

WHAT IS CLAIMED IS:

1. A composition comprising an interleukin-22 (IL-22) Fc fusion protein, wherein the IL-22 Fc fusion protein comprises a glycosylated IL-22 polypeptide linked to an antibody Fc region by a linker, and wherein the composition has an average sialic acid content in the range of 8 to 12 moles of sialic acid per mole of the IL-22 Fc fusion protein.
2. The composition of claim 1, wherein the IL-22 polypeptide is N-glycosylated.
3. The composition of claim 1 or 2, wherein the IL-22 polypeptide is glycosylated at one or more locations corresponding to amino acid residues Asn21, Asn35, Asn64, and/or Asn143 of SEQ ID NO: 4.
4. A composition comprising an IL-22 Fc fusion protein, wherein the IL-22 Fc fusion protein comprises a glycosylated IL-22 polypeptide linked to an antibody Fc region by a linker, wherein the IL-22 polypeptide is glycosylated at one or more locations corresponding to amino acid residues Asn21, Asn35, Asn64, and/or Asn143 of SEQ ID NO: 4, and wherein:
 - (a) the percent N-glycosylation site occupancy at residue Asn21 is in the range of 70 to 90;
 - (b) the percent N-glycosylation site occupancy at residue Asn35 is in the range of 90 to 100;
 - (c) the percent N-glycosylation site occupancy at residue Asn64 is in the range of 90 to 100;
 - and/or
 - (d) the percent N-glycosylation site occupancy at residue Asn143 is in the range of 25 to 35.
5. The composition of any one of claims 1-3, wherein the composition has an average sialic acid content in the range of 8 to 9 moles of sialic acid per mole of the IL-22 Fc fusion protein.
6. The composition of claim 4, wherein the composition has an average sialic acid content of 8 or 9 moles of sialic acid per mole of the IL-22 Fc fusion protein.
7. The composition of any one of claims 1-5, wherein the sialic acid glycosylation comprises N-acetylneuraminic acid (NANA).
8. The composition of any one of claims 1-5, wherein the composition has an average N-glycolylneuraminic acid (NGNA) content of less than 1 mole of NGNA per mole of the IL-22 Fc fusion protein.
9. The composition of any one of claims 1-8, wherein the composition is a liquid composition.
10. The composition of any one of claims 1-9, wherein:
 - (i) the IL-22 Fc fusion protein has a maximum observed concentration (C_{max}) of about 8,000 ng/mL to about 19,000 ng; and/or

(ii) the IL-22 Fc fusion protein has an area under the serum concentration-time curve from time 0 to the last measureable time point (AUC_{last}) of about 7,000 day·ng/mL to about 25,000 day·ng/mL; and/or

(iii) the IL-22 Fc fusion protein has a clearance (CL) of about 40 mL/kg/day to about 140 mL/kg/day.

11. The composition of claim 10, wherein the C_{max} , AUC_{last} , and/or CL is assessed following intravenous administration of about 1,000 µg/kg of the IL-22 Fc fusion protein to a CD1 mouse.

12. The composition of any one of claims 2-11, wherein the IL-22 polypeptide comprises N-glycans having monoantennary, biantennary, triantennary, and/or tetraantennary structure.

13. The composition of claim 12, wherein:

- (i) about 0.1% to about 2% of the N-glycans have monoantennary structure;
- (ii) about 10% to about 25% of the N-glycans have biantennary structure;
- (iii) about 25% to about 40% of the N-glycans have triantennary structure; and/or
- (iv) about 30% to about 51% of the N-glycans have tetraantennary structure.

14. The composition of any one of claims 2-13, wherein the IL-22 Fc fusion protein comprises N-glycans comprising zero, one, two, three, or four galactose moieties.

15. The composition of claim 14, wherein:

- (i) about 9% to about 32% of the N-glycans comprise zero galactose moieties;
- (ii) about 10% to about 20% of the N-glycans comprise one galactose moiety;
- (iii) about 8% to about 25% of the N-glycans comprise two galactose moieties;
- (iv) about 12% to about 25% of the N-glycans comprise three galactose moieties; and/or
- (v) about 12% to about 30% of the N-glycans comprise four galactose moieties.

16. The composition of any one of claims 2-15, wherein the IL-22 Fc fusion protein comprises N-glycans comprising zero, one, two, three, or four sialic acid moieties.

17. The composition of claim 16, wherein:

- (i) about 12% to about 35% of the N-glycans comprise zero sialic acid moieties;
- (ii) about 10% to about 30% of the N-glycans comprise one sialic acid moiety;
- (iii) about 10% to about 30% of the N-glycans comprise two sialic acid moieties;
- (iv) about 10% to about 30% of the N-glycans comprise three sialic acid moieties; and/or
- (v) about 1% to about 20% of the N-glycans comprise four sialic acid moieties.

18. The composition of any one of claims 2-17, wherein (i) the IL-22 polypeptide comprises about 0% to about 10% N-glycans comprising a terminal mannose moiety; and/or (ii) the IL-22 polypeptide comprises about 30% to about 55% N-glycans comprising a terminal N-acetylglucosamine (GlcNAc) moiety.

19. The composition of claim 18, wherein the N-glycans comprise one, two, three, or four terminal GlcNAc moieties.

20. The composition of claim 19, wherein:

- (i) about 1% to about 20% of the N-glycans comprise one terminal GlcNAc moiety;
- (ii) about 1% to about 20% of the N-glycans comprise two terminal GlcNAc moieties;
- (iii) about 5% to about 25% of the N-glycans comprise three terminal GlcNAc moieties; and/or
- (iv) about 0% to about 15% of the N-glycans comprise four terminal GlcNAc moieties.

21. The composition of any one of claims 2-20, wherein (i) the IL-22 polypeptide comprises about 20% to about 45% N-glycans comprising a terminal galactose (Gal) moiety; and/or (ii) the N-glycans comprise one, two, or three terminal Gal moieties.

22. The composition of claim 21, wherein:

- (i) about 15% to about 30% of the N-glycans comprise one terminal Gal moiety;
- (ii) about 1% to about 15% of the N-glycans comprise two terminal Gal moieties; and/or
- (iii) about 0.1% to about 6% of the N-glycans comprise three terminal Gal moieties.

23. The composition of any one of claims 2-22, wherein: (i) the IL-22 polypeptide comprises N-glycans comprising galactose N-acetylglucosamine (LacNAc) repeats; (ii) the IL-22 polypeptide comprises N-glycans comprising fucosylated N-glycans; and/or (iii) the IL-22 polypeptide comprises N-glycans comprising afucosylated N-glycans.

24. The composition of any one of claims 1-23, wherein the Fc region is not glycosylated.

25. The composition of claim 24, wherein: (i) the amino acid residue at position 297 as in the EU index of the Fc region is Gly or Ala; and/or (ii) the amino acid residue at position 299 as in the EU index of the Fc region is Ala, Gly, or Val.

26. The composition of any one of claims 1-25, wherein the Fc region comprises the CH2 and CH3 domain of IgG1 or IgG4.

27. The composition of claim 26, wherein the Fc region comprises the CH2 and CH3 domain of IgG4.

28. The composition of any one of claims 1-27, wherein the IL-22 Fc fusion protein comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:8.

29. The composition of any one of claims 1-28, wherein the IL-22 Fc fusion protein comprises or consists of the amino acid sequence of SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:16.

30. The composition of any one of claims 1-29, wherein the IL-22 polypeptide is a human IL-22 polypeptide.
31. The composition of claim 30, wherein the IL-22 polypeptide comprises the amino acid sequence of SEQ ID NO:4.
32. The composition of any one of claims 1-31, wherein the linker comprises or consists of the amino acid sequence RVESKYGPP (SEQ ID NO: 44).
33. The composition of any one of claims 1-32, wherein the IL-22 Fc fusion protein binds to IL-22 receptor.
34. The composition of claim 33, wherein the IL-22 receptor is human IL-22 receptor.
35. The composition of claim 34, wherein the human IL-22 receptor comprises a heterodimer consisting of an IL-22R1 polypeptide and an IL-10R2 polypeptide.
36. The composition of claim 35, wherein the IL-22R1 polypeptide comprises the amino acid sequence of SEQ ID NO:82 and the IL-10R2 polypeptide comprises the amino acid sequence of SEQ ID NO:84.
37. The composition of any one of claims 1-36, wherein the IL-22 Fc fusion protein consists of two single-chain units linked by two inter-chain disulfide bridges, wherein each single chain unit consists of a human IL-22 fusion protein comprising IL-22 fused with the Fc region of a human immunoglobulin IgG4.
38. The composition of any one of claims 1-37, wherein the composition is a pharmaceutical composition.
39. The composition of claim 38, wherein the composition is aqueous and/or sterile.
40. The composition of claim 38 or 39, further comprising an additional therapeutic agent.
41. The composition of any one of claims 38-40, further comprising a gelling agent.
42. A method of treating inflammatory bowel disease (IBD) in a subject in need thereof, the method comprising administering to the subject the composition of any one of claims 1-41.
43. The method of claim 42, wherein the IBD is ulcerative colitis or Crohn's disease.
44. The method of claim 43, wherein the IBD is ulcerative colitis.
45. The method of claim 44, wherein the ulcerative colitis is moderate to severe ulcerative colitis.
46. The method of claim 43, wherein the IBD is Crohn's disease.

47. A composition comprising an interleukin (IL)-22 Fc fusion protein of any one of claims 1 to 41 for use as a medicament.

48. A composition comprising an interleukin (IL)-22 Fc fusion protein of any one of claims 1 to 41 for use in

- (i) treating inflammatory bowel disease (IBD),
- (ii) inhibiting microbial infection in the intestine, preserving goblet cells in the intestine during a microbial infection, enhancing epithelial cell integrity, epithelial cell proliferation, epithelial cell differentiation, epithelial cell migration or epithelial wound healing in the intestine,
- (iii) treating acute kidney injury or acute pancreatitis,
- (iv) accelerating or improving wound healing in a subject in need thereof,
- (v) preventing or treating a cardiovascular disease such as coronary artery disease, coronary microvascular disease, stroke, carotid artery disease, peripheral artery disease, or chronic kidney disease,
- (vi) treating metabolic syndrome, or
- (vii) treating acute endotoxemia or sepsis.

49. Use of a composition comprising an interleukin (IL)-22 Fc fusion protein of any one of claims 1 to 41 for the preparation of a medicament for use in

- (i) treating inflammatory bowel disease (IBD),
- (ii) inhibiting microbial infection in the intestine, preserving goblet cells in the intestine during a microbial infection, enhancing epithelial cell integrity, epithelial cell proliferation, epithelial cell differentiation, epithelial cell migration or epithelial wound healing in the intestine,
- (iii) treating acute kidney injury or acute pancreatitis,
- (iv) accelerating or improving wound healing in a subject in need thereof,
- (v) preventing or treating a cardiovascular disease such as coronary artery disease, coronary microvascular disease, stroke, carotid artery disease, peripheral artery disease, or chronic kidney disease,
- (vi) treating metabolic syndrome, or
- (vii) treating acute endotoxemia or sepsis.

50. A method of inhibiting microbial infection in the intestine, preserving goblet cells in the intestine during a microbial infection, enhancing epithelial cell integrity, epithelial cell proliferation, epithelial cell differentiation, epithelial cell migration or epithelial wound healing in the intestine, of a subject in need thereof, the method comprising administering to the subject the composition of any one of claims 1-41.

51. A method of treating acute kidney injury or acute pancreatitis in a subject in need thereof, the method comprising administering to the subject the composition of any one of claims 1-41.

52. A method of accelerating or improving wound healing in a subject in need thereof, the method comprising administering to the subject the composition of any one of claims 1-41.

53. A method for preventing or treating a cardiovascular condition in a subject in need thereof, which condition includes a pathology of atherosclerotic plaque formation, the method comprising administering to the subject the composition of any one of claims 1-41.

54. A method for treating metabolic syndrome in a subject in need thereof, the method comprising administering to the subject the composition of any one of claims 1-41.

55. A method of treating acute endotoxemia, sepsis, or both, in a subject in need thereof, the method comprising administering to the subject the composition of any one of claims 1-41.

56. The method, composition, or use of any one of claims 42-55, wherein the composition is administered intravenously, subcutaneously, intraperitoneally, or topically.

57. The method, composition, or use of any one of claims 42-56, wherein the subject is co-administered with at least one additional therapeutic agent.

58. A method of making a composition comprising an IL-22 Fc fusion protein, the method comprising:

culturing an inoculum train culture comprising a plurality of host cells in a production medium under conditions suitable to form a production culture for at least about 10 days, wherein the host cells comprise a nucleic acid encoding an IL-22 Fc fusion protein, the IL-22 Fc fusion protein comprising an IL-22 polypeptide linked to an Fc region by a linker, wherein the host cells express the IL-22 Fc fusion protein, thereby making the composition comprising an IL-22 Fc fusion protein, wherein the IL-22 polypeptide is glycosylated, and wherein the composition has an average sialic acid content in the range of 6 to 12 moles of sialic acid per mole of the IL-22 Fc fusion protein.

59. The method of claim 58, wherein the duration of the culturing is at least 11 days, at least 12 days, or at least 13 days.

60. The method of claim 58 or 59, wherein the duration of the culturing is 12 days.

61. The method of any one of claims 58-60, further comprising generating a seed train culture by culturing a host cell comprising a nucleic acid encoding the IL-22 Fc fusion protein in a seed train medium under conditions suitable to form the seed train culture prior to culturing the inoculum train culture in the production medium.

62. The method of claim 61, further comprising inoculating the seed train culture in an inoculum medium under conditions suitable to form an inoculum train culture prior to culturing the inoculum train culture in the production medium.

63. The method of any one of claims 58-62, wherein the host cells are eukaryotic host cells.

64. The method of claim 63, wherein the eukaryotic host cells are mammalian host cells.

65. The method of claim 64, wherein the mammalian host cells are Chinese hamster ovary (CHO) cells.

66. The method of any one of claims 58-65, further comprising:
harvesting a cell culture fluid comprising the IL-22 Fc fusion protein from the production culture.

67. The method of claim 66, wherein harvesting the cell culture fluid comprises: (i) cooling the production culture; (ii) removing the host cells from the production medium by centrifugation to form the cell culture fluid; and/or (iii) filtering the cell culture fluid.

68. The method of any one of claims 58-67, further comprising:
purifying the IL-22 Fc fusion protein in the cell culture fluid.

69. The method of claim 68, wherein purifying the IL-22 Fc fusion protein comprises the following substeps:

(i) contacting the cell culture fluid to an affinity chromatographic support, optionally washing the affinity chromatographic support with a wash buffer, eluting the IL-22 Fc fusion protein from the affinity chromatographic support with a first elution buffer to form an affinity pool, and optionally inactivating viruses in the affinity pool;

(ii) contacting the affinity pool to an anion-exchange chromatographic support, optionally washing the anion-exchange chromatographic support with a first equilibration buffer, eluting the IL-22 Fc fusion protein from the anion-exchange chromatographic support with a second elution buffer to form an anion-exchange pool, and optionally filtering the anion-exchange pool to remove viruses; and

(iii) contacting the anion-exchange pool to a hydrophobic-interaction chromatographic support and collecting the flow-through to form a purified product pool comprising the IL-22 Fc fusion protein, and optionally washing the hydrophobic-interaction chromatographic support with a second equilibration buffer, collecting the flow-through, and adding it to the purified product pool.

70. The method of claim 69, wherein purifying the IL-22 Fc fusion protein further comprises one or more of the following substeps:

(iv) concentrating the purified product pool to form a concentrated product pool;

(v) ultrafiltering the purified product pool;

(vi) exchanging the buffer of the concentrated product pool to form a ultrafiltration and diafiltration (UFDF) pool comprising the IL-22 Fc fusion protein; and/or

(vii) conditioning the UFDF pool with a formulation buffer to form a conditioned UFDF pool comprising the IL-22 Fc fusion protein.

71. The method of claim 69 or 70, wherein substep (i) further comprises inactivating viruses by adding a detergent to the cell culture fluid prior to contacting the cell culture fluid to the affinity column.

72. The method of any one of claims 58-71, wherein the method further comprises enriching the sialic acid content of the composition.

73. The method of claim 72, wherein the composition has an initial average sialic acid content in the range of 6 to 8 moles of sialic acid per mole of the IL-22 Fc fusion protein.

74. The method of claim 72, wherein the composition has an initial average sialic acid content of 6, 7, or 8 moles of sialic acid per mole of the IL-22 Fc fusion protein.

75. The method of any one of claims 72-74, wherein the method comprises enriching the average sialic acid content to the range of 8 to 12 moles of sialic acid per mole of the IL-22 Fc fusion protein.

76. The method of any one of claims 62-75, wherein the method further comprises enriching the average sialic acid content to the range of 8 to 9 moles of sialic acid per mole of the IL-22 Fc fusion protein.

77. The method of any one of claims 70-76, wherein the affinity chromatographic support comprises a protein A resin, a protein G resin, or an IL-22 receptor resin.

78. The method of claim 77, wherein the protein A resin is a MABSELECT SURE® resin.

79. The method of any one of claims 70-78, wherein the anion-exchange chromatographic support comprises a strong anion exchanger with multimodal functionality resin.

80. The method of claim 79, wherein the anion-exchange chromatographic support comprises a CAPTO™ adhere resin.

81. The method of any one of claims 58-80, wherein the composition has an average sialic acid content of 8 to 12 moles of sialic acid per mole of the IL-22 Fc fusion protein.

82. The method of any one of claims 58-81, wherein the composition has an average sialic acid content of 8 or 9 moles of sialic acid per mole of the IL-22 Fc fusion protein.

83. The method of any one of claims 58-82, wherein the IL-22 Fc fusion protein consists of two single-chain units linked by two inter-chain disulfide bridges, wherein each single chain unit consists of a human IL-22 fusion protein comprising IL-22 fused with the Fc region of a human immunoglobulin IgG4.

84. A composition produced by the method of any one of claims 58-83.

85. The composition of claim 84, wherein the composition is a pharmaceutical composition.

86. A method of selecting a batch comprising an IL-22 Fc fusion protein for release, the method comprising the following steps:

- (a) providing a batch comprising IL-22 Fc fusion proteins;
- (b) assessing the levels of sialic acid in the batch; and
- (c) selecting the batch for release if the batch has an average sialic acid content in the range of 8 to 12 moles of sialic acid per mole of the IL-22 Fc fusion protein.

87. The method of claim 86, wherein step (c) comprises selecting the batch for release if the batch has an average sialic acid content of 8 to 9 moles of sialic acid per mole of the IL-22 Fc fusion protein.

88. The method of claim 86 or 87, wherein step (c) comprises selecting the batch for release if the batch has an average sialic acid content of 8 moles of sialic acid per mole of the IL-22 Fc fusion protein.

89. The method of claim 86 or 87, wherein step (c) comprises selecting the batch for release if the batch has an average sialic acid content of 9 moles of sialic acid per mole of the IL-22 Fc fusion protein.

90. The method of any one of claims 86-89, wherein step (b) comprises using high-performance liquid chromatography (HPLC), ultra-high performance liquid chromatography (UHPLC), capillary electrophoresis, or a colorimetric assay to assess the levels of sialic acid in the batch.

91. The method of claim 90, wherein step (b) comprises assessing the levels of sialic acid using HPLC.

92. A method for controlling sialic acid content of a composition comprising an IL-22 Fc fusion protein, the IL-22 Fc fusion protein comprising a glycosylated IL-22 polypeptide linked by a linker to an antibody Fc region, the method comprising:

culturing an inoculum train culture comprising a plurality of host cells in a production medium under conditions suitable to form a production culture for at least 10 days, wherein the host cells comprise a nucleic acid encoding the IL-22 Fc fusion protein and express the IL-22 Fc fusion protein, wherein the composition has an average sialic acid content in the range of 6 to 12 moles of sialic acid per mole of the IL-22 Fc fusion protein; and

enriching the average sialic acid content of the composition to the range of 8 to 12 moles of sialic acid per mole of the IL-22 Fc fusion protein, thereby controlling the sialic acid content of the composition.

93. The method of claim 92, wherein the method comprises enriching the average sialic acid content of the composition to the range of 8 to 9 moles of sialic acid per mole of the IL-22 Fc fusion protein.

94. The method of claim 92 or 93, wherein enriching the average sialic acid content comprises harvesting a cell culture fluid comprising the IL-22 Fc fusion protein from the production culture.

95. The method of claim 94, wherein harvesting the cell culture fluid comprises: (i) cooling the production culture; (ii) removing the host cells from the production medium by centrifugation to form the cell culture fluid; and/or (iii) filtering the cell culture fluid.

96. The method of claim 94 or 95, wherein enriching the average sialic acid content of the composition further comprises purifying the IL-22 Fc fusion protein in the cell culture fluid.

97. The method of claim 96, wherein purifying the IL-22 Fc fusion protein comprises the following substeps:

(i) contacting the cell culture fluid to an affinity chromatographic support, optionally washing the affinity chromatographic support with a wash buffer, eluting the IL-22 Fc fusion protein from the affinity chromatographic support with a first elution buffer to form an affinity pool, and optionally inactivating viruses in the affinity pool;

(ii) contacting the affinity pool to an anion-exchange chromatographic support, optionally washing the anion-exchange chromatographic support with a first equilibration buffer, eluting the IL-22 Fc fusion protein from the anion-exchange chromatographic support with a second elution buffer to form an anion-exchange pool, and optionally filtering the anion-exchange pool to remove viruses; and

(iii) contacting the anion-exchange pool to a hydrophobic-interaction chromatographic support and collecting the flow-through to form a purified product pool comprising the IL-22 Fc fusion protein, and optionally washing the hydrophobic-interaction chromatographic support with a second equilibration buffer, collecting the flow-through, and adding it to the purified product pool.

98. The method of claim 97, wherein purifying the IL-22 Fc fusion protein further comprises one or more of the following substeps:

(iv) concentrating the purified product pool to form a concentrated product pool;

(v) ultrafiltering the purified product pool;

(vi) exchanging the buffer of the concentrated product pool to form a ultrafiltration and diafiltration (UFDF) pool comprising the IL-22 Fc fusion protein; and/or

(vii) conditioning the UFDF pool with a formulation buffer to form a conditioned UFDF pool comprising the IL-22 Fc fusion protein.

99. The method of claim 97 or 98, wherein substep (i) further comprises inactivating viruses by adding a detergent to the cell culture fluid prior to contacting the cell culture fluid to the affinity column.

100. The method of any one of claims 97-99, wherein the affinity chromatographic support comprises a protein A resin, a protein G resin, or an IL-22 receptor resin.

101. The method of claim 100, wherein the protein A resin is a MABSELECT SURE® resin.

102. The method of any one of claims 97-101, wherein the anion-exchange chromatographic support comprises a strong anion exchanger with multimodal functionality resin.

103. The method of claim 102, wherein the anion-exchange chromatographic support comprises a CAPTO™ adhere resin.