CLAIMS

1. 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.

2. The method of claim 2, wherein the aliphatic amino acid at position 241 is an Ala (F241A).

3. The method of claim 1 or 2, wherein the second expression cassette further comprises a polynucleotide encoding a beta-1,4-galactosyltransferase 1 (B4GALT1) enzyme.

4. The method of any one of claims 1-3, wherein at least 60%, at least 70%, at least 80%, or at least 90% of the modified Fc polypeptides have a SA moiety attached to an N-glycan of the modified Fc polypeptide via an α(2,6) linkage.

5. The method of any one of claims 1-4, wherein the N-glycan of the modified Fc polypeptides is mono-sialylated or di-sialylated.

6. The method of claim 5, wherein:

(a) about 30% of the modified Fc polypeptides comprise mono-sialylated N-glycans comprising a SA moiety attached via the α(2,6) linkage;

(b) about 30% or about 90% of the modified Fc polypeptides comprise di-sialylated N-glycans comprising two SA moieties attached via the α(2,6) linkage; and/or

(c) at least about 60%, at least about 70%, at least about 80%, at least about 90%, or about 100% of the modified Fc polypeptides comprise a galactose moiety.

7. The method of claim 6, wherein:

(a) the galactose moiety is attached to an α(1,3) arm and/or α(1,6) arm of the N-glycan; and/or

(b) the galactose moiety is a branched galactose moiety.

8. The method of any one of claims 3-7, wherein 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).

9. The method of any one of claims 1-8, wherein 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 after between 10 days and 20 days following the start of step (a).

10. The method of any one of claims 1-9, further comprising contacting the mammalian host cell with an additive that enhances sialylation of the modified Fc polypeptides; optionally wherein 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-Bu3ManNAc, α(2,3)-Dehydro-2-deoxy-N-acetylneuraminic acid (DANA), siastatin B, fetuin, and glycerol.

11. The method of any one of claims 4-10, wherein glycosylation of the N-glycan is determined using high-performance liquid chromatography (HPLC), mass spectrometry (MS), or a combination thereof; optionally wherein the HPLC is hydrophilic interaction liquid chromatography (HILIC).

12. 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.

13. The population of claim 12, wherein the N-glycan is attached to the asparagine (Asn) at amino acid residue 297 of the modified Fc polypeptide (Asn297; numbered according to Kabat; corresponding to amino acid residue 88 of SEQ ID NO: 2).

14. The population of claim 12 or 13, wherein at least 70%, at least 80%, or at least 90% of the modified Fc polypeptides comprise the SA moiety attached to the N-glycan of the modified Fc polypeptides via the α(2,6) linkage.

15. The population of any one of claims 12-14, wherein:

(a) the N-glycan of the Fc polypeptides is mono-sialylated or di-sialylated;

(b) at least 30% of the modified Fc polypeptides comprise mono-sialylated N-glycans comprising a SA moiety attached via the α(2,6) linkage;

(c) at least 30% of the modified Fc polypeptides comprise di-sialylated N-glycans comprising two SA moieties attached via the α(2,6) linkage;

(d) about 90% of the modified Fc polypeptides comprise di-sialylated N-glycans comprising two SA moieties attached via the α(2,6) linkage; and/or

(e) at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 100% of the modified Fc polypeptides comprise a galactose moiety.

16. The population of claim 15, wherein:

a) the galactose moiety is attached to an α(1,3) arm and/or α(1,6) arm of the N-glycan; and/or

b) the galactose moiety is a branched galactose moiety.

17. 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.

18. A pharmaceutical composition, comprising: (a) the population of any one of claims 12-17 and (b) a pharmaceutically acceptable carrier, diluent, or excipient.

19. Use of a population of any one of claims 12-17 or the pharmaceutical composition of claim 18 in the manufacture of a medicament for the treatment of an inflammatory disease or condition in a subject in need thereof.

20. The use of claim 19, wherein:

a) the inflammatory disease or condition is an autoimmune disease or condition;

b) the inflammatory disease or condition is arthritis; and/or

c) the inflammatory disease or condition is immune thrombocytopenia (ITP).

21. The use of claim 19 or 20, wherein:

a) the population or the pharmaceutical composition has a half-life of at least 3.5 days or at least 4 days following administration of the population or the pharmaceutical composition to the subject;

b) 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, 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;

c) the concentration of the modified 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 the subject, optionally wherein 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 following administration of the population or the pharmaceutical composition to the subject.

22. The use of any one of claims 19-21, wherein the medicament is to be further administered with an additional therapeutic agent to the subject, optionally wherein the

additional therapeutic agent is to be administered to the subject prior to, concurrently with, or subsequent to administration of the population or the pharmaceutical composition; further optionally wherein 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.

23. 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.

24. A mammalian host cell, comprising: the nucleic acid expression vector according to claim 23.