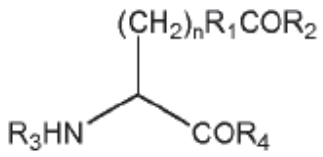
CLAIMS

1. An IFN beta polypeptide comprising an amino acid sequence as set forth in SEQ ID NOs: 1, 3 or 4, except that at least one amino acid is substituted by a non-naturally encoded amino acid at positions selected from positions 36 and 111 of SEQ ID NO: 1 or the corresponding amino acid positions in SEQ ID NOs: 3 or 4, wherein the polypeptide is linked to a water soluble polymer and wherein the non-naturally encoded amino acid has the structure:



wherein n is 0-10, R_1 is an alkyl, aryl, substituted alkyl, or substituted aryl; R_2 is H, an alkyl, aryl, substituted alkyl, or substituted aryl; and R_3 is H, an amino acid, a polypeptide, or a carboxy terminus modification group.

2. The IFN beta polypeptide of claim 1, wherein a non-naturally encoded amino acid is substituted at position 36 of SEQ ID NO: or the corresponding amino acid position in SEQ ID NOs: 3 or 4.

3. The IFN beta polypeptide of claim 1, wherein the polypeptide is linked to a bifunctional polymer, bifunctional linker, or at least one additional IFN beta polypeptide.

4. The IFN beta polypeptide of claim 2, wherein the bifunctional linker or polymer is linked to a second polypeptide.

5. The IFN beta polypeptide of claim 3, wherein the second polypeptide is a IFN beta polypeptide.

6. The IFN beta polypeptide of claim 1, wherein said water soluble polymer is linked to a non-naturally encoded amino acid present in said IFN beta polypeptide.

7. The IFN beta polypeptide of claim 1, wherein the water soluble polymer has a molecular weight of between about 0.1 kDa and about 100 kDa, more specifically wherein the water soluble polymer has a molecular weight of between about 0.1 kDa and about 50 kDa.

8. The IFN beta polypeptide of claim 1, which is made by reacting a IFN beta polypeptide comprising a carbonyl-containing amino acid with a water soluble polymer comprising an aminooxy, hydrazine, hydrazide or semicarbazide group, more specifically wherein the aminooxy, hydrazine, hydrazide or semicarbazide group is linked to the water soluble polymer through an amide linkage.

9. The IFN beta polypeptide of claim 1, which is made by reacting a water soluble polymer comprising a carbonyl group with a polypeptide comprising a non-naturally encoded amino acid that comprises an aminooxy, a hydrazine, a hydrazide or a semicarbazide group, or;

the IFN beta polypeptide of claim 1, which is made by reacting a IFN beta polypeptide comprising an alkyne-containing amino acid with a water soluble polymer comprising an azide moiety, or;

the IFN beta polypeptide of claim 1, which is made by reacting a IFN beta polypeptide comprising an azide-containing amino acid with a water soluble polymer comprising an alkyne moiety.

10. The IFN beta polypeptide of claim 1, wherein the non-naturally encoded amino acid comprises an azide or an alkyne group, wherein said group is linked to a water soluble polymer through an amide linkage.

11. The IFN beta polypeptide of claim 1, wherein the water soluble polymer is poly(ethylene glycol) and the poly(ethylene glycol) moiety is a branched or multiarmed polymer and wherein each branch of the water soluble polymer has a molecular weight of between about 1 kDa and about 100 kDa.

CLAIMS

1. An IFN beta polypeptide comprising an amino acid sequence set forth in SEQ ID NOs: 1, 3 or 4 or variant of SEQ ID NO: 1 comprising a leader or signal peptide consisting of the sequence of residues 1-21 of SEQ ID NO: 4, wherein said IFN beta polypeptide sequence is modified by the presence of one or more non-naturally encoded amino acids therein at a residue position of SEQ ID NO: 1 or 3 selected from the group consisting of residues 36 and 111 or corresponding residue position(s) of SEQ ID NO: 4 or said variant of SEQ ID NO: 1, wherein the non-naturally encoded amino acid is p-acetyl-phenylalanine (pAF), and wherein said IFN beta polypeptide is linked to a water-soluble polymer comprising a poly(ethylene glycol) moiety and having a molecular weight of about 30 kDa or about 40 kDa.

2. The IFN beta polypeptide according to claim 1, wherein the non-naturally encoded amino acids is at residue position 36 of SEQ ID NO: 1 or 3 or corresponding residue position of SEQ ID NO: 4 or variant of SEQ ID NO: 1.

3. The IFN beta polypeptide according to claim 1 , wherein the one or more non-naturally encoded amino acids is at residue position 111 of SEQ ID NO: 1 or 3 or corresponding residue position of SEQ ID NO: 4 or variant of SEQ ID NO: 1.

4. The IFN beta polypeptide according to any one of claims 1 to 3, wherein said IFN beta polypeptide comprises a plurality of the non-naturally encoded amino acids at residue positions 36 and 111 of SEQ ID NO: 1 or 3 or corresponding residue positions of SEQ ID NO: 4 or variant of SEQ ID NO: 1.

5. The IFN beta polypeptide according to any one of claims 1 to 4, wherein the IFN beta polypeptide comprises SEQ ID NO: 1 or 3 modified by the presence of the one or more non-naturally encoded amino acids.

6. The IFN beta polypeptide according to any one of claims 1 to 4, wherein the IFN beta polypeptide comprises SEQ ID NO: 4 modified by the presence of the one or more non-naturally encoded amino acids.

7. The IFN beta polypeptide according to any one of claims 1 to 4, wherein the IFN beta polypeptide comprises the variant of SEQ ID NO: 1, wherein said variant comprises residues 1-21 of SEQ ID NO: 4 positioned before the methionine residue at position 1 of SEQ ID NO: 1 and comprises a serine residue at position 38 thereof and is modified by the presence of the one or more non-naturally encoded amino acids.

8. The IFN beta peptide according to any one of claims 1 to 7, wherein the IFN beta polypeptide has improved antiviral activity relative to SEQ ID NO: 1 .

9. The IFN beta peptide according to claim 8, wherein the improved antiviral activity is antiviral activity against Vesicular Stomatitis Virus (VSV).

10. The IFN beta peptide according to claim 8 or 9, wherein the improved antiviral activity relative to SEQ ID NO: 1 is determined by infection of isolated human WISH cells by Vesicular Stomatitis Virus (VSV).

11. The IFN beta polypeptide according to any one of claims 1 to 10, wherein the IFN beta polypeptide further comprises one or more amino acid substitutions, additions or deletions that modulate affinity of the IFN beta polypeptide for an IFN receptor and/or increase stability of the IFN beta polypeptide and/or increase solubility of the IFN beta polypeptide and/or modulate immunogenicity of the IFN beta polypeptide and/or modulate serum half-life or circulation time of the IFN beta polypeptide and/or enhance anti-viral activity of the IFN beta polypeptide and/or increase expression of the IFN beta polypeptide when it is produced in a recombinant host cell or synthesized in vitro and/or increase protease resistance of the IFN beta polypeptide.

12. The IFN beta polypeptide according to any one of claims 1 to 11 when produced by a process comprising ribosomal incorporation of p-acetyl-phenylalanine (pAF) ex vivo or in a recombinant cell comprising: (i) mRNA encoding said IFN beta polypeptide and comprising a selector codon that is selective for the p-acetyl-phenylalanine (pAF); (ii) the p-acetyl-phenylalanine (pAF); (iii) an orthogonal tRNA (O-tRNA) molecule capable of being aminoacylated with the p-acetyl-phenylalanine (pAF); (iv) an orthogonal RNA synthetase (O-RS) capable of aminoacylating the O-tRNA.

13. The IFN beta polypeptide according to any one of claims 1 to 11 when produced by a process comprising reacting an IFN beta polypeptide with one or more water-soluble polymers each comprising a poly(ethylene glycol) moiety to thereby form an amide linkage or oxime bond between a p-acetyl-phenylalanine (pAF) residue and a poly(ethylene glycol) moiety.

14. The IFN beta polypeptide according to any one of claims 1 to 11 when produced by a process comprising:

(a) producing an IFN beta polypeptide by ribosomal incorporation of the p-acetyl-phenylalanine (pAF) ex vivo or in a recombinant cell comprising: (i) mRNA encoding said IFN beta polypeptide and comprising a selector codon that is selective for the p-acetyl-phenylalanine (pAF); (ii) the p-acetyl-phenylalanine (pAF); (iii) an orthogonal tRNA (O-tRNA) molecule capable of being aminoacylated with the p-acetyl-phenylalanine (pAF); (iv) an orthogonal RNA synthetase (O-RS) capable of aminoacylating the O-tRNA; and

(b) reacting the produced IFN beta polypeptide with one or more water-soluble polymers each comprising a poly(ethylene glycol) moiety to thereby form an amide linkage or oxime bond between a p-acetyl-phenylalanine (pAF) residue and a poly(ethylene glycol) moiety.

15. The IFN beta polypeptide according to any one of claims 1 to 14, when modified post-translationally by linkage to a linker or biologically active molecule.

16. The IFN beta polypeptide of claim 15, wherein the biologically active molecule is a second polypeptide.

17. The IFN beta polypeptide according to claim 16, wherein the second polypeptide is a second IFN beta polypeptide.

18. The IFN beta polypeptide according to claim 16 or 17, wherein the second polypeptide is linked to polymer.

19. The IFN beta polypeptide according to claim 18, wherein the polymer is a water soluble polymer.

20. The IFN beta polypeptide according to claim 18, wherein the water soluble polymer comprises a poly(ethylene glycol) moiety.

21. The IFN beta polypeptide according to any one of claims 15 to 20, wherein the linker or biologically active molecule is linked to the IFN beta polypeptide via a saccharide moiety.

22. An isolated nucleic acid comprising a polynucleotide that encodes the IFN beta polypeptide according to any one of claims 1 to 14, wherein said polynucleotide comprises at least one selector codon encoding a p-acetyl-phenylalanine (pAF) residue of said IFN beta polypeptide.

23. The isolated nucleic acid according to claim 22, wherein the selector codon is selected from the group consisting of an amber codon, ochre codon, opal codon, a unique codon, a rare codon, and a four-base codon.

24. A method of making an IFN beta polypeptide, said method comprising contacting an IFN beta polypeptide with a water-soluble polymer comprising a poly(ethylene glycol) moiety and having a molecular weight of about 30 kDa or about 40 kDa to thereby form a linkage between the IFN beta polypeptide and the poly(ethylene glycol) moiety, wherein said IFN beta polypeptide comprises an amino acid sequence set forth in SEQ ID NOs: 1, 3 or 4 or variant of SEQ ID NO: 1 comprising a leader or signal peptide consisting of the sequence of residues 1-21 of SEQ ID NO: 4, and wherein said IFN beta polypeptide sequence is modified by the presence of one or more p-acetyl-phenylalanine (pAF) residues

therein at a residue position of SEQ ID NO: 1 or 3 selected from the group consisting of residues 36 and 111 or corresponding residue position(s) of SEQ ID NO: 4 or said variant of SEQ ID NO: 1.

25. The method according to claim 24, wherein the poly(ethylene glycol) moiety comprises an aminooxy, a hydrazine, a hydrazide or a semicarbazide functional group, and wherein reacting the IFN beta polypeptide with the poly(ethylene glycol) moiety produces an amide linkage between a p-acetyl-phenylalanine (pAF) residue of the IFN beta polypeptide and the poly(ethylene glycol) moiety.

26. The method according to claim 24, wherein contacting an IFN beta polypeptide with a poly(ethylene glycol) moiety produces an oxime bond between a p-acetyl-phenylalanine (pAF) residue of the IFN beta polypeptide and the poly(ethylene glycol) moiety.

27. A cell comprising the isolated nucleic acid according to claim 22 or 23.

28. The cell according to claim 27, wherein the cell further comprises an orthogonal tRNA synthetase or an orthogonal tRNA.

29. A method of making an IFN beta polypeptide comprising a non-naturally encoded amino acid, the method comprising:

(i) culturing cells comprising the nucleic acid according to claim 22 or 23 in the presence of p-acetyl-phenylalanine (pAF) for incorporation into the IFN beta polypeptide, an orthogonal RNA synthetase, and an orthogonal tRNA under conditions sufficient to permit expression of the IFN beta polypeptide comprising the p-acetyl-phenylalanine (pAF) ; and

(ii) purifying the IFN beta polypeptide.

30. The method according to claim 29, further comprising:

(i) obtaining the cells, wherein the cells comprise or are capable of expressing the nucleic acid according to claim 22 or 23 and the orthogonal RNA synthetase and the orthogonal tRNA, and wherein the culturing comprises providing the p-acetyl-phenylalanine (pAF) to the cells thus obtained; or

(ii) producing recombinant cells for the culturing at (i), wherein said recombinant cells are capable of expressing the nucleic acid according to claim 22 or 23 and the orthogonal RNA synthetase and orthogonal tRNA, and wherein the culturing comprises providing the p-acetyl-phenylalanine (pAF) to the cells thus produced.

31. A recombinant IFN beta polypeptide comprising p-acetyl-phenylalanine (pAF) when produced by performing the method according to claim 29 or 30.

32. A pharmaceutical composition comprising the IFN beta polypeptide according to any one of claims 1 to 21 or 31 and a pharmaceutically acceptable carrier.

33. Use of the IFN beta polypeptide according to any one of claims 1 to 21 or 31 in the preparation of a medicament for treatment or prophylaxis of malignancy, cancer, tumor, tumor angiogenesis, multiple sclerosis (MS), ischemia reperfusion injury, multi-organ failure, intestinal ischemia- reperfusion injury (IRI), demyelinating optic neuritis (DON), stroke, transient ischemic attack, inflammatory bowel disease, an autoimmune disease, Guillain-Barre syndrome, or abnormal cell growth.

34. Use of the IFN beta polypeptide according to any one of claims 1 to 21 or 31 in the preparation of a medicament for treatment or prophylaxis of a viral infection or a viral condition associated with a viral infection.