

CLAIMS

~~We claim:~~

1. 1. A composition comprising an intact activatable antibody and a clipped variant thereof, wherein the composition includes at least 95 % intact activatable antibody and 0.05 to 5 % clipped variant thereof, as determined by SDS-cGE, wherein the percentages of intact activatable antibody and the clipped variant thereof total 100%.

~~2. 2.~~ The composition of claim 1, ~~wherein the clipped variant comprises an antigen binding domain (AB) and at least a portion of a cleavable moiety (CM).~~

~~3. The composition of claim 1 or 2,~~ further comprising less than 150 ppm host cell proteins (HCP) as determined by a corresponding HCP ELISA and/or 0.1 to 5 % high molecular weight species (HMWS) as determined by SE-HPLC.

4. ~~The composition of claim 1;~~ or optionally, further comprising- about 0.5 ppm to 100 ppm host cell proteins (HCP) as determined by a corresponding HCP ELISA and 0.1 to 3 % HMWS as determined by SE-HPLC.

~~5. 3.~~ The composition of claim 1, ~~comprising~~ wherein the composition comprises at least 97 % intact activatable antibody, 0.05 to 3 % clipped variant, -as determined by SDS-cGE, about 0.5 ppm to 100 ppm host cell proteins (HCP) as determined by a corresponding HCP ELISA, and 0.1 to 3 % HMWS as determined by SE-HPLC, wherein the percentages of intact activatable antibody and the clipped variant thereof total 100%.

~~6. The %;~~ or optionally, wherein the composition of claim 1, comprising comprises at least 97 % of the intact activatable antibody, 0.05 to 3 % clipped variant as determined by SDS-cGE, and 0.1 to 3% HMWS as determined by SE-HPLC, wherein the percentages of intact activatable antibody and the clipped variant thereof total 100%.

~~7. An aqueous composition comprising an intact activatable antibody and a clipped variant thereof and an anion selected from Cl^- , Br^- , H_2PO_4^- , I^- , NO_3^- , ClO_4^- , and SCN^- , a cation selected from $\text{N}(\text{CH}_3)_4^+$, NH_4^+ , Ba^+ , Ca^{2+} , Mg^{2+} , Cs^+ , Rb^+ , K^+ , Na^+ , or a combination of the anion and the cation.~~

8. ~~The composition of any one or combination of claims 1-7, comprising 2 (N-morpholino)ethanesulfonic acid (MES), MOPS, HEPES, (NH₄)₂SO₄, Na₂SO₄, Na₃PO₄, K₃PO₄, NaCl, KCl, and CH₃COONH₄, or a combination thereof.~~
9. ~~The composition of any one or combination of claims 1-9, comprising 10 mM to 100 mM of the anion, cation, MES, MOPS, HEPES, (NH₄)₂SO₄, Na₂SO₄, Na₃PO₄, K₃PO₄, NaCl, KCl, and CH₃COONH₄, or combination thereof.~~
10. ~~The composition of any one or combination of claims 1-7, comprising arginine, tryptophan, asparagine, glutamine, lysine, histidine, serine, proline, or a salt thereof.~~
11. ~~The composition of claim 10, comprising 10 mM to 150 mM of the arginine, tryptophan, asparagine, glutamine, lysine, histidine, serine, proline, or salt thereof.~~
12. ~~The composition of any one or combination of claims 1-11, wherein the composition is an aqueous composition having a pH of about 5.0 to about 8.0, or about 5.0 to about 7.5, or about 5.0 to about 7.0, or about 5.5 to about 6.5.~~
13. ~~The composition of any one or combination of claims 1-12, wherein the composition is an aqueous composition having a conductivity of from 110 to 150 mS/cm, 115 to 145 mS/cm, 120 to 140 mS/cm, 125 to 135 mS/cm or about 130 mS/cm.~~
14. ~~The composition of any one or combination of claims 1-7, wherein the composition is free of buffering agents.~~
15. ~~The composition of any one or combination of claims 1-7, wherein the composition has a pH outside of the buffering capacity of a buffering agent present in the composition.~~
16. ~~The composition of any one or combination of claims 1-13, comprising a buffering agent having a pKa of 6.0 to 9.0.~~
17. ~~A container, vial, syringe, or injector device containing the composition of any one or combination of claims 1-16.~~
18. ~~An aqueous feedstock for an HIC or MMC chromatography column, comprising water, intact activatable antibody, at least 2% clipped variant of the intact activatable antibody as determined by SDS-cGE, at least 10 ppm HCP as determined by a corresponding HCP ELISA, and at least 1% HMWS as determined by SE-HPLC.~~
19. ~~The aqueous feedstock for an HIC or MMC chromatography column of claim 18, comprising at least 90% intact activatable antibody as determined by SDS-cGE, at~~

- least 2 to 15% of the clipped variant of the intact activatable antibody as determined by SDS-cGE, at least 10 ppm HCP as determined by ELISA, and 1 to 6% HMWS as determined by SE-HPLC.
20. ~~A HIC eluate of the aqueous feedstock of claim 18 or claim 19, comprising at least 95% intact activatable antibody as determined by SDS-cGE, less than 2% of the clipped variant of the intact activatable antibody as determined by SDS-cGE, less than 5 ppm HCP as determined by ELISA, and less than 1% HMWS as determined by SE-HPLC, wherein the percentages of intact activatable antibody and the clipped variant total 100%.~~
21. ~~The HIC eluate of claim 20, comprising at least 97% intact activatable antibody as determined by SDS-cGE, less than 1% of the clipped variant of the intact activatable antibody as determined by SDS-cGE, less than 2 ppm HCP as determined by ELISA, and less than 0.5% HMWS as determined by SE-HPLC, wherein the percentages of intact activatable antibody and the clipped variant total 100%.~~
22. ~~A MMC eluate of the aqueous feedstock of claim 18 or claim 19, comprising at least 97% intact activatable antibody as determined by SDS-cGE, less than 1% of the clipped variant of the intact activatable antibody as determined by SDS-cGE, less than 6 ppm HCP as determined by ELISA, and less than 1% HMWS as determined by SE-HPLC, wherein the percentages of intact activatable antibody and the clipped variant total 100%.~~
23. ~~The MMC eluate of claim 22, comprising at least 98% intact activatable antibody as determined by SDS-cGE, less than 0.5% of the clipped variant of the intact activatable antibody as determined by SDS-cGE, less than 5 ppm HCP as determined by ELISA, and less than 0.5% HMWS as determined by SE-HPLC, wherein the percentages of intact activatable antibody and the clipped variant total 100%.~~
24. ~~The HIC eluate of claim 20 or 21 or the MMC eluate of claim 22 or claim 23, having the intact activatable antibody in a yield of at least 75% of the intact activatable antibody present in the aqueous feedstock of claim 18 or claim 19.~~
25. ~~The HIC eluate of claim 20 or 21 or the MMC eluate of claim 22 or claim 23, wherein the level of reduction in relative quantity of clipped variant in the aqueous~~

~~feedstock compared to the relative quantity of clipped variant in the eluate is between two fold and twenty fold.~~

~~26. The HIC eluate of claim 20 or 21 or the MMC eluate of claim 22 or claim 23, wherein the level of reduction in relative quantity of clipped variant in the aqueous feedstock compared to the relative quantity of clipped variant in the eluate is between two fold and ten fold.~~

~~27. The HIC eluate of claim 20 or 21 or the MMC eluate of claim 22 or claim 23, wherein the level of reduction in relative quantity of clipped variant in the aqueous feedstock compared to the relative quantity of clipped variant in the eluate is between five fold and ten fold.~~

~~28.~~ 4. _____ A process for producing a composition comprising: (A) greater than 95 % intact activatable antibody comprising a masking moiety (MM₅), a cleavable moiety (CM₅), and a an antigen binding domain (AB₅); and (B) 0.05 to 5 % clipped variant thereof as determined by SDS-cGE, the process comprising:

(+) (i) _____ loading an aqueous feedstock comprising water, (A), (B), and a first salt onto a chromatography column, wherein the chromatography column comprises a stationary phase that comprises a support matrix and hydrophobic ligands bound thereto; and

(ii) _____ eluting the chromatography column with an eluent comprising water and a second salt to obtain the composition.

~~29. 5. The process of claim 28, comprising reducing the relative quantity of clipped variant in the aqueous feedstock by 75 to 90%.~~

~~30. The process of claim 28, comprising reducing the amount of HCP in the aqueous feedstock by 75 to 90%.~~

~~31. The process of claim 28, comprising reducing the amount of HMWS in the aqueous feedstock by 75 to 90%.~~

~~32. The process of claim 28, comprising reducing the amount of active clipped variant, HCP, and HMWS in the aqueous feedstock by 70 to 95%.~~

33. The process of claim 28, wherein the composition comprises at least 95% intact activatable antibody, and 0.1 to 5 % clipped variant, as determined by SDS-cGE, wherein the percentages of intact activatable antibody and the clipped variant total 100%.

34. The process of any one or combination of claims 28-33, wherein the clipped variant shares at least 95% sequence identity with the intact activatable antibody.

35. The process of any one or combination of claims 28-34, wherein the clipped variant has a molecular weight that is 85-98% of the intact activatable antibody.

36. The process of any one or combination of claims 28-33, wherein the clipped variant differs from the intact activatable antibody only in that it lacks the MM and a portion of the CM.

37. The process of any one or combination of claims 28-36, wherein the activatable antibody is a homodimer that comprises two identical AB, two identical CM, and two identical MM, and wherein the clipped variant is a single arm clipped variant.

38. 6. A method for producing a composition comprising an intact activatable antibody and less than 5 % clipped variant thereof as determined by SDS-cGE, less than 5 % HMWS as determined by SE-HPLC, and less than 100 ppm HCP as determined by a corresponding HCP ELISA, or a combination thereof, the method comprising (i) loading an aqueous feedstock comprising water, the intact activatable antibody, the clipped variant thereof, HMWS, HCP, and a first salt onto a chromatography column, wherein the chromatography column comprises a stationary phase that comprises a support matrix and hydrophobic ligands bound thereto, and (ii) eluting the chromatography column with an eluent comprising water and a second salt to obtain the composition.

39. A method of separating an intact activatable antibody from a clipped variant thereof that has an amino acid sequence that is at least 90% identical to the amino acid sequence of the intact activatable antibody, comprising (i) loading an aqueous feedstock comprising water, the intact activatable antibody, the clipped variant thereof, and a first salt onto a chromatography column, wherein the chromatography column comprises a stationary phase that comprises a support matrix and hydrophobic ligands bound thereto, and (ii) eluting the chromatography column with an eluent comprising water and a second salt to obtain a composition wherein the

~~relative quantity of the clipped variant in the aqueous feedstock is reduced by at least 90%.~~

40. ~~7.~~ 7. A pharmaceutical composition comprising the composition of any one or combination of claims 1-~~163~~, or a composition produced by the process or method of any one or combination of claims ~~28-394-6~~, and a pharmaceutically acceptable carrier.

41. ~~8.~~ 8. A method of making a pharmaceutical composition comprising combining the composition of any one or combination of claims 1-~~163~~, or a composition produced by the process or method of any one or combination of claims ~~28-394-6~~ with a pharmaceutically acceptable carrier.

42. ~~A method of~~ 9. Use of the pharmaceutical composition of claim 7 in the manufacture of a medicament for treating a subject suffering from a cancer, an inflammatory disease, an autoimmune disease, or a combination thereof, comprising administering the pharmaceutical composition of claim 40 to the subject.

43. ~~A method of treating a subject suffering from a cancer, an inflammatory disease, an autoimmune disease, or a combination thereof, comprising administering a composition comprising a subtoxic dose of a clipped variant of an activatable antibody and a dose of an intact activatable antibody, wherein the relative quantity of intact activatable antibody is at least 95% and the relative quantity of clipped variant thereof is less than 5%, as determined by SDS-cGE.~~

44. 10. A process for reducing the amount of clipped variant of an intact activatable antibody by 7- to 10-fold as determined by SDS-cGE, reducing the amount of HCP by 8- to 10-fold as determined by ELISA, and reducing the amount of HMWS by 7- to 13-fold as determined by SE-HPLC in a bioharvest composition or protein affinity chromatography eluate while obtaining a yield of 75-85% of the intact activatable antibody comprising:

(a) loading bioharvest composition or protein affinity chromatography eluate onto a chromatography column comprising a stationary phase that comprises a support matrix and ligands comprising a hydrophobic substituent bound thereto and loading a first salt and water onto the chromatography column; and

——(b) eluting the chromatography column with an eluent comprising water and a second salt to generate an eluate.

~~45.~~ 11. The process of claim ~~44~~10, wherein the eluate comprises at least 95% of the intact activatable antibody as determined by SDS-cGE, less than 2% of the clipped variant of the intact activatable antibody as determined by SDS-cGE, less than 5 ppm HCP as determined by ELISA, and less than 1% HMWS as determined by SE-HPLC, wherein the percentages of intact activatable antibody and the clipped variant total ~~100%.~~100% or optionally,

~~46.~~ The process of claim ~~44~~, wherein the eluate comprises at least 97% intact activatable antibody as determined by SDS-cGE, less than 1% of the clipped variant of the intact activatable antibody as determined by SDS-cGE, less than 2 ppm HCP as determined by ELISA, and less than 0.5% HMWS as determined by SE-HPLC, wherein the percentages of intact activatable antibody and the clipped variant total ~~100%.~~

~~47. The process of claim 44, wherein the eluate comprises at least 97% intact activatable antibody as determined by SDS-cGE, less than 1% of the clipped variant of the intact activatable antibody as determined by SDS-cGE, less than 6 ppm HCP as determined by ELISA, and less than 1% HMWS as determined by SE-HPLC wherein the percentages of intact activatable antibody and the clipped variant total 100%.~~

~~48.~~ The process of claim ~~44~~12. The process of claim 10, wherein the eluate comprises at least 98% intact activatable antibody as determined by SDS-cGE, less than 0.5% of the clipped variant of the intact activatable antibody as determined by SDS-cGE, less than 5 ppm HCP as determined by ELISA, and less than 0.5% HMWS as determined by SE-HPLC wherein the percentages of intact activatable antibody and the clipped variant total 100%.

~~49. The process of any one or combination of claims 44-48, wherein the clipped variant shares at least 95% sequence identity with the intact activatable antibody.~~

~~50.~~ 13. A method for determining or monitoring a relative percentage of an activatable antibody and a clipped variant thereof during a composition production process, the method comprising:

- a) a) subjecting a sample composition comprising a population of activatable antibody and a population of clipped variants thereof to a gel capillary electrophoresis procedure;
- ~~b)~~ b) separating the population of activatable antibody from the population of clipped variants thereof in the gel capillary electrophoresis procedure; and
- e) quantifying the relative amounts of the population of activatable antibody and the population of clipped variants thereof by determining the peak area corresponding to intact prodomain-containing polypeptide and the peak area corresponding to -clipped prodomain-containing polypeptide(s) thereof.

~~51.~~ 14. The method of claim ~~50~~13, wherein the sample composition is a cell harvest composition of a cell expressing the activatable antibody; or optionally,
wherein the sample composition has been subjected to protein affinity chromatography;
or optionally,

~~52. The method of any one of claims 50-51, wherein the sample composition has been subjected to protein affinity chromatography.~~

~~53. The method of any one of claims 50-52, wherein the sample composition has been subjected to ion exchange chromatography.~~

~~54. The method of any one of claims 50-53; or optionally,~~
wherein the sample composition has been subjected to anion exchange chromatography.

~~55. The method of any one of claims 50-54; or optionally,~~
wherein the sample composition has been subjected to hydrophobic interaction chromatography.

~~56. The method of any one of claims 50-55; or optionally,~~
wherein the sample composition has been subjected to multimodal chromatography.

~~57. The method of claim 50, wherein the relative percentage is monitored by quantifying the relative percentages of the population of activatable antibody and the population of clipped variants thereof after each of two or more of cell harvest, protein affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography, and multimodal chromatography.~~

58. ~~The method of claim 50~~ 15. The method of claim 13, wherein step a) comprises contacting the activatable antibody and clipped variant thereof in the sample composition with a reducing agent.

59. 16. ~~The method pharmaceutical composition of claim 58, wherein the reducing agent is dithioerythritol (DTE), dithiothreitol (DTT), beta-mercaptoethanol~~ 7 for use in the treatment of a cancer, an inflammatory disease, an autoimmune disease, or a combination thereof.

60. ~~The method of claim 50 or claim 58, wherein step a) comprises contacting the activatable antibody and clipped variant thereof in the sample composition with a denaturing agent.~~

61. ~~The method of claim 60, wherein the denaturing agent is sodium dodecyl sulfate (SDS).~~

62. ~~The method of claim 61, further comprising performing a gel capillary electrophoresis procedure using SDS protein molecular weight standards and producing a molecular weight standard calibration curve.~~

63. ~~The method of any one of claims 50-62, wherein step c) comprises obtaining an electropherogram containing a peak corresponding to each of the activatable antibody and clipped variant thereof subjected to the gel capillary electrophoresis procedure.~~

64. ~~The method of claim 63, further comprising performing peak integration for each peak corresponding to each of the activatable antibody and clipped variant thereof to obtain the peak area of clipped prodomain-encoding polypeptide and the peak area of intact prodomain-encoding polypeptide.~~