Claims:

1. A method of improving the manufacturability of an immunoglobulin, comprising;

identifying a parent immunoglobulin having a VL domain and a VH domain,

introducing a substitution into a framework aggregation-prone segment and/or a CDR3 aggregation-prone segment in the VL domain of the parent immunoglobulin, and/or

introducing a substitution into a framework region aggregation-prone segment and/or a CDR3 aggregation-prone segment in the VH domain of the parent immunoglobulin, and/or;

introducing a substitution into a constant region aggregation-prone segment in the CH1 domain of the parent immunoglobulin,

thereby producing a variant immunoglobulin having a VL domain and a VH domain which displays improved manufacturability relative to the parent immunoglobulin.

2. A variant immunoglobulin comprising;

a light chain variable (VL) domain which comprises a substitution at framework (FR) aggregation-prone segment and/or a complementary determining region 3 (CDR3) aggregation-prone segment;

a heavy chain variable (VH) domain which comprises a substitution at a framework region aggregation-prone segment and/or a CDR3 aggregation-prone segment; and/or,

a heavy chain constant region 1 (CH1) domain which comprises a substitution at a CH1 aggregation-prone segment.

3. A method according to claim 1 or a variant immunoglobulin according to claim 2 wherein the variant immunoglobulin displays both reduced aggregation propensity and increased productivity upon expression, relative to the parent immunoglobulin

4. A variant immunoglobulin or a method according to claim 3 wherein the variant immunoglobulin displays an increase in productivity of at least 20% and a decrease in aggregation propensity of at least 30% relative to the parent immunoglobulin.

5. A variant immunoglobulin or a method according to claim 3 or claim 4 wherein a substitution is introduced in the VL domain of the parent immunoglobulin at the VL position 37 aggregation-prone segment and/or the VL position 45 aggregation-prone segment; and/or

a substitution is introduced in the VH domain of the parent immunoglobulin at the VH FR1 aggregation-prone segment; the VH position 95 aggregation-prone segment; and the VH CDR3 aggregation-prone segment.

6. A variant immunoglobulin or a method according to claim 5 wherein the substitution is introduced at a position selected from the group consisting of 1, 17, 94, 95, 96, 100, 100d and 102 of the VH domain sequence and/or a position selected from the group consisting of 37, 45 and 46 of the VL domain sequence.

7. A variant immunoglobulin or a method according to claim 6 wherein the residue at position 37 in the VL domain sequence is substituted for Q or N.

8. A variant immunoglobulin or a method according to claim 7 wherein the VL domain comprises a L37Q substitution.

9. A variant immunoglobulin or a method according to any one of claims 6 to 8 wherein the residue at position 45 in the VL domain sequence is substituted for E, K, R, Q or V.

10. A variant immunoglobulin or a method according to claim 9 wherein the VL domain comprises a T45E, T45K, T45R, T45Q or T45V substitution.

11. A variant immunoglobulin or a method according to any one of claims 6 to 10 wherein the residue at position 46 in the VL domain sequence is substituted for L or Y.

12. A variant immunoglobulin or a method according to claim 11 wherein the VL domain comprises a T46L or T46Y substitution.

13. A variant immunoglobulin or a method according to any one of claims 6 to 12 wherein the residue at position 1 in the VH domain sequence is substituted for E, Q, D, A or V.

14. A variant immunoglobulin or a method according to claim 13 wherein the VH domain comprises a Q1E, Q1D, Q1A or Q1V substitution or an E1Q, E1D, E1A or E1V substitution.

15. A variant immunoglobulin or a method according to any one of claims 6 to 14 wherein the residue at position 17 in the VH domain sequence is substituted for R.

16. A variant immunoglobulin or a method according to claim 15 wherein the VH domain comprises a G17R substitution.

17. A variant immunoglobulin or a method according to any one of claims 6 to 16 wherein the residue at position 94 in the VH domain sequence is substituted for R.

18. A variant immunoglobulin or a method according to claim 17 wherein the VH domain comprises a H94R substitution.

19. A variant immunoglobulin or a method according to any one of claims 6 to 18 wherein the residue at position 95 in the VH domain sequence is substituted for D.

20. A variant immunoglobulin or a method according to claim 19 wherein the VH domain comprises a R95D substitution.

21. A variant immunoglobulin or a method according to any one of claims 6 to 20 wherein the residue at position 96 in the VH domain sequence is substituted for A.

22. A variant immunoglobulin or a method according to claim 21 wherein the VH domain comprises a G96A substitution.

23. A variant immunoglobulin or a method according to any one of claims 6 to 22 wherein the residue at position 100d in the VH domain sequence is substituted for F, G, L, P or M.

24. A variant immunoglobulin or a method according to claim 23 wherein the VH domain comprises a S100dF, S100dG, S100dL, S100dP or S100dM substitution.

25. A variant immunoglobulin or a method according to any one of claims 6 to 24 wherein the residue at position 102 in the VH domain sequence is substituted for A, F, H, I, L, V or Y.

26. A variant immunoglobulin or a method according to claim 25 wherein the VH domain comprises a P102A, P102F, P102H, P102I, P102L, P102Y, P102V or S102V substitution.

27. A variant immunoglobulin or a method according to any one of claims 3 to 26 wherein the sequence of the variant immunoglobulin has up to 10 amino acid substitutions relative to the parent immunoglobulin sequence.

28. A variant immunoglobulin or a method according to any one of claims 3 to 27 wherein the sequence of the VH domain comprises substitutions in the VH FR1 aggregation-prone segment and the VH CDR3 aggregation-prone segment.

29. A variant immunoglobulin or a method according to claim 28 wherein the sequence of the VH domain comprises substitutions at position 1 and position 102.

30. A variant immunoglobulin or a method according to claim 29 wherein the residue at position 102 is substituted for A, F, H, I, L, V or Y and wherein the residue at position 1 is substituted for E, Q, D, A or V.

31. A variant immunoglobulin or a method according to claim 30 wherein the VH domain comprises a P102Y substitution and a Q1A or Q1E substitution.

32. A variant immunoglobulin or a method according to any one of claims 3 to 31 wherein the sequence of the VH domain comprises substitutions in the VH position 95 aggregation-prone segment and the VH CDR3 aggregation-prone segment.

33. A variant immunoglobulin or a method according to claim 32 wherein the sequence of the VH domain comprises substitutions at position 95 and position 102.

34. A variant immunoglobulin or a method according to claim 33 wherein the residue at position 102 is substituted for A, F, H, I, L, V or Y and wherein the residue at position 95 is substituted for E or D.

35. A variant immunoglobulin or a method according to claim 34 wherein the VH domain comprises a S102V substitution and a R95D substitution.

36. A variant immunoglobulin or a method according to any one of claims 33 to 35 wherein the VH domain further comprises substitutions at positions 94 and 100.

37. A variant immunoglobulin or a method according to claim 36 wherein the residue at position 94 is substituted for R and the residue at position 100 is substituted for F.

38. A variant immunoglobulin or a method according to claim 37 wherein the VH domain comprises a H94R substitution and a V100F substitution.

39. A variant immunoglobulin or a method according to any one of claims 3 to 38 wherein the sequence of the VL domain of the variant immunoglobulin comprises substitutions in the position 37 aggregation-prone segment and the position 45 aggregation-prone segment.

40. A variant immunoglobulin or a method according to claim 39 wherein the sequence of the VL domain of the variant immunoglobulin comprises substitutions at position 37 and position 45.

41. A variant immunoglobulin or a method according to claim 40 wherein the residue at position 37 is substituted for Q or N and wherein the residue at position 45 is substituted for R, K or E.

42. A variant immunoglobulin or a method according to claim 41 wherein the VL domain comprises a L37Q substitution and a Q45R substitution.

43. A variant immunoglobulin or a method according to any to any one of claims 3 to 42 wherein the VL domain comprises a S102V substitution and a R95D substitution and the VL domain comprises a L37Q substitution and a Q45R substitution.

44. A variant immunoglobulin or a method according to any one of claims 3 to 43 wherein the VH domain comprises one or more substitutions in the VH position 95 aggregation-prone segment and the VL domain comprises one or more substitutions in the position 45 aggregation-prone segment.

45. A variant immunoglobulin or a method according to claim 44 wherein the VH domain comprises substitutions at positions 94 and 95 and the VL domain comprises a substitution at position 46.

46. A variant immunoglobulin or a method according to claim 45 wherein the residue at position 94 in the VH domain is substituted for R, K or T and the residue at position 95 in the VH domain is substituted for E or D and the residue at position 46 in the VL domain is substituted for K, R, H, F or S.

47. A variant immunoglobulin or a method according to claim 46 wherein the VH domain comprises a H94R and an R95D substitution and the VL domain comprises a L46R substitution.

48. A variant immunoglobulin or a method according to any one of claims 3 to 47 wherein the VH domain of the variant immunoglobulin comprises two or more substitutions in the VH CDR3 aggregation-prone segment.

49. A variant immunoglobulin or a method according to claim 48 wherein the VH domain comprises substitutions at positions 100d and 102.

50. A variant immunoglobulin or a method according to claim 49 wherein the VH domain comprises S100dF and P102V substitutions; S100dM and P102V substitutions; S100dF and P102Y substitutions or S100dM and P102Y substitutions.

51. A variant immunoglobulin or a method according to any one of the preceding claims wherein the variant immunoglobulin is humanised.

52. A variant immunoglobulin or a method according to any one of the preceding claims wherein the variant immunoglobulin is expressed in a recombinant system.

53. A variant immunoglobulin or a method according to any one of the preceding claims wherein the variant immunoglobulin is isolated and/or purified following expression.

54. A variant immunoglobulin or a method according to any one of the preceding claims wherein the variant immunoglobulin is formulated with a pharmaceutically acceptable excipient.

55. A method of assessing the manufacturability of an immunoglobulin comprising;

identifying the amino acid residue at one or more positions selected from the group consisting of positions 37, 45 and 46 in the VL domain of the immunoglobulin, positions 1, 17, 94, 95, 96, 100, 100d and 102, in the VH domain,

wherein the presence of a residue other than Q at position 37; E, K, R, Q or V, preferably K or R, at position 45, L or Y, preferably L, at position 46, in the VL domain of the immunoglobulin and/or;

the presence of a residue other than A, D, E, Q or V at position 1, R at position 17, R at position 94, A at position 96, F, G, L, M or P, preferably F or M at position 100d, A, F, H, I, L, V or Y, preferably V or Y, at position 102, in the VH domain of the immunoglobulin,

is indicative that the manufacturability of the immunoglobulin are sub-optimal.

56. A method according to claim 55 comprising that the manufacturability of the immunoglobulin is sub-optimal and improving the manufacturability of the immunoglobulin by a method according to any one of claims 1 and 3 to 54.

57. A method of preparing an immunoglobulin with improved manufacturability comprising;

providing a nucleic acid encoding a variant immunoglobulin produced by a method according to any one of claims 1 and 3 to 46,

expressing the nucleic acid under conditions to bring about production of said variant immunoglobulin, and recovering it.

58. A method according to claim 57 comprising formulating the variant immunoglobulin into a composition including a pharmaceutically acceptable excipient.

Claims:

1. A method of improving the manufacturability of an immunoglobulin, comprising;
identifying a parent immunoglobulin having a VL domain and a VH domain,
introducing a substitution into a framework aggregation-prone segment and/or a CDR3 aggregation-prone segment in the VL domain of the parent immunoglobulin, and/or
introducing a substitution into a framework region aggregation-prone segment and/or a CDR3 aggregation-prone segment in the VH domain of the parent immunoglobulin, and/or;
introducing a substitution into a constant region aggregation-prone segment in the CH1 domain of the parent immunoglobulin,
thereby producing a variant immunoglobulin having a VL domain and a VH domain which displays improved manufacturability relative to the parent immunoglobulin.

2. A variant immunoglobulin comprising;
a light chain variable (VL) domain which comprises a substitution at framework (FR) aggregation-prone segment and/or a complementary determining region 3 (CDR3) aggregation-prone segment;
a heavy chain variable (VH) domain which comprises a substitution at a framework region aggregation-prone segment and/or a CDR3 aggregation-prone segment; and/or,
a heavy chain constant region 1 (CH1) domain which comprises a substitution at a CH1 aggregation-prone segment.

3. A method according to claim 1 or a variant immunoglobulin according to claim 2 wherein the variant immunoglobulin displays both reduced aggregation propensity and increased productivity upon expression, relative to the parent immunoglobulin

4. A variant immunoglobulin or a method according to claim 3 wherein a substitution is introduced in the VL domain of the parent immunoglobulin at the VL position 37 aggregation-prone segment and/or the VL position 45 aggregation-prone segment; and/or
a substitution is introduced in the VH domain of the parent immunoglobulin at the VH FR1 aggregation-prone segment; the VH position 95 aggregation-prone segment; and the VH CDR3 aggregation-prone segment.

5. A variant immunoglobulin or a method according to claim 4 wherein the substitution is introduced at a position selected from the group consisting of 1, 17, 94, 95, 96, 100, 100d and 102 of the VH domain sequence and/or a position selected from the group consisting of 37, 45 and 46 of the VL domain sequence;
optionally wherein the residue at position 37 in the VL domain sequence is substituted for Q or N;
optionally wherein the residue at position 45 in the VL domain sequence is substituted for E, K, R, Q or V;
optionally wherein the residue at position 46 in the VL domain sequence is substituted for L or Y;

optionally wherein the residue at position 1 in the VH domain sequence is substituted for E, Q, D, A or V;

optionally 6 to 14 wherein the residue at position 17 in the VH domain sequence is substituted for R;

optionally wherein the residue at position 94 in the VH domain sequence is substituted for R;

optionally wherein the residue at position 95 in the VH domain sequence is substituted for D;

optionally wherein the residue at position 96 in the VH domain sequence is substituted for A;

optionally wherein the residue at position 100d in the VH domain sequence is substituted for F, G, L, P or M; and,

optionally wherein the residue at position 102 in the VH domain sequence is substituted for A, F, H, I, L, V or Y.

6. A variant immunoglobulin or a method according to any one of claims 3 to 5 wherein the sequence of the variant immunoglobulin has up to 10 amino acid substitutions relative to the parent immunoglobulin sequence.

7. A variant immunoglobulin or a method according to any one of claims 3 to 6 wherein the sequence of the VH domain comprises substitutions in the VH FR1 aggregation-prone segment and the VH CDR3 aggregation-prone segment; substitutions in the VH position 95 aggregation-prone segment and the VH CDR3 aggregation-prone segment; or two or more substitutions in the VH CDR3 aggregation-prone segment.

8. A variant immunoglobulin or a method according to claim 7 wherein the sequence of the VH domain comprises substitutions at position 1 and position 102 or substitutions at position 95 and position 102; or substitutions at positions 100d and 102,

optionally wherein the residue at position 102 is substituted for A, F, H, I, L, V or Y and the residue at position 1 is substituted for E, Q, D, A or V; or,

wherein the residue at position 102 is substituted for A, F, H, I, L, V or Y and the residue at position 95 is substituted for E or D.

9. A variant immunoglobulin or a method according to claim 8 wherein the VH domain further comprises substitutions at positions 94 and 100;

optionally wherein the residue at position 94 is substituted for R and the residue at position 100 is substituted for F.

10. A variant immunoglobulin or a method according to any one of claims 3 to 9 wherein the sequence of the VL domain of the variant immunoglobulin comprises substitutions in the position 37 aggregation-prone segment and the position 45 aggregation-prone segment;

optionally wherein the residue at position 37 is substituted for Q or N and wherein the residue at position 45 is substituted for R, K or E.

11. A variant immunoglobulin or a method according to any one of claims 3 to 10 wherein the VH domain comprises one or more substitutions in the VH position 95 aggregation-prone segment and the VL domain comprises one or more substitutions in the position 45 aggregation-prone segment;

optionally wherein the residue at position 94 in the VH domain is substituted for R, K or T and the residue at position 95 in the VH domain is substituted for E or D and the residue at position 46 in the VL domain is substituted for K, R, H, F or S.

12. A variant immunoglobulin or a method according to any one of the preceding claims wherein the variant immunoglobulin is humanised; expressed in a recombinant system; isolated and/or purified following expression; and/or formulated with a pharmaceutically acceptable excipient.

13. A method of assessing the manufacturability of an immunoglobulin comprising;

identifying the amino acid residue at one or more positions selected from the group consisting of positions 37, 45 and 46 in the VL domain of the immunoglobulin, positions 1, 17, 94, 95, 96, 100, 100d and 102, in the VH domain,

wherein the presence of a residue other than Q at position 37; E, K, R, Q or V, preferably K or R, at position 45, L or Y, preferably L, at position 46, in the VL domain of the immunoglobulin and/or;

the presence of a residue other than A, D, E, Q or V at position 1, R at position 17, R at position 94, A at position 96, F, G, L, M or P, preferably F or M at position 100d, A, F, H, I, L, V or Y, preferably V or Y, at position 102, in the VH domain of the immunoglobulin,

is indicative that the manufacturability of the immunoglobulin is sub-optimal.

14. A method according to claim 13 comprising that the manufacturability of the immunoglobulin is sub-optimal and improving the manufacturability of the immunoglobulin by a method according to any one of claims 1 and 3 to 12.

15. A method of preparing an immunoglobulin with improved manufacturability comprising;

providing a nucleic acid encoding a variant immunoglobulin produced by a method according to any one of claims 1 and 3 to 12,

expressing the nucleic acid under conditions to bring about production of said variant immunoglobulin, and recovering it; and,

optionally formulating the variant immunoglobulin into a composition including a pharmaceutically acceptable excipient.