

CLAIMS

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 sodium dodecyl sulfate-capillary gel electrophoresis (SDS-cGE), wherein the intact activatable antibody comprises (i) at least a first antigen binding domain (AB) that has a specific binding affinity for a first biological target, and (ii) a first prodomain,

wherein the at least first AB comprises a first antibody light variable domain (VL) and a first antibody heavy variable domain (VH),

wherein the first prodomain comprises a first masking moiety (MM) and a first cleavable moiety (CM),

wherein the first AB is coupled to the first prodomain,

wherein the clipped variant of the intact activatable antibody comprises the AB of the activatable antibody but lacks all or a portion of the MM, and

wherein the percentages of intact activatable antibody and the clipped variant thereof total 100%.

2. The composition of claim 1, further comprising less than 150 ppm host cell proteins (HCP) as determined by a corresponding HCP enzyme-linked immunosorbent assay (ELISA) and/or 0.1 to 5 % high molecular weight species (HMWS) as determined by size exclusion-high-performance liquid chromatography (SE-HPLC); or optionally, further comprising 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.

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

4. A process for producing a composition comprising: (A) greater than 95 % intact activatable antibody comprising a masking moiety (MM), a cleavable moiety (CM), and an antigen binding domain (AB); and (B) 0.05 to 5 % clipped variant thereof as determined by SDS-cGE, wherein the clipped variant of the intact activatable antibody comprises the AB of the activatable antibody but lacks all or a portion of the MM, 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;

wherein the first salt and the second salt are the same salt or different salts;

wherein the aqueous feedstock is a bulk intermediate product composition produced from subjecting a bioharvest composition comprising the intact activatable antibody and the clipped impurity to one or more intervening unit operations selected from the group consisting of a centrifugation step, a filtration step, an affinity chromatography step, a virus inactivation step, a size exclusion chromatography step, a virus filtration step, an ion exchange chromatography step, or a combination of any two or more thereof.

5. The process of claim 4, 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%.

6. A method for producing a composition comprising an intact activatable antibody and 0.05 to 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;

wherein the aqueous feedstock is a bulk intermediate product composition produced from subjecting a bioharvest composition comprising the intact activatable antibody and the clipped impurity to one or more intervening unit operations selected from the group consisting of a centrifugation step, a filtration step, an affinity chromatography step, a virus inactivation step, a size exclusion chromatography step, a virus filtration step, an ion exchange chromatography step, or a combination of any two or more thereof;

wherein the intact activatable antibody comprises (i) at least a first antigen binding domain (AB) that has a specific binding affinity for a first biological target, and (ii) a first prodomain,

wherein the at least first AB comprises a first antibody light variable domain (VL) and a first antibody heavy variable domain (VH),

wherein the first prodomain comprises a first masking moiety (MM) and a first cleavable moiety (CM),

wherein the first AB is coupled to the first prodomain, and

wherein the clipped variant of the intact activatable antibody comprises the AB of the activatable antibody but lacks all or a portion of the MM.

7. A pharmaceutical composition comprising the composition of any one or combination of claims 1-3, or a composition produced by the process or method of any one or combination of claims 4-6, and a pharmaceutically acceptable carrier.

8. A method of making a pharmaceutical composition comprising combining the composition of any one or combination of claims 1-3, or a composition produced by the process or method of any one or combination of claims 4-6 with a pharmaceutically acceptable carrier.

9. Use of the pharmaceutical composition of claim 7 in the manufacture of a medicament for treating a cancer, an inflammatory disease, an autoimmune disease, or a combination thereof.

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 a 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, wherein the intact activatable antibody comprises (i) at least a first antigen binding domain (AB) that has a specific binding affinity for a first biological target, and (ii) a first prodomain,

wherein the at least first AB comprises a first antibody light variable domain (VL) and a first antibody heavy variable domain (VH),

wherein the first prodomain comprises a first masking moiety (MM) and a first cleavable moiety (CM),

wherein the first AB is coupled to the first prodomain,

wherein the clipped variant of the intact activatable antibody comprises the AB of the activatable antibody but lacks all or a portion of the MM.

11. The process of claim 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%; or optionally,

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%.

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%.

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) subjecting a sample composition comprising a population of activatable antibody and a population of clipped variants thereof to a gel capillary electrophoresis procedure;

b) separating the population of activatable antibody from the population of clipped variants thereof in the gel capillary electrophoresis procedure; and 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, wherein the intact activatable antibody comprises (i) at least a first antigen binding domain (AB) that has a specific binding affinity for a first biological target, and (ii) a first prodomain,

wherein the at least first AB comprises a first antibody light variable domain (VL) and a first antibody heavy variable domain (VH),

wherein the first prodomain comprises a first masking moiety (MM) and a first cleavable moiety (CM),

wherein the first AB is coupled to the first prodomain,

wherein the clipped variant of the intact activatable antibody comprises the AB of the activatable antibody but lacks all or a portion of the MM.

14. The method of claim 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,

wherein the sample composition has been subjected to ion exchange chromatography; or
optionally,

wherein the sample composition has been subjected to anion exchange chromatography;
or optionally,

wherein the sample composition has been subjected to hydrophobic interaction
chromatography; or optionally,

wherein the sample composition has been subjected to multimodal chromatography.

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.

16. The pharmaceutical composition of claim 7 for use in the treatment of a cancer,
an inflammatory disease, an autoimmune disease, or a combination thereof.