CLAIMS:

WHAT IS CLAIMED IS:

1. A fusion protein, comprising a growth hormone (GH) sequence selected from human growth hormone of SEQ ID NO: 1 and an agonist thereof that is at least 90% identical to the amino acid sequence of SEQ ID NO: 1, wherein said GH is linked to an extended recombinant polypeptide (XTEN) of at least about 200 amino acid residues, wherein the XTEN is characterized in that:

(a) the XTEN sequence comprises at least about 200 contiguous amino acids that exhibits at least 90% sequence identity to a comparable length of an amino acid sequence selected from SEQ ID NOs 59-79;

(b) the XTEN sequence lacks a predicted T-cell epitope when analyzed by TEPITOPE algorithm, wherein the TEPITOPE algorithm prediction for epitopes within the XTEN sequence is based on a score of –9 or greater;

(c) it has a subsequence score of less than 10; and

(d) the sum of glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) residues constitutes more than about 90% of the total amino acid residues of the XTEN.

2. The fusion protein of claim 1, further comprising a second XTEN sequence, and wherein the fusion protein adopts a multiple-XTEN configurationwhich is XTEN-GH-XTEN, GH-XTEN-XTEN, XTEN-XTEN-GH, XTEN-GH-XTEN-XTEN, XTEN-XTEN-GH-XTEN, XTEN-XTEN-GH-XTEN, GH-XTEN-GH-XTEN, XTEN-GH-XTEN-GH, XTEN-XTEN-GH-XTEN-GH, XTEN-XTEN-GH-GH, GH-XTEN-XTEN-GH, GH-GH-XTEN-XTEN, GH-GH-XTEN-XTEN-GH, or GH-XTEN-GH-XTEN-GH.

3. The fusion protein of claim 1 or claim 2, wherein the fusion protein binds to growth hormone receptor(s) with an affinity lower than that of the corresponding GH that lacks the XTEN.

4. The fusion protein of claim 1 or 2, comprising an XTEN amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%,95%,96%,97%,98%, or 99% sequence identity to an amino acid sequence selected from SEQ ID NOs 669-844.

5. The fusion protein of claim 1 or 2, comprising an amino acid sequence that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to an amino

acid sequence selected from SEQ ID NO: 689, SEQ ID NO: 695, SEQ ID NO: 697, SEQ ID NO: 757, and SEQ ID NO: 791.

6. The fusion protein of claim 5, comprising an amino acid sequence selected from SEQ ID NO: 689, SEQ ID NO: 695, SEQ ID NO: 697, SEQ ID NO: 757, and SEQ ID NO: 791.

7. The fusion protein of claim 5, comprising an amino acid sequence that has at least 90% sequence identity to the amino acid sequence of SEQ ID NO: 757.

8. The fusion protein of claim 7, wherein the fusion protein comprises an amino acid sequence that has at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 757.

9. The fusion protein of claim 7, comprising the amino acid sequence of SEQ ID NO: 757.

10. The fusion protein of claim 1 or 2, comprising an amino acid sequence encoded by a nucleic acid that has at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to a nucleic acid sequence selected from SEQ ID NOs 669-796.

11. The fusion protein of claim 10, wherein the nucleic acid is selected from SEQ ID NO:690, SEQ ID NO: 696, SEQ ID NO: 698, SEQ ID NO: 758 and SEQ ID NO: 792.

12. The fusion protein of claim 10, wherein the nucleic acid is SEQ ID NO: 758.

13. A fusion protein comprising an amino acid sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 1 linked to an extended recombinant polypeptide (XTEN) sequence having at least 90% sequence identity to the sequence of SEQ ID NO: 75.

14. The fusion protein of claim 13 wherein said XTEN sequence has at least 99% sequence identity to the sequence of SEQ ID NO: 75.

15. The fusion protein of claim 1, configured according to formula I:

$(XTEN)_x$-GH-$(XTEN)_y$ (I)

wherein independently for each occurrence:

(a) x is either 0 or 1; and

(b) y is either 0 or 1, wherein $x+y \geq 1$.

16. The fusion protein of claims 1, 2, 13, 14 or 15, characterized in that it has a longer terminal half-life when administered to a subject compared to the corresponding GH that lacks the XTEN when administered to a subject at a comparable molar dose.

17. The fusion protein of claims 1, 2, 13, 14 or 15, characterized in that the binding affinity of the fusion protein to the growth hormone receptor is reduced by at least about 10-fold as compared to the binding affinity of the corresponding GH that lacks the XTEN.

18. The fusion protein of claims 1, 2, 13, 14 and 15, characterized in that

(i) when a smaller molar amount of the fusion protein than the corresponding GH that lacks the XTEN is administered to a subject , the fusion protein achieves a prolonged biologically active concentration as compared to the corresponding GH that lacks the XTEN;

(ii) when a smaller molar amount of the fusion protein than the corresponding GH that lacks the XTEN is administered to a subject, the fusion protein achieves a comparable therapeutic effect as the corresponding GH that lacks the XTEN;

(iii) when the fusion protein is administered to a subject less frequently in comparison to the corresponding GH that lacks the XTEN administered to a subject using an otherwise equivalent molar amount, the fusion protein achieves a similar biologically active concentration as compared to the corresponding GH that lacks the XTEN;

(iv) when the fusion protein is administered to a subject less frequently in comparison to the corresponding GH that lacks the XTEN administered to a subject using an otherwise equivalent molar amount, the fusion protein achieves a comparable therapeutic effect as the corresponding GH that lacks the XTEN.

19. A nucleic acid comprising a nucleic acid sequence that has at least 90% sequence identity to a sequence selected from SEQ ID NOs 669-796, or the complement thereof.

20. The nucleic acid of claim 19, wherein the nucleic acid has at least 90% sequence identity to a sequence selected from SEQ ID NO:690, SEQ ID NO: 696, SEQ ID NO: 698, SEQ ID NO: 758 and SEQ ID NO: 792.

21. The nucleic acid of claim 20, wherein the nucleic acid has at least 90% sequence identity to the sequence of SEQ ID NO:690.

22. The nucleic acid of claim 21, wherein the nucleic acid comprises the sequence of SEQ ID NO: 690.

23. A method of producing a fusion protein comprising GH fused to one or more extended recombinant polypeptides (XTEN), comprising:

(a) providing a host cell comprising a recombinant polynucleotide molecule of any one of claims 19-22;

(b) culturing the host cell under conditions permitting the expression of the fusion protein; and

(c) recovering the fusion protein.

24. The method of claim 23, wherein the one or more XTEN of the expressed fusion protein has at least 90% sequence identity to a sequence selected from SEQ ID NOs 59-79.

25. The method of claim 23, wherein the recombinant polynucleotide molecule encoding the fusion protein comprises a nucleic acid sequence exhibiting at least 90% sequence identity to a nucleic acid sequence selected from SEQ ID NOs 669-844.

26. The method of claim 23, wherein the recombinant polynucleotide molecule is SEQ ID NO: 758.

27. The method of claim 23, wherein the host cell is a prokaryotic cell.

28. The method of claim 23, wherein the fusion protein is recovered from the host cell cytoplasm in substantially soluble form.

29. A fusion protein made by the method of claim 23, wherein the recombinant polynucleotide molecule comprises a nucleic acid sequence exhibiting at least 90% sequence identity to the sequence of SEQ ID NO: 758.

30. The fusion protein of claim 29, wherein the recombinant polynucleotide molecule is SEQ ID NO: 758.

31. A nucleic acid comprising a nucleotide sequence encoding the fusion protein of claim 1or the complement thereof.

32. A nucleic acid comprising a nucleotide sequence encoding the fusion protein of claim 13, or the complelemt thereof.

33. An expression vector comprising the nucleic acid of any one of claims19-22 and 31-32.

34. An isolated host cell comprising the nucleic acid of any one of claims 19-22 and 31-32, wherein the host cell is a prokaryotic host cell.

35. Use of a pharmaceutical composition for the manufacture of a medicament for treating a growth-hormone related condition in a subject, the pharmaceutical composition comprising a therapeutically effective amount of a fusion protein of claim, 1, 2, 13, 14 or 15 and a pharmaceutically acceptable carrier.

36. The use of claim 35, wherein the growth-hormone related condition is selected from growth-hormone deficiency, Turner's Syndrome, Prader-Willi Syndrome, chronic renal failure, intrauterine growth retardation, idiopathic short stature, AIDS wasting, obesity, multiple sclerosis, fibromyalgia, Crohn's disease, ulcerative colitis, muscular dystrophy, low muscle mass, and osteoporosis, myocardial ischaemia, low bone density.

37. The use of claim 35, wherein administration of two or more consecutive doses of the medicament using a therapeutically effective dose regimen to a subject results in a prolonged period between consecutive C_{max} peaks and/or C_{min} troughs for blood levels of the fusion protein

compared to the corresponding GH that lacks the XTEN and is administered using a therapeutically effective dose regimen established for the GH.

38. The use of claim 35, wherein:

(i) the medicament is suitable for administration of a smaller molar amount of the fusion protein to a subject in comparison to the corresponding GH that lacks the XTEN administered to a subject under an otherwise equivalent dose regimen, and the medicament comprising the fusion protein achieves a comparable therapeutic effect as the corresponding GH that lacks the XTEN;

(ii) the medicament is suitable for less frequent administration to a subject in comparison to the corresponding GH that lacks the XTEN administered to a subject using an otherwise equivalent molar dose, and the medicament comprising the fusion protein achieves a comparable therapeutic effect as the corresponding GH that lacks the XTEN.

39. The use of claim 35, wherein the therapeutic effect is a measured parameter selected from IGF-1 concentrations, IGFBP3 concentration, height velocity, lean body mass, total body fat, trunk fat, response to insulin challenge, rate of division of chondrocytes, chondrocyte numbers, bone density, bone growth, and increase in epiphyseal plate width.

40. The use of claim 35, wherein a greater increase of blood IGF-1 concentration is achieved in the subject administered said medicament with at least 14 day intervals compared to corresponding GH that lacks the XTEN and administered daily at the same total molar dose.

41. The use of claim 35, wherein the therapeutic effect is evidenced by an increased weight gain and bone elongation in a mouse subject.

42. The use of claim 35, wherein plasma concentrations of said fusion protein in the subject administered with a single dose of 0.3 mg/kg of said fusion protein are detectable at least 400 h after said administration.

43. The use of claim 35, wherein administration of said composition to a subject yields (a) in the subject a plasma concentration of said fusion protein of at least about 100 ng/ml after said administration; and/ or (b) an increased IGF-1 concentration compared to the IGF-1 concentration prior to dosing.

WHAT IS CLAIMED IS:

1. An isolated fusion protein, comprising a growth hormone (GH) that is at least 90% identical to an amino acid sequence selected from Table 1 , wherein said growth hormone is linked to an extended recombinant polypeptide (XTEN) of at least 200 amino acids residues, wherein the XTEN is characterized in that:

(a) the XTEN sequence comprises at least 200 contiguous amino acids that exhibits at least 90% sequence identity to a comparable length of an amino acid sequence selected from Table 3;

(b) the XTEN sequence lacks a predicted T-cell epitope when analyzed by TEPITOPE algorithm, wherein the TEPITOPE algorithm prediction for epitopes within the XTEN sequence is based on a score of -9 or greater;

(c) it has a subsequence score of less than 10; and

(d) the sum of glycine (G), alanine (A), serine (S), threonine (T), glutamate (E) and proline (P) residues constitutes more than 90% of the total amino acid residues of the XTEN.

(e) the XTEN sequence is substantially non-repetitive such that (i) at least 80% of the XTEN sequence consists of non-overlapping sequence motifs wherein each of the sequence motifs has at least 9 to 14 amino acid residues and wherein the sequence of any two contiguous amino acid residues does not occur more than twice in each of the sequence motifs; or (ii) the XTEN sequence contains no three contiguous amino acids that are identical unless the amino acids are serine residues.

2. The isolated fusion protein of claim 1 , wherein the growth hormone is a human growth hormone.

3. The isolated fusion protein of claim 1 or 2, further comprising a second XTEN sequence, and wherein the fusion protein adopts a multiple -XTEN configuration shown in Table 5.

4. The isolated fusion protein of any one of claims 1 to 3, wherein the growth hormone peptide and the XTEN is linked via a spacer, wherein the spacer sequence comprises between 1 to 50 amino acid residues, and wherein the spacer optionally comprises a cleavage sequence.

5. The isolated fusion protein of any of claims 1 to 4, wherein the fusion protein binds to the same target receptor of the corresponding native growth hormone that lacks the XTEN, and wherein said fusion protein retains at least 0.1%-30% of the binding affinity of the corresponding growth hormone that lacks the XTEN.

6. The isolated fusion protein of any of claims 1 to 5, comprising an amino acid sequence that has at least 90% sequence identity to an amino acid sequence selected from Table 35, Table 36, and Table 37.

7. A pharmaceutical composition comprising the isolated fusion protein of any of claims 1 to 6, and a pharmaceutically acceptable carrier.

8. The isolated protein of any of claims 1 to 6 that is configured according to formula I: $(XTEN)_x$-GH-$(XTEN)_5$, (I) wherein independently for each occurrence:

(a) x is either 0 or 1 ; and

(b) y is either 0 or 1, wherein x+y>l.

9. The isolated fusion protein of any of claims 1 to 6, wherein the XTEN is fused to the growth hormone on an N- or C-terminus of the growth hormone.

10. The isolated fusion protein of any of claims 1 to 6 and 8 to 9, comprising a human growth hormone of Table 1 and a first and a second XTEN selected from AE912, AM923, AE144, and AE288 of Table 3.

11. The isolated fusion protein of any of claims 1 to 6 and 8 to 10, characterized in that:

(i) it has a longer terminal half- life when administered to a subject compared to the corresponding growth hormone that lacks the XTEN when administered to a subject at a comparable molar dose;

(ii) when a smaller molar amount of the fusion protein is administered to a subject in comparison to the corresponding growth hormone that lacks the XTEN administered to a subject under an otherwise equivalent dose regimen, the fusion protein achieves a comparable area under the curve (AUC) as the corresponding growth hormone that lacks the XTEN;

(iii) when a smaller molar amount of the fusion protein is administered to a subject in comparison to the corresponding growth hormone that lacks the XTEN administered to a subject under an otherwise equivalent dose regimen, the fusion protein achieves a comparable therapeutic effect as the corresponding growth hormone that lacks the XTEN;

(iv) when the fusion protein is administered to a subject less frequently in comparison to the corresponding growth hormone that lacks the XTEN administered to a subject using an otherwise equivalent molar amount, the fusion protein achieves a comparable area under the curve (AUC) as the corresponding growth hormone that lacks the XTEN;

(v) when the fusion protein is administered to a subject less frequently in comparison to the corresponding growth hormone that lacks the XTEN administered to a subject using an otherwise equivalent molar amount, the fusion protein achieves a comparable therapeutic effect as the corresponding growth hormone that lacks the XTEN;

(vi) when an accumulatively smaller molar amount of the fusion protein is administered to a subject in comparison to the corresponding growth hormone that lacks the XTEN administered to a subject under an otherwise equivalent dose period, the fusion protein achieves comparable area under the curve (AUC) as the corresponding growth hormone that lacks the XTEN; or

(vii) when an accumulatively smaller molar amount of the fusion protein is administered to a subject in comparison to the corresponding growth hormone that lacks the XTEN administered to a subject under an otherwise equivalent dose period, the fusion protein achieves comparable therapeutic effect as the corresponding growth hormone that lacks the XTEN.

12. A method of producing a fusion protein comprising a growth hormone (GH) fused to one or more extended recombinant polypeptides (XTEN), comprising:

(a) providing host cell comprising a recombinant polynucleotide molecule encoding the fusion protein of claim 1 or 6;

(b) culturing the host cell under conditions permitting the expression of the fusion protein; and

(c) recovering the fusion protein.

13. The method of claim 12, wherein the growth hormone of the fusion protein has at least 90% sequence identity to:

(a) a human growth hormone; or

(b) the human growth hormone sequence selected from Table 1.

14. The method of claim 12 or 13, wherein the one or more XTEN of the expressed fusion protein has at least 90% sequence identity to a sequence selected from Table 3.

15. The method of any of claims 12 to 14, wherein the polynucleotide molecule encoding the fusion protein comprises a nucleic acid sequence exhibiting at least 90% sequence identity of a nucleic acid sequence selected from Table 35, Table 36, and Table 37.

16. The method of any of claims 12 to 15, wherein the polynucleotide is codon optimized for enhanced expression of said fusion protein in the host cell.

17. The method any of claims 12 to 16, wherein the host cell is a prokaryotic cell.

18. The method of any of claims 12 to 17, wherein the isolated fusion protein is recovered from the host cell cytoplasm in substantially soluble form.

19. An isolated nucleic acid comprising a nucleotide sequence encoding the fusion protein of any of claims 1 to 6 and 8 to 11 or the complement thereof.

20. An expression vector comprising the nucleic acid of claim 19.

21. An isolated host cell comprising the nucleic acid of claim 19.

22. A fusion protein comprising a growth hormone (GH)for treating a growth-hormone related condition in a subject, comprising the fusion protein of any of claims 1 to 6 and 8 to 11.

23. The fusion protein of claim 22, wherein the growth-hormone related condition is selected from growth-hormone deficiency, Turner's Syndrome, Prader-Willi Syndrome, idiopathic short stature, AIDS wasting, multiple sclerosis, Crohn's disease, ulcerative colitis, and muscular dystrophy.

24. The fusion protein of claim 22 or 23, wherein a therapeutically effective amount of the fusion protein results in maintaining blood concentrations of the fusion protein within a therapeutic window for the fusion protein at least three-fold longer compared to the corresponding native growth hormone that lacks the XTEN administered at a comparable amount to a subject.

25. The fusion protein of any of claims 22 to 24, wherein two or more consecutive doses of the fusion protein administered under a therapeutically effective dose regimen results in a gain in time between consecutive C_{max} peaks and/or C_{mn} troughs for blood levels of the fusion protein compared to the corresponding growth hormone not linked to the fusion protein and administered using a therapeutically dose regimen established for the GH.

26. The fusion protein of any of claims 22 to 25, wherein

(i) a smaller molar amount of the fusion protein in comparison to the corresponding growth hormone that lacks the XTEN achieves a comparable therapeutic effect as the corresponding growth hormone that lacks the XTEN;

(ii) the fusion protein is administered less frequently to a subject in comparison to the corresponding growth hormone that lacks the XTEN administered to a subject using an otherwise equivalent molar dose, and the fusion protein achieves a comparable therapeutic effect as the corresponding growth hormone that lacks the XTEN; or

(iii) an accumulatively smaller molar amount of the fusion protein is administered to a subject in comparison to the corresponding growth hormone that lacks the XTEN administered to a subject under the otherwise equivalent dose period, the fusion protein achieves a comparable therapeutic effect as the corresponding growth hormone that lacks the XTEN.

27. The fusion proteinof claim 26, wherein the therapeutic effect is a measured parameter selected from IGF- 1 concentrations, IGFBP3 concentration, height velocity, lean body mass, total body fat, trunk fat, response to insulin challenge, rate of division of chondrocytes, chondrocyte numbers, bone density, bone growth, and increase in epiphyseal plate width.

28. A kit, comprising the pharmaceutical composition of claim 7, a label identifying the pharmaceutical composition, and an instruction for storage, reconstitution and/or administration of the pharmaceutical compositions to a subject.

29. A fusion protein, comprising an amino acid sequence that is at least 90% identical to the amino acid sequence of AE912-hGH-AE144 as shown in Table 36.

30. The fusion protein of claim 29, comprising an amino acid sequence that is at least 95% identical to the amino acid sequence of AE912-hGH-AE144 as shown in Table 36.

31. The fusion protein of claim 29, comprising an amino acid sequence that is at least 99% identical to the amino acid sequence of AE912-hGH-AE144 as shown in Table 36.

32. A fusion protein, comprising the amino acid sequence of AE912-hGH-AE144 as shown in Table 36.

33. An isolated nucleic acid comprising a nucleotide sequence encoding the fusion protein of any one of claims 29-32, or the complement thereof.

34. An expression vector comprising the nucleic acid of claim 33.

35. An isolated host cell comprising the nucleic acid of claim 33.

36. The isolated host cell of claim 21 or claim 35, wherein the host cell is a prokaryotic host cell.

37. Use of a therapeutically effective amount of a fusion protein of any one of claims 29-32, in the manufacture of a medicament for treating a growth-hormone related condition in a subject.

38. The use of claim 37, wherein the growth-hormone related condition is selected from growth-hormone deficiency, Turner's Syndrome, Prader-Willi Syndrome, idiopathic short stature, AIDS wasting, multiple sclerosis, Crohn's disease, ulcerative colitis, and muscular dystrophy.

39. A pharmaceutical composition comprising the isolated fusion protein of any one of claims 29-32, and a pharmaceutically acceptable carrier.

40. A kit, comprising the pharmaceutical composition of claim 39, a label identifying the pharmaceutical composition, and an instruction for storage, reconstitution and/or administration of the pharmaceutical compositions to a subject.

41. The fusion protein of claim 1 or 2, further comprising an additional XTEN polypeptide sequence having at least 90% sequence identity to the amino acid sequence of amino acids 1106 to 1251 of AE912-hGH-AE144 as shown in Table 36.

42. The fusion protein of claim 41, wherein the additional XTEN polypeptide sequence has at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the sequence of amino acids 1106 to 1251 of AE912-hGH-AE144 as shown in Table 36.

43. The fusion protein of claim 41, wherein the additional XTEN polypeptide sequence has at least 98% or 99% identity to the sequence of amino acids 1106 to 1251 of AE912-hGH-AE144 as shown in Table 36.

44. The fusion protein of claim 41, wherein the additional XTEN polypeptide sequence comprises the sequence of amino acids 1106 to 1251 of AE912-hGH-AE144 as shown in Table 36.