MODIFIED FC POLYPEPTIDES WITH ENHANCED SIALYLATION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application Nos. 63/375,490 (filed on September 13, 2022) and 63/512,013 (filed on July 5, 2023), the disclosures of each of which are incorporated by reference herein in their entirety for all purposes.

SEQUENCE LISTING

[0002] The present specification makes reference to a Sequence listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML file, created on September 12, 2023, is named NVG_003WO_ST26.xml and is 8 kb in size.

BACKGROUND

[0003] Inflammatory disorders, including autoimmune diseases, are disorders involving abnormal activation and subsequent migration of white blood cells to affected areas of the body. These conditions encompass a wide range of ailments that affect the lives of millions of people throughout the world. Although various treatments are presently available, many possess significant side effects or are insufficiently effective in alleviating symptoms.

[0004] Immunoglobulin G (IgG) has long been appreciated to mediate both pro- and anti-inflammatory activities through interactions mediated by its fragment crystallizable (Fc) region. While Fc-FcγR interactions are responsible for the pro-inflammatory properties of immune complexes and cytotoxic antibodies, intravenous gamma globulin (IVIG) and its constituent Fc fragments are anti-inflammatory and are widely used to suppress inflammation in disease states.

[0005] Sialylation of recombinant therapeutic glycoproteins, such as Fc polypeptides, is generally performed in mammalian cell lines capable of reproducing mammalian or mammalian-like glycosylation profiles.

SUMMARY

[0006] The present disclosure provides compositions comprising Fc polypeptides having a high degree of sialylation at their N-glycan and methods of producing and using the same.

[0007] In one aspect, the disclosure provides a population of modified Fc polypeptides, each modified Fc polypeptide having (i) an amino acid sequence at least 75% identical to the sequence of SEQ ID NO: 2 and (ii) an aliphatic amino acid residue at position 241 (numbered according to Kabat; corresponding to amino acid residue 32 of SEQ ID NO: 2), comprising: at least 60% of the modified Fc polypeptides having a sialic acid (SA) moiety attached to an N-glycan of the modified Fc polypeptide via an α(2,6) linkage. Disclosed herein, in certain examples, are populations of modified Fc polypeptides, each modified Fc polypeptide having (i) an amino acid sequence at least 75%

(*e.g.*, at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identical to the sequence of SEQ ID NO: 2 and (ii) an aliphatic amino acid residue (*e.g.*, alanine, glycine, isoleucine, leucine, proline, valine, and methionine) at position 241 (numbered according to Kabat; corresponding to amino acid residue 32 of SEQ ID NO: 2), comprising: at least 60% (*e.g.*, at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the modified Fc polypeptides having a sialic acid (SA) moiety attached to an N-glycan of the Fc polypeptide via an α(2,6) linkage. In some examples, the aliphatic amino acid at position 241 is an alanine (Ala; F241A). In some examples, the aliphatic amino acid at position 241 is a glycine (Gly; F241G). In some examples, the aliphatic amino acid at position 241 is an isoleucine (Ile; F241I). In some examples, the aliphatic amino acid at position 241 is a leucine (Leu; F241L). In some examples, the aliphatic amino acid at position 241 is a proline (Pro; F241P). In some examples, the aliphatic amino acid at position 241 is a valine (Val; F241V). In some examples, the aliphatic amino acid at position 241 is a methionine (Met; F241M). In some examples, the N-glycan is attached to the asparagine (Asn) at amino acid residue 297 of the polypeptide (Asn297; numbered according to Kabat; corresponding to amino acid residue 88 of SEQ ID NO: 2). In some examples, at least 70% (*e.g.*, at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the Fc polypeptides comprise the SA moiety attached to the N-glycan of the Fc polypeptides via the α(2,6) linkage. In some examples, at least 80% (*e.g.*, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the Fc polypeptides comprise the SA moiety attached to the N-glycan of the Fc polypeptides via the α(2,6) linkage. In some examples, at least 90% (*e.g.*, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) of the Fc polypeptides comprise the SA moiety attached to the N-glycan of the Fc polypeptides via the α(2,6) linkage. In some examples, the N-glycan of the Fc polypeptides is mono-sialylated or di-sialylated. In some examples, at least 30% (*e.g.*, at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more) of the Fc polypeptides comprise mono-sialylated N-glycans comprising a SA moiety attached via the α(2,6) linkage. In some examples, at least 30% (*e.g.*, at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more) of the Fc polypeptides comprise di-sialylated N-glycans comprising two SA moieties attached via

the α(2,6) linkage. In some examples, about 90% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) of the Fc polypeptides comprise di-sialylated N-glycans comprising two SA moieties attached via the α(2,6) linkage. In some examples, at least about 60% (*e.g.*, at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the Fc polypeptides comprise a galactose moiety. In some examples, at least about 70% (*e.g.*, at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the Fc polypeptides comprise a galactose moiety. In some examples, at least about 80% (*e.g.*, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the Fc polypeptides comprise a galactose moiety. In some examples, at least about 90% (*e.g.*, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) of the Fc polypeptides comprise a galactose moiety. In some examples, about 100% of the Fc polypeptides comprise a galactose moiety. In some examples, the galactose moiety is attached to an α(1,3) arm and/or α(1,6) arm of the N-glycan. In some examples, the galactose moiety is a branched galactose moiety.

[0008] In one aspect, the disclosure provides a population of modified Fc polypeptides, each modified Fc polypeptide having (i) an amino acid sequence at least 75% identical to the sequence of SEQ ID NO: 2 and (ii) an aliphatic amino acid residue at position 241 (numbered according to Kabat; corresponding to amino acid residue 32 of SEQ ID NO: 2), comprising: about 40% of the modified Fc polypeptides having a SA moiety attached to the N-glycan of the modified Fc polypeptide via an α(2,3) linkage. Disclosed herein, in certain examples, are populations of modified Fc polypeptides, each modified Fc polypeptide having (i) an amino acid sequence at least 75% (*e.g.*, at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identical to the sequence of SEQ ID NO: 2 and (ii) an aliphatic amino acid residue (*e.g.*, alanine, glycine, isoleucine, leucine, proline, valine, and methionine) at position 241 (numbered according to Kabat; corresponding to amino acid residue 32 of SEQ ID NO: 2), comprising: about 40% (*e.g.*, 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, and 50%) of the modified Fc polypeptides having a SA moiety attached to the N-glycan of the Fc polypeptide via an α(2,3) linkage. In some examples, the aliphatic amino acid at position 241 is an alanine (Ala; F241A). In some examples, the aliphatic amino acid at position 241 is a glycine (Gly; F241G). In some examples, the aliphatic amino acid at position 241 is an isoleucine (Ile; F241I). In some examples, the aliphatic amino acid at position 241 is a leucine (Leu; F241L). In some examples, the aliphatic amino acid at position 241 is a proline (Pro; F241P). In some examples, the aliphatic amino acid at position 241 is a valine (Val; F241V). In some examples, the

aliphatic amino acid at position 241 is a methionine (Met; F241M). In some examples, the N-glycan is attached to the asparagine (Asn) at amino acid residue 297 of the polypeptide (Asn297; numbered according to Kabat; corresponding to amino acid residue 88 of SEQ ID NO: 2). In some examples, the Fc polypeptides are IgG1. In some examples, the Fc polypeptides are IgG3.

[0009] In one aspect, the disclosure provides a pharmaceutical composition, comprising: (a) the population as disclosed herein and (b) a pharmaceutically acceptable carrier, diluent, or excipient. Disclosed herein, in certain examples, are pharmaceutical compositions, comprising: (a) a population of modified Fc polypeptides of any of the foregoing examples, and (b) a pharmaceutically acceptable carrier, diluent, or excipient. In some examples, the pharmaceutically acceptable carrier, diluent, or excipient is selected from the group consisting of: a stabilizer, buffer, surfactant, filler, solvent, tonicity or osmolarity adjusting agent, antioxidant, adjuvant, and antimicrobial agent.

[0010] Disclosed herein, in certain examples, are methods of treating an inflammatory disease or condition in a subject (*e.g.*, a human) in need thereof, comprising: administering to the subject a therapeutically effective amount of a population of modified Fc polypeptides of any of the foregoing examples. In some examples, the inflammatory disease or condition is an autoimmune disease or condition. In some examples, the inflammatory disease or condition is arthritis. In some examples, the inflammatory disease or condition is immune thrombocytopenia (ITP). In some examples, the population or the pharmaceutical composition has a half-life of at least 3.5 days (*e.g.*, at least 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10, or more days) following administration of the population or the pharmaceutical composition to the subject. In some examples, the population or the pharmaceutical composition has a half-life of at least 4 days (*e.g.*, at least 4, 4.5, 5, 6, 7, 8, 9, 10, or more days) following administration of the population or the pharmaceutical composition to the subject. In some examples, the population or the pharmaceutical composition is cleared from the circulation of the subject at a rate of no greater than 45 mL/day/kg (*e.g.*, no greater than 45, 40, 35, 30, 25, 20 mL/day/kg, or less). In some examples, the population or the pharmaceutical composition is cleared from the circulation of the subject at a rate of no greater than 40 mL/day/kg (*e.g.*, no greater than 40, 35, 30, 25, 20 mL/day/kg, or less). In some examples, the population or the pharmaceutical composition is cleared from the circulation of the subject at a rate of no greater than 35 mL/day/kg (*e.g.*, no greater than 35, 30, 25, 20 mL/day/kg, or less). In some examples, the population or the pharmaceutical composition is cleared from the circulation of the subject at a rate of no greater than 30 mL/day/kg (*e.g.*, no greater than 30, 25, 20 mL/day/kg, or less). In some examples, the population or the pharmaceutical composition is cleared from the circulation of the subject at

a rate of no greater than 25 mL/day/kg (*e.g.*, no greater than 25, 25, 20 mL/day/kg, or less). In some examples, the concentration of the Fc polypeptide over time (AUC) is at least 480 day×mg/mL between 1 and 35 days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 days) following administration of the population or the pharmaceutical composition to the subject. In some examples, the AUC is at least 500 day×mg/mL, at least 550 day×mg/mL, at least 600 day×mg/mL, at least 650 day×mg/mL, at least 700 day×mg/mL, at least 750 day×mg/mL, at least 800 day×mg/mL, or at least 850 day×mg/mL between 1 and 35 days (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, or 35 days) following administration of the population or the pharmaceutical composition to the subject. In some examples, the method further comprises administration of an additional therapeutic agent to the subject. In some examples, the additional therapeutic agent is administered to the subject prior to, concurrently with, or subsequent to administration of the composition or the pharmaceutical composition. In some examples, the additional therapeutic agent is a second modified Fc polypeptide comprising one or more amino acid substitutions selected from the group consisting of M252Y, S254T, T256E, H433K, and N434F. In some examples, the second modified Fc polypeptide comprises amino acid substitutions M252Y, S254T, T256E, H433K, and N434F. In some examples, the second modified Fc polypeptide has an amino acid sequence set forth as SEQ ID NO: 5. In some examples, the second modified Fc polypeptide is administered at a dose of 1 mg/kg to 20 mg/kg (*e.g.*, 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, 6 mg/kg, 7 mg/kg, 8 mg/kg, 9 mg/kg, 10 mg/kg, 11 mg/kg, 12 mg/kg, 13 mg/kg, 14 mg/kg, 15 mg/kg, 16 mg/kg, 17 mg/kg, 18 mg/kg, 19 mg/kg, or 20 mg/kg). In some examples, the second modified Fc polypeptide is administered at a dose of 10 mg/kg. In some examples, the second modified Fc polypeptide is administered once weekly for four weeks. In some examples, the additional therapeutic agent is selected from the group consisting of an anti-inflammatory agent, an immune-suppressive agent, an analgesic, a disease-modifying antirheumatic drug (DMARD), a counterirritant, a platelet-boosting drug, a thrombopoietin receptor (TPOR) agonist, physical therapy, and surgery. In some examples, the anti-inflammatory agent is selected from the group consisting of non-steroidal anti-inflammatory drug (NSAID), corticosteroid, anti-inflammatory antibody or an antigen-binding fragment thereof, anti-inflammatory cytokine, kinase inhibitor, and intravenous immunoglobulin (IVIG).

[0011] In one aspect, the disclosure provides a nucleic acid expression vector, comprising: (a) a first expression cassette, comprising: a first mammalian promoter operably linked to a polynucleotide encoding a modified Fc polypeptide having: (i) an amino acid sequence at least 75% identical to the sequence of SEQ ID NO: 2 and (ii) an aliphatic amino acid residue at position 241 (numbered according to Kabat; corresponding to amino acid residue 32 of SEQ ID NO: 2); and (b) a second expression cassette, comprising: a second mammalian promoter operably linked to a polynucleotide encoding a beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) enzyme. Disclosed herein, in certain examples, are nucleic acid expression vectors, comprising: (a) a first expression cassette, comprising: a first mammalian promoter operably linked to a polynucleotide encoding a modified Fc polypeptide having: (i) an amino acid sequence at least 75% (*e.g.*, at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identical to the sequence of SEQ ID NO: 2 and (ii) an aliphatic amino acid residue (*e.g.*, alanine, glycine, isoleucine, leucine, proline, valine, and methionine) at position 241 (numbered according to Kabat; corresponding to amino acid residue 32 of SEQ ID NO: 2); and (b) a second expression cassette, comprising: a second mammalian promoter operably linked to a polynucleotide encoding a beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) enzyme. In some examples, the aliphatic amino acid at position 241 is an Ala (F241A). In some examples, the aliphatic amino acid at position 241 is an alanine (Ala; F241A). In some examples, the aliphatic amino acid at position 241 is a glycine (Gly; F241G). In some examples, the aliphatic amino acid at position 241 is an isoleucine (Ile; F241I). In some examples, the aliphatic amino acid at position 241 is a leucine (Leu; F241L). In some examples, the aliphatic amino acid at position 241 is a proline (Pro; F241P). In some examples, the aliphatic amino acid at position 241 is a valine (Val; F241V). In some examples, the aliphatic amino acid at position 241 is a methionine (Met; F241M). In some examples, the second expression cassette further comprises a polynucleotide encoding a beta-1,4-galactosyltransferase 1 (B4GALT1) enzyme. In some examples, the polynucleotide encoding the B4GALT1 enzyme is operatively linked to the second promoter. In some examples, the second expression cassette further comprises an internal ribosome entry site (IRES) sequence positioned between the polynucleotide encoding the ST6GAL1 enzyme and the polynucleotide encoding the B4GALT1 enzyme. In some examples, the first promoter and the second promoter are each independently selected from the group consisting of a murine cytomegalovirus (CMV) promoter, elongation factor 1α (EF1α) promoter, eukaryotic elongation factor 2 (EEF2) promoter, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) promoter, phosphoglycerate kinase (PGK) promoter, actin promoter, and ubiquitin promoter.

[0012] In one aspect, the disclosure provides a mammalian host cell, comprising: the nucleic acid expression vector as disclosed herein. Disclosed herein, in certain examples, are mammalian host cells, comprising: an expression vector of the foregoing examples. In some examples, the mammalian host cell is a Chinese hamster ovary (CHO) cell. In some examples, the mammalian host cell is a human embryonic kidney 293 (HEK293) cell.

[0013] In one aspect, the disclosure provides a method of manufacturing a population of modified Fc polypeptides, each modified Fc polypeptide having: (i) an amino acid sequence having at least 75% identity to SEQ ID NO: 2, and (ii) an aliphatic amino acid residue at position 241 (numbered according to Kabat; corresponding to amino acid residue 32 of SEQ ID NO: 2), the method comprising: (a) culturing a mammalian host cell under conditions and for a time that induces expression of a first expression cassette and a second expression cassette, thus producing the population of modified Fc polypeptides; and (b) purifying the population of modified Fc polypeptides, wherein the mammalian host cell comprises a nucleic acid expression vector comprising: (1) a first expression cassette comprising: a first mammalian promoter operably linked to a polynucleotide encoding a modified Fc polypeptide having: (i) an amino acid sequence at least 75% identical to the sequence of SEQ ID NO: 2 and (ii) an aliphatic amino acid residue at position 241 (numbered according to Kabat; corresponding to amino acid residue 32 of SEQ ID NO: 2); and (2) a second expression cassette comprising: a second mammalian promoter operably linked to a polynucleotide encoding a beta-galactoside alpha-2,6-sialyltransferase 1 (ST6GAL1) enzyme. Disclosed herein, in certain examples, are methods of manufacturing a population of modified Fc polypeptides, each modified Fc polypeptide having: (i) an amino acid sequence having at least 75% (*e.g.*, at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) identity to SEQ ID NO: 2, and (ii) an aliphatic amino acid residue (*e.g.*, alanine, glycine, isoleucine, leucine, proline, valine, and methionine) at position 241 (numbered according to Kabat; corresponding to amino acid residue 32 of SEQ ID NO: 2), the method comprising: (a) culturing a mammalian host cell of the foregoing examples under conditions and for a time that induces expression of the first expression cassette and the second expression cassette, thus producing the population of modified Fc polypeptides; and (b) purifying the population of modified Fc polypeptides. In some examples, the N-glycan is attached to the asparagine (Asn) at amino acid residue 297 of the polypeptide (Asn297; numbered according to Kabat; corresponding to amino acid residue 88 of SEQ ID NO: 2). In some examples, at least 70% (*e.g.*, at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the Fc polypeptides comprise the SA moiety attached to the N-glycan of the Fc polypeptides via the α(2,6) linkage. In some examples, at least 80% (*e.g.*, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the Fc polypeptides comprise the SA moiety attached to the N-glycan of the Fc polypeptides via the α(2,6) linkage. In some examples, at least 90% (*e.g.*, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) of the Fc polypeptides comprise the SA moiety attached to the N-glycan of the Fc polypeptides via the α(2,6) linkage. In some examples, the first promoter and the second promoter are each independently selected from the group consisting of a murine CMV promoter, EF1α promoter, EEF2 promoter, GAPDH promoter, PGK promoter, actin promoter, and ubiquitin promoter. In some examples, the first expression cassette and/or the second expression cassette each independently comprise one or more regulatory sequences selected from the group consisting of a 5' untranslated region (UTR), 3' UTR, enhancer, insulator, intron, RNA export element, polyadenylation signal, internal ribosome entry site (IRES), and transcription terminator. In some examples, the N-glycan of the Fc polypeptides is mono-sialylated or di-sialylated. In some examples, at least 30% (*e.g.*, at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 99%, or more) of the Fc polypeptides comprise mono-sialylated N-glycans comprising a SA moiety attached via the α(2,6) linkage. In some examples, at least 30% (*e.g.*, at least 30%, 40%, 50%, 60%,

70%, 80%, 90%, 95%, 99%, or more) of the Fc polypeptides comprise di-sialylated N-glycans comprising two SA moieties attached via the α(2,6) linkage. In some examples, about 90% (*e.g.*, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) of the Fc polypeptides comprise di-sialylated N-glycans comprising two SA moieties attached via the α(2,6) linkage. In some examples, at least about 60% (*e.g.*, at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the Fc polypeptides comprise a galactose moiety. In some examples, at least about 70% (*e.g.*, at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the Fc polypeptides comprise a galactose moiety. In some examples, at least about 80% (*e.g.*, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) of the Fc polypeptides comprise a galactose moiety. In some examples, at least about 90% (*e.g.*, at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) of the Fc polypeptides comprise a galactose moiety. In some examples, about 100% of the Fc polypeptides comprise a galactose moiety. In some examples, the galactose moiety is attached to an α(1,3) arm and/or α(1,6) arm of the N-glycan. In some examples, the galactose moiety is a branched galactose moiety. In some examples, the mammalian host cell comprises ST6GAL1 and B4GALT1 at a ratio of 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, 1:8, 1:9, 1:10, 1:11, 1:12, 1:13, 1:14, 1:15, 1:16, 1:17, 1:18, 1:19, or 1:20 (mol:mol). In some examples, the mammalian host cell is in a population of mammalian host cells in which at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or more of the mammalian host cells remain viable between 10 days and 20 days (*e.g.*, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 days) following the start of step (a). In some examples, the method further comprises contacting the mammalian host cell with an additive that enhances sialylation of the Fc polypeptides. In some examples, the additive is selected from the group consisting of uridine, manganese, copper, dexamethasone, hydrocortisone, N-acetyl mannosamine, tetraacetylated ManNAc, N-azidoacetyl D-mannosamine, 1,3,4-O-Bu_3ManNAc, α(2,3)-Dehydro-2-deoxy-N-acetylneuraminic acid (DANA), siastatin B, fetuin, and glycerol. In some examples, glycosylation of the N-glycan is determined using HPLC, MS, or a

combination thereof. In some examples, the HPLC is hydrophilic interaction liquid chromatography (HILIC).

[0014] In one aspect, the disclosure provides use of a population as disclosed herein or the pharmaceutical composition as disclosed herein in the manufacture of a medicament for the treatment of an inflammatory disease or condition in a subject in need thereof. Disclosed herein, in certain examples, are population or pharmaceutical compositions (as disclosed here) for use as a medicament.

[0015] Disclosed herein, in certain examples, are population or pharmaceutical compositions (as disclosed here) for in the treatment of an inflammatory disease or condition, e.g., an autoimmune disease or condition, in a subject in need thereof. For example, the inflammatory disease or condition may be arthritis or immune thrombocytopenia (ITP).

[0016] In some examples, the population or the pharmaceutical composition has a half-life of at least 3.5 or at least 4 days following administration of the population or the pharmaceutical composition to a subject.

[0017] In some examples, the population or the pharmaceutical composition, following administration to a subject, is cleared from the circulation of the subject at a rate of no greater than 45 mL/day/kg, no greater than 40 mL/day/kg, no greater than 35 mL/day/kg, no greater than 30 mL/day/kg, or no greater than 25 mL/day/kg.

[0018] In some examples, the concentration of the Fc polypeptide over time (AUC) is at least 480 day×mg/mL between 1 and 35 days following administration of the population or the pharmaceutical composition to a subject, e.g., at least 500 day×mg/mL, at least 550 day×mg/mL, at least 600 day×mg/mL, at least 650 day×mg/mL, at least 700 day×mg/mL, at least 750 day×mg/mL, at least 800 day×mg/mL, or at least 850 day×mg/mL between 1 and 35 days following administration of the population or the pharmaceutical composition to a subject.

[0019] In some examples, an additional therapeutic agent is administered to the subject. In some examples, the additional therapeutic agent is administered to the subject prior to, concurrently with, or subsequent to administration of the population or the pharmaceutical composition.

[0020] In some examples, the additional therapeutic agent is a second modified Fc polypeptide comprising one or more amino acid substitutions selected from the group consisting of M252Y, S254T, T256E, H433K, and N434F. In some examples, the second modified Fc polypeptide comprises amino acid substitutions M252Y, S254T, T256E, H433K, and N434F. In some examples, the second modified Fc polypeptide has an amino acid sequence set forth as SEQ ID NO: 5.

[0021] In some examples, the second modified Fc polypeptide is administered to a subject at a dose of 1 mg/kg to 20 mg/kg, e.g., at a dose of 10 mg/kg. In some examples, the second modified Fc polypeptide is administered to a subject once weekly for four weeks.

[0022] In some examples, the additional therapeutic agent is selected from the group consisting of an anti-inflammatory agent, an immune-suppressive agent, an analgesic, a disease-modifying antirheumatic drug (DMARD), a counterirritant, a platelet-boosting drug, a thrombopoietin receptor (TPOR) agonist, physical therapy, and surgery.

[0023] In some examples, the anti-inflammatory agent is selected from the group consisting of non-steroidal anti-inflammatory drug (NSAID), corticosteroid, anti-inflammatory antibody or an antigen-binding fragment thereof, anti-inflammatory cytokine, kinase inhibitor, and intravenous immunoglobulin (IVIG).

DEFINITIONS

[0024] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. Generally, nomenclatures utilized in connection with and techniques of immunology, oncology, cell and tissue culture, molecular biology, and protein chemistry described herein are those well-known and commonly used in the art. It is to be understood that the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of any subject matter claimed. The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0025] As used herein, singular forms “a,” “and,” and “the” include plural referents unless the context clearly indicates otherwise. Thus, *e.g.*, reference to “a polypeptide” includes a plurality of polypeptides.

[0026] As used herein, all numerical values or numerical ranges include whole integers within or encompassing such ranges and fractions of the values or the integers within or encompassing ranges unless the context clearly indicates otherwise. Thus, *e.g.*, reference to a range of 90-100%, includes 91%, 92%, 93%, 94%, 95%, 96%, 97%, *etc.*, as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, *etc.*, 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, *etc.*, and so forth. In another example, reference to a range of 1-5,000 fold includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 fold, *etc.*, as well as 1.1, 1.2, 1.3, 1.4, 1.5 fold, *etc.*, 2.1, 2.2, 2.3, 2.4, 2.5 fold, *etc.*, and so forth.