

CLAIMS

WHAT IS CLAIMED:

1. A method for ~~improving recombinant protein titer in production of a recombinant protein by culturing recombinant eukaryotic cells, the method comprising:~~

~~(a) providing a defined selecting a cell culture medium having with reduced impurities, the defined for use in cell culture to improve cell culture performance, the method comprising:~~

~~(a) providing a cell culture medium having comprising a 4-hydroxyethyl piperazine ethanesulfonic acid (HEPES) buffer;~~

~~(b) analyzing the cell culture medium comprising the HEPES buffer to determine the amount of HEPES related impurities HEPES+[O₂]-[H₂] and HEPES-[CH₄] present in the cell culture medium;~~

~~(c) selecting the cell culture medium comprising the HEPES buffer for use in cell culture if the cell culture medium comprising the HEPES buffer is determined to have less than about 4000 μmol of a HEPES related impurity having a molecular weight of 267.07 HEPES+[O₂]-[H₂] per mole of total HEPES, and less than about 400 μmol of a HEPES related impurity having a molecular weight of 221.06 HEPES-[CH₄] per mole of total HEPES;~~

~~wherein the use of the cell culture medium comprising the HEPES buffer having less than about 4000 μmol of HEPES+[O₂]-[H₂] per mole of total HEPES, and less than about 400 μmol of HEPES-[CH₄] per mole of total HEPES improves cell culture performance, as compared to cell culture performance in non-HEPES related impurity reduced media.~~

2. The method of claim 1, wherein the improved cell culture performance comprises at least one selected from the group consisting of improved protein titer and cell growth.

3. A cell culture comprising (i) at least one recombinant eukaryotic cell that can express a recombinant protein and (ii) a cell culture medium with reduced impurities, wherein the cell culture is produced by a method comprising the steps of:

(a) providing a cell culture medium comprising a 4-hydroxyethyl piperazine ethanesulfonic acid (HEPES) buffer having reduced HEPES related impurities, wherein the cell culture medium has less than about 4000 μmol of HEPES+[O2]-[H2] per mole of total HEPES, and less than about 400 μmol of HEPES-[CH4] per mole of total HEPES, wherein the cell culture medium is provided by analysis of cell culture medium to determine the amount of HEPES+[O2]-[H2] and HEPES-[CH4] present in the cell culture medium, and selection of cell culture medium having less than about 4000 μmol of HEPES+[O2]-[H2] per mole of total HEPES, and less than about 400 μmol of HEPES-[CH4] per mole of total HEPES;

(b) culturing said recombinant eukaryotic cells in said ~~defined~~ cell culture medium having reduced impurities; and

(c) expressing a recombinant protein of interest from said recombinant eukaryotic cells; and

~~(d) producing a higher titer of the recombinant protein in the defined cell culture medium having reduced impurities relative to the that of similar or identical cells cultured in non-reduced impurity media.~~

2. ~~The method of claim 14.~~ The cell culture of claim 3, wherein the eukaryotic cell is selected from the group consisting of mammalian cell, avian cell, insect cell, and yeast cell.

35. The ~~method~~cell culture of claim 14, wherein the eukaryotic cell is selected from the group consisting of CHO, COS, retinal cell, Vero, CV1, kidney, HeLa, HepG2, WI38, MRC 5, Colo25, HB 8065, HL-60, lymphocyte, A431, CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT cell, stem cell, tumor cell, and a cell line derived from an aforementioned cell.

46. The ~~method~~cell culture of claim 35, wherein the eukaryotic cell is a CHO cell.

5.7. The ~~method~~cell culture of claim 13, wherein said expressing a recombinant protein of interest occurs during production phase, growth phase, or both.

~~6.8.~~ The methodcell culture of claim ~~13~~, wherein said culturing of the recombinant eukaryotic cells in said ~~defined~~ cell culture medium having reduced impurities occurs during production phase, growth phase, or both.

~~7.9.~~ The methodcell culture of claim ~~13~~, wherein the cell growth during said culturing of the recombinant eukaryotic cells is higher than the cell growth of similar or identical recombinant eukaryotic cells in non-impurity reduced media.

~~8.10.~~ The methodcell culture of claim ~~13~~, wherein the higher titer of the recombinant protein is increased ~~by at least about 5%~~, as compared to that of similar or identical cells cultured in non-reduced impurity media.

~~9.—11.~~ The methodcell culture of claim ~~13~~, wherein the recombinant protein is an antibody, a human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a multispecific antibody, a bispecific antibody, an antigen binding antibody fragment, a single chain antibody, a diabody, triabody or tetrabody, a Fab fragment or a F(ab')₂ fragment, an IgD antibody, an IgE antibody, an IgM antibody, an IgG antibody, an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody.

~~1012.~~ The methodcell culture of claim ~~13~~, wherein the recombinant protein comprises an Fc domain.

~~1113.~~ The methodcell culture of claim ~~1012~~, wherein the recombinant protein is selected from the group consisting of an Fc-fusion protein, a receptor-Fc-fusion protein (TRAP), an antibody, an antibody fragment, and a ScFv-Fc fusion protein.

~~1214.~~ The methodcell culture of claim ~~1113~~, wherein the recombinant protein is selected from the group consisting of an anti-PD1 antibody, an anti-PDL-1 antibody, an anti-Dll4 antibody, an anti-ANG2 antibody, an anti-AngPt13 antibody, an anti-PDGFR antibody, an anti-Erb3 antibody, an anti-PRLR antibody, an anti-TNF antibody, an anti-EGFR antibody, an anti-PCSK9 antibody, an anti-GDF8 antibody, an anti-GCGR antibody, an anti-VEGF antibody, an anti-IL1R antibody, an anti-IL4R antibody, an anti-IL6R antibody, an anti-IL1 antibody, an anti-IL2 antibody, an anti-IL3 antibody, an anti-IL4 antibody, an anti-IL5 antibody, an anti-IL6

antibody, an anti-IL7 antibody, an anti-RSV antibody, an anti-NGF antibody, an anti-CD3 antibody, an anti-CD20 antibody, an anti-CD19 antibody, an anti-CD28 antibody, an anti-CD48 antibody, an anti-CD3/anti-CD20 bispecific antibody, an anti-CD3/anti-MUC16 bispecific antibody, and an anti-CD3/anti-PSMA bispecific antibody.

~~13~~15. The ~~method~~cell culture of claim ~~11~~13, wherein the recombinant protein is selected from the group consisting of alirocumab, atoltivimab, maftivimab, odesivimab, odesivivmab-ebgn, casirivimab, imdevimab, cemiplimab, cemiplimab-rwlc, dupilumab, evinacumab, evinacumab-dgnb, fasimumab, nesvacumab, trevogrumab, rinucumab and sarilumab.

~~14~~16. The ~~method~~cell culture of claim ~~13~~15, wherein the recombinant protein is dupilumab.

~~15. — A cell culture medium having reduced impurities, the medium comprising a defined cell culture medium having reduced impurities, the defined cell culture medium comprising a 4-hydroxyethyl-piperazine-ethanesulfonic acid (HEPES) buffer, and having less than about 800 μ mol of a HEPES-related impurity having a molecular weight of 267.07 per mole of total HEPES, and less than about 80 μ mol of a HEPES-related impurity having a molecular weight of 221.06 per mole of total HEPES.~~

~~16. — The cell culture medium of claim 15, wherein the medium is hydrolysate-free.~~

~~17. The cell culture medium of claim 15, wherein the medium is chemically defined.~~

~~18. — The cell culture medium of claim 15, further comprising insulin.~~

~~19. — The cell culture medium of claim 15, further comprising ≥ 0.09 mM ± 0.014 mM ornithine, $\geq 0.20 \pm 0.03$ mM putrescine, or combinations thereof.~~

~~20. — The cell culture medium of claim 15, further comprising $\geq 40 \pm 6$ mM of a mixture of amino acids or salts thereof.~~

~~21. — The cell culture medium of claim 20, wherein the mixture of amino acids consists of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.~~

22. — ~~The cell culture medium of claim 15, further comprising one or more fatty acids and tocopherol.~~

23. — ~~The cell culture medium of claim 22, wherein the one or more fatty acids are selected from the group consisting of linoleic acid, linolenic acid, thioctic acid, oleic acid, palmitic acid, stearic acid, arachidic acid, arachidonic acid, lauric acid, behenic acid, decanoic acid, dodecanoic acid, hexanoic acid, lignoceric acid, myristic acid, and octanoic acid.~~

24. — ~~The cell culture medium of claim 15, further comprising a mixture of nucleosides.~~

25. — ~~The cell culture medium of claim 24, wherein the mixture of nucleosides comprises one or more of adenosine, guanosine, cytidine, uridine, thymidine, and hypoxanthine.~~

26. — ~~The cell culture medium of claim 25, comprising adenosine, guanosine, cytidine, uridine, thymidine, and hypoxanthine.~~

27. — ~~The cell culture medium of claim 15, further comprising one or more salts of divalent cations.~~

28. — ~~The cell culture medium of claim 27, wherein the divalent cation is magnesium, calcium, or both.~~

29. — ~~The cell culture medium of claim 28, comprising Ca^{2+} and Mg^{2+} .~~

30. — ~~The cell culture medium of claim 15, wherein the medium is chemically defined media and comprises: HEPES buffer; a mixture of amino acids; optionally a mixture of nucleosides; one or more fatty acids and tocopherol; one or more salts of divalent cations; and one or more vitamins.~~

31. — ~~The cell culture medium of claim 30, further comprising insulin.~~

32. — ~~A method for selecting a defined cell culture medium for use in cell culture to improve cell culture performance, the method comprising:~~

~~(a) providing a defined cell culture medium comprising a 4-hydroxyethyl-piperazine ethanesulfonic acid (HEPES) buffer;~~

~~(b) analyzing the defined cell culture medium comprising the HEPES buffer to determine the amount of a HEPES-related impurity having a molecular weight of 267.07 and the amount of~~

a HEPES related impurity having a molecular weight of 221.06 present in the defined cell culture medium;

(c) selecting the defined cell culture medium comprising the HEPES buffer for use in cell culture if the defined cell culture medium comprising the HEPES buffer is determined to have less than about 4000 μmol of a HEPES related impurity having a molecular weight of 267.07 per mole of total HEPES, and less than about 400 μmol of a HEPES related impurity having a molecular weight of 221.06 per mole of total HEPES;;

wherein the use of the defined cell culture medium comprising the HEPES buffer having less than about 4000 μmol of a HEPES related impurity having a molecular weight of 267.07 per mole of total HEPES, and less than about 400 μmol of a HEPES related impurity having a molecular weight of 221.06 per mole of total HEPES improves cell culture performance, as compared to cell culture performance in non-HEPES related impurity reduced media.

33.—The method of claim 32, wherein the improved cell culture performance includes improved cell culture titer and/or cell growth.

34.—A method for selecting a HEPES buffer for use in cell culture to improve cell culture performance, the method comprising:

(a) providing a 4-hydroxyethyl-piperazine-ethanesulfonic acid (HEPES) buffer;

(b) analyzing the HEPES buffer to determine the amount of a HEPES related impurity having a molecular weight of 267.07 and the amount of a HEPES related impurity having a molecular weight of 221.06 present in the HEPES buffer;

(c) selecting the HEPES buffer for use in cell culture if the HEPES buffer is determined to have less than about 4000 μmol of a HEPES related impurity having a molecular weight of 267.07 per mole of total HEPES, and less than about 400 μmol of a HEPES related impurity having a molecular weight of 221.06 per mole of total HEPES;;

wherein the use of the HEPES buffer having less than about 4000 μmol of a HEPES related impurity having a molecular weight of 267.07 per mole of total HEPES, and less than about 400 μmol of a HEPES related impurity having a molecular weight of 221.06 per mole of total HEPES to be used in connection with the cell culture improves cell culture performance, as

compared to cell culture performance in the presence of HEPES buffer having higher amounts of said impurities.

35. — The method of claim 34, wherein the improved cell culture performance includes improved cell culture titer and/or cell growth.

36. — A cell culture comprising a cell and a cell culture medium according to any of claims 15-31, claim 13, wherein the recombinant protein is aflibercept.

37. — The cell culture according to claim 36, wherein the cell is a eukaryotic cell.

38. — The cell culture according to claim 37, wherein the eukaryotic cell is selected from the group consisting of a mammalian cell, an avian cell, an insect cell and a yeast cell.

39. — The cell culture according to claim 36, when the cell can express an antibody selected from the group consisting of alirocumab, atoltivimab, maftivimab, odesivimab, odesivimab-ebgn, casirivimab, imdevimab, cemiplimab, cemiplimab-rwlc, dupilumab, evinacumab, evinacumab-dgnb, fasimumab, nesvacumab, trevogrumab, rinucumab and sarilumab.

40. — The cell culture according to claim 39, when the cell can express dupilumab.

41. The method of claim 8, wherein the higher titer of the recombinant protein is increased by at least about 10%, as compared to that of similar or identical cells cultured in non-reduced impurity media.

42. The method of claim 8, wherein the higher titer of the recombinant protein is increased by at least about 15%, as compared to that of similar or identical cells cultured in non-reduced impurity media.

~~43. — The method of claim 8, wherein the higher titer of the recombinant protein is increased by at least about 25%, as compared to that of similar or identical cells cultured in non-reduced impurity media.~~

~~44. 18. The method of claim 1, wherein the doubling rate of viable cells in media having reduced impurities is at least 5% greater than the doubling rate of cells cultured in non-impurity reduced media; a eukaryotic cell is selected from the group consisting of mammalian cell, avian cell, insect cell, and yeast cell.~~

~~45. — The method of claim 44, wherein the doubling rate of viable cells in media having reduced impurities is at least 10% greater than the doubling rate of cells cultured in non-impurity reduced media.~~

~~46. — The method of claim 44, wherein the doubling rate of viable cells in media having reduced impurities is at least 15% greater than the doubling rate of cells cultured in non-impurity reduced media.~~

~~47. — The method of claim 44, wherein the doubling rate of viable cells in media having reduced impurities is at least 25% greater than the doubling rate of cells cultured in non-impurity reduced media.~~

~~48. — A cell culture medium according to claim 15, wherein the cell culture medium comprises at least one compound selected from the group consisting of vinylsulfonic acid, HEPES+[O]⁻[H⁺], acetamidomethane-sulfonic acid, HEPES+[O]⁻, 2,2-Dihydroxyethane-sulfonic acid, HEPES-[C₂H₆]⁺[O]⁻ [SO₃-containing] species.~~

~~49. — A cell culture comprising (i) at least one recombinant eukaryotic cell that can express a recombinant protein and (ii) a cell culture medium, wherein the cell culture is produced by a method comprising the steps of:~~

~~(a) providing a defined cell culture medium having reduced impurities, the defined cell culture medium having less than about 4000 μ mol of a HEPES related impurity having a molecular weight of 267.07 per mole of total HEPES, and less than about 400 μ mol of a HEPES related impurity having a molecular weight of 221.06 per mole of total HEPES;~~

~~(b) culturing said recombinant eukaryotic cells in said defined cell culture medium having reduced impurities;~~

~~(c) expressing a recombinant protein of interest from said recombinant eukaryotic cells; and~~

~~(d) producing a higher titer of the recombinant protein in the defined cell culture medium having reduced impurities relative to the that of similar or identical cells cultured in non-reduced impurity media.~~

~~50. — The cell culture of claim 49, wherein the eukaryotic cell is selected from the group consisting of mammalian cell, avian cell, insect cell, and yeast cell.~~

~~51. — The cell culture of claim 50~~19. The method of claim 1, wherein the eukaryotic cell is selected from the group consisting of CHO, COS, retinal cell, Vero, CV1, kidney, HeLa, HepG2, WI38, MRC 5, Colo25, HB 8065, HL-60, lymphocyte, A431, CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT cell, stem cell, tumor cell, and a cell line derived from an aforementioned cell.

~~52~~20. The ~~cell culture~~method of claim ~~51~~1, wherein the eukaryotic cell is a CHO cell.

~~53. — The cell culture of claim 49, wherein said expressing a recombinant protein of interest occurs during production phase, growth phase, or both.~~

~~54. — The cell culture of claim 49, wherein said culturing of the recombinant eukaryotic cells in said defined cell culture medium having reduced impurities occurs during production phase, growth phase, or both.~~

~~55. — The cell culture of claim 49, wherein the cell growth during said culturing of the recombinant eukaryotic cells is higher than the cell growth of similar or identical recombinant eukaryotic cells in non-purity reduced media.~~

~~56. — The cell culture of claim 49, wherein the higher titer of the recombinant protein is increased by at least about 5%, as compared to that of similar or identical cells cultured in non-reduced purity media.~~

~~57. — The cell culture of claim 49~~21. The method of claim 1, wherein the recombinant protein is an antibody, a human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a multispecific antibody, a bispecific antibody, an antigen binding antibody fragment, a single chain antibody, a diabody, triabody or tetrabody, a Fab fragment or a F(ab')₂ fragment, an IgD antibody, an IgE antibody, an IgM antibody, an IgG antibody, an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody.

~~58~~22. The ~~cell culture method~~ of claim ~~49~~1, wherein the recombinant protein comprises an Fc domain.

~~59~~23. The ~~cell culture method~~ of claim ~~58~~1, wherein the recombinant protein is selected from the group consisting of an Fc-fusion protein, a receptor-Fc-fusion protein (TRAP), an antibody, an antibody fragment, and a ScFv-Fc fusion protein.

~~60~~24. The ~~cell culture method~~ of claim ~~59~~23, wherein the recombinant protein is selected from the group consisting of an anti-PD1 antibody, an anti-PDL-1 antibody, an anti-Dll4 antibody, an anti-ANG2 antibody, an anti-AngPt13 antibody, an anti-PDGFR antibody, an anti-Erb3 antibody, an anti-PRLR antibody, an anti-TNF antibody, an anti-EGFR antibody, an anti-PCSK9 antibody, an anti-GDF8 antibody, an anti-GCGR antibody, an anti-VEGF antibody, an anti-IL1R antibody, an anti-IL4R antibody, an anti-IL6R antibody, an anti-IL1 antibody, an anti-IL2 antibody, an anti-IL3 antibody, an anti-IL4 antibody, an anti-IL5 antibody, an anti-IL6 antibody, an anti-IL7 antibody, an anti-RSV antibody, an anti-NGF antibody, an anti-CD3 antibody, an anti-CD20 antibody, an anti-CD19 antibody, an anti-CD28 antibody, an anti-CD48 antibody, an anti-CD3/anti-CD20 bispecific antibody, an anti-CD3/anti-MUC16 bispecific antibody, and an anti-CD3/anti-PSMA bispecific antibody.

~~6125.~~ The ~~cell culture method~~ of claim ~~5923~~, wherein the recombinant protein is selected from the group consisting of alirocumab, atoltivimab, maftivimab, odesivimab, odesivivmab-ebgn, casirivimab, imdevimab, cemiplitmab, cemplitmab-rwlc, dupilumab, evinacumab, evinacumab-dgnb, fasimumab, nesvacumab, trevogrumab, rinucumab and sarilumab.

~~62.26.~~ The ~~cell culture method~~ of claim ~~6125~~, wherein the recombinant protein is dupilumab.

~~63.~~—A recombinant protein produced in a cell culture comprising (i) at least one recombinant eukaryotic cell that can express said recombinant protein and (ii) a cell culture medium, wherein the recombinant protein is produced by a method comprising the steps of:

~~(a) providing a defined cell culture medium having reduced impurities, the defined cell culture medium having less than about 4000 μ mol of a HEPES related impurity having a molecular weight of 267.07 per mole of total HEPES, and less than about 400 μ mol of a HEPES related impurity having a molecular weight of 221.06 per mole of total HEPES;~~

~~(b) culturing said recombinant eukaryotic cells in said defined cell culture medium having reduced impurities;~~

~~(c) expressing a recombinant protein of interest from said recombinant eukaryotic cells; and~~

~~(d) producing a higher titer of the recombinant protein in the defined cell culture medium having reduced impurities relative to the that of similar or identical cells cultured in non-reduced impurity media.~~

~~64.~~—The recombinant protein of claim ~~63~~, wherein the eukaryotic cell is ~~27.~~ The method of claim ~~23~~, wherein the recombinant protein is aflibercept.

~~28.~~ The method of claim ~~1~~, wherein the analyzing is performed by at least one selected from the group consisting of ~~mammalian cell, avian cell, insect cell, and yeast cell~~ HPLC and LC-MS.

~~65.~~—The recombinant protein of claim ~~64~~, wherein the eukaryotic cell is selected from the group consisting of CHO, COS, retinal cell, Vero, CV1, kidney, HeLa, HepG2, WI38, MRC 5, Colo25, HB 8065, HL 60, lymphocyte, A431, CV 1, U937, 3T3, L cell, C127 cell, SP2/0, NS 0, MMT cell, stem cell, tumor cell, and a cell line derived from an aforementioned cell.

~~66. 29. The recombinant protein cell culture of claim 653, wherein the eukaryotic cell analysis is a CHO cell.~~

~~67. —The recombinant protein of claim 63, wherein said expressing a recombinant protein of interest occurs during production phase, growth phase, or both.~~

~~68. —The recombinant protein of claim 63, wherein said culturing of the recombinant eukaryotic cells in said defined cell culture medium having reduced impurities occurs during production phase, growth phase, or both.~~

~~69. —The recombinant protein of claim 63 wherein the cell growth during said culturing of the recombinant eukaryotic cells is higher than the cell growth of similar or identical recombinant eukaryotic cells in non impurity reduced media.~~

~~70. —The recombinant protein of claim 63, wherein the higher titer of the recombinant protein is increased performed by at least about 5%, as compared to that of similar or identical cells cultured in non reduced impurity media.~~

~~71. —The recombinant protein of claim 63, wherein the recombinant protein is an antibody, a human antibody, a humanized antibody, a chimeric antibody, a monoclonal antibody, a multispecific antibody, a bispecific antibody, an antigen binding antibody fragment, a single chain antibody, a diabody, triabody or tetrabody, a Fab fragment or a F(ab')₂ fragment, an IgD antibody, an IgE antibody, an IgM antibody, an IgG antibody, an IgG1 antibody, an IgG2 antibody, an IgG3 antibody, or an IgG4 antibody.~~

~~72. —The recombinant protein of claim 63, wherein the recombinant protein comprises an Fc domain.~~

~~73. —The recombinant protein of claim 72, wherein the recombinant protein is one selected from the group consisting of an Fc fusion protein, a receptor Fc fusion protein (TRAP), an antibody, an antibody fragment, HPLC and LC-MS, a ScFv-Fc fusion protein.~~

~~74. —The recombinant protein of claim 73, wherein the recombinant protein is selected from the group consisting of an anti-PD1 antibody, an anti-PDL-1 antibody, an anti-Dll4 antibody, an anti-ANG2 antibody, an anti-AngPt13 antibody, an anti-PDGFR antibody, an anti-Erb3 antibody, an anti-PRLR antibody, an anti-TNF antibody, an anti-EGFR antibody, an anti-PCSK9 antibody, an~~

~~anti-GDF8 antibody, an anti-GCGR antibody, an anti-VEGF antibody, an anti-IL1R antibody, an anti-IL4R antibody, an anti-IL6R antibody, an anti-IL1 antibody, an anti-IL2 antibody, an anti-IL3 antibody, an anti-IL4 antibody, an anti-IL5 antibody, an anti-IL6 antibody, an anti-IL7 antibody, an anti-RSV antibody, an anti-NGF antibody, an anti-CD3 antibody, an anti-CD20 antibody, an anti-CD19 antibody, an anti-CD28 antibody, an anti-CD48 antibody, an anti-CD3/anti-CD20 bispecific antibody, an anti-CD3/anti-MUC16 bispecific antibody, and an anti-CD3/anti-PSMA bispecific antibody.~~

75. — ~~The recombinant protein of claim 73, wherein the recombinant protein is selected from the group consisting of alirocumab, atoltivimab, maftivimab, odesivimab, odesivivmab-ebgn, casirivimab, imdevimab, cemiplimab, cemiplimab-rwlc, dupilumab, evinacumab, evinacumab-dgnb, fasimumab, nesvacumab, trevogrumab, rinucumab and sarilumab.~~

76. — ~~The recombinant protein of claim 75, wherein the recombinant protein is dupilumab.~~

77. — ~~A cell according to any of the above claims.~~

78. — ~~A cell culture according to any of the above claims.~~

79. — ~~A method according to any of the above claims.~~

80. — ~~A recombinant protein according to any of the above claims.~~