

sepsis, as well as methods of selecting a batch comprising IL-22 Fc fusion proteins for release. Also provided herein are methods of controlling sialic acid content of an IL-22 Fc fusion protein and methods of reducing in vivo clearance and/or increasing half-life by adjusting the sialic acid content of an IL-22 Fc fusion protein or a composition thereof.

In one example, the disclosure features 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 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. In one aspect, the present disclosure refers to a liquid 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. In some examples, the IL-22 polypeptide is N-glycosylated. In some examples, 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.

In another example, the disclosure features 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 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. In one [aspect example](#), the present disclosure refers to a liquid 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 the composition comprises one or more of the properties selected from:

- (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
- (d) the percent N-glycosylation site occupancy at residue Asn143 is in the range of 25 to 35.

In some examples of any of the preceding aspects, 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. In some examples, 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. In some examples, the composition has an average sialic acid content of 8 moles of sialic acid per mole of the IL-22 Fc fusion protein. In other examples, the composition has an average sialic acid content of 9 moles of sialic acid per mole of the IL-22 Fc fusion protein.

In some examples of any of the preceding aspects, the sialic acid glycosylation comprises N-acetylneuraminic acid (NANA).

In some examples of any of the preceding aspects, 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.

In some examples of any of the preceding aspects, the composition is a liquid composition.

In some examples of any of the preceding aspects: (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; (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. In some examples, 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.

described herein. In some examples, the IBD is ulcerative colitis or Crohn's disease. In some examples, the IBD is ulcerative colitis. In some examples, the ulcerative colitis is moderate to severe ulcerative colitis. In some examples, the IBD is Crohn's disease.

In another example, the disclosure features any of the compositions described herein for use as a medicament.

In another example, the disclosure features any of the compositions described herein 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, (vii) treating acute endotoxemia or sepsis, or (viii) treating GVHD.

In another example, the disclosure features any of the compositions described herein 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, (vii) treating acute endotoxemia or sepsis, or (viii) treating GVHD.

In one aspect, the present disclosure refers to use of any of the compositions described herein in the manufacture of a medicament for treating inflammatory bowel disease (IBD) in a subject in need thereof.

In one aspect, the present disclosure refers to use of any of the compositions described herein in the manufacture of a medicament for 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.

In one aspect, the present disclosure refers to use of any of the compositions described herein in the manufacture of a medicament for treating acute kidney injury or acute pancreatitis in a subject in need thereof.

In one aspect, the present disclosure refers to use of any of the compositions described herein in the manufacture of a medicament for accelerating or improving wound healing in a subject in need thereof.

In one aspect, the present disclosure refers to use of any of the compositions described herein in the manufacture of a medicament for treating a cardiovascular condition that includes a pathology of atherosclerotic plaque formation in a subject in need thereof.

In one aspect, the present disclosure refers to use of any of the compositions described herein in the manufacture of a medicament for treating metabolic syndrome in a subject in need thereof.

In one aspect, the present disclosure refers to use of any of the compositions described herein in the manufacture of a medicament for treating acute endotoxemia, sepsis, or both, in a subject in need thereof.

In one aspect, the present disclosure refers to use of any of the compositions described herein in the manufacture of a medicament for treating or preventing acute graft-versus-host disease (GVHD) in a subject in need thereof.

In another example, the disclosure features 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 any of the compositions described herein.

In another example, the disclosure features a method of treating acute kidney injury or acute pancreatitis in a subject in need thereof, the method comprising administering to the subject any of the compositions described herein.

In another example, the disclosure features a method of accelerating or improving wound healing in a subject in need thereof, the method comprising administering to the subject any of the compositions described herein.

In another example, the disclosure features 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 any of the compositions described herein.

In another example, the disclosure features a method for treating metabolic syndrome in a subject in need thereof, the method comprising administering to the subject any of the compositions described herein.

In another example, the disclosure features a method of treating acute endotoxemia, sepsis, or both, in a subject in need thereof, the method comprising administering to the subject any of the compositions described herein.

In another example, the disclosure features a method of treating GVHD in a subject in need thereof, the method comprising administering to the subject any of the compositions described herein.

In some examples of any of the preceding aspects, the composition is administered intravenously, subcutaneously, intraperitoneally, or topically.

In some examples of any of the preceding aspects, the subject is co-administered with at least one additional therapeutic agent.

In another aspect, the present disclosure refers to a method of making a liquid composition comprising an IL-22 Fc fusion protein, the method comprising the following steps:

(a) culturing for ~~about~~ 9 days to no more than ~~about~~ 13 days a plurality of Chinese hamster ovary (CHO) cells in a production medium under conditions suitable to form a production culture, wherein the CHO 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 CHO cells express and secrete the IL-22 Fc fusion protein into the production medium, and wherein the IL-22 polypeptide is glycosylated; and

(b) collecting the production medium, thereby making the composition comprising an IL-22 Fc fusion protein, wherein the composition has an average sialic acid content in the range of ~~about~~ 5 to ~~about~~ 12 moles of sialic acid per mole of the IL-22 Fc fusion protein.

In another example, the disclosure features a method of making a composition comprising an IL-22 Fc fusion protein, the method comprising the following steps: (a) providing a host cell comprising a nucleic acid encoding a IL-22 Fc fusion protein, the IL-22 Fc fusion protein comprising an IL-22 polypeptide linked to an Fc region by a linker; (b) culturing the host cell in a seed train medium under conditions suitable to form a seed train culture; (c) inoculating the seed train in an inoculum medium under conditions suitable to form an inoculum train culture; and (d) culturing the inoculum train in a production medium under conditions suitable to form a production culture, wherein the host cells of the production culture express the IL-22 Fc fusion protein, and wherein the duration of step (d) is at least 10 days, 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. In some examples, the duration of step (d) is at least 11 days, at least 12 days, or at least 13 days. In some examples, the duration of step (d) is 12 days.

In some examples of any of the preceding aspects, the method further comprises the following step: (e) harvesting a cell culture fluid comprising the IL-22 Fc fusion protein from the production culture. In some examples, step (e) 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.

In some examples of any of the preceding aspects, the method further comprises the following step: (f) purifying the IL-22 Fc fusion protein in the cell culture fluid. In some examples, step (f) 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

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. In some examples, 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. In some examples, 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.

In some examples of any of the preceding aspects, the method further comprises enriching the sialic acid content of the composition. In some examples, 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. In some examples, 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. In some examples, the method further 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. In some examples, 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.

In some examples of any of the preceding aspects, the affinity chromatographic support comprises a protein A resin, a protein G resin, or an IL-22 receptor resin. In some examples, the protein A resin is a MABSELECT SURE® resin.

In some examples of any of the preceding aspects, the anion-exchange chromatographic support comprises a strong anion exchanger with multimodal functionality resin. In some examples, the anion-exchange chromatographic support comprises a CAPTO™ adhere resin.

In some examples of any of the preceding aspects, 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.

In some examples of any of the preceding aspects, 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.

In another example, the disclosure features a composition produced by any of the methods described herein. In one [aspectexample](#), the present disclosure refers to a liquid composition produced by any of the methods described herein. In some examples, the composition is a pharmaceutical composition.

WHAT IS CLAIMED IS:

1. A liquid 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 composition has an average sialic acid content in the range of 8 to 10 moles of sialic acid per mole of the IL-22 Fc fusion protein.
3. The composition of claim 1 or 2, 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.
4. The composition of any one of claims 1-3, wherein the sialic acid glycosylation comprises N-acetylneuraminic acid (NANA).
5. The composition of any one of claims 1-4, 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.
6. The composition of any one of claims 1-5, wherein the IL-22 polypeptide comprises N-glycans having monoantennary, biantennary, triantennary, and tetraantennary structure.
7. The composition of any one of claims 1-6, wherein the IL-22 Fc fusion protein comprises N-glycans comprising zero, one, two, three, or four galactose moieties.
8. The composition of any one of claims 1-7, wherein the IL-22 Fc fusion protein comprises N-glycans comprising zero, one, two, three, or four sialic acid moieties.
9. The composition of any one of claims 1-8, wherein the IL-22 polypeptide comprises ~~about~~ 0% to ~~about~~ 10% N-glycans comprising a terminal mannose moiety.
10. The composition of any one of claims 1-9, wherein the IL-22 polypeptide comprises ~~about~~ 30% to ~~about~~ 55% N-glycans comprising a terminal N-acetylglucosamine (GlcNAc) moiety.
11. The composition of any one of claims 1-10, wherein the IL-22 polypeptide comprises N-glycans ~~comprise-comprising~~ one, two, three, or four terminal GlcNAc moieties.
12. The composition of any one of claims 1-11, wherein the IL-22 polypeptide comprises ~~about~~ 20% to ~~about~~ 45% N-glycans comprising a terminal galactose (Gal) moiety.
13. The composition of any one of claims 1-12, wherein the IL-22 polypeptide comprises N-glycans ~~comprise-comprising~~ one, two, or three terminal Gal moieties.
14. The composition of any one of claims 1-13, wherein the IL-22 polypeptide comprises N-glycans comprising galactose N-acetylglucosamine (LacNAc) repeats.

15. The composition of any one of claims 1-14, wherein the composition has one or more of the following properties:

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

16. The composition of any one of claims 1-15, wherein the composition has one or more of the following properties:

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

17. The composition of any one of claims 1-16, wherein the composition has one or more of the following properties:

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

18. The composition of any one of claims 1-17, wherein the composition has one or more of the following properties:

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

19. The composition of any one of claims 1-18, wherein the composition has one or more of the following properties:

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

20. The composition of any one of claims 1-19, 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.

21. The composition of any one of claims 1-20, wherein the IL-22 polypeptide is a human IL-22 polypeptide.

22. The composition of any one of claims 1-21, wherein the IL-22 Fc fusion protein binds to human IL-22 receptor.

23. The composition of any one of claims 1-22, wherein the IL-22 polypeptide comprises the amino acid sequence of SEQ ID NO:4.

24. The composition of any one of claims 1-23, wherein the linker comprises the amino acid sequence RVESKYGPP (SEQ ID NO: 44).

~~25. A liquid 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 the composition comprises one or more of the properties selected from:~~

- ~~(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~~
- ~~(d) the percent N-glycosylation site occupancy at residue Asn143 is in the range of 25 to 35.~~

26-25. A method of making a liquid composition comprising an IL-22 Fc fusion protein, the method comprising the following steps:

(a) culturing for ~~about~~ 9 days to no more than ~~about~~ 13 days a plurality of Chinese hamster ovary (CHO) cells in a production medium under conditions suitable to form a production culture, wherein the CHO 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 CHO cells express and secrete the IL-22 Fc fusion protein into the production medium, and wherein the IL-22 polypeptide is glycosylated; and

(b) collecting the production medium, thereby making the composition comprising an IL-22 Fc fusion protein, wherein the composition has an average sialic acid content in the range of ~~about~~ 5 to ~~about~~ 12 moles of sialic acid per mole of the IL-22 Fc fusion protein.

27-26. The method of claim 26-25, wherein the method further comprises enriching the sialic acid content of the composition.

28-27. The method of claim 26-25 or 27-26, wherein the method further comprises harvesting a cell culture fluid comprising the IL-22 Fc fusion protein from the production medium collected in step (b) and purifying the IL-22 Fc fusion protein from the cell culture fluid.

29-28. The method of claim 28-27, wherein purifying the IL-22 Fc fusion protein comprises the following steps:

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

(ii) contacting the affinity pool to an anion-exchange chromatographic support and 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

(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.

30-29. The method of claim 29-28, wherein step (i) further comprises washing the affinity chromatographic support with a wash buffer prior to eluting the IL-22 Fc fusion protein.

31-30. The method of claim 29-28 or 30-29, wherein step (i) further comprises inactivating viruses in the affinity pool.

32-31. The method of any one of claims 29-31-28-30, wherein step (ii) further comprises washing the anion-exchange chromatographic support with a first equilibration buffer prior to eluting the IL-22 Fc fusion protein from the anion-exchange chromatographic support.

33-32. The method of any one of claims 29-32-28-31, wherein step (ii) further comprises filtering the anion-exchange pool to remove viruses.

34-33. The method of any one of claims 29-33-28-32, wherein step (iii) further comprises washing the hydrophobic-interaction chromatographic support with a second equilibration buffer, collecting the flow-through, and adding it to the purified product pool.

35-34. The method of any one of claims 29-34-28-33, wherein purifying the IL-22 Fc fusion protein further comprises:

(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

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

36-35. The method of any one of claims 29-35-28-34, wherein the affinity chromatographic support comprises a protein A resin and the anion-exchange chromatographic support comprises a multimodal anion exchange resin.

37-36. The method of claim 36-35, wherein the multimodal anion-exchange resin is a CAPTO™ adhere resin.

38-37. The method of any one of claims 26-37-25-36, wherein the CHO cells are cultured for ~~about~~ 9 days to no more than ~~about~~ 12 days.

39-38. The method of any one of claims 26-38-25-37, wherein the CHO cells are cultured for ~~about~~ 10 days to no more than ~~about~~ 11 days.

~~40. A liquid composition produced by the method of any one of claims 26-39.~~

41-39. 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.

42-40. 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 for at least 9 days to no more than 12 days an inoculum train culture comprising a plurality of CHO cells in a production medium under conditions suitable to form a production culture, 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 5 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.

43-41. The method of claim 42-40, wherein the CHO cells are cultured for ~~about~~ 9 days to no more than ~~about~~ 12 days.

44-42. The method of claim 43-41, wherein the CHO cells are cultured for ~~about~~ 10 days to no more than ~~about~~ 11 days.

45-43. Use of the composition of any one of claims 1-24 in the manufacture of a medicament for treating inflammatory bowel disease (IBD) in a subject in need thereof.

46-44. Use of the composition of any one of claims 1-24 in the manufacture of a medicament for 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.

47-45. Use of the composition of any one of claims 1-24 in the manufacture of a medicament for treating acute kidney injury or acute pancreatitis in a subject in need thereof.

48-46. Use of the composition of any one of claims 1-24 in the manufacture of a medicament for accelerating or improving wound healing in a subject in need thereof.

49-47. Use of the composition of any one of claims 1-24 in the manufacture of a medicament for treating a cardiovascular condition that includes a pathology of atherosclerotic plaque formation in a subject in need thereof.

50.48. Use of the composition of any one of claims 1-24 in the manufacture of a medicament for treating metabolic syndrome in a subject in need thereof.

51.49. Use of the composition of any one of claims 1-24 in the manufacture of a medicament for treating acute endotoxemia, sepsis, or both, in a subject in need thereof.

52.50. Use of the composition of any one of claims 1-24 in the manufacture of a medicament for treating or preventing acute graft-versus-host disease (GVHD) in a subject in need thereof.