyproline. In another embodiment, the modified compounds exhibit substantially longer half-lives in blood following intravenous injection than do the corresponding unmodified compounds. In one embodiment, modifications also increase the compound's solubility in aqueous solution, eliminate aggregation, enhance the physical and chemical stability of the compound, and greatly reduce the immunogenicity and reactivity of the compound. In another embodiment, the desired *in vivo* biological activity is achieved by the administration of such polymer-compound abducts less frequently or in lower doses than with the unmodified compound.

In some embodiments, preparation of effective amount or dose can be estimated initially from in vitro assays. In one embodiment, a dose can be formulated in animal models and such information can be used to more accurately determine useful doses in humans.

In one embodiment, toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. In one embodiment, the data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. In one embodiment, the dosages vary depending upon the dosage form employed and the route of administration utilized. In one embodiment, the exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. [See e.g., Fingl, et al., (1975) "The Pharmacological Basis of Therapeutics", Ch. 1 p.1].

In one embodiment, depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

In one embodiment, the amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

In one embodiment, compositions including the preparation of the present invention formulated in a compatible pharmaceutical carrier are also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

In one embodiment, compositions of the present invention are presented in a pack or dispenser device, such as an FDA approved kit, which contain one or more unit dosage forms containing the active ingredient. In one embodiment, the pack, for example, comprises metal or plastic foil, such as a blister pack. In one embodiment, the pack or dispenser device is accompanied by instructions for administration. In one embodiment, the pack or dispenser is accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, in one embodiment, is labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert.

In one embodiment, it will be appreciated that the polypeptides of the present invention can be provided to the individual with additional active agents to achieve an improved therapeutic effect as compared to treatment with each agent by itself. In another embodiment, measures (e.g., dosing and selection of the complementary agent) are taken to adverse side effects which are associated with combination therapies.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation"

Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference. Other general references are provided throughout this document. .

EXAMPLE 1

Generation of EPO constructs

MATERIALS AND METHODS:

Construction of expression vector pCI-dhfr: pCI-neo mammalian expression vector was purchased from Promega (catalog no.E1841). The vector contains a CMV IE enhancer/promoter and neomycin phosphotransferase gene. The pSV2-dhfr clone was purchased from ATCC (Catalog No.37146). The plasmid contains the murine dhfr gene. The construction of pCI-dhfr vector was performed as follows:

a. The pSV2-dhfr plasmid was digested with restriction enzyme BglII (3' end of the dhfr gene). DNA polymerase I, Large (Klenow) Fragment was used to fill-in the 5' overhangs to form blunt ends. The DNA was then digested with restriction enzyme AvrII (5' end of the dhfr gene). The dhfr gene (AvrII –blunt end) fragment was isolated.

b. The pCI-neo vector was digested with restriction enzyme BstXI (3' end of the neo gene). DNA polymerase I, Large (Klenow) Fragment was used to remove the 3' overhangs to form blunt ends. The DNA was then digested with restriction enzyme AvrII (5' end of the neo gene). The expression vector (AvrII –blunt end) was isolated.

c. The dhfr gene was ligated into pCI vector to form an expression vector containing the dhfr gene (pCI-dhfr).

Construction of hEPO-CTP variants: A cassette gene containing the C-Terminal peptide (CTP) of the beta subunit of hCG was fused to the coding sequence of human EPO

(NP_000790.2) at different locations. Four EPO-CTP variants were constructed as illustrated in Figures 1A-D. The proEPO signal peptide was used for the construction of the secreted EPO-CTP variants. XbaI – NotI fragments containing Epo sequences were ligated into the pCI-dhfr expression vector of the present invention.

[0207] Table 2 hereinbelow summarizes the primer sequences used for constructing the CTP –containing polypeptides of the present invention.

Table 2

Primer number	SEQ ID NO	sequence	Restriction site (underlined ir sequence)
1	7	5' AATCTAGAGGTCATCATGGGGGTGC 3'	XbaI
2	8	5'ATTGCGGCCGCGGATCCAGAAGACCTTTATTG 3'	NotI
17^R	9	5' TAAATATTGGGGTGTCCGAGGGCCC 3'	SspI
10	10	5' CCAATATTACCACAAGCCCCACCACGCCTCAT 3'	SspI
11^R	11	5'TGCGGCCGCGGATCCTTATCTGTCCCCTGTCCTGC 3'	NotI
15	12	5' GCCCTGCTGTCGGAAGC 3'	
2^R	13	5' ATTGCGGCCGCGGATCCAGAAGACCTTTATTG	NotI
23^R	14	5'CTTTGAGGAAGAGGAGCCCAGGACTGGGAGGC3'	
24	15	5' CCTGGGCTCCTCTTCCTCAAAGGC 3'	
38^R	16	5' GCTTCCGACAGCAGGGC 3'	

EPO-1 701-1-p17-6 (Epo-1 – SEQ ID NO: 1): The XbaI–NotI 702 bp fragment was constructed by PCR using the above primers (SEQ ID NOs: 7-16). Then the XbaI–NotI PCR fragment containing Epo-ctp sequence was ligated into pCI-dhfr expression vector.

EPO-2 701-2-p24-2 (Epo-2- SEQ ID NO: 2): The XbaI/ApaI fragment (hGH-ctp) of pCI-dhfr-401-2-p21-2 (hGH-ctpx2) was replaced by the XbaI/ApaI fragment (EPO-ctp) of 701-1-p17-6 to create an Epo-ctpx2.

EPO-4-701-4-p42-1(Epo-4 – SEQ ID NO: 4): Firstly, a fragment from pCI-dhfr- EPO-ctp (701-1-p17-6) was constructed by PCR using primers 1 and 17 followed by XbaI/SspI digestion. This resulted in a fragment containing EPO and partial 5' CTP.

Secondly, a new fragment was constructed by overlapping PCR, on pGT123-hEpo as a template, using primer 10 and primer 11. SspI/NotI digestion resulted in fragment containing 3' partial CTP and Epo.

The two fragments were ligated into pCI-dhfr to construct the p701-4-p42-1 clone.

EPO-3-p56-6 (Epo-3 SEQ ID NO; 3): Primers were purchased from Sigma-Genosys. PCR reaction was performed using primer 15 (SEQ ID NO: 12) and primer 2^R (SEQ ID NO: 13) and plasmid DNA of pCI-dhfr- EPO-ctp x2 (701-2-p24-2) as a template. As a result of the PCR amplification, a 486 bp product was formed and ligated into TA cloning vector (Invitrogen, catalog K2000-01). Stu I –NotI fragment containing *Epo-ctp x2 sequence was isolated (209 bp).

Three sequential PCR reactions were performed. The first reaction was conducted with primer 1 (SEQ ID NO: 7) and primer 23^R (SEQ ID NO: 14) and plasmid DNA of pGT123-epo-ctp as a template; as a result of the PCR amplification, an 80 bp product was formed (signal peptide).

The second reaction was conducted with primer 24 (SEQ ID NO: 15) and primer 11^R (SEQ ID NO: 11) and plasmid DNA of 701-4-p42-1 as a template; as a result of the PCR amplification, a 610 bp product was formed.

[0208] The last reaction was conducted with primers 1 (SEQ ID NO: 7) and 11^R (SEQ ID NO: 11) and a mixture of the products of the previous two reactions as a template; as a result of the PCR amplification, a 700 bp product was formed and the XbaI-StuI fragment was isolated.

[0209] The two fragments (XbaI-StuI and StuI –NotI) were inserted into the eukaryotic expression vector pCI-dhfr (triple ligation) to yield the 701-3-p56-6 clone.

[0210] ***EPO-5-p91-4 (Epo-5 SEQ ID NO; 5 - (ctp- Epo):*** Primers were ordered from Sigma-Genosys. A PCR reaction was performed using primer 1 (SEQ ID NO: 7) and primer 11^R (SEQ ID NO: 11) and plasmid DNA of pCI-dhfr- ctp-EPO-ctp x2 (701-3-p56-6) as a template; as a result of the PCR amplification, a 670 bp product was formed and ligated into TA cloning vector (Invitrogen, catalog K2000-01) . XbaI –NotI fragment containing ctp-Epo sequence was ligated into our eukaryotic expression vector pCI-dhfr to yield the 701-5-p91-4 clone.

EPO-6-p90-1 (Epo-6 SEQ ID NO: 6 - (ctp- Epo-ctp): Three PCR reactions were performed. The first reaction was conducted with primer 1 (SEQ ID NO: 7) and primer 38^R (SEQ ID NO: 16) and plasmid DNA of 701-3-p56-6 as a template; as a result of the PCR amplification, a 400 bp product was formed.

The second reaction was conducted with primer 15 (SEQ ID NO: 12) and primer 2^R (SEQ ID NO: 13) and plasmid DNA of 701-1-p17-6 as a template; as a result of the PCR amplification, a 390 bp product was formed.

The last reaction was conducted with primers 1 (SEQ ID NO: 7) and 2^R (SEQ ID NO: 13) and a mixture of the products of the previous two reactions as a template; as a result of the PCR amplification, a 787 bp product was formed and ligated into TA cloning vector (Invitrogen, catalog K2000-01). The XbaI –NotI fragment containing ctp-Epo-ctp sequence was ligated into the eukaryotic expression vector pCI-dhfr to yield the 701-6-p90-1 clone.

EXAMPLE 2

Expression and Isolation of EPO-CTP polypeptides

MATERIALS AND METHODS

DNA transfection and clone selection: DG44 cells were transfected with pCI-DHFR expression vectors containing EPO-CTP variants using FuGENE6 Reagent (FuGENE Transfection Reagent – Roche Cat.11 815 091 001). 48 hr following transfection, cells were diluted and seeded at 50-200 cells per well in a selective medium (CD DG44 Medium w/o HT (Gibco: Scotland part: #07990111A) Sku num.:ME060027 supplemented with 8 mM L-Glutamine Biological Industries: Cat: 03-020-1A) and 18 mL/L of 10 % Pluronic F-68 solution (Gibco: Cat: 240040-032). Selected clones were screened for highest protein production using commercial ELISA. 3-5 producing clones per each variant were frozen for

a backup cell bank. A selected clone for each variant was adapted to growth in larger scale cultures up to 1L flasks on an orbital shaker platform. Supernatants were collected and analyzed by ELISA, SDS-PAGE and western blot. Following the withdrawal of aliquots, the protein-containing supernatants were kept frozen until further use.

Cell culture: DG44 cells were maintained in DG44 medium with HT (cat# 12610-010, Gibco) supplemented with 8 mM L-Glutamine (Biological Industries: Cat: 03-020-1A) and 18 mL/L of 10 % Pluronic F-68 solution (Gibco: Cat: 240040-032), at 37 ^{0}C in humidified 8 % CO_2 incubator. Transfected clones were maintained in DG44 basal medium without HT supplement, hypoxanthine and thymidine, with pluronic acid and L-glutamine.

Sample preparation: Supernatants were collected, filtrated and analyzed by ELISA to determine protein concentration. SDS-PAGE and western blot were used to determine purity and identity. Following ELISA, sample concentrations were defined and the solution was dialyzed against PBS. Following the withdrawal of aliquots, the protein-contained supernatants were kept frozen at -20 ^{0}C until further use.

Western Blotting: Samples were electrophoresed on nondenaturing 15 % SDS-polyacrylamide gels. Gels were allowed to equilibrate for 10 min in 25 mM Tris and 192 mM glycine in 20 % (vol/vol) methanol). Proteins were transferred to a 0.2 μm pore size nitrocellulose membrane (Sigma, Saint Louis, MO) at 250 mA for 3h, using a Mini Trans-Blot electrophoresis cell (Biorad Laboratories, Richmond, CA). The nitrocellulose membrane was incubated in 5 % non-fat dry milk for 2 h at room temperature. The membrane was incubated with EPO anti-serum (1:1000 titer) overnight at 4 °C followed by three consecutive washes in PBS containing 0.1 % Tween (10 min/wash). The membrane was incubated with secondary antibody conjugated to Horse Radish Peroxidase (HRP) (Zymed, San Francisco, CA) for 2 h at room temperature, followed by three washes. Finally, the nitrocellulose paper was reacted with enhanced chemiluminescent substrate (ECL) (Pierce, Rockford, IL) for 5 min, dried with a Whatman sheet, and exposed to X-ray film.

RESULTS

Table 3 hereinbelow shows the concentrations of the various CTP-modified EPO forms obtained from 5 selected clones and their preparation for further testing.

Table 3

#Version	*# Clone*	*Stock Titer IU/ml*[1]	*Post dilution in Mock sup. according to Epo3 titer IU/ml*[2]	*Post ultrafiltration IU/ml* [3]
Epo0 SEQ ID NO: 16	17	3093	102	335
Epo1 SEQ ID NO: 1	47	1049	104	291
Epo2 SEQ ID NO: 2	67	2160	110	303
Epo3 SEQ ID NO: 3	85	105	119	392
Epo4 SEQ ID NO: 4	112	6100	ND	342

1. EPO variants stock concentration were determined by ELISA (Quantikine IVD Epo ELISA, DEP00, R&D Systems)

2. Samples EPO-0, 1, 2 and 4 were diluted to 105 IU/ml in mock sup (Adjusted to Epo3 titer). Epo0 = wild type EPO expressed in the same system as the CTP modified EPOs

3. All samples were concentrated and dialyzed by ultrafiltration against PBS to a final concentration of 180 IU/ml.

All proteins were detected by Western blot as illustrated in Figure 2.

EXAMPLE 3

Biological activity of the EPO-CTP polypeptides of the present invention

The TF-1 bioactivity test represents the ability of the EPO-CTP variants to bind its receptor and then stimulate activity which results in cell proliferation. Therefore, this test was used as a first step in evaluating the biological potency of the EPO-CTP polypeptides of the present invention.

MATERIALS AND METHODS

Cell Proliferation Analysis: Proliferation assay was performed with the cell line TF-1, measuring levels of MTT cellular stain as a function of EPO activity (Kitamura *et al.*, Kitamura, T. *et al.* (1989) *Establishment and characterization of a unique human cell line that proliferates*; Hammerling U. et al. In vitro bioassay for human erythropoietin based on proliferative stimulation of an erythroid cell line and analysis of carbohydrate-dependent microheterogeneity. Journal of Pharm. Biomed. Analysis 14(11): 1455-1469 (1996). Exponentially growing TF-1 cells were washed twice, plated at about 10^4 cells/well in microtiter plates, and incubated in basal medium with a titrated dilution series of EPO (Recormon), EPO standard (NIBSC standard), rhEPO (MOD-7010), MOD-701 variants (EPO-1, EPO-2, EPO-3 and EPO-4) for 48 hours. 4 hours prior to assaying for cell proliferation, MTT reagent was added to the wells, and absorbance was measured by ELISA reader. A calculated protein concentration value for each variant protein was obtained from Eprex's (Epoetin (EPO)-man-made form of the human hormone) dose-response standard curve.

RESULTS

The *in vitro* biological activity of EPO polypeptides was determined with an Epo-dependent cell line, human erythroleukemia TF-1 (DSMZ Cell Bank) [Dong et al., Biochemical and Biophysical Research Communications, Volume 339, Issue 1, 6 January 2006, Pages 380-385]. The MTT assay was performed [Hammerling U. et al. In vitro bioassay for human erythropoietin based on proliferative stimulation of an erythroid cell line and analysis of carbohydrate-dependent microheterogeneity. Journal of Pharm. Biomed. Analysis 14(11): 1455-1469 (1996);], and the laboratory standard of EPO used to generate the standard curve was calibrated against the International Standard (Epo ampoule code 87/684 of NIBSC).

The results are summarized in Table 4 hereinbelow. The results indicate that the highest potency was achieved by using EPO 3 and EPO 0 in both 2 and 0.5 IU/ml concentrations.

Table 4

Eprex STD	*TF-1 Bioactivity IU/ml*						
IU/ml	EPO 0 SEQ ID NO: 16	EPO 1 SEQ ID NO: 1	EPO 2 SEQ ID NO: 2	EPO 3 SEQ ID NO: 3	EPO 4 SEQ ID NO: 4	Recormon	EPO st
2	4.93	2.32	2.13	6.91	3.55	3.44	7.40
0.5	1.60	0.76	0.53	1.34	0.84	0.87	1.53

CONCLUSION

As summarized in Table 4 hereinabove, different levels of potency were exerted by EPO-CTP polypeptides, indicating differences in receptor binding. EPO-CTP polypeptides differ by the number of CTP cassettes and the location to which they are fused. EPO-1 and EPO-2 contain 1 CTP sequence or 2 CTP sequences at the C-terminal of EPO, while EPO-3 contains 1 CTP at N-terminal and 2 CTP sequences at C-terminal. EPO-4 is a dimer of two EPO molecules linked by CTP sequence. EPO-3 demonstrated potency level quite similar to WT-EPO, while EPO-1 and EPO-4 were about 50 % less potent than WT-EPO, and EPO-2 potency was even less than 50 %.

EXAMPLE 4

Evaluation of the EPO-CTP polypeptides of the present invention in a mouse model

The following experiment was performed in order to compare the bio-activity of the EPO-CTP polypeptides of the present invention and commercial EPO

MATERIALS AND METHODS

Animals:

Species/Strain: ICR or CD-1 Mice of either sex about 20-25g

Group Size: n=7

No. Groups: 9

Total No. Animals: n=63

Experimental design of the study: The experiment was set up as summarized in Table 5 hereinbelow.

Table 5

Group No.	*No. Mice per Group*	*TREATMENT*		
		Compound	*Dose Level*	*Dosing Regimen*
1	*n*=7	Vehicle	0	1x weekly
2		MOCK		
3		MOD-7010	15 µg/kg	
4		MOD-7011		
5		MOD-7012		
6		MOD-7013		
7		MOD-7014		
8		Commercial rhEPO	15 µg/kg	
9			5 µg/kg	3 x weekly

Animal treatment: All animals were administered with either control or the test EPO polypeptides of the present invention by bolus injection. The injection volume did not exceed 10 ml/kg. The length of the experiment was 22 days. A morbidity and mortality check was performed daily.

Reticulocyte count and hematocrit (hct) examination: Reticulocyte count was carried out in all test animals at day 2 and 14 hrs following the 1st respective vehicle or treatment injection. HCT was determined in all animals once prior to initial treatment ("0" Baseline control) and at 24 hrs after the 1st respective vehicle or treatment injection, and thereafter twice weekly until study termination (Day-22).

RESULTS

The hematocrit results which are illustrated in Figures 3-5 show that EPO 3 has the highest hematocrit percentage change from baseline compared to EPO 1, EPO 2, Recormon 1, Recormon 3, rhEPO, and vehicle. The results demonstrating the percentage of reticulocytes in mice treated with the EPO-CTP polypeptides are summarized in Table 6 hereinbelow. These results show that EPO-3 is the most potent stimulator of erythropoiesis.

Table 6

	% reticulocytes	
Days	***2***	***14***
Control	3.72	3.46
	1.08	0.8
Mock	3.5	3.64
	0.6	1.13
7010 SEQ ID NO: 16	3.5	3.9
	0.6	1.54
7011 SEQ ID NO: 1	3.52	1.94
	1.38	1.08
7012 SEQ ID NO: 2	3.82	3.0
	1.02	0.88
7013 SEQ ID NO: 3	2.66	5.20
	0.97	2.96
7014 SEQ ID NO: 4	3.48	3.82
	0.71	0.90

Recormon 1/W	3.23	3.27
	0.73	0.59
Recormon 3/w	4.13	4.24
	1.21	1.14

CONCLUSION

The *in vivo* experiment was designed to measure two parameters; the first was to measure erythropoiesis parameters such as percentage of reticulocytes and increase of hemoglobin, RBC and hematocrit levels. The second was to measure the durability of the biological activity of each variant by injecting once weekly doses.

A superior performance of EPO-3 in its ability to stimulate erythropoiesis was observed in normal mice.

EXAMPLE 5

Comparison of the EPO-CTP polypeptides of the present invention to Aranesp

The following experiment was performed in order to compare the biological activity of a single bolus dose of some EPO-CTP polypeptides of the present invention, commercial EPO and Aranesp. Aranesp is a commercial long-acting recombinant erythropoietin in which two site mutations were introduced, resulting in two additional N-glycosylation sites and an increase in the number of incorporated sialic acid residues.

MATERIALS AND METHODS

Animals:

Species/Strain: Female CD-1 Mice of either sex about 20-25g

Group Size: n=3

Experimental design of the study: The experiment was set up as summarized in Table 7 hereinbelow.

Table 7

Group #	Test Article	animals/ group/ time-point	Dose Solution Conc. (µg/mL)	Dose Volume (mL/kg)	Time-Points * (hours post-administration)
1	MOD-7010 SEQ ID NO: 11	3	1.5	10	0 (Pre-dose), 0.25, 0.5, 1, 2, 6, 24, 48, 96, 168, 216, 264 and 336 hr post-dose administration
2	MOD-7013 SEQ ID NO: 3	3	1.5	10	0.25, 0.5, 1, 2, 6, 24, 48, 96, 168, 216, 264 and 336 hr post-dose administration
3	Aranesp	3	1.5	10	0.25, 0.5, 1, 2, 6, 24, 48, 96, 168, 216, 264 and 336 hr post-dose administration

Animal treatment: All animals were administered with either control or the test EPO polypeptides of the present invention by bolus injection. The injection volume did not exceed 10 ml/kg. The length of the experiment was 14 days. A morbidity and mortality check was performed daily.

Reticulocyte count and hematocrit (hct) examination: Reticulocyte count and hematocrit examination were performed as described above.

RESULTS

The results are illustrated in Figures 6-9. Following a single I.V. injection of 15 µg/kg of EPO 3, all three blood parameters associated with erythropoietin i.e. number of reticulocytes, hemoglobin level and hematocrit, were improved relative to those obtained with similar injected dose of rhEPO or Aranesp.

EXAMPLE 6

Comparison of the pharmacokinetics of EPO-CTP polypeptides of the present invention to Aranesp

The following experiment was performed in order to compare the pharmacokinetics of EPO-CTP polypeptide of the present invention, commercial EPO and Aranesp.

MATERIALS AND METHODS

Serum samples were analyzed in order to determine specific concentration levels for each sample. Concentration and time-point data were processed using WinNonLin noncompartmental analysis. Parameters determined included: AUC, CL, Ke, t1/2, Cmax, Tmax, and Vdz.

Serum concentrations were determined using two ELISA kits in parallel. EPO-3 serum concentration was measured using StemCell ELISA kit in comparison to EPO-0 and Aranesp serum concentration which were determined using R&D system ELISA kit.

RESULTS

The results of the pharmacokinetic analysis are summarized in Table 8, hereinbelow. These results show that EPO 3 exhibited favorable pharmacokinetic measures as indicated for example in AUC measures, t1/2, and Cmax. Tmax measures were equal to EPO-0, EPO-3, and Aranesp.

Table 8

Parameters	Units	EPO-0	EPO-3	Aranesp
AUClast	hr*mIU/mL	31739	306072	178661
CL^	mL/hr/kg	1.1152	0.2188	0.1207
Ke	1/hr	0.157	0.0529	0.0639
t1/2	hr	4.4139	13.1141	10.84
Cmax	mIU/mL	10766	16466	13266

Tmax	Hr	0.25	0.25	0.25
Vdz	mL/kg	7.1017	4.1394	1.8877

The results of the serum concentration analysis are illustrated in Figure 9. These results show that EPO-3 was still detectable in the serum after about 190 hours. Both EPO-0 and Aranesp were not detectable in the serum after about 140 hours and 50 hours, respectively.

CONCLUSION

Clearance of EPO-3 (MOD-7013) from the blood of CD-1 mice was significantly slower than that for rhEPO or Aranesp. The corresponding calculated half life times were: rhEPO - 4.41 h; Aranesp - 0.84 h; and MOD-7013 - 13.11 h.

EXAMPLE 7

Generation of hGH constructs

MATERIALS AND METHODS

Four hGH clones (variants of 20kD protein) were synthesized. Xba I –Not I fragments containing hGH sequences from the four variants were ligated into the eukaryotic expression vector pCI-dhfr previously digested with XbaI –NotI. DNA from the 4 clones (401-0, 1, 2, 3 and 4) was prepared. Another partial hGH clone (1-242 bp) from 22kD protein was also synthesized (0606114). Primers were ordered from Sigma-Genosys. The primer sequences used to generate the hGH-CTP polypeptides of the present invention are summarized in Table 9, hereinbelow.

Table 9

Primer number	SEQ ID NO	*sequence*	Restriction site (underlined in sequence)
25	27	5' CTCTAGAGGACATGGCCAC 3'	XbaI

32^R	28	5' ACAGGGAGGTCTGGGGGTTCTGCA 3'	
33	29	5' TGCAGAACCCCCAGACCTCCCTGTGC 3'	
4^R	30	5' CCAAACTCATCAATGTATCTTA 3'	
25	31	5' CTCTAGAGGACATGGCCAC 3'	XbaI
35^R	32	5' CGAACTCCTGGTAGGTGTCAAAGGC 3'	
34	33	5' GCCTTTGACACCTACCAGGAGTTCG 3'	
37^R	34	5'ACGCGGCCGCATCCAGACCTTCATCACTGAGGC 3'	NotI
39^R	35	5' GCGGCCGCGGACTCATCAGAAGCCGCAGCTGCCC 3'	

Construction of 402-0-p69-1 (hGH) SEQ ID NO: 36: MOD-4020 is the wild type recombinant human growth hormone (without CTP) which was prepared for use as control in the below described experiments.

Three PCR reactions were performed. The first reaction was conducted with primer 25 and primer 32^R and plasmid DNA of 0606114 (partial clone of hGH 1-242 bp) as a template; as a result of the PCR amplification, a 245 bp product was formed.

The second reaction was conducted with primer 33 and primer 4^R and plasmid DNA of 401-0-p57-2 as a template; as a result of the PCR amplification, a 542 bp product was formed.

The last reaction was conducted with primers 25 and 4^R and a mixture of the products of the previous two reactions as a template; as a result of the PCR amplification, a 705 bp product was formed and ligated into the TA cloning vector (Invitrogen, catalog K2000-01). The XbaI –NotI fragment containing hGH-0 sequence was ligated into the eukaryotic expression vector pCI-dhfr. The vector was transfected into DG-44 CHO cells. Cells were grown in protein-free medium.

Construction of 402-1-p83-5 (hGH-CTP) - SEQ ID NO: 37 and 402-2-p72-3(hGH-CTPx2) – SEQ ID NO: 38: MOD-4021 is a recombinant human growth hormone which was fused to 1 copy of the C-terminal peptide of the beta chain of human Chorionic Gonadotropin (CTP). The CTP cassette of MOD-4021 was attached to the C-terminus (one cassette). MOD-4022 is a recombinant human growth hormone which was fused to 2 copies of the C-terminal peptide of the beta chain of human Chorionic Gonadotropin (CTP). The two CTP cassettes of MOD-4022 were attached to the C-terminus (two cassettes).

Construction of hGH-CTP and hGH-CTP-CTP was performed in the same way as the construction of hGH-0. pCI-dhfr-401-1-p20-1 (hGH*-ctp) and pCI-dhfr-401-2-p21-2 (hGH*-ctp x2) were used as templates in the second PCR reaction.

MOD-4021 and MOD-4022 were expressed in DG-44 CHO cells. Cells were grown in protein-free medium. The molecular weight of MOD-4021 is ~30.5Kd since hGH has a MW of 22 Kd while each "CTP cassette" contributes 8.5 Kd to the overall molecular weight (see Figure 10). The molecular weight of MOD-4022 is ~39 Kd (see Figure 10).

Construction of 402-3-p81-4 (CTP-hGH-CTP-CTP) - SEQ ID NO: 39 and 402-4-p82-9(CTP*hGH-CTP-CTP) – SEQ ID NO: 40: MOD-4023 is a recombinant human growth hormone which was fused to 3 copies of the C-terminal peptide of the beta chain of human Chorionic Gonadotropin (CTP). The three CTP cassettes of MOD-4023 were attached to both N-terminus (one cassette) and the C-terminus (two cassettes). MOD-4024 is a recombinant human growth hormone which is fused to 1 truncated and 2 complete copies of the C-terminal peptide of the beta chain of human Chorionic Gonadotropin (CTP). The truncated CTP cassette of MOD-4024 was attached to the N-terminus and two CTP cassettes were attached to the C-terminus (two cassettes).

Three PCR reactions were performed. The first reaction was conducted with primer 25 and primer 35^R and plasmid DNA of p401-3-p12-5 or 401-4-p22-1as a template; as a result of the PCR amplification, a 265 or 220 bp product was formed. The second reaction was conducted with primer 34 and primer 37^R and plasmid DNA of TA-hGH-2-q65-1 as a template; as a result of the PCR amplification, a 695 bp product was formed. The last reaction was conducted with primers 25 and 37^R and a mixture of the products of the previous two reactions as a template; as a result of the PCR amplification, a 938 or 891bp product was formed and ligated into TA cloning vector (Invitrogen, catalog K2000-01). Xba I –Not I

fragment containing hGH sequence was ligated into our eukaryotic expression vector pCI-dhfr.

MOD-4023 and MOD-4024 were expressed in DG-44 CHO cells. Cells were grown in protein-free medium. The molecular weight of MOD-4023 is ~47.5Kd (see Figure 10) and the molecular weight of MOD-4024 is ~43.25Kd (see Figure 10).

Construction of 402-6-p95a-8 (CTP-hGH-CTP) - SEQ ID NO: 41: Construction of hGH-6 was performed in the same way as the construction of hGH-3. pCI-dhfr-402-1-p83-5 (hGH-ctp) was used as a template in the second PCR reaction.

Construction of 402-5-p96-4 (CTP-hGH) - SEQ ID NO: 42: PCR reaction was performed using primer 25 and primer 39^R and plasmid DNA of pCI-dhfr- ctp-EPO-ctp (402-6-p95a-8) as a template; as a result of the PCR amplification , a 763 bp product was formed and ligated into TA cloning vector (Invitrogen, catalog K2000-01) . Xba I –Not I fragment containing ctp-hGH sequence was ligated into our eukaryotic expression vector pCI-dhfr to yield 402-5-p96-4 clone.

EXAMPLE 8

In vivo bioactivity tests of hGH-CTP polypeptides of the present invention

The following experiment was performed in order to test the potential long acting biological activity of hGH-CTP polypeptides in comparison with commercial recombinant human GH and MOD-4020.

MATERIALS AND METHODS

Female hypophysectomized rats (60 -100 g) received a weekly S.C. injection of 21.7 μg hGH-CTP polypeptides or a once daily 5 μg S.C. injection of control commercial rhGH.

Weight was measured in all animals before treatment, 24 hours following first injection and then every other day until the end of the study on day 21. Each point represents the group's average weight gain percentage ((Weight day 0-weight last day)/Weight day 0). Average weight gain was normalized against once-daily injection of commercial hGH. The treatment schedule is summarized in Table 10.

Table 10

No.	*Drug*	*N*	*Route*	*Treatment Schedule*	*Equimolar Dose (μg/rat)*	*Accumulate Dosage (μg/rat)*	*Dose Vol.(ml)*
1	Vehicle	7	s.c.	days 1, 7 and 13; 1/W	NA	NA	0.25
2	Mock	7	s.c	days 1, 7 and 13;1/W	NA	NA	0.25
3	MOD-4020 SEQ ID NO: 36	7	s.c	days 1, 7 and 13; 1/W	21.7	65	0.25
4	MOD-4021 SEQ ID NO: 37	7	s.c.	days 1, 7 and 13; 1/W	21.7	65	0.25
5	MOD-4022 SEQ ID NO: 38	7	s.c.	days 1, 7 and 13; 1/W	21.7	65	0.25
6	MOD-4023 SEQ ID NO: 39	7	s.c.	days 1, 7 and 13; 1/W	21.7	65	0.25
7	MOD-4024 SEQ ID NO: 40	7	s.c.	days 1, 7 and 13; 1/W	21.7	65	0.25
8	Commercial	7	s.c.	days 1, 7	21.7	65	0.25

	hGH v.1			and 13; 1/W			
9	Commercial hGH v.1	7	s.c.	days 1–13; d/W	5	65	0.25

RESULTS

Results are summarized in Figure 11. These results show that MOD-4023 (SEQ ID NO: 39) and MOD-4024 (SEQ ID NO: 40) induced over 120% weight gain compared to commercial rhGH which induced 100% weight gain.

CONCLUSION

3 weekly doses (Days of injections;1, 7, and 13) of 21.7μg of MOD-4023 (SEQ ID NO: 39) and MOD-4024 (SEQ ID NO: 40) induced a 30 % greater weight increase in hypophysectomised rats compared to commercial rhGH injected at the same accumulated dose which was administered once per day at a dose of 5 μg for 13 days.

WHAT IS CLAIMED IS:

1. A polypeptide comprising a peptide of interest and at least two chorionic gonadotrophin carboxy terminal peptides, wherein a first chorionic gonadotrophin carboxy terminal peptide of said at least two chorionic gonadotrophin carboxy terminal peptides is attached to an amino terminus of said peptide of interest, and a second chorionic gonadotrophin carboxy terminal peptide of said at least two chorionic gonadotrophin carboxy terminal peptides is attached to a carboxy terminus of said peptide of interest.

2. The polypeptide of claim 1, wherein said polypeptide further comprises a third chorionic gonadotrophin carboxy terminal peptide attached in tandem to said second chorionic gonadotrophin carboxy terminal peptide.

3. The polypeptide of any one of claims 1-2, wherein said peptide of interest is an hGH peptide.

4. The polypeptide of any one of claims 1-2, wherein said peptide of interest is selected from an interferon peptide and a GLP-1 peptide.

5. The polypeptide of one of claims 1-4, wherein said peptide of interest is glycosylated.

6. The polypeptide of one of claims 1-4, wherein said peptide of interest is non-glycosylated.

7. The polypeptide of claim 3, wherein the sequence of said peptide comprises an amino acid sequence selected from the sequences set forth in SEQ ID NO: 39 - 41.

8. A polynucleotide comprising a coding portion that encodes a polypeptide, said polypeptide comprising a peptide of interest and at least two chorionic gonadotrophin carboxy terminal peptides, wherein a first CTP chorionic gonadotrophin amino acid sequence of said at least two chorionic gonadotrophin carboxy terminal peptides is attached to an amino terminus of said peptide of interest, and a second chorionic gonadotrophin carboxy terminal peptide of

said at least two chorionic gonadotrophin carboxy terminal peptides is attached to the carboxy terminus of said peptide of interest.

9. The polynucleotide of claim 8, wherein said polypeptide further comprises a third chorionic gonadotrophin carboxy terminal peptide attached in tandem to said second chorionic gonadotrophin carboxy terminal peptide.

10. The polynucleotide of any one of claims 8-9, wherein said peptide of interest is an hGH peptide.

11. The polynucleotide of any one of claims 9-11, wherein said peptide of interest is said selected from an interferon peptide and a GLP-1 peptide.

12. The polynucleotide of claim 10, wherein the sequence of said polynucleotide comprises a sequence selected from the sequences set forth in SEQ ID NO: 44-46.

13. A method of treating a growth, weight-related or metabolic condition in a subject, said method comprising the step of administering to said subject a therapeutically effective amount of the polypeptide of claim 3 or the polynucleotide of claim 10, thereby treating a subject having a growth, weight-related or metabolic condition.

14. A method of improving a biological half life of a peptide of interest, comprising the step of attaching a first chorionic gonadotrophin carboxy terminal peptide to an amino terminus of said peptide of interest and a second chorionic gonadotrophin carboxy terminal peptide to the carboxy terminus of said peptide of interest, thereby improving a biological half life of a peptide of interest.

15. A method of administering a peptide of interest to a subject in need thereof, comprising the step of attaching a first chorionic gonadotrophin carboxy terminal peptide to an amino terminus of said peptide of interest and a second chorionic gonadotrophin carboxy terminal

peptide to the carboxy terminus of said peptide of interest, thereby generating an improved polypeptide for administration to said subject, thereby administering a peptide of interest to a subject in need thereof.

16. A polypeptide comprising an EPO peptide and at least two chorionic gonadotrophin carboxy terminal peptides, wherein a first chorionic gonadotrophin carboxy terminal peptide of said at least two chorionic gonadotrophin carboxy terminal peptides is attached to an amino terminus of said EPO peptide, and a second chorionic gonadotrophin carboxy terminal peptide of said at least two chorionic gonadotrophin carboxy terminal peptides is attached to a carboxy terminus of said EPO peptide.

17. The polypeptide of claim 16, wherein said polypeptide further comprises a third chorionic gonadotrophin carboxy terminal peptide attached in tandem to said second chorionic gonadotrophin carboxy terminal peptide.

18. The polypeptide of any one of claims 16-17, wherein the sequence of at least one of said at least two chorionic gonadotrophin carboxy terminal peptides comprises an amino acid sequence selected from the sequences set forth in SEQ ID NO: 17 and SEQ ID NO: 18.

19. The polypeptide of any one of claims 1-7 or 16-18, wherein at least one of said at least two chorionic gonadotrophin carboxy terminal peptides is truncated.

20. The polypeptide of any one of claims 1-7 or 16-19, wherein at least one of said at least two chorionic gonadotrophin carboxy terminal peptides is glycosylated.

21. The polypeptide of any one of claims 1-7 or 16-20, further comprising a signal peptide.

22. The polypeptide of claim 21, wherein the sequence of said signal peptide is as set forth in SEQ ID NO: 19.

23. The polypeptide of any one of claims 16-22, wherein the sequence of said polypeptide comprises an amino acid sequence selected from the sequences set forth in SEQ ID NO: 3 and SEQ ID NO: 6.

24. A pharmaceutical composition comprising the polypeptide of any one of claims 1-7 or 16-23.

25. A polynucleotide comprising a coding sequence, said coding sequence encoding a polypeptide comprising an EPO peptide and at least two chorionic gonadotrophin carboxy terminal peptides, wherein a first chorionic gonadotrophin carboxy terminal peptide of said at least two chorionic gonadotrophin carboxy terminal peptides is attached to an amino terminus of said EPO peptide, and a second chorionic gonadotrophin carboxy terminal peptide of said at least two chorionic gonadotrophin carboxy terminal peptides is attached to the carboxy terminus of said EPO peptide.

26. The polynucleotide of claim 25, wherein said polypeptide further comprises a third chorionic gonadotrophin carboxy terminal peptide attached in tandem to said second chorionic gonadotrophin carboxy terminal peptide.

27. The polynucleotide of any one of claims 8-12 and 25-26, wherein said polypeptide further comprises a signal peptide.

28. The polynucleotide of claim 27, wherein the sequence of said signal peptide is as set forth in SEQ ID NO: 19.

29. An expression vector comprising the polynucleotide of any one of claims 8-12 and 25-28.

30. A cell comprising the expression vector of claim 29.

31. A pharmaceutical composition comprising the expression vector of claim 29.

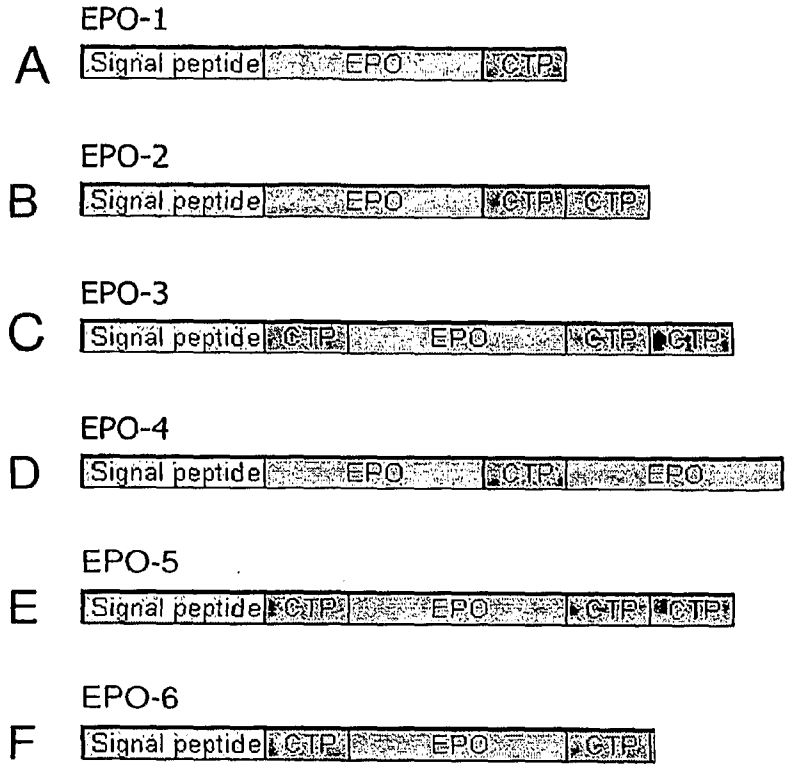
32. A method of treating or reducing the incidence of anemia in a subject, said method comprising the step of administering to said subject a therapeutically effective amount of the polypeptide of any of claims 26-23 or the polynucleotide of any one of claims 25-28, thereby treating or reducing the incidence of anemia in a subject.

33. A method of improving a biological half life of an EPO peptide, comprising the step of attaching a first chorionic gonadotrophin carboxy terminal peptide to an amino terminus of said EPO peptide and a second chorionic gonadotrophin carboxy terminal peptide to a carboxy terminus of said EPO peptide, thereby improving a biological half life of an EPO peptide.

34. A method of administering an EPO peptide to a subject in need thereof, comprising the step of attaching a first chorionic gonadotrophin carboxy terminal peptide to an amino terminus of said EPO peptide and a second chorionic gonadotrophin carboxy terminal peptide to a carboxy terminus of said EPO peptide, thereby generating an improved polypeptide for administration to said subject, thereby administering an EPO peptide to a subject in need thereof.

35. The method of any one of claims 13-15 or 32-34, further comprising the step of attaching a third chorionic gonadotrophin carboxy terminal peptide in tandem to said second chorionic gonadotrophin carboxy terminal peptide.

36. The method of any one of claims 13-15 or 32-35, wherein the sequence of at least one of said at least two chorionic gonadotrophin carboxy terminal peptides comprises an amino acid sequence selected from the sequences set forth in SEQ ID NO: 17-18.



FIGURES 1A-F

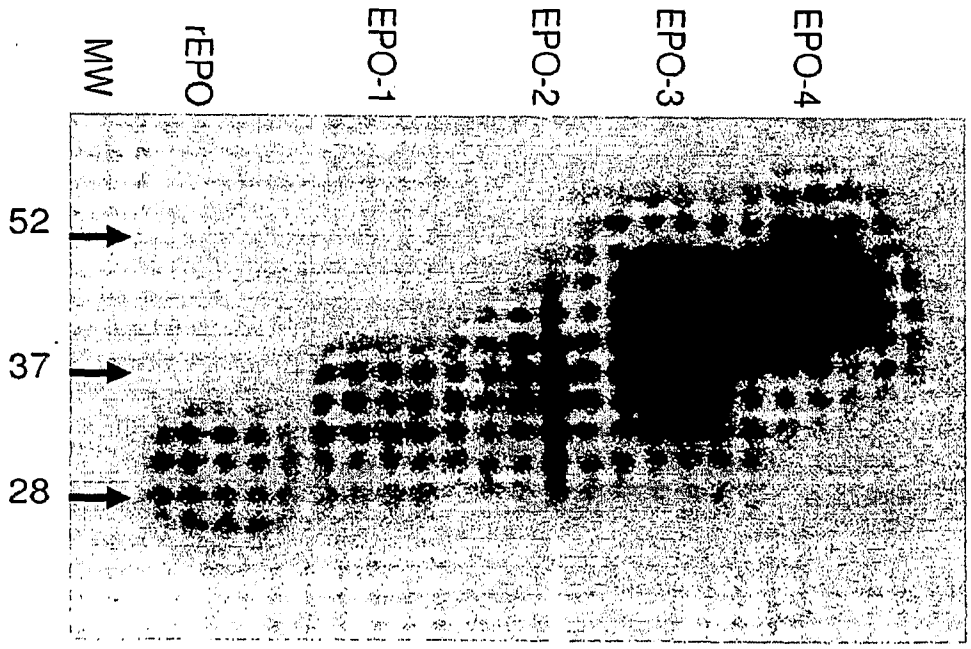


FIGURE 2

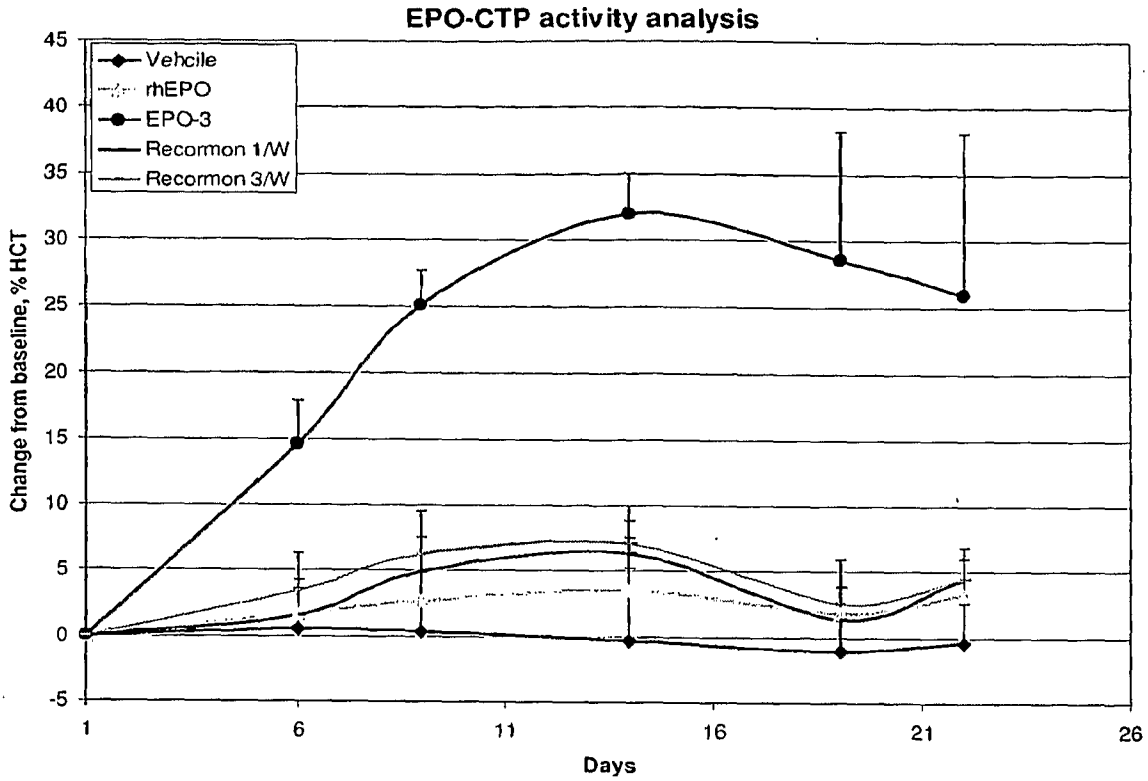


FIGURE 3

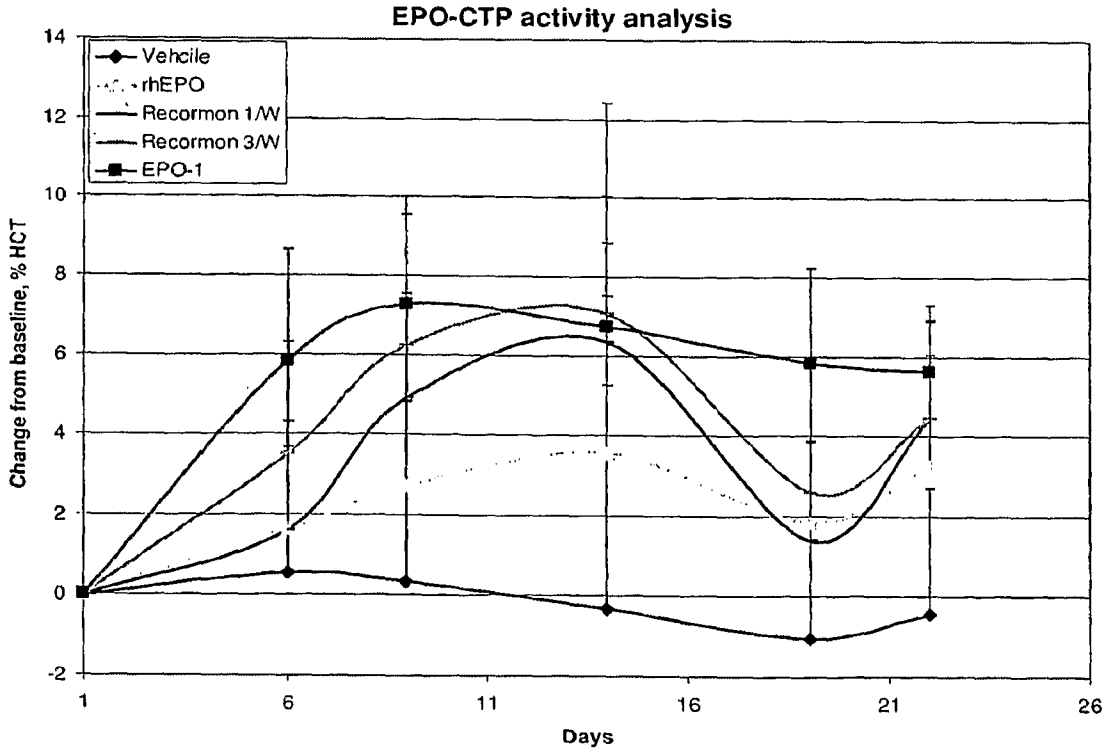


FIGURE 4

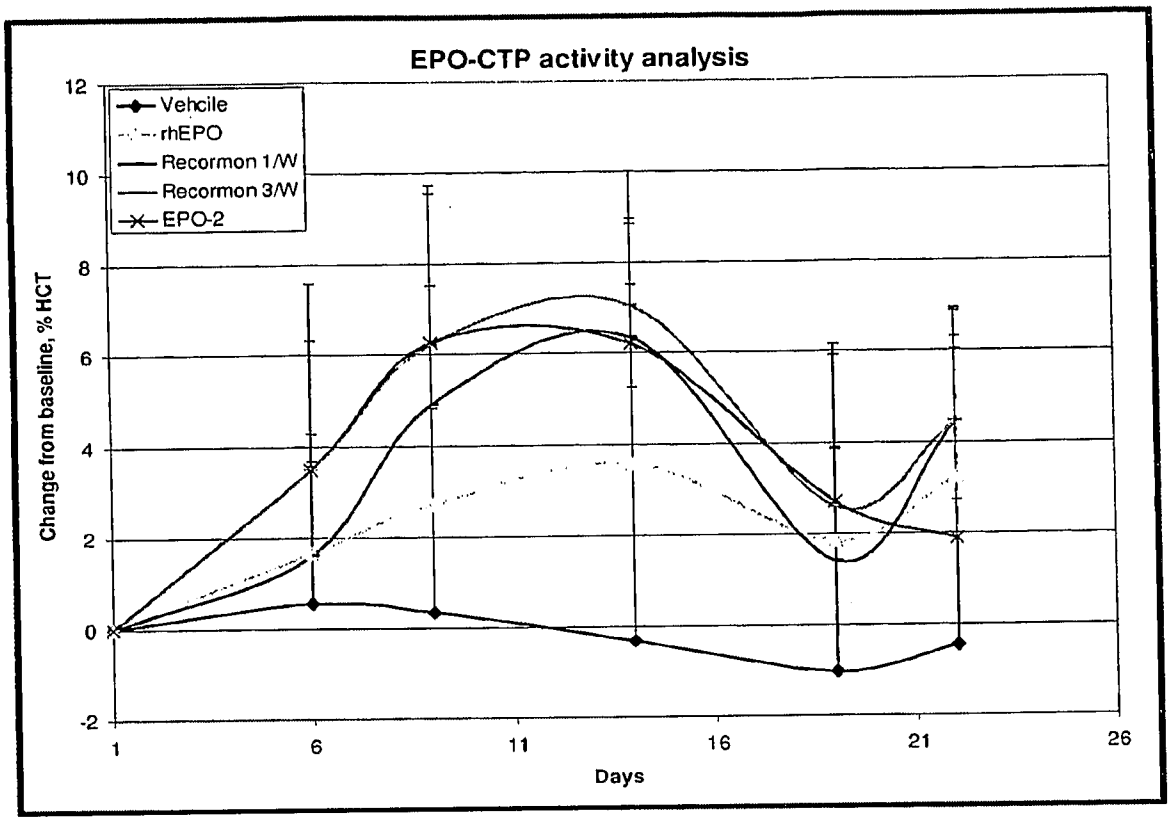


FIGURE 5

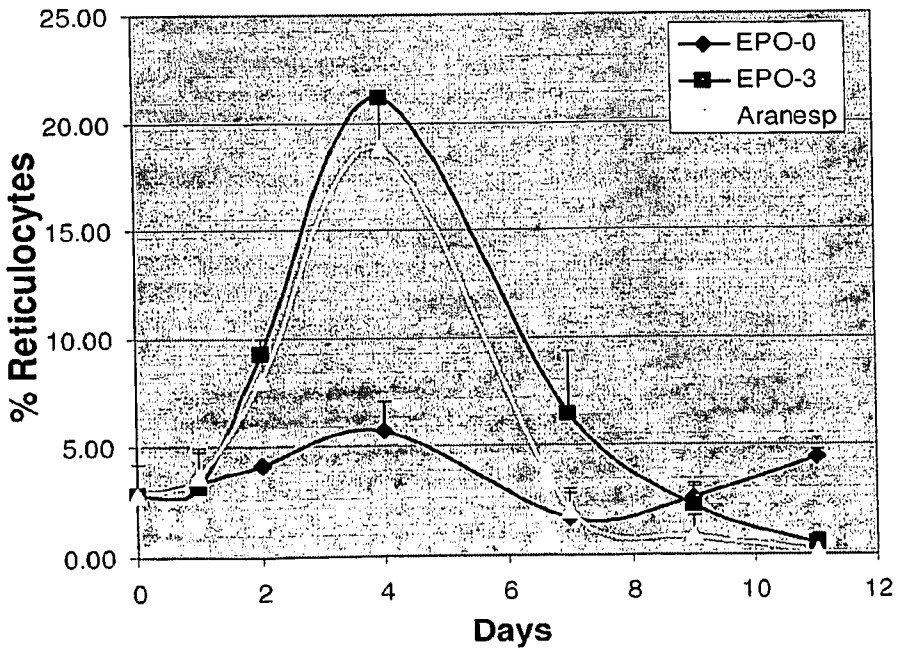


FIGURE 6

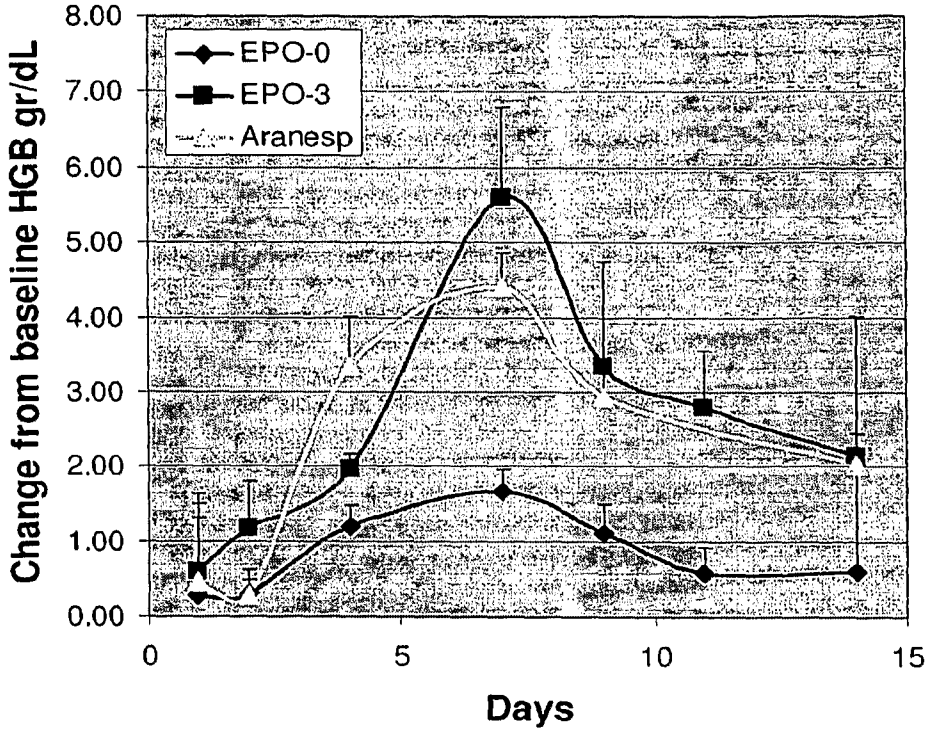


FIGURE 7

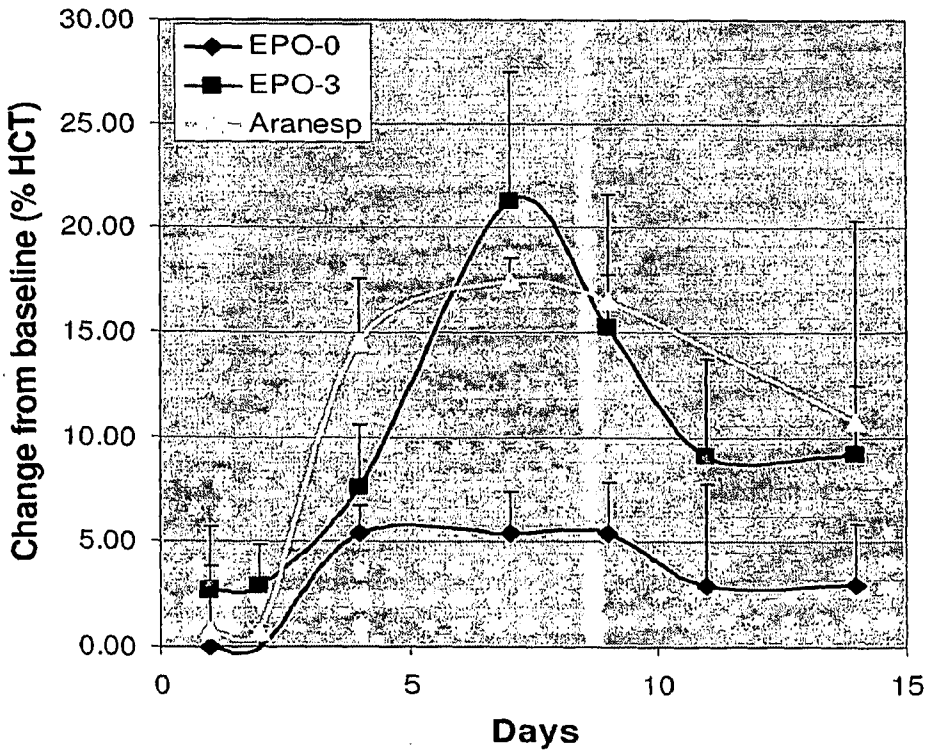


FIGURE 8

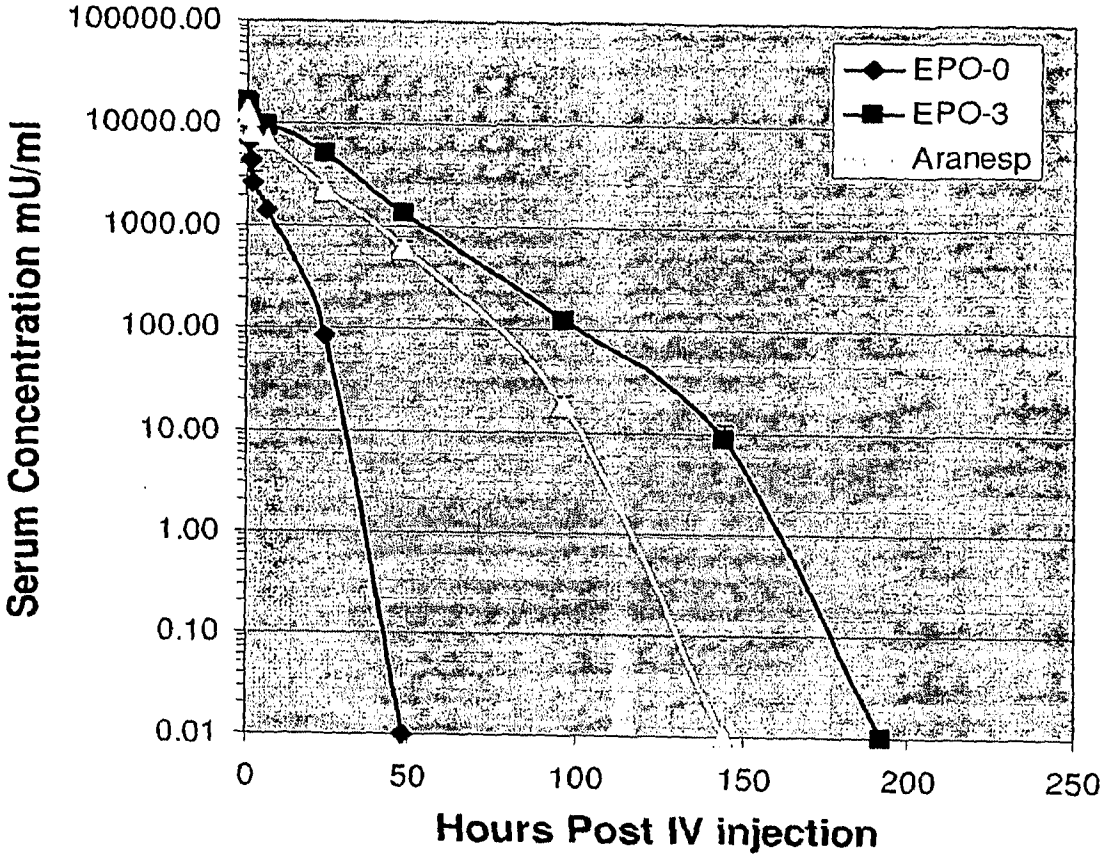


FIGURE 9

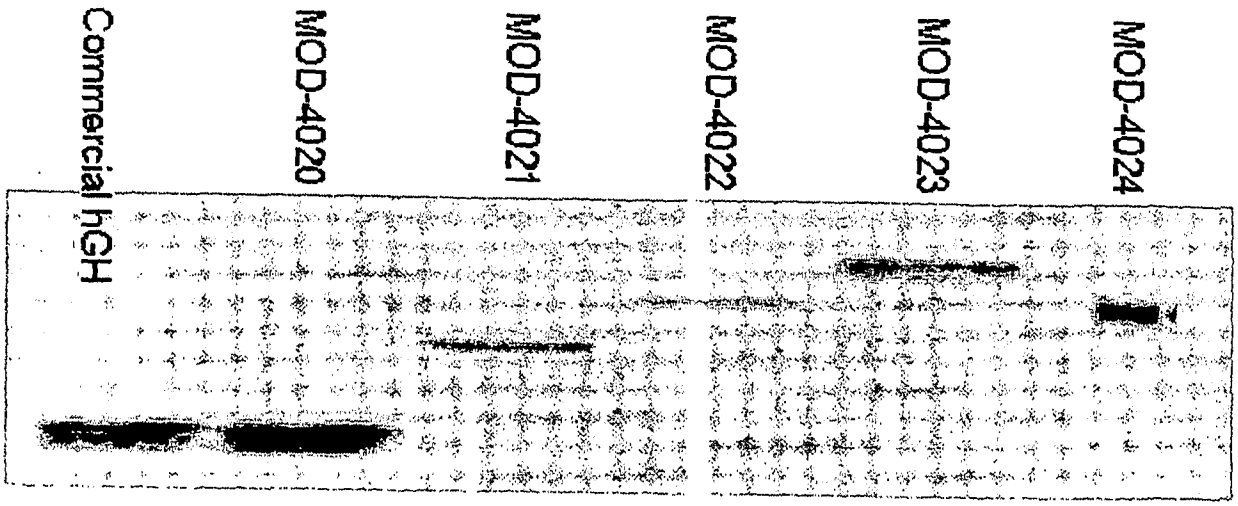


FIGURE 10

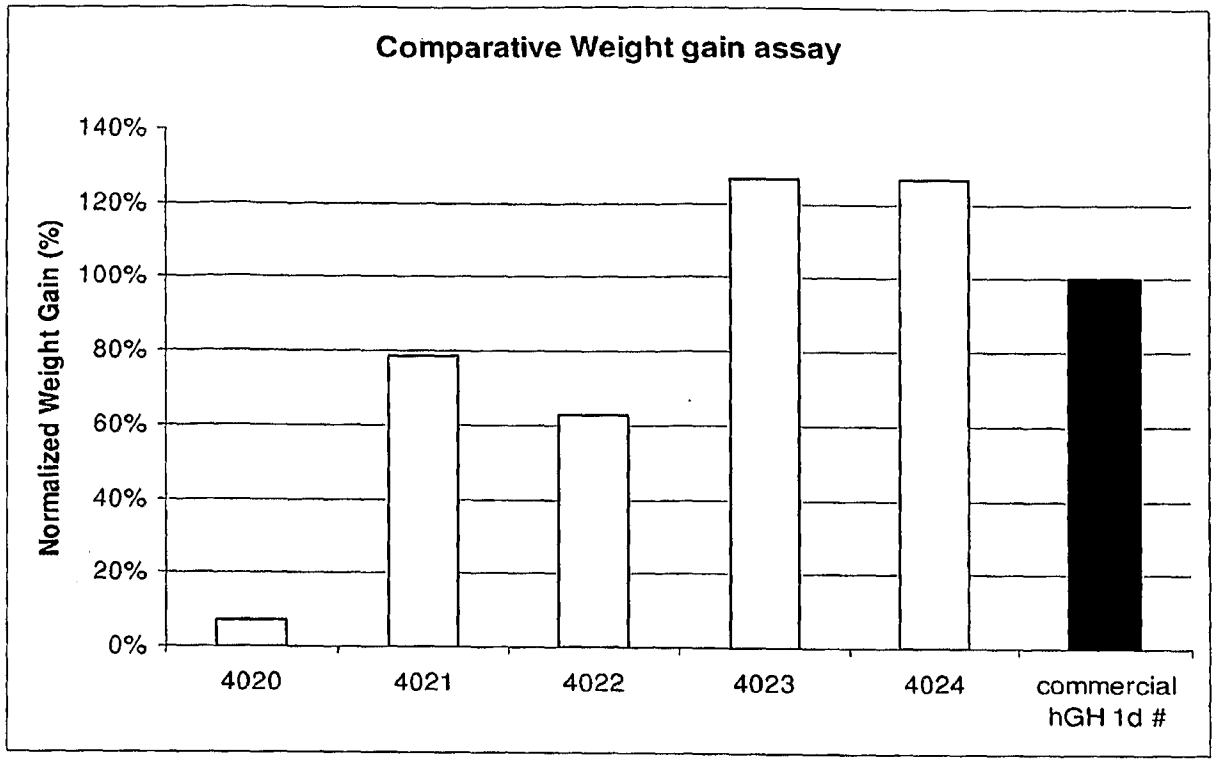

Comparative Weight gain assay
Normalized Weight Gain (%)
140%
120%
100%
80%
60%
40%
20%
0%
4020
4021
4022
4023
4024
commercial hGH 1d #

FIGURE 11