

CLAIMS

What is claimed:

- 5 1. A method of producing a composition comprising a humanized anti- $\alpha 4\beta 7$ antibody, said method comprising
- culturing a mammalian host cell in a production medium, and
- adding a supplement comprising uridine, manganese, and galactose to the
- 10 production medium, thereby producing a composition comprising the humanized anti- $\alpha 4\beta 7$ antibody,
- wherein the mammalian host cell is genetically engineered to express a humanized anti- $\alpha 4\beta 7$ antibody which is an IgG1 antibody, comprises a heavy chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 4, a CDR2 domain as set forth in SEQ ID NO: 3, and a CDR1 domain as set forth in SEQ ID NO: 2; and comprises a light
- 15 chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 8, a CDR2 domain as set forth in SEQ ID NO: 7, and a CDR1 domain as set forth in SEQ ID NO: 6.
2. The method of claim 1, wherein the composition comprises a decreased amount of basic isoform (as determined by Cation Exchange Chromatography (CEX)) of the
- 20 humanized anti- $\alpha 4\beta 7$ antibody in comparison to a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under substantially similar conditions but in the absence of the supplement; or
- the composition comprises about 16% or less, about 14% or less, or about 13% or less basic isoform (as determined by CEX) of the humanized anti- $\alpha 4\beta 7$ antibody.
- 25 3. The method of claim 1 or 2, wherein the supplement is added to the production medium or is added to a feed medium which is subsequently added to the production medium.
- 30 4. The method of any one of claims 1 to 3, wherein the cumulative concentration of uridine added in the production medium between supplementation and harvest is about 1 to about 7 mM; wherein the cumulative concentration of manganese in the production medium between supplementation and harvest is about 0.002 to about 0.015 mM; and/or

wherein the cumulative concentration of galactose in the production medium between supplementation and harvest is about 3 to about 20 mM.

5. The method of any one of claims 1 to 4, wherein manganese is added as a supplement multiple times to the production medium by about 0.1 to 10 μ M, about 0.2 to 1.5 μ M, about 0.2 to 5 μ M, about 0.25 to 2 μ M, about 0.3 to 1.2 μ M, or about 0.3 to 0.8 μ M with each addition;

uridine is added as a supplement multiple times to the production medium by about 25 to 1000 μ M, about 75 to 750 μ M, about 55 to 620 μ M, about 100 to 600 μ M, about 150 to 450 μ M, about 100 to 700 μ M, about 100 to 600 μ M, or about 170 to 630 μ M with each addition; and/or

wherein galactose is added as a supplement multiple times to the production medium by about 0.1 to 10 mM, 0.2 to 7.5 mM, 0.5 to 5 mM, 0.4 to 2.8 mM, 0.5 to 3.5 mM, 0.7 to 2.9 mM, 0.75 to 2.5 mM or by about 1.2 mM or 1.4 mM with each addition.

6. The method of claim 5, wherein the supplement is added daily or every two days, and/or the supplement is added beginning on day 4 of the production phase culture.

7. The method of any one of claims 1-6, wherein the feed medium further comprises zinc.

8. The method of claim 7, wherein the cumulative concentration of zinc added to the production medium between supplementation and harvest is about 0.005 mM to about 0.045 mM.

9. The method of any one of claims 1-8, wherein the method further decreases the percentage of acidic species of the humanized anti- $\alpha 4\beta 7$ antibody relative to the percentage of acidic species produced in a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under substantially similar conditions but in the absence of the supplement.

10. The method of any one of claims 1-9, wherein the method is a fed batch method.

11. A method of producing a composition comprising a humanized anti- $\alpha 4\beta 7$ antibody, said method comprising culturing a mammalian host cell in a production medium comprising zinc, thereby producing a composition comprising the humanized anti- $\alpha 4\beta 7$ antibody,

wherein the mammalian host cell is genetically engineered to express a humanized anti- $\alpha 4\beta 7$ antibody which is an IgG1 antibody, comprises a heavy chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 4, a CDR2 domain as set forth in SEQ ID NO: 3, and a CDR1 domain as set forth in SEQ ID NO: 2; and comprises a light chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 8, a CDR2 domain as set forth in SEQ ID NO: 7, and a CDR1 domain as set forth in SEQ ID NO: 6.

12. The method of claim 11, wherein the composition comprises a decreased amount of basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody in comparison to a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under the same conditions in the absence of zinc; or

the composition comprises about 16% or less, about 14% or less, about 13% or less basic isoform of the humanized anti- $\alpha 4\beta 7$ antibody.

13. The method of claim 11 or 12, wherein the concentration of zinc in the production medium is 2 μ M to 60 μ M.

14. The method of any one of claims 11-13, wherein the method comprises supplementing the production medium with zinc by adding a feed medium comprising zinc to the production medium, and/or wherein the feed medium is added to the production medium beginning on about day four of a production phase.

15. The method of any one of claims 1-14, wherein the production medium comprises 5.0 to 8.8 g/L lysine and 3.0 to 12.0 g/L arginine.

16. The method of claim 15, wherein the production medium comprises 4.5 to 5.5 g/L lysine or 5.5 to 8.8 g/L lysine.

17. The method of claim 15 or 16, wherein the production medium comprises 5.4 to 7.4 g/L arginine or 7.4 to 12 g/L arginine.

18. A method of producing a composition comprising a humanized anti- $\alpha 4\beta 7$ antibody, said method comprising

culturing a mammalian host cell in a production medium in a production phase, such that a composition comprising the humanized anti- $\alpha 4\beta 7$ antibody is produced, wherein the production medium has an average temperature of about 37 degrees Celsius, wherein the host cell is genetically engineered to express a humanized IgG1 anti- $\alpha 4\beta 7$ antibody, wherein the humanized anti- $\alpha 4\beta 7$ comprises a heavy chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 4, a CDR2 domain as set forth in SEQ ID NO: 3, and a CDR1 domain as set forth in SEQ ID NO: 2; and comprises a light chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 8, a CDR2 domain as set forth in SEQ ID NO: 7, and a CDR1 domain as set forth in SEQ ID NO: 6.

19. The method of claim 18, wherein the method is a method producing a composition comprising 2.5% or less HMW species (as determined by SEC) of the humanized anti- $\alpha 4\beta 7$ antibody.

20. A method of producing a composition comprising a humanized anti- $\alpha 4\beta 7$ antibody, said method comprising

culturing a mammalian host cell in a growth medium in an expansion phase, wherein the mammalian host cell is genetically engineered to express a humanized anti- $\alpha 4\beta 7$ antibody, and

culturing the mammalian host cell in a production medium in a production phase, such that a composition comprising the humanized anti- $\alpha 4\beta 7$ antibody is produced,

wherein the mammalian host cell is cultured at a temperature that is approximately the same in both the expansion phase and the production phase, and

wherein the humanized anti- $\alpha 4\beta 7$ antibody is an IgG1 antibody; comprises a heavy chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 4, a CDR2 domain as set forth in SEQ ID NO: 3, and a CDR1 domain as set forth in SEQ ID NO: 2; and comprises a light chain variable region comprising a CDR3 domain as set forth

in SEQ ID NO: 8, a CDR2 domain as set forth in SEQ ID NO: 7, and a CDR1 domain as set forth in SEQ ID NO: 6, wherein the temperature is from 36 to 38 degrees Celsius, and wherein the method is a method of producing a composition comprising high levels of monomer (as determined by SEC) of the humanized anti- $\alpha 4\beta 7$ antibody, relative to a control culture cultured under substantially similar conditions but with a different temperature between the expansion and the production phases.

21. The method of claim 20, wherein the average temperature is from 36.5 to 37.5 degrees Celsius or the temperature is an average temperature of about 37 degrees Celsius.

22. The method of any one of claims 1-21, wherein the production medium has a temperature ranging from 36 to 38 degrees Celsius.

23. The method of any one of claims 1-22, wherein the production medium has a pH ranging from 6.5 to 7;
has a pH ranging from 6.8 to 7.0; and/or
has a glucose level that is maintained at about 7 g/L or less during the production phase.

24. The method of any one of claims 1-23, wherein the production phase is 14 days or less or the production phase ranges from 10 days to 17 days.

25. The method of any one of claims 1-24, which is performed in a large scale bioreactor.

26. The method of any one of claims 1-25, wherein the production phase results in a titer of the humanized anti- $\alpha 4\beta 7$ antibody of greater than 3 g/L; about 3 to about 8 g/L, or about 5 to about 7 g/L.

27. The method of any one of claims 1-26, wherein the mammalian host cell is a Chinese Hamster Ovary (CHO) cell.

28. The method of any one of claims 1-27, wherein the humanized anti- $\alpha 4\beta 7$ antibody comprises a heavy chain variable domain comprising an amino acid sequence as set forth

in SEQ ID NO: 1, and comprises a light chain variable domain comprising an amino acid sequence as set forth in SEQ ID NO: 5; or is vedolizumab.

29. The method of any one of claims 1-28, wherein the method comprises harvesting and purification of the antibody.

30. The method of claim 29, wherein the method further comprises preparing a pharmaceutical formulation of the purified antibody which is suitable for human therapeutic use.

31. A composition comprising humanized anti- $\alpha 4\beta 7$ antibodies, wherein the composition is produced using or is obtainable by any one of the methods of claims 1-30.

32. The composition of claim 31, comprising a population of humanized anti- $\alpha 4\beta 7$ antibodies having (i) 90% or more, or (ii) 92-95%, total asialo-, agalacto, core fucosylated biantennary glycan (G0F), asialo-, monogalacto, core fucosylated biantennary glycan (G1F), and/or asialo-, digalacto, core fucosylated biantennary glycan (G2F) glycosylation variants.

33. The method of any one of claims 1-10, wherein the method is a method of producing a composition having a decreased amount of a G0F glycoform (as determined by Hydrophilic Interaction Chromatography (HILIC) of the humanized anti- $\alpha 4\beta 7$ antibody, in comparison to a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under substantially similar conditions but in the absence of the supplement.

34. The method of claim 33, wherein the composition comprises at least about a 15% decreased or a 20% decreased level of the G0F glycoform of the humanized anti- $\alpha 4\beta 7$ antibody in comparison to a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under substantially similar conditions in the absence of the supplement.

35. The method of any one of claims 1-10, wherein the method is a method of producing a composition having about 65% or less, about 60% or less, or about 55% or less G0F glycoform (as determined by HILIC) of the humanized anti- α 4 β 7 antibody.

5 36. The method of any one of claims 1-10, wherein the method is a method of producing a composition having an increased amount of a G1F glycoform (as determined by HILIC) of the humanized anti- α 4 β 7 antibody in comparison to a control mammalian host cell expressing the humanized anti- α 4 β 7 antibody that is cultured under substantially similar conditions in the absence of the supplement.

10 37. The method of claim 36, wherein the composition comprises at least about a 2-fold or at least about a 3-fold increase in the G1F glycoform of the humanized anti- α 4 β 7 antibody in comparison to the control.

15 38. The method of any one of claims 1-10, wherein the method is a method of producing a composition having about 25% or more or about 30% or more G1F glycoform (as determined by HILIC) of the humanized anti- α 4 β 7 antibody.

20 39. The method of any one of claims 1-10, wherein the method is a method of producing a composition having an increased amount of a G2F glycoform (as determined by HILIC) of the humanized anti- α 4 β 7 antibody in comparison to a control mammalian host cell expressing the humanized anti- α 4 β 7 antibody that is cultured under substantially similar conditions in the absence of the supplement.

25 40. The method of claim 39, wherein the composition comprises at least about a 3-fold increase or about a 4-fold increase in the G2F glycoform of the humanized anti- α 4 β 7 antibody in comparison to the control.

30 41. The method of any one of claims 1-10, wherein the method is a method of producing a composition having about 3% or more or about 4% or more G2F glycoform (as determined by HILIC) of the humanized anti- α 4 β 7 antibody.

42. The method of any one of claims 33-41, wherein the method further decreases the percentage of acidic species of the humanized anti- $\alpha 4\beta 7$ antibody relative to the percentage of acidic species produced in a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under substantially similar conditions in the absence of the supplement; or

wherein the method further increases the percentage of main isoform species of the humanized anti- $\alpha 4\beta 7$ antibody relative to the percentage of main isoform species produced in a control mammalian host cell expressing the humanized anti- $\alpha 4\beta 7$ antibody that is cultured under substantially similar conditions in the absence of a feed medium comprising uridine, manganese, and galactose added to the production medium.

43. The method of any one of claims 33-42, wherein the feed medium is added to the production medium beginning on about day four of the production phase; or wherein the production medium has a pH of about 6.8 to about 7.1.

44. The method of any one of claims 33-43, wherein the method comprises harvesting and purification of the antibody.

45. The method of claim 44, wherein the method further comprises preparing a pharmaceutical formulation of the purified antibody which is suitable for human therapeutic use.

46. A composition comprising a humanized anti- $\alpha 4\beta 7$ antibody, wherein the composition is obtainable by any one of the methods of claims 33-43.

47. A cell culture comprising a host cell genetically engineered to express a humanized anti- $\alpha 4\beta 7$ antibody, and a production medium supplemented with uridine, manganese, and galactose (UMG), wherein the humanized anti- $\alpha 4\beta 7$ antibody is an IgG1 antibody and comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:1, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5.

48. The cell culture of claim 47, wherein the production medium comprises supplemented uridine at a concentration of about 1 to about 7 mM, supplemented manganese at a concentration of about 0.002 to about 0.015 mM, and supplemented galactose at a concentration of about 3 to about 20 mM on the day of harvest.

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49. The cell culture of claim 47 or 48, wherein the production medium further comprises zinc.

50. The cell culture of claim 49, wherein the production medium comprises supplemented zinc at a concentration of about 0.005 mM to 0.045 mM on the day of harvest.

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51. The cell culture of any one of claims 47-50, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:1, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5.

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52. A cell culture comprising a host cell genetically engineered to express a humanized anti- $\alpha 4\beta 7$ antibody, and a production medium supplemented with zinc, wherein the humanized anti- $\alpha 4\beta 7$ antibody is an IgG1 antibody and comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:1, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5.

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53. The cell culture of claim 52, wherein the production medium comprises supplemented zinc at a concentration of about 0.005 mM to 0.045 mM on the day of harvest and/or wherein the production medium further comprises uridine, manganese, and galactose (UMG).

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54. The cell culture of claim 50, wherein the production medium comprises supplemented uridine at a concentration of about 2 to about 5 mM, supplemented manganese at a concentration of about 0.001 to about 0.01 mM, and supplemented galactose at a concentration of about 10 to about 15 mM on the day of harvest.

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55. The cell culture of any one of claims 47-54, wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has an isoform distribution comprising:

a. 16% or less, 15% or less, 14% or less, 13% or less, or 12% or less basic isoform; and/or

b. at least 65%, at least 68%, at least 70%, at least 72%, or at least 75% major isoform; and/or

wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has a fucosylated N-glycan content comprising:

c. 65% or less, 60% or less, or 55% or less G0F;

d. 25% or more, 27% or more, or 30% or more G1F; and/or

e. 2.5% or more, 3% or more, 3.5% or more, 4% or more, or 4.5% or more G2F.

56. The cell culture of any one of claims 47-55, wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has a total fucosylated N-glycan content (G0F + G1F + G2F) of at least 88%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, or at least 95%.

57. The cell culture of any one of claims 47-55, wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has a total fucosylated N-glycan content (G0F + G1F + G2F) of 92-95%; and/or wherein the expressed humanized anti- $\alpha 4\beta 7$ antibody has a total fucosylated N-glycan content (G0F + G1F + G2F) of 91-92%, 91-92.5%, or 91-93%.

58. The cell culture of any one of claims 47-57, wherein the host cell is a CHO cell.

59. A humanized anti- $\alpha 4\beta 7$ antibody produced by the cell culture of any one of claims 47-58.

60. A method of producing a composition comprising a humanized anti- $\alpha 4\beta 7$ antibody, said method comprising

culturing a mammalian host cell genetically engineered to express the humanized anti- $\alpha 4\beta 7$ antibody in a first production medium having a first pH; and

culturing the mammalian host cell in a second production medium having a second pH;

wherein the second pH is lower than the first pH, and

wherein the humanized anti- $\alpha 4\beta 7$ antibody is an IgG1 antibody; comprises a heavy chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 4, a CDR2 domain as set forth in SEQ ID NO: 3, and a CDR1 domain as set forth in SEQ ID NO: 2; and comprises a light chain variable region comprising a CDR3 domain as set forth in SEQ ID NO: 8, a CDR2 domain as set forth in SEQ ID NO: 7, and a CDR1 domain as set forth in SEQ ID NO: 6.

61. The method of claim 60, wherein the second pH is 0.1 to 0.5 pH units lower than the first pH and/or the first pH is in the range of pH 6.8-7.2, and wherein the second pH is in the range of pH 6.7-6.95.

62. The method of claim 60 or 61, wherein the mammalian host cell is cultured at the first pH for 120 hours or less, 85-110 hours; or 90-100 hours.

63. The method of any one of claims 60-62, further comprising harvesting the anti- $\alpha 4\beta 7$ antibody from the second production medium.

64. The method of any one of claims 60-63, wherein the composition has an increased level of the anti- $\alpha 4\beta 7$ antibody major isoform, relative to a control composition in which the mammalian host cell is cultured at the first pH without a pH shift.