

IL-22 Fc FUSION PROTEINS AND METHODS OF USE

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in
5 ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on
January 24, 2019, is named 50474-180WO2_Sequence_Listing_1.24.19_ST25 and is 121,827 bytes in
size.

FIELD OF THE INVENTION

10 The present invention relates to IL-22 Fc fusion proteins, compositions (e.g., pharmaceutical
compositions) comprising the same, and methods of making, purifying, and using the same.

BACKGROUND OF THE INVENTION

Interleukin (IL)-22 is a member of the IL-10 family of cytokines that is produced, e.g., by Th22
15 cells, NK cells, lymphoid tissue inducer (LTi) cells, dendritic cells, and Th17 cells. IL-22 binds to the IL-
22R1/IL-10R2 receptor complex, which is expressed in innate cells (e.g., epithelial cells, hepatocytes, and
keratinocytes) and in barrier epithelial tissues of several organs (e.g., dermis, pancreas, intestine, and the
respiratory system).

IL-22 plays an important role in mucosal immunity, mediating early host defense against attaching
20 and effacing bacterial pathogens. IL-22 promotes the production of anti-microbial peptides and pro-
inflammatory cytokines from epithelial cells and stimulates proliferation and migration of colonic epithelial
cells in the gut. Upon bacterial infection, IL-22 knock-out mice displayed impaired gut epithelial
regeneration, high bacterial load, and increased mortality. Similarly, infection of IL-22 knock-out mice
with influenza virus resulted in severe weight loss and impaired regeneration of tracheal and bronchial
25 epithelial cells. Thus, IL-22 plays a pro-inflammatory role in suppressing microbial infection as well as an
anti-inflammatory protective role in epithelial regeneration in inflammatory responses.

There remains a need for improved therapeutic agents and methods for treatment of
inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, as well as other
disorders including microbial infection, acute kidney injury, acute pancreatitis, wounds, cardiovascular
30 conditions, metabolic syndrome, acute endotoxemia, graft-versus-host disease (GVHD), and sepsis.
There also remains a need for improved methods for making and purifying such therapeutic agents.

SUMMARY

The present disclosure provides, *inter alia*, interleukin (IL)-22 Fc fusion proteins, compositions
35 (e.g., pharmaceutical compositions) comprising the same, and methods of making, purifying, and using
the same, e.g., for treatment of disorders including IBD, microbial infection, acute kidney injury, acute
pancreatitis, wounds, cardiovascular conditions, metabolic syndrome, acute endotoxemia, GVHD, and

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.

5 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.

15 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, 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:

- 30 (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).

40 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.

45 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.

In some examples of any of the preceding aspects, the IL-22 polypeptide comprises N-glycans having monoantennary, biantennary, triantennary, and/or tetraantennary structure. In some examples: (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. In some examples: (i) 0.1% to 2% of the N-glycans have monoantennary structure; (ii) 10% to 25% of the N-glycans have biantennary structure; (iii) 25% to 40% of the N-glycans have triantennary structure; and/or (iv) 30% to 51% of the N-glycans have tetraantennary structure.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein comprises N-glycans comprising zero, one, two, three, or four galactose moieties. In some examples: (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. In some examples: (i) 9% to 32% of the N-glycans comprise zero galactose moieties; (ii) 10% to 20% of the N-glycans comprise one galactose moiety; (iii) 8% to 25% of the N-glycans comprise two galactose moieties; (iv) 12% to 25% of the N-glycans comprise three galactose moieties; and/or (v) 12% to 30% of the N-glycans comprise four galactose moieties.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein comprises N-glycans comprising zero, one, two, three, or four sialic acid moieties. In some examples: (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. In some examples: (i) 12% to 35% of the N-glycans comprise zero sialic acid moieties; (ii) 10% to 30% of the N-glycans comprise one sialic acid moiety; (iii) 10% to 30% of the N-glycans comprise two sialic acid moieties; (iv) 10% to 30% of the N-glycans comprise three sialic acid moieties; and/or (v) 1% to 20% of the N-glycans comprise four sialic acid moieties.

In some examples of any of the preceding aspects, (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. In some examples, (i) the IL-22 polypeptide comprises 0% to 10% N-glycans comprising a terminal mannose moiety; and/or (ii) the IL-22 polypeptide comprises 30% to 55% N-glycans comprising a terminal GlcNAc moiety. In some examples, the IL-22 polypeptide comprises 0% to 10% N-glycans comprising a terminal mannose moiety. In some examples, the IL-22 polypeptide comprises 30% to 55% N-glycans comprising a terminal GlcNAc moiety.

In some examples of any of the preceding aspects, the N-glycans comprise one, two, three, or four terminal GlcNAc moieties. In some examples: (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. In some examples: (i) 1% to 20% of the N-glycans comprise one terminal GlcNAc moiety; (ii) 1% to 20% of the N-glycans comprise two terminal GlcNAc moieties; (iii) 5% to 25% of the N-glycans comprise three terminal GlcNAc moieties; and/or (iv) 0% to 15% of the N-glycans comprise four terminal GlcNAc moieties.

In some examples of any of the preceding aspects, (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. In some examples, (i) the IL-22 polypeptide comprises 20% to 45% N-glycans comprising a terminal Gal moiety; and/or (ii) the N-glycans comprise one, two, or three terminal Gal moieties.

In some examples of any of the preceding aspects: (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. In some examples: (i) 15% to 30% of the N-glycans comprise one terminal Gal moiety; (ii) 1% to 15% of the N-glycans comprise two terminal Gal moieties; and/or (iii) 0.1% to 6% of the N-glycans comprise three terminal Gal moieties.

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

In another example, the disclosure provides a composition comprising an IL-22 Fc fusion protein having the N-glycan distribution shown in Table 12 or 13.

In some examples of any of the preceding aspects, the concentration of the IL-22 Fc fusion protein is about 0.5 mg/mL to about 20 mg/mL. In some examples, the concentration of the IL-22 Fc fusion protein is about 0.5 mg/mL to about 5 mg/mL. In some examples, the concentration of the IL-22 Fc fusion protein is about 1 mg/mL. In some examples, the concentration of the IL-22 Fc fusion protein is about 8 mg/mL to about 12 mg/mL. In some examples, the concentration of the IL-22 Fc fusion protein is about 10 mg/mL.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein has been produced from a production culture having a volume of at least about 500 L. In some examples of any of the preceding aspects, the IL-22 Fc fusion protein has been produced from a production culture having a volume of about 500 L to about 5,000 L. In some examples, the IL-22 Fc fusion protein has been produced from a production culture having a volume of about 1,000 L to about 3,000 L. In some

examples the IL-22 Fc fusion protein has been produced from a production culture having a volume of about 1,500 L to about 2,500 L. In some examples, the IL-22 Fc fusion protein has been produced from a production culture having a volume of about 2000 L.

5 In some examples of any of the preceding aspects, the Fc region is not glycosylated. In some examples: (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. In some examples, the amino acid residue at position 297 as in the EU index of the Fc region is Gly or Ala. In some examples, the amino acid residue at position 297 as in the EU index of the Fc region is Gly. In other examples, the amino acid residue at position 297 as in the EU index of the Fc region is Ala.

10 In some examples of any of the preceding aspects, the Fc region comprises the CH2 and CH3 domain of IgG1 or IgG4. In some examples, the Fc region comprises the CH2 and CH3 domain of IgG4.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein comprises an amino acid sequence having at least 95% (e.g., at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%) sequence identity to the amino acid sequence of SEQ ID NO:8.

15 In some examples of any of the preceding aspects, 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.

In some examples of any of the preceding aspects, the IL-22 polypeptide is a human IL-22 polypeptide. In some examples, the IL-22 polypeptide comprises the amino acid sequence of SEQ ID NO:4.

20 In some examples of any of the preceding aspects, the linker comprises or consists of the amino acid sequence RVESKYGPP (SEQ ID NO: 44).

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein binds to IL-22 receptor. In some examples, the IL-22 receptor is human IL-22 receptor. In some examples, the human IL-22 receptor comprises a heterodimer consisting of an IL-22R1 polypeptide and an IL-10R2 polypeptide. In some examples, 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.

25 In some examples of any of the preceding aspects, 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.

30 In some examples of any of the preceding aspects, the composition is a pharmaceutical composition. In some examples, the composition is aqueous and/or sterile. In some examples, the composition further comprises an additional therapeutic agent. In some examples, the composition further comprises a gelling agent.

In another example, the disclosure features a method of treating inflammatory bowel disease (IBD) in a subject in need thereof, the method comprising administering to the subject any of the compositions

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

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, step (f) 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 another example, the disclosure features 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. In some examples, the duration of the culturing is at least 11 days, at least 12 days, or at least 13 days. In some examples, the duration of the culturing is 12 days.

In some examples of any of the preceding aspects, the method further comprises 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. In some examples, the method further comprises 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.

In some examples of any of the preceding aspects, the host cells are eukaryotic host cells. In some examples, the eukaryotic host cells are mammalian host cells. In some examples, the mammalian host cells are Chinese hamster ovary (CHO) cells. In some examples, 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.

In some examples of any of the preceding aspects, the method further comprises purifying the IL-22 Fc fusion protein in the cell culture fluid. In some examples, 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. 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 aspect, 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.

In some examples of any of the preceding aspects, 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.

In another aspect, the present disclosure refers to 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. In some examples, 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. In some examples, 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. In some examples, 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. In some examples, 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. In some examples, step (b) comprises assessing the levels of sialic acid using HPLC.

In another example, the disclosure features 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. In one aspect, the present disclosure refers to 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. In some examples, 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.

In another example, the disclosure features 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.

In some examples of any of the preceding aspects, 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.

In some examples of any of the preceding aspects, enriching the average sialic acid content comprises harvesting a cell culture fluid comprising the IL-22 Fc fusion protein from the production culture. In some examples, 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.

In some examples of any of the preceding aspects, enriching the average sialic acid content of the composition further comprises purifying the IL-22 Fc fusion protein in the cell culture fluid. In some examples, 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. 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, 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, 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 one example, the disclosure features an IL-22 Fc fusion protein comprising an IL-22 polypeptide linked to an Fc region by a linker, wherein the IL-22 polypeptide is glycosylated, and wherein the IL-22 Fc

fusion protein has a sialic acid content in the range of from 8 to 12 moles of sialic acid per mole of the IL-22 Fc fusion protein. In certain examples, 8 to 12 moles of sialic acid per mole of the IL-22 Fc fusion protein means that 8 to 12 sialic acid moieties are comprised in one mole of the IL-22 fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content in the range of 8 to 9 moles of sialic acid per mole of the IL-22 Fc fusion protein.

In another example, the disclosure features an IL-22 Fc fusion protein comprising an IL-22 polypeptide linked to an Fc region by a linker, wherein the IL-22 polypeptide is glycosylated, and wherein the IL-22 Fc fusion protein has a potency of about 40% to about 130% relative to a reference IL-22 Fc fusion protein having a sialic acid content of about 8 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a potency of about 80% to about 120% relative to a reference IL-22 Fc fusion protein having a sialic acid content of about 8 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a potency of about 60% to about 110% relative to a reference IL-22 Fc fusion protein having a sialic acid content of about 8 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a potency of about 80% to about 100% relative to a reference IL-22 Fc fusion protein having a sialic acid content of about 8 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, potency is assessed in a receptor binding assay or a cell-based binding assay. In some examples, the reference IL-22 Fc fusion protein has the N-glycan distribution shown in Table 12 and/or Table 13.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein has a sialic acid content of from about 8 to about 12 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of from about 8 to about 11 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of from about 8 to about 10 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of from about 8 to about 9 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of about 8 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of 8 moles of sialic acid per mole of the IL-22 Fc fusion protein.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein has a sialic acid content of about 9 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of 9 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the sialic acid is N-acetylneuraminic acid (NANA). In some examples, the IL-22 Fc fusion protein has a maximum observed concentration ($C_{\max}$) of about 9,000 ng/mL to about 18,000 ng/mL. In some examples, the $C_{\max}$ is assessed following intravenous administration of about 1,000 μ g/kg of the IL-22 Fc fusion protein to a CD1 mouse. In some examples, 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. In some examples, the AUC_{last} is assessed following intravenous administration of about 1,000 µg/kg of the IL-22 Fc fusion protein to a CD1 mouse. In some examples, 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 CL is assessed following intravenous administration of about 1,000 µg/kg of the IL-22 Fc fusion protein to a CD1 mouse.

In some examples of any of the preceding aspects, the IL-22 polypeptide is N-glycosylated.

In some examples of any of the preceding aspects, the IL-22 polypeptide comprises N-glycans having monoantennary, biantennary, triantennary, and/or tetraantennary structure. In some examples, about 0.1% to about 2% of the N-glycans have monoantennary structure. In some examples, about 0.5% to about 1.5% of the N-glycans have monoantennary structure. In some examples, about 1% of the N-glycans have monoantennary structure. In some examples, about 10% to about 25% of the N-glycans have biantennary structure. In some examples, about 12% to about 21% of the N-glycans have biantennary structure. In some examples, about 17% of the N-glycans have biantennary structure. In some examples, about 25% to about 40% of the N-glycans have triantennary structure. In some examples, about 28% to about 35% of the N-glycans have triantennary structure. In some examples, about 31% of the N-glycans have triantennary structure. In some examples, about 30% to about 51% of the N-glycans have tetraantennary structure. In some examples, about 35% to about 48% of the N-glycans have tetraantennary structure. In some examples, about 42% of the N-glycans have tetraantennary structure.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein comprises N-glycans comprising zero, one, two, three, or four galactose moieties. In some examples, about 9% to about 32% of the N-glycans comprise zero galactose moieties. In some examples, about 15% to about 25% of the N-glycans comprise zero galactose moieties. In some examples, about 21% of the N-glycans comprise zero galactose moieties. In some examples, about 10% to about 20% of the N-glycans comprise one galactose moiety. In some examples, about 12% to about 16% of the N-glycans comprise one galactose moiety. In some examples, about 14% of the N-glycans comprise one galactose moiety. In some examples, about 8% to about 25% of the N-glycans comprise two galactose moieties. In some examples, about 10% to about 16% of the N-glycans comprise two galactose moieties. In some examples, about 13% of the N-glycans comprise two galactose moieties. In some examples, about 12% to about 25% of the N-glycans comprise three galactose moieties. In some examples, about 15% to about 22% of the N-glycans comprise three galactose moieties. In some examples, about 19% of the N-glycans comprise three galactose moieties. In some examples, about 12% to about 30% of the N-glycans comprise four galactose moieties. In some examples, about 15% to about 25% of the N-glycans comprise four galactose moieties. In some examples, about 24% of the N-glycans comprise four galactose moieties.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein comprises N-glycans comprising zero, one, two, three, or four sialic acid moieties. In some examples, about 12% to about 35% of the N-glycans comprise zero sialic acid moieties. In some examples, about 20% to about 30% of the N-glycans comprise zero sialic acid moieties. In some examples, about 24% of the N-glycans
5 comprise zero sialic acid moieties. In some examples, about 10% to about 30% of the N-glycans comprise one sialic acid moiety. In some examples, about 15% to about 25% of the N-glycans comprise one sialic acid moiety. In some examples, about 20% of the N-glycans comprise one sialic acid moiety. In some examples, about 10% to about 30% of the N-glycans comprise two sialic acid moieties. In some examples, about 15% to about 25% of the N-glycans comprise two sialic acid moieties. In some
10 examples, about 21% of the N-glycans comprise two sialic acid moieties. In some examples, about 10% to about 30% of the N-glycans comprise three sialic acid moieties. In some examples, about 12% to about 24% of the N-glycans comprise three sialic acid moieties. In some examples, about 17% of the N-glycans comprise three sialic acid moieties. In some examples, about 1% to about 20% of the N-glycans comprise four sialic acid moieties. In some examples, about 5% to about 15% of the N-glycans comprise
15 four sialic acid moieties. In some examples, about 9% of the N-glycans comprise four sialic acid moieties.

In some examples of any of the preceding aspects, the IL-22 polypeptide comprises about 0% to about 10% N-glycans comprising a terminal mannose moiety. In some examples, about 1% to about 4% of the N-glycans comprise a terminal mannose moiety. In some examples, about 2% of the N-glycans
20 comprise a terminal mannose moiety.

In some examples of any of the preceding aspects, the IL-22 polypeptide comprises about 30% to about 55% N-glycans comprising a terminal N-acetylglucosamine (GlcNAc) moiety. In some examples, about 35% to about 50% of the N-glycans comprise a terminal GlcNAc moiety. In some examples, about 42% of the N-glycans comprise a terminal GlcNAc moiety.

In some examples of any of the preceding aspects, the N-glycans comprise one, two, three, or four terminal GlcNAc moieties. In some examples, about 1% to about 20% of the N-glycans comprise one terminal GlcNAc moiety. In some examples, about 5% to about 15% of the N-glycans comprise one terminal GlcNAc moiety. In some examples, about 10% of the N-glycans comprise one terminal GlcNAc moiety. In some examples, about 1% to about 20% of the N-glycans comprise two terminal GlcNAc
30 moieties. In some examples, about 5% to about 15% of the N-glycans comprise two terminal GlcNAc moieties. In some examples, about 10% of the N-glycans comprise two terminal GlcNAc moieties. In some examples, about 5% to about 25% of the N-glycans comprise three terminal GlcNAc moieties. In some examples, about 10% to about 20% of the N-glycans comprise three terminal GlcNAc moieties. In some examples, about 14% of the N-glycans comprise three terminal GlcNAc moieties. In some
35 examples, about 0% to about 15% of the N-glycans comprise four terminal GlcNAc moieties. In some examples, about 4% to about 12% of the N-glycans comprise four

terminal GlcNAc moieties. In some examples, about 7% of the N-glycans comprise four terminal GlcNAc moieties.

5 In some examples of any of the preceding aspects, the IL-22 polypeptide comprises about 20% to about 45% N-glycans comprising a terminal galactose (Gal) moiety. In some examples, about 25% to about 35% of the N-glycans comprise a terminal Gal moiety. In some examples, about 32% of the N-glycans comprise a terminal Gal moiety.

10 In some examples of any of the preceding aspects, the N-glycans comprise one, two, or three terminal Gal moieties. In some examples, about 15% to about 30% of the N-glycans comprise one terminal Gal moiety. In some examples, about 20% to about 25% of the N-glycans comprise one terminal Gal moiety. In some examples, about 23% of the N-glycans comprise one terminal Gal moiety. In some examples, about 1% to about 15% of the N-glycans comprise two terminal Gal moieties. In some examples, about 2% to about 12% of the N-glycans comprise two terminal Gal moieties. In some examples, about 7% of the N-glycans comprise two terminal Gal moieties. In some examples, about 0.1% to about 6% of the N-glycans comprise three terminal Gal moieties. In some examples, about 1% to about 3% of the N-glycans comprise three terminal Gal moieties. In some examples, about 2% of the N-glycans comprise three terminal Gal moieties.

20 In some examples of any of the preceding aspects, the IL-22 polypeptide comprises N-glycans comprising galactose N-acetylglucosamine (LacNAc) repeats. In some examples, about 1% to about 10% of the N-glycans comprise LacNAc repeats. In some examples, about 3% to about 6% of the N-glycans comprise LacNAc repeats. In some examples, about 5% of the N-glycans comprise LacNAc repeats.

25 In some examples of any of the preceding aspects, the IL-22 polypeptide comprises N-glycans comprising fucosylated N-glycans. In some examples, about 60% to about 80% of the N-glycans are fucosylated. In some examples, about 65% to about 75% of the N-glycans are fucosylated. In some examples, about 70% of the N-glycans are fucosylated.

In some examples of any of the preceding aspects, the IL-22 polypeptide comprises N-glycans comprising afucosylated N-glycans. In some examples, about 10% to about 30% of the N-glycans are afucosylated. In some examples, about 15% to about 25% of the N-glycans are afucosylated. In some examples, about 20% of the N-glycans are afucosylated.

30 In some examples of any of the preceding aspects, the IL-22 polypeptide is glycosylated on amino acid residues Asn21, Asn35, Asn64, and/or Asn143 of SEQ ID NO:4. In some examples, the IL-22 polypeptide is glycosylated on amino acid residues Asn21, Asn35, Asn64, and Asn143 of SEQ ID NO:4. In some examples, the glycosylation occupancy on amino acid residue Asn21 of SEQ ID NO:4 is about 70% to about 90%. In some examples, the glycosylation occupancy on amino acid residue Asn21 of SEQ ID NO:4 is about 75% to about 85%. In some examples, the glycosylation occupancy on amino acid residue Asn21 of SEQ ID NO:4 is about 81% to about 84%. In some examples, the glycosylation occupancy on amino acid residue Asn21 of SEQ ID NO:4 is about 82%. In some

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examples, the glycosylation occupancy on amino acid residue Asn35 of SEQ ID NO:4 is about 90% to about 100%. In some examples, the glycosylation occupancy on amino acid residue Asn35 of SEQ ID NO:4 is about 95% to about 100%. In some examples, the glycosylation occupancy on amino acid residue Asn35 of SEQ ID NO:4 is about 100%. In some examples, the glycosylation occupancy on amino acid residue Asn64 of SEQ ID NO:4 is about 90% to about 100%. In some examples, the glycosylation occupancy on amino acid residue Asn64 of SEQ ID NO:4 is about 95% to about 100%. In some examples, the glycosylation occupancy on amino acid residue Asn64 of SEQ ID NO:4 is about 100%. In some examples, the glycosylation occupancy on amino acid residue Asn143 of SEQ ID NO:4 is about 15% to about 45%. In some examples, the glycosylation occupancy on amino acid residue Asn143 of SEQ ID NO:4 is about 25% to about 35%. In some examples, the glycosylation occupancy on amino acid residue Asn143 of SEQ ID NO:4 is about 32% to about 35%. In some examples, the glycosylation occupancy on amino acid residue Asn143 of SEQ ID NO:4 is about 33%.

In some examples, the IL-22 polypeptide is glycosylated on amino acid residues Asn21, Asn35, Asn64, and Asn143 of SEQ ID NO:4, wherein the glycosylation occupancy on amino acid residue Asn21 of SEQ ID NO:4 is about 81% to about 84%, the glycosylation occupancy on amino acid residue Asn35 of SEQ ID NO:4 is about 100%, the glycosylation occupancy on amino acid residue Asn64 of SEQ ID NO:4 is about 100% and the glycosylation occupancy on amino acid residue Asn143 of SEQ ID NO:4 is about 32% to about 35%. In some examples, the IL-22 polypeptide is glycosylated on amino acid residues Asn21, Asn35, Asn64, and Asn143 of SEQ ID NO:4, wherein the glycosylation occupancy on amino acid residue Asn21 of SEQ ID NO:4 is 81% to 84%, the glycosylation occupancy on amino acid residue Asn35 of SEQ ID NO:4 is 100%, the glycosylation occupancy on amino acid residue Asn64 of SEQ ID NO:4 is 100% and the glycosylation occupancy on amino acid residue Asn143 of SEQ ID NO:4 is 32% to 35%.

In another example, the disclosure an IL-22 Fc fusion protein having the N-glycan distribution shown in Table 12 or 13.

In some examples of any of the preceding aspects, the Fc region is not glycosylated. In some examples, the amino acid residue at position 297 as in the EU index of the Fc region is glycine (Gly). In some examples, the amino acid residue at position 297 as in the EU index of the Fc region is alanine (Ala). In some examples, the amino acid residue at position 299 as in the EU index of the Fc region is Ala, Gly, or valine (Val). In some examples, the Fc region comprises the CH2 and CH3 domain of IgG1 or IgG4. In some examples, the Fc region comprises the CH2 and CH3 domain of IgG4.

In some examples of any of the preceding aspects, 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. In some examples, the IL-22 Fc fusion protein comprises an amino acid sequence having at least 96% sequence identity to the amino acid sequence of SEQ ID NO:8. In some examples, the IL-22 Fc fusion protein comprises an amino acid sequence having at least 97% sequence identity to the amino

acid sequence of SEQ ID NO:8. In some examples, the IL-22 Fc fusion protein comprises an amino acid sequence having at least 98% sequence identity to the amino acid sequence of SEQ ID NO:8. In some examples, the IL-22 Fc fusion protein comprises an amino acid sequence having at least 99% sequence identity to the amino acid of SEQ ID NO:8. In some examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:16. In some examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8. In some examples, the IL-22 Fc fusion protein consists of the amino acid sequence of SEQ ID NO:8. In some examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:10. In some examples, the IL-22 Fc fusion protein consists of the amino acid sequence of SEQ ID NO:10. In some examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:16. In some examples, the IL-22 Fc fusion protein consists of the amino acid sequence of SEQ ID NO:16. In some examples, the Fc region is not N-glycosylated.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein is a dimeric IL-22 Fc fusion protein. In other examples of any of the preceding aspects, the IL-22 Fc fusion protein is a monomeric IL-22 Fc fusion protein. In some examples, the IL-22 polypeptide is a human IL-22 polypeptide. In some examples, the IL-22 polypeptide comprises the amino acid sequence of SEQ ID NO:4.

In some examples of any of the preceding aspects, the linker comprises the amino acid sequence RVESKYGPP (SEQ ID NO: 44). In some examples, the linker consists of the amino acid sequence RVESKYGPP (SEQ ID NO: 44).

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein binds to IL-22 receptor. In some examples, the IL-22 receptor is human IL-22 receptor. In some examples, the IL-22 Fc fusion protein binds to IL-22RA1 and/or IL-10R2. In some examples, the IL-22 Fc fusion protein binds to IL-22RA1.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein is produced by the method comprising the step of culturing a host cell capable of expressing the IL-22 Fc fusion protein under conditions suitable for expression of the IL-22 Fc fusion protein. In some examples, the method further comprises the step of obtaining the IL-22 Fc fusion protein from the cell culture or culture medium. In some examples, the host cell is a CHO cell.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein has an N-glycolylneuraminic acid (also known as Neu5Gc or NGNA) content of less than about 5 moles of NGNA per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has an NGNA content of less than 1 mole of NGNA per mole of the IL-22 Fc fusion protein.

In another example, the disclosure features a pharmaceutical composition comprising any of the IL-22 Fc fusion proteins described herein and at least one pharmaceutically acceptable carrier. In some examples, the IL-22 Fc fusion protein has a 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 Fc fusion protein has a

sialic acid content in the range of 8 to 10 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a 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 IL-22 Fc fusion protein has a sialic acid content of about 8 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of 8 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of about 9 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of 9 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the sialic acid is N-acetylneuraminic acid (NANA). In some examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:16. In some examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8. In some examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:16.

In some examples of any of the preceding aspects, the pharmaceutical composition further comprises an additional therapeutic agent. In some examples, the pharmaceutical composition further comprises a gelling agent. In some examples, the gelling agent is a polysaccharide. In some examples, the gelling agent is a cellulosic agent. In some examples, the gelling agent is methylcellulose, hydroxyethyl cellulose, carboxymethyl cellulose, hydroxypropyl cellulose, POE-POP block polymers, alginate, hyaluronic acid, polyacrylic acid, hydroxyethyl methylcellulose or hydroxypropyl methylcellulose. In some examples, the gelling agent is a hydroxypropyl methylcellulose. In some examples, the pharmaceutical composition is for topical administration.

In another example, the disclosure features a method of treating inflammatory bowel disease (IBD) in a subject in need thereof, the method comprising administering to the any of the IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions 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 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 IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein. In some examples, the epithelial cell is an intestinal epithelial cell.

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 IL-22 Fc fusion proteins described herein or any of the pharmaceutical 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 IL-22 Fc fusion

proteins described herein or any of the pharmaceutical compositions described herein. In some examples, the wound is a chronic wound or an infected wound. In some examples, the subject is diabetic. In some examples, the diabetic subject has type II diabetes. In some examples, the wound is a diabetic foot ulcer. In some examples, the IL-22 Fc fusion protein or the pharmaceutical composition is administered until there is complete wound closure.

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 IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein. In some examples, the cardiovascular disease is coronary artery disease, coronary microvascular disease, stroke, carotid artery disease, peripheral artery disease, or chronic kidney disease. In some examples, the method further comprises slowing down the progression of atherosclerotic plaque formation or preventing indicia of atherosclerosis. In some examples, the indicia of atherosclerosis include plaque accumulation and/or vascular inflammation.

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 IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein. In some examples, the method further comprises reducing one or more risk factors associated with metabolic syndrome, including one or more of abdominal obesity, hyperglycemia, dyslipidemia, and hypertension. In some examples, the method further comprises reducing the level of bacterial lipopolysaccharide in the subject.

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 the subject any of the IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein. In some examples, the subject is in need of a change in HDL/LDL lipid profile.

In another example, the disclosure features a method of treating GVHD in a subject in need thereof, the method comprising administering the subject any of the IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein.

In a further example, the disclosure features a composition comprising any of the IL-22 Fc fusion proteins described herein or the pharmaceutical compositions described herein for use as medicament.

In another example, the disclosure features a composition comprising any of the IL-22 Fc fusion proteins described herein or the pharmaceutical 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 yet another example, the disclosure features the use of a composition comprising any of the IL-22 Fc fusion proteins described herein or the pharmaceutical 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 some examples of any of the preceding aspects, the IL-22 Fc fusion protein has a sialic acid content of from about 8 to about 12 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of from about 8 to about 10 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of from about 8 to about 9 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of about 8 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid content of about 9 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the sialic acid is N-acetylneuraminic acid (NANA). In some examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8, SEQ ID NO:10, or SEQ ID NO:16. In some examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:8. In some examples, the IL-22 Fc fusion protein comprises the amino acid sequence of SEQ ID NO:16.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein or the pharmaceutical composition is administered intravenously, subcutaneously, intraperitoneally, or topically. In some examples, the IL-22 Fc fusion protein or the pharmaceutical composition is administered intravenously. In some examples, the IL-22 Fc fusion protein or the pharmaceutical composition is administered subcutaneously.

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

In some examples of any of the preceding aspects, the subject is a human.

In another example, the disclosure features a method of making any of the IL-22 Fc fusion proteins described herein, the method comprising the following steps: (a) providing a host cell comprising a nucleic acid encoding any of the IL-22 Fc fusion proteins described herein; (b) culturing the host cell in a seed train medium under conditions suitable to form a seed train; (c) inoculating the seed train into an

inoculum medium and culturing under conditions suitable to form an inoculum train; 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, thereby making the IL-22 Fc fusion protein.

5 In another example, the disclosure features a method of making 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; (c) inoculating the seed train in an inoculum medium under conditions suitable to form an inoculum train; 10 and (d) culturing the inoculum train in a production medium under conditions time suitable to form a production culture, wherein the host cells of the production culture express the IL-22 Fc fusion protein, thereby making the IL-22 Fc fusion protein, wherein the IL-22 polypeptide is glycosylated, and wherein the IL-22 Fc fusion protein has a sialic acid content of from about 8 to about 12 moles of sialic acid per mole of the IL-22 Fc fusion protein. In some examples, the IL-22 Fc fusion protein has a sialic acid 15 content in 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 host cell is a frozen host cell, and step (a) further comprises thawing the frozen host cell in a seed train medium.

In some examples of any of the preceding aspects, the method further comprises passaging the inoculum train about 1 to about 10 times prior to step (d). In some examples, the inoculum train is 20 passaged about 2 to about 6 times prior to step (d). In some examples, the inoculum train is passaged about 5 times prior to step (d).

In some examples of any of the preceding aspects, the seed train medium comprises a selection agent capable of selecting for the host cell. In some examples, the selection agent is methionine sulfoximine, methotrexate, or an antibiotic. In some examples, the selection agent is methionine 25 sulfoximine. In some examples, the selection agent is an antibiotic. In some examples, the antibiotic is selected from blasticidin, geneticin, hygromycin B, puromycin, mycophenolic acid, or zeocin.

In some examples of any of the preceding aspects, the seed train medium, the inoculum medium, and/or the production medium comprises an antifoaming agent. In some examples, the antifoaming agent is simethicone emulsion, antifoam 204, antifoam A, antifoam B, antifoam C, antifoam Y-30, or 30 antifoam SE-15. In some examples, the antifoaming agent is simethicone emulsion.

In some examples of any of the preceding aspects, the seed train medium, the inoculum medium, and/or the production medium includes a buffering agent, a cell protective agent, a polysaccharide, and/or an osmolality adjustment agent.

In some examples of any of the preceding aspects, step (b) is performed at a temperature of 35 about 25°C to about 40°C. In some examples, step (b) is performed at a temperature of about 35°C to about 39°C. In some examples, step (b) is performed at a temperature of about 37°C.

In some examples of any of the preceding aspects, step (b) is performed in a spinner, a spin tube, a shake flask, a single-use bioreactor (e.g., a WAVE BIOREACTOR™ or an AMBR® bioreactor (e.g., an AMBR® 15 bioreactor)), or a seed train bioreactor. In some examples, step (b) is performed in a seed train spinner or a shake flask. In other examples, step (b) is performed in a single-use bioreactor (e.g., a WAVE BIOREACTOR™ or an AMBR® bioreactor (e.g., an AMBR® 15 bioreactor or an AMBR® 250 bioreactor)). In some examples, step (b) has a duration of about 1 day to about 12 days per passage. In some examples, step (b) has a duration of about 2 days to about 7 days per passage. In some examples, step (b) is performed in a seed train bioreactor.

In some examples of any of the preceding aspects, the pH of the seed train medium is about 6 to about 8. In some examples, the pH of the seed train medium is about 6.5 to about 7.5. In some examples, the pH of the seed train medium is about 7.15.

In some examples of any of the preceding aspects, the dissolved oxygen of the seed train medium is about 15% to about 50%. In some examples, the dissolved oxygen of the seed train medium is about 20% to about 40%. In some examples, the dissolved oxygen of the seed train medium is about 30%.

In some examples of any of the preceding aspects, step (b) has a duration of about 1 day to about 10 days. In some examples, step (b) has a duration of about 2 days to about 5 days.

In some examples of any of the preceding aspects, step (c) is performed at a temperature of about 25°C to about 40°C. In some examples, step (c) is performed at a temperature of about 35°C to about 39°C. In some examples, step (c) is performed at a temperature of about 37°C.

In some examples of any of the preceding aspects, step (c) is performed in one or more bioreactors. In some examples, step (c) is performed in three or four bioreactors.

In some examples of any of the preceding aspects, the pH of the inoculum medium is about 6 to about 8. In some examples, the pH of the inoculum medium is about 6.5 to about 7.5. In some examples, the pH of the inoculum medium is about 7.1.

In some examples of any of the preceding aspects, the dissolved oxygen of the inoculum medium is about 15% to about 50%. In some examples, the dissolved oxygen of the inoculum medium is about 20% to about 40%. In some examples, the dissolved oxygen of the inoculum medium is about 30%.

In some examples of any of the preceding aspects, step (c) has a duration of about 1 day to about 5 days. In some examples, step (c) has a duration of about 2 days to about 3 days.

In some examples of any of the preceding aspects, step (d) includes a temperature shift from an initial temperature to a post-shift temperature. In some examples, the initial temperature is about 25°C to about 40°C. In some examples, the initial temperature is about 35°C to about 39°C. In some examples, the initial temperature is about 37°C. In some examples, the post-shift temperature is about 25°C to about 40°C. In some examples, the post-shift temperature is about 30°C to about 35°C. In some examples, the post-shift temperature is about 33°C. In some examples, the

temperature shift occurs over a period of about 12 h to about 120 h. In some examples, the temperature shift occurs over a period of about 48 h to about 96 h. In some examples, the temperature shift occurs over a period of about 72 h.

5 In some examples of any of the preceding aspects, the pH of the production medium is about 6 to about 8. In some examples, the pH of the production medium is about 6.5 to about 7.5. In some examples, the pH of the production medium is about 7.0. In some examples, step (d) is performed in a production bioreactor. In some examples, the dissolved oxygen of the production medium is about 15% to about 50%. In some examples, the dissolved oxygen of the production medium is about 20% to about 40%. In some examples, the dissolved oxygen of the production medium is about 30%.

10 In some examples of any of the preceding aspects, step (d) has a duration of about 5 days to about 25 days. In some examples, step (d) has a duration of about 7 days to about 16 days. In some examples, step (d) has a duration of about 8 days to about 16 days. In some examples, step (d) has a duration of about 12 days. In some examples, step (d) further comprises adding nutrients to the production medium by a nutrient feed.

15 In some examples of any of the preceding aspects, the host cell is a prokaryotic cell or a eukaryotic cell. In some examples, the host cell is a eukaryotic cell. In some examples, the eukaryotic cell is a mammalian cell. In some examples, the mammalian cell is a Chinese hamster ovary (CHO) cell. In some examples, the CHO cell is a suspension-adapted CHO cell.

20 In some examples of any of the preceding aspects, the method 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 cooling the production culture. In some examples, step (e) comprises cooling the production culture to about 2°C to about 8°C. In some examples, step (e) comprises removing the host cells from the production medium by centrifugation to form the cell culture fluid. In some examples, step (e) further comprises filtering the cell culture fluid.

25 In some examples of any of the preceding aspects, the method 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
30 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
35 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, step (f) further comprises the following substep: (iv) concentrating the purified product pool to form a concentrated product pool. In some examples, step (f) further comprises the following substep: (v) ultrafiltering the purified product pool. In some examples, ultrafiltering comprises filtering the purified product pool with a 10-kDa composite regenerated cellulose ultrafiltration membrane. In some examples, step (f) further comprises the following substep: (vi) exchanging the buffer of the concentrated product pool to form a ultrafiltration and diafiltration (UFDF) pool comprising the IL-22 Fc fusion protein. In some examples, the buffer of the concentrated product pool is exchanged with a diafiltration buffer comprising 0.01 M sodium phosphate, pH 7.2, final concentration. In some examples, step (f) further comprises the following substep: (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, substep (i) comprises inactivating viruses by adding a detergent to the affinity pool. In some examples, the detergent is TRITON® X-100 or TRITON® CG110. In some examples, the final concentration of the detergent is about 0.01% to about 2% (v/v). In some examples, the final concentration of the detergent is about 0.1% to about 1% (v/v). In some examples, the final concentration of the detergent is about 0.3% to about 0.5% (v/v). In some examples, the final concentration of the detergent is about 0.5%. In some examples, the virus inactivation is performed at about 12° to about 25°C. In some examples, inactivating viruses has a duration of greater than about 0.5 h.

In another example, the disclosure features a method of purifying an IL-22 Fc fusion protein, the method comprising: (a) providing a cell culture fluid comprising an IL-22 Fc fusion protein and optionally inactivating viruses in the cell culture fluid; (b) contacting the cell culture fluid to an affinity chromatographic support, optionally washing the affinity chromatographic support with a wash buffer, and 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; (c) 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 (d) 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, the IL-22 polypeptide is glycosylated, and wherein the IL-22 Fc fusion protein has a sialic acid content of from about 8 to about 12 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, the wash buffer comprises 0.4 M potassium phosphate, pH 7.0, final concentration.

5 In some examples of any of the preceding aspects, the first elution buffer comprises 0.3 M L-arginine hydrochloride, 0.013 M sodium phosphate, pH 3.8, final concentration.

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, the first
10 equilibration buffer comprises 0.04 M sodium acetate, pH 5.8, final concentration. In some examples, the second elution buffer is a gradient elution buffer. In some examples, the gradient elution buffer comprises 0.04 M sodium acetate, pH 5.8 as Buffer A of the gradient elution buffer and 0.04 M sodium acetate, 0.3M sodium sulfate pH 5.8 as Buffer B of the gradient, wherein the gradient starts at 10% of Buffer B. In some examples, the second equilibration buffer comprises 0.025 M MOPS, 0.3 M sodium sulfate, pH 7.0, final
15 concentration.

In another example, any of the IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein can be used in a method of treating IBD in a subject in need thereof. In some examples, the IBD is ulcerative colitis (UC) or Crohn's disease. In some examples, the IBD is ulcerative colitis (UC). In some examples, the ulcerative colitis is moderate to
20 severe ulcerative colitis. In some examples, the IBD is Crohn's disease.

In another example, any of the IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein can be used in 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
25 wound healing in the intestine, of a subject in need thereof. In some examples, the epithelial cell is an intestinal epithelial cell.

In another example, any of the IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein can be used in a method of treating acute kidney injury or acute pancreatitis in a subject in need thereof.

30 In another example, any of the IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein can be used in a method of accelerating or improving wound healing in a subject in need thereof. In some examples, the wound is a chronic wound or an infected wound. In some examples, the subject is diabetic. In some examples, the diabetic subject has type II diabetes. In some examples, the wound is a diabetic foot ulcer. In some examples, the IL-22 Fc
35 fusion protein or the pharmaceutical composition is administered until there is complete wound closure.

In another example, any of the IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein can be used in a method for preventing or treating a cardiovascular condition in a subject in need thereof, which condition includes a pathology of atherosclerotic plaque formation. In some examples, the cardiovascular disease is coronary artery disease, coronary microvascular disease, stroke, carotid artery disease, peripheral artery disease, or chronic kidney disease. In some examples, the method comprises slowing down the progression of atherosclerotic plaque formation or preventing indicia of atherosclerosis. In some examples, the indicia of atherosclerosis include plaque accumulation and/or vascular inflammation.

In another example, any of the IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein can be used in a method for treating metabolic syndrome in a subject in need thereof. In some examples, the method further comprises reducing one or more risk factors associated with metabolic syndrome, including one or more of abdominal obesity, hyperglycemia, dyslipidemia, and hypertension. In some examples, the method further comprises reducing the level of bacterial lipopolysaccharide in the subject.

In another example, any of the IL-22 Fc fusion proteins described herein or any of the pharmaceutical compositions described herein can be used in a method of treating acute endotoxemia, sepsis, or both, in a subject in need thereof.

In some examples of any of the preceding aspects, the subject is in need of a change in HDL/LDL lipid profile.

In some examples of any of the preceding aspects, the IL-22 Fc fusion protein or the pharmaceutical composition is to be administered intravenously, subcutaneously, intraperitoneally, or topically. In some examples, the IL-22 Fc fusion protein or the pharmaceutical composition is to be administered intravenously. In some examples, the IL-22 Fc fusion protein or the pharmaceutical composition is to be administered subcutaneously.

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

Each and every example can be combined unless the context clearly suggests otherwise. Each and every example can be applied to each and every aspect of the disclosure unless the context clearly suggests otherwise.

Specific examples of the present disclosure will become evident from the following more detailed description of certain preferred examples and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A is a schematic diagram showing a schematic design configuration of an exemplary dimeric IL-22 Fc fusion protein having two human interleukin-22 (IL-22) polypeptides each fused to a

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 N-glycans comprise 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 N-glycans comprise 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 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
- (iv) about 30% to about 51% of the N-glycans 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 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
- (v) about 12% to about 30% of the N-glycans 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 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
- (v) about 1% to about 20% of the N-glycans 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 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
- (iv) about 0% to about 15% of the N-glycans 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 comprise one terminal Gal moiety;
- (ii) about 1% to about 15% of the N-glycans comprise two terminal Gal moieties; and
- (iii) about 0.1% to about 6% of the N-glycans 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. 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. The method of claim 26, wherein the method further comprises enriching the sialic acid content of the composition.

28. The method of claim 26 or 27, 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. The method of claim 28, 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. The method of claim 29, wherein step (i) further comprises washing the affinity chromatographic support with a wash buffer prior to eluting the IL-22 Fc fusion protein.

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

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

34. The method of any one of claims 29-33, 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. The method of any one of claims 29-34, 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. The method of any one of claims 29-35, wherein the affinity chromatographic support comprises a protein A resin and the anion-exchange chromatographic support comprises a multimodal anion exchange resin.

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

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

39. The method of any one of claims 26-38, 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. 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. 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. The method of claim 42, wherein the CHO cells are cultured for about 9 days to no more than about 12 days.

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

45. 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. 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. 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. 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. 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. 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. 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. 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.