MODULATED LYSINE VARIANT SPECIES COMPOSITIONS AND METHODS FOR PRODUCING AND USING THE SAME

RELATED APPLICATIONS

This application claims priority to International Patent Application Serial No. PCT/US2013/031389, filed on March 14, 2013, the contents of which is hereby incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Large-scale production of antibodies for biopharmaceutical applications involves the use of cell cultures that are known to produce antibodies, and antigen-binding portions thereof, exhibiting varying levels of heterogeneity, which may lead to either decreased product efficacy and stability or just the opposite depending upon the nature of the heterogeneity. One source of antibody heterogeneity involves C-terminal lysine residues, such as those found on the heavy chains of antibody molecules. For example, C-terminal lysines can potentially be present on both the heavy chains of an antibody (Lys 2), on either one of the heavy chains (Lys 1), or neither of them (Lys 0). Since lysine can carry a positive charge, antibodies lacking the basic C-terminal lysine(s) differ in their charge state from ones that contain the lysine, so that the distribution of lysine variants (%Lys 0, %Lys 1, %Lys 2 of the total lysine sum) can be detected using ion-exchange chromatographic methods, for example, using a ProPac WCX-10 Weak Cation-Exchange column for high-resolution separation of protein isoforms (Dionex, CA), or other methods known in the art, and subsequently quantified.

C-terminal lysine heterogeneity is commonly observed in biopharmaceutical antibody and protein compositions. However, the development of compositions comprising antibodies, or antigen-binding portions thereof, with lower or higher levels of certain lysine variants is an important, need in the biopharmaceutical industry.

SUMMARY ~~OF THE INVENTION~~

In one aspect, there is provided a method for producing a modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, comprising implementing a change to a cell culture condition used to produce said modulated lysine variant species composition, wherein the change in the cell culture condition results in a decrease in the amount of a lysine variant species that lacks a C-terminal lysine (Lys 0) as compared to the amount of Lys 0 in a control composition comprising an antibody, or antigen-binding portion thereof, produced without the change to the cell culture condition.

In one aspect, there is provided a composition comprising human anti-TNFα antibodies, wherein said human anti-TNFα antibodies comprise C-terminal lysine variant species, wherein less than 65% of said lysine variant species in said composition have zero C-terminal lysines (Lys 0), and wherein said human anti-TNFα antibodies comprise a light chain variable region (LCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO:7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO:5, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO:3; and a heavy chain variable region (HCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO:8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO:4.

In one aspect, there is provided a composition comprising human anti-TNFα antibodies, wherein said human anti-TNFα antibodies comprise C-terminal lysine variant species, wherein greater than 35% of said lysine variant species in said composition have one C-terminal lysine (Lys 1) or two C terminal lysines (Lys 2), and wherein said human anti-TNFα antibodies comprise a light chain variable region (LCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO:7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO:5, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO:3; and a heavy chain variable region (HCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO:8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO:4.

In one aspect, there is provided a pharmaceutical formulation comprising the composition as disclosed herein and a pharmaceutically acceptable carrier.

In one aspect, there is provided the use of the formulation as disclosed herein in the manufacture of a medicament for treating a subject having a disease or disorder in which TNFα activity is detrimental, wherein a therapeutically effective amount of the formulation is to be administered to the subject, thereby treating the TNFα-associated disease or disorder.

In one aspect, there is provided a method for producing the composition as disclosed herein, comprising culturing cells expressing said human anti-TNFα antibodies in a culture media.

The present ~~invention~~ disclosure is based on the identification and optimization of processes for the production of antibodies or antigen-binding portions thereof (*e.g.*, cell culture processes), which result in the production of compositions comprising antibodies, or antigen-binding portions thereof, that comprise modified distributions of C-terminal lysine variants. C-

terminal lysines can be present on both the heavy chains of an antibody (Lys 2), on either one of them (Lys 1), or neither of them (Lys 0). Since antibodies lacking basic C-terminal lysines differ in the charge state from the ones that contain one or two lysines, the distribution of lysine variants (%Lys 0, %Lys 1, %Lys 2 of the total lysine sum in the composition) can be detected by ion-exchange chromatographic methods such as, for example, weak cation-exchange chromatography (WCX-10) and subsequently quantified (Figure 1).

The present ~~invention~~ disclosure provides methods for producing compositions comprising modulated amounts (or percentages) of Lys 1, Lys 2, and/or Lys 0 variants as compared to the amount (or percentage) of Lys 1, Lys 2, and/or Lys 0 variants in a starting population of proteins (referred to herein as "modulated lysine variant species compositions"). In one embodiment, the methods of the ~~invention~~ disclosure are used to produce a protein composition comprising an increased amount of Lys 1 and/or Lys 2 variants and a decreased amount of Lys 0 variants, *e.g.*, as compared to the amount (or percentage) of Lys 1, Lys 2, and/or Lys 0 variants in a starting population of proteins, *e.g.*, a control composition. In another embodiment, the methods of the ~~invention~~ disclosure are used to produce a protein composition comprising a decreased amount of Lys 1 and/or Lys 2 variants and an increased amount of Lys 0 variants, *e.g.*, as compared to the amount (or percentage) of Lys 1, Lys 2, and/or Lys 0 variants in a starting population of proteins, *e.g.*, a control composition.

For example, a modulated lysine variant species composition of the ~~invention~~ disclosure may comprise more than about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% Lys 1 and/or Lys 2 antibodies, or antigen-binding portions thereof relative to the lysine sum (*i.e.*, the sum of Lys 0, Lys 1 and Lys 2 in the composition). In one embodiment, a modulated lysine variant species composition of the ~~invention~~ disclosure may comprise more than about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 60% to about 70%, or about 70% to about 80%, about 80% to about 90%, or about 90% to about 100% Lys 1 and/or Lys 2 antibodies, or antigen-binding portions thereof relative to the lysine sum. Ranges within one or more of any of the preceding percentages are also included.

A modulated lysine variant species composition of the ~~invention~~ disclosure may also comprise less than about 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1%, or zero antibodies, or antigen-binding portions thereof that are Lys 0, relative to the lysine sum. In one embodiment, a modulated lysine variant species composition of the ~~invention~~ disclosure may also comprise less than about 0% to about 10%, about 10% to about 20%, about 20% to about 30%, about 30% to about 40%, about 50% to about 60%,

about 60% to about 70%, or about 70% to about 80%, antibodies or antigen-binding portions thereof that are Lys 0, relative to the lysine sum. Ranges within one or more of any of the preceding percentages are also included.

A modulated lysine variant species composition also includes a composition comprising an antibody, or antigen-binding portion thereof, that comprises more than about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% antibodies, or antigen-binding portions thereof that have one C-terminal lysine (Lys 1) relative to the lysine sum. Ranges within one or more of any of the preceding percentages are also included. In one embodiment, a modulated lysine variant species composition of the ~~invention~~ disclosure may comprise more than about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 60% to about 70%, or about 70% to about 80%, about 80% to about 90%, or about 90% to about 100% Lys 1 antibodies, or antigen-binding portions thereof relative to the lysine sum. Ranges within one or more of any of the preceding percentages are also included.

A modulated lysine variant species composition also includes a composition comprising an antibody, or antigen-binding portion thereof, that comprises more than about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% antibodies, or antigen-binding portions thereof that have two C-terminal lysines (Lys 2) relative to the lysine sum. Ranges within one or more of any of the preceding percentages are also included. In one embodiment, a modulated lysine variant species composition of the ~~invention~~ disclosure may comprise more than about 20% to about 30%, about 30% to about 40%, about 40% to about 50%, about 60% to about 70%, or about 70% to about 80%, about 80% to about 90%, or about 90% to about 100% Lys 2 antibodies, or antigen-binding portions thereof relative to the lysine sum. Ranges within one or more of any of the preceding percentages are also included.

In one embodiment, the methods of the ~~invention~~ disclosure, alone or in combination, may reduce the amount or percentage of one or more C-terminal lysine variants (Lys 0, Lys 1, or Lys 2) in a protein or antibody sample by about 1%, 1.2%, 1.5%, 2%, 2.2%, 2.5%, 3%, 3.2%, 3.5%, 4%, 4.2%, 4.5%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, and ranges within one or more of the preceding, as compared to a non-modulated lysine variant species composition. In one aspect of this embodiment, the percentage of Lys 0, Lys 1, or Lys 2 variants are reduced by about 1% to about 25%, about 5% to about 20%, about 5% to about 15%, or about 5% to about

10%, and ranges within one or more of the preceding, as compared to a non-modulated lysine variant species composition.

In one embodiment, the methods of the ~~invention~~ disclosure, alone or in combination, may increase the amount or percentage of one or more C-terminal lysine variants (Lys 0, Lys 1, or Lys 2) in a protein or antibody sample by about 1%, 1.2%, 1.5%, 2%, 2.2%, 2.5%, 3%, 3.2%, 3.5%, 4%, 4.2%, 4.5%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, and ranges within one or more of the preceding, as compared to a non-modulated lysine variant species composition. In one aspect of this embodiment, the percentage of Lys 0, Lys 1, or Lys 2 variants are increased by about 1% to about 25%, about 5% to about 20%, about 5% to about 15%, or about 5% to about 10%, and ranges within one or more of the preceding, as compared to a non-modulated lysine variant species composition,

As demonstrated herein, modulated lysine variant species compositions of the ~~invention~~ disclosure comprising adalimumab (*e.g.*, modulated lysine variant species compositions that comprise only Lys 1 and Lys 2 variants, and not Lys 0 variants) have increased therapeutic efficacy and improved biological properties, such as, for example, increased tissue penetration (such as cartilage), reduced tissue destruction (such as cartilage), reduced synovial proliferation, reduced bone erosion, increased protection against the development of arthritic scores and/or histopathology scores, reduced cell infiltration, reduced proteoglycan loss, reduced chondrocyte death, and/or increased TNFα affinity, *e.g.*, as compared to a control composition wherein the Lys distribution has not been modified by the methods described herein (see Example 5).

In one embodiment, a modulated lysine variant species composition of the ~~invention~~ disclosure comprises about 0.0% to about 10% acidic species (AR), about 0.0% to about 5% AR, about 0.0% to about 4% AR, about 0.0% to about 3% AR, about 0.0% to about 2% AR, about 3% to about 5% AR, about 5% to about 8% AR, or about 8% to about 10% AR, or about 10% to about 15% AR, and ranges within one or more of the preceding. In another embodiment, a modulated lysine variant species composition of the ~~invention~~ disclosure comprises, *e.g.*, about 15% or less AR. In one aspect of this embodiment, a modulated lysine variant species composition comprises about 14% or less AR, 13% or less AR, 12% or less AR, 11% or less AR, 10% or less AR, 9% or less AR, 8% or less AR, 7% or less AR, 6% or less AR, 5% or less AR, 4.5% or less AR, 4% or less AR, 3.5% or less AR, 3% or less AR, 2.5% or less AR, 2% or less AR, 1.9% or less AR, 1.8% or less AR, 1.7% or less AR, 1.6% or less AR, 1.5% or less AR, 1.4% or less AR, 1.3% or less AR, 1.2% or less AR, 1.1% or less AR, 1% or less AR, 0.9% or less

AR, 0.8% or less AR, 0.7% or less AR, 0.6% or less AR, 0.5% or less AR, 0.4% or less AR, 0.3% or less AR, 0.2% or less AR, 0.1% or less AR, or 0.0% AR, and ranges within one or more of the preceding.

In one embodiment, the acidic species comprise acidic region 1 (AR1) and acidic region 2 (AR2). In one aspect of this embodiment, a modulated lysine variant species composition comprises about 0.1% or less AR1 and about 3% or less AR2. In another aspect of this embodiment, the composition comprises about 0.0% AR1 and about 1.4% AR2. In another aspect of this embodiment, the composition comprises about 0% to about 3% AR, *e.g.,* about 3%, 2.5%, 2%, 1.5%, 1.4%, 1.3%, 1.2%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, or less AR1. In another aspect of this embodiment, the composition comprises about 0% to about 3% AR, *e.g.,* about 3%, 2.5%, 2%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, or less AR2.

In one embodiment, the distribution or amount of C-terminal lysine variants produced by cell culture is modulated by employing certain media components during production of a protein, for example, an antibody, of interest. In some embodiments, the modulated lysine variant species compositions of the ~~invention~~ disclosure are produced by supplementing the media of cells expressing the protein of interest with one or more amino acids during cell culture. In certain embodiments, one or more of the amino acids belong to a group of basic amino acids. In certain embodiments, the one or more amino acids is arginine, lysine, histidine, or combinations thereof, including combinations of arginine and/or lysine with ornithine. In certain embodiments, supplementing the media of cells expressing the protein of interest with one or more amino acids reduces the relative amount of a Lys 0 lysine variant, and increases the relative amount of a Lys 1 and/or Lys 2 lysine variant in the cell culture or a composition comprising the protein or antibody purified from the cell culture. In certain embodiments, these amino acids may be supplemented as dipeptides or tri-peptides of different combinations for lysine variant modulation

In some embodiments, the modulated lysine variant species compositions of the ~~invention~~ disclosure are produced by supplementing the media of cells expressing the protein of interest with zinc during cell culture. In certain embodiments, supplementing the media of cells expressing the protein of interest with zinc to an overall concentration of less than about 10 μM reduces the relative amount of a Lys 0 lysine variant, and increases the relative amount of a Lys 1 and/or Lys 2 lysine variant in the cell culture or a composition comprising the protein

or antibody purified from the cell culture. In certain embodiments, modulation of concentration of zinc in combination with that of the basic amino acids is used to modulate the lysine variant distribution.

In another embodiment, the modulated lysine variant species compositions of the ~~invention~~ disclosure are produced by adjusting the pH, and/or temperature of a cell culture expressing a protein or antibody of interest. In certain embodiments, increasing the pH of the cell culture expressing the protein or antibody of interest reduces the amount of a Lys 0 lysine variant, and increases the amount of a Lys 1 and/or Lys 2 lysine variant in the cell culture, or a composition comprising the protein or antibody purified from the cell culture, compared to a control cell culture at a lower pH. In certain embodiments, the final pH of the cell culture is adjusted to a pH of about 6.7 to about 7.1. In certain embodiments, the temperature of the cell culture is adjusted to a temperature of about 31°C to about 37 °C. In certain embodiments, decreasing the temperature of the cell culture expressing the protein or antibody of interest reduces the amount of a Lys 0 lysine variant, and increases the amount of a Lys 1 and/or Lys 2 lysine variant in the cell culture, or a composition comprising the protein or antibody purified from the cell culture, compared to a control cell culture at a higher temperature.

In certain embodiments, the methods of the ~~invention~~ disclosure modulate the distribution, reduce the amount, or increase the amount of lysine variants present in the resulting composition. In certain embodiments, the resulting composition has a reduced amount of Lys 0 lysine variant, and an increased amount of Lys 1 and Lys 2 lysine variants compare to a composition that was not prepared according to the methods of the present application. In one aspect, the sample comprises a cell harvest wherein the cell line is employed to produce specific proteins of the present ~~invention~~disclosure. In a particular aspect, the sample is prepared from a cell line used to produce anti-TNF-α antibodies.

In certain embodiments, the methods of the ~~invention~~ disclosure for modulating the C-terminal lysine variants in a protein composition described herein is exerted by employing one or more of the foregoing methods during the production and purification of the desired protein, such as antibodies or antigen-binding portions thereof.

The purity/heterogeneity of the proteins of interest in the resultant sample product can be analyzed using methods well known to those skilled in the art, *e.g.*, weak cation exchange chromatography (WCX), capillary isoelectric focusing (cIEF), size-exclusion

chromatography, Poros™ A HPLC Assay, Host Cell Protein ELISA, Protein A ELISA, and western blot analysis.

In one embodiment, the antibody, or antigen-binding portion thereof, of the compositions disclosed herein is an anti-TNFα antibody, or antigen-binding portion thereof. For example, in one aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, dissociates from human TNFα with a K_d of about 1 x 10^{-8} M or less and a K_{off} rate constant of 1x10^{-3} S^{-1} or less. In another aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, comprises a light chain variable region (LCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 5, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 3; and a heavy chain variable region (HCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 6, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4. In still another aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2. In yet another aspect of this embodiment, the anti-TNFα antibody, or antigen-binding portion thereof, is adalimumab, or an antigen binding-portion thereof.

In one embodiment, a modulated lysine variant species composition of the ~~invention~~ disclosure comprises adalimumab, and has a percentage of Lys 1/Lys 2 variants that is not the same as the percentage of Lys 1/Lys 2 variants present in adalimumab formulated as HUMIRA® as currently approved and described in the "Highlights of Prescribing Information" for HUMIRA® (adalimumab) Injection (Revised Jan. 2008), the contents of which are hereby incorporated herein by reference.

In another embodiment, a modulated lysine variant species composition of the ~~invention~~ disclosure comprises adalimumab, and has a percentage of Lys 1/Lys 2 variants that is higher than the percentage of Lys 1/Lys 2 variants present in adalimumab formulated as HUMIRA® as currently approved and described in the "Highlights of Prescribing Information" for HUMIRA® (adalimumab) Injection (Revised Jan. 2008).

As demonstrated herein, a modulated lysine variant species composition of the present ~~invention~~ disclosure exhibits increased efficacy in treating or preventing diseases in a subject compared to a corresponding non-modulated lysine variant species composition. In particular,

modulated lysine variant species compositions comprising anti-TNFα antibodies, or antigen binding portions thereof, *e.g.,* adalimumab, exhibit increased cartilage tissue penetration, reduced cartilage destruction, reduced synovial proliferation, and/or reduced bone erosion as compared to a non-modulated lysine variant species composition. In another aspect of this embodiment, modulated lysine variant species compositions comprising anti-TNFα antibodies, or antigen binding portions thereof, *e.g.,* adalimumab, exhibit increased protection against the development of arthritic scores and/or increased protection against the development of histopathology scores as compared to a non-modulated lysine variant species composition, when administered to an animal model of arthritis.

In still another embodiment, a modulated lysine variant species composition comprising an anti-TNFα antibody, or antigen binding portion thereof, *e.g.,* adalimumab, exhibits reduced cell infiltration, reduced proteoglycan loss, and/or reduced chondrocyte death as compared to a non-modulated lysine variant species composition.

In another embodiment, a modulated lysine variant species composition of the ~~invention~~ disclosure comprises anti-TNFα antibodies, or antigen-binding portions thereof, *e.g.,* adalimumab, exhibit increased TNFα affinity as compared to a non-modulated lysine variant species composition.

Another aspect of the ~~invention~~ disclosure provides methods for treating a subject having a disorder in which TNFα activity is detrimental, *e.g.,* rheumatoid arthritis, juvenile idiopathic arthritic, psoriatic arthritis, ankylosing spondylitis, Crohn's Disease, plaque psoriasis, active axial spondyloarthritis (active axSpA) and non-radiographic axial spondyloarthritis (nr-axSpA), comprising administering a therapeutically effective amount of the composition of a modulated lysine variant species composition to the subject, thereby treating the TNFα-associated disease or disorder.

Other features and advantages of the ~~invention~~ disclosure will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a representation of the lysine variants in a sample WCX-10 chromatogram and quantification scheme of each of the variants.

Figure 2 depicts the effect of zinc modulation in adalimumab producing cell line 1, media 1 on viable cell density (n=2).

CLAIMS

1. A modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, wherein the amount of lysine variant species that have one C-terminal lysine (Lys 1) and/or two C-terminal lysines (Lys 2) in said composition is about 10% or more greater than the amount of Lys 1 and/or Lys 2 in a non-modified lysine variant species composition.

2. The modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, of claim 1, wherein the amount of lysine variant species that have one C-terminal lysine (Lys 1) and/or two C-terminal lysines (Lys 2) in said composition is about 15% or more greater than the amount of Lys 1 and/or Lys 2 in a non-modified lysine variant species composition.

3. The modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, of claim 1, wherein the amount of lysine variant species that have one C-terminal lysine (Lys 1) and/or two C-terminal lysines (Lys 2) in said composition is about 20% or more greater than the amount of Lys 1 and/or Lys 2 in a non-modified lysine variant species composition.

4. The modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, of claim 1, wherein the amount of lysine variant species that have one C-terminal lysine (Lys 1) and/or two C-terminal lysines (Lys 2) in said composition is about 25% or more greater than the amount of Lys 1 and/or Lys 2 in a non-modified lysine variant species composition.

5. The modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, of claim 1, wherein the amount of lysine variant species that have one C-terminal lysine (Lys 1) and/or two C-terminal lysines (Lys 2) in said composition is about 30% or more greater than the amount of Lys 1 and/or Lys 2 in a non-modified lysine variant species composition.

6. The modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, of claim 1, wherein the amount of lysine variant species that have

one C-terminal lysine (Lys 1) and/or two C-terminal lysines (Lys 2) in said composition is about 40% or more greater than the amount of Lys 1 and/or Lys 2 in a non-modified lysine variant species composition.

7. A modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, wherein the amount of lysine variant species that have zero C-terminal lysines (Lys 0) in said composition is about 40% or less lower than the amount of Lys 0 in a non-modified lysine variant species composition.

8. A modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, of claim 7, wherein the amount of lysine variant species that have zero C-terminal lysines (Lys 0) in said composition is about 30% or less lower than the amount of Lys 0 in a non-modified lysine variant species composition.

9. A modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, of claim 7, wherein the amount of lysine variant species that have zero C-terminal lysines (Lys 0) in said composition is about 25% or less lower than the amount of Lys 0 in a non-modified lysine variant species composition.

10. A modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, of claim 7, wherein the amount of lysine variant species that have zero C-terminal lysines (Lys 0) in said composition is about 20% or less lower than the amount of Lys 0 in a non-modified lysine variant species composition.

11. A modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, of claim 7, wherein the amount of lysine variant species that have zero C-terminal lysines (Lys 0) in said composition is about 15% or less lower than the amount of Lys 0 in a non-modified lysine variant species composition.

12. A modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, of claim 7, wherein the amount of lysine variant species that have zero C-terminal lysines (Lys 0) in said composition is about 10% or less lower than the amount of Lys 0 in a non-modified lysine variant species composition.

13. The composition of any one of claims 1-12, wherein the composition comprises less than about 10% acidic species.

14. The composition of claim 13, wherein the composition comprises about 5% or less acidic species (AR).

15. The composition of claim 13, wherein the composition comprises about 4% or less acidic species (AR).

16. The composition of claim 13, wherein the composition comprises about 3% or less acidic species (AR).

17. The composition of claim 16, wherein the composition comprises about 0.1% or less AR1 and about 3% or less AR2.

18. The composition of claim 16, wherein the composition comprises about 1% or less AR1.

19. The composition of claim 18, wherein the composition comprises about 0.1% or less AR1.

20. The composition of claim 18, wherein the composition comprises about 3% or less AR2.

21. The composition of claim 13, wherein the composition comprises about 2% or less acidic species (AR).

22. The composition of claim 21, wherein the composition comprises about 1.4% or less acidic species (AR)

23. The composition of claim 22, wherein the composition comprises about 1.4% AR2 and about 0.0% AR1.

24. The composition of any one of claims 1-12, wherein the antibody, or antigen-binding portion thereof, is an anti-TNFα antibody, or antigen-binding portion thereof.

25. The composition of claim 24, wherein the antibody, or antigen-binding portion thereof, comprises a light chain variable region (LCVR) having a CDR1 domain comprising

the amino acid sequence of SEQ ID NO:7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO:5, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO:3; and a heavy chain variable region (HCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO:8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO:4.

26. The composition of claim 25, wherein the antibody, or antigen-binding portion thereof, comprises a LCVR comprising the amino acid sequence set forth in SEQ ID NO:1 and a HCVR comprising the amino acid sequence set forth in SEQ ID NO:2.

27. The composition of claim 26, wherein the antibody, or antigen-binding portion thereof, comprises adalimumab, or an antigen-binding portion thereof.

28. A composition comprising the antibody, or antigen-binding portion thereof, of any one of claims 1-12, and a pharmaceutically acceptable carrier.

29. A method of treating a subject having a disorder in which TNFα activity is detrimental, the method comprising administering a therapeutically effective amount of the composition of any one of claims 1-12 to the subject, thereby treating the TNFα-associated disease or disorder.

30. The method of claim 29, wherein the disorder in which TNFα activity is detrimental is selected from the group consisting of rheumatoid arthritis, juvenile idiopathic arthritic, psoriatic arthritis, ankylosing spondylitis, Crohn's Disease and plaque psoriasis.

31. A method for producing a modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, comprising implementing a change to a cell culture condition used to produce said modulated lysine variant species composition, wherein the change in the cell culture condition results in a decrease in the amount of a lysine variant species that lacks a C-terminal lysine (Lys 0) as compared to the amount of Lys 0 in a control composition comprising an antibody, or antigen-binding portion thereof, produced without the change to the cell culture condition.

32. The method of claim 31, wherein the change to a cell culture condition is a change to the cell culture media.

33. The method of claim 31, wherein the change to the cell culture media is an increase in the amount of an amino acid selected from the group consisting of arginine, lysine, histidine, and combinations thereof, in the cell culture media.

34. The method of claim 33, wherein the change to the cell culture media further comprises an increase in the amount of ornithine.

35. The method of claim 33, wherein the amino acid concentration is increased to a concentration of between about 0.025 and 20 g/L.

36. The method of claim 33, wherein the amino acid concentration is increased to a total concentration of about: arginine 9g/L, lysine 11 g/L, and histidine 10 g/L.

37. The method of claim 31, wherein the change to a cell culture condition is modulation of the amount of zinc in the cell culture media.

38. The method of claim 37, wherein the zinc concentration in the cell culture media is below 60 μM.

39. The method of claim 38, wherein the change to a cell culture condition further comprises an increase in the amount of arginine, lysine, and histidine.

40. The method of claim 31, wherein the change to a cell culture condition is a change to the cell culture pH.

41. The method of claim 31, wherein the change to a cell culture condition is a change to the concentration of dissolved oxygen (DO) in the cell culture media.

42. The method of claim 31, wherein the change to a cell culture condition is a change to the concentration of phosphate in the cell culture media.

43. The method of claim 42, wherein the phosphate concentration is increased to a total concentration of about 30mM.

44. The method of claim 31, wherein the change to a cell culture condition is a change to the cell culture temperature.

45. The method of claim 42, wherein the temperature is changed to a temperature of between about 30 and 50 °C.

46. The method of claim 42, wherein the temperature is changed to a temperature of between about 30 and 37 °C.

47. The method of claim 42, wherein the temperature is changed to a temperature of about 31 °C.

CLAIMS

1. A method for producing a modulated lysine variant species composition comprising an antibody, or antigen-binding portion thereof, comprising implementing a change to a cell culture condition used to produce said modulated lysine variant species composition, wherein the change in the cell culture condition results in a decrease in the amount of a lysine variant species that lacks a C-terminal lysine (Lys 0) as compared to the amount of Lys 0 in a control composition comprising an antibody, or antigen-binding portion thereof, produced without the change to the cell culture condition.

2. The method of claim 1, wherein the change to a cell culture condition is a change to the cell culture media.

3. The method of claim 1, wherein the change to the cell culture media is an increase in the amount of an amino acid selected from the group consisting of arginine, lysine, histidine, and combinations thereof, in the cell culture media.

4. The method of claim 3, wherein the change to the cell culture media further comprises an increase in the amount of ornithine.

5. The method of claim 3, wherein the amino acid concentration is increased to a concentration of between about 0.025 and 20 g/L.

6. The method of claim 3, wherein the amino acid concentration is increased to a total concentration of about: arginine 9g/L, lysine 11 g/L, and histidine 10 g/L.

7. The method of claim 1, wherein the change to a cell culture condition is modulation of the amount of zinc in the cell culture media.

8. The method of claim 7, wherein the zinc concentration in the cell culture media is below 60 µM.

9. The method of claim 8, wherein the change to a cell culture condition further comprises an increase in the amount of arginine, lysine, and histidine.

10. The method of claim 1, wherein the change to a cell culture condition is a change to the cell culture pH.

11. The method of claim 1, wherein the change to a cell culture condition is a change to the concentration of dissolved oxygen (DO) in the cell culture media.

12. The method of claim 1, wherein the change to a cell culture condition is a change to the concentration of phosphate in the cell culture media.

13. The method of claim 12, wherein the phosphate concentration is increased to a total concentration of about 30mM.

14. The method of claim 1, wherein the change to a cell culture condition is a change to the cell culture temperature.

15. The method of claim 12, wherein the temperature is changed to a temperature of between about 30 and 50 °C.

16. The method of claim 12, wherein the temperature is changed to a temperature of between about 30 and 37 °C.

17. The method of claim 12, wherein the temperature is changed to a temperature of about 31 °C.

18. A composition comprising human anti-TNFα antibodies, wherein said human anti-TNFα antibodies comprise C-terminal lysine variant species, wherein less than 65% of said lysine variant species in said composition have zero C-terminal lysines (Lys 0), and wherein said human anti-TNFα antibodies comprise a light chain variable region (LCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO:7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO:5, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO:3; and a heavy chain variable region (HCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO:8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO:4.

19. The composition of claim 18, wherein less than 60%, less than 55%, less than 50%, less than 45%, less than 40%, less than 30%, less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, less than 4%, less than 3%, less than 2%, or less than 1% of said lysine variant species in said composition have zero C-terminal lysines (Lys 0).

20. A composition comprising human anti-TNFα antibodies, wherein said human anti-TNFα antibodies comprise C-terminal lysine variant species, wherein greater than 35% of said lysine variant species in said composition have one C-terminal lysine (Lys 1) or two C terminal lysines (Lys 2), and wherein said human anti-TNFα antibodies comprise a light chain variable region (LCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO:7, a CDR2 domain comprising the amino acid sequence of SEQ ID NO:5, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO:3; and a heavy chain variable region (HCVR) having a CDR1 domain comprising the amino acid sequence of SEQ ID NO:8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 domain comprising the amino acid sequence of SEQ ID NO:4.

21. The composition of claim 20, wherein greater than 40%, greater than 45%, greater than 50%, greater than 55%, greater than 60%, greater than 65%, greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater than 90%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, or 100% of said lysine variant species in said composition have one C terminal lysine (Lys 1) or two Cterminal lysines (Lys 2).

22. The composition of claim 20 or 21, wherein greater than 20%, greater than 25%, greater than 30%, greater than 35%, greater than 40%, greater than 45%, greater than 50%, greater than 55%, greater than 60%, greater than 65%, greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater than 90%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, or 100% of said lysine variant species in said composition have one C-terminal lysine (Lys 1).

23. The composition of claim 20 or 21, wherein greater than 20%, greater than 25%, greater than 30%, greater than 35%, greater than 40%, greater than 45%, greater than 50%, greater than 55%, greater than 60%, greater than 65%, greater than 70%, greater than 75%, greater than 80%, greater than 85%, greater than 90%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, greater than 99%, or 100% of said lysine variant species in said composition have two C-terminal lysines (Lys 2).

24. The composition of any one of claims 18 to 23, wherein said human anti-TNFα antibodies are adalimumab.

25. A pharmaceutical formulation comprising the composition of any one of claims 18 to 24 and a pharmaceutically acceptable carrier.

26. The formulation of claim 25, wherein the formulation is selected from the group consisting of a liquid formulation, a lyophilized formulation, and a reconstituted lyophilized formulation.

27. The formulation of claim 25 or 26, wherein the antibodies are present in said formulation at a concentration of between 1 mg/ml and 25 mg/ml, between 1 mg/ml and 200 mg/ml, between 25 mg/ml and 200 mg/ml, between 50 mg/ml and 200 mg/ml, between 75 mg/ml and 200 mg/ml, between 100 mg/ml and 200 mg/ml, between 125 mg/ml and 200 mg/ml, between 150 mg/ml and 200 mg/ml, between 25 mg/ml and 150 mg/ml, between 50 mg/ml and 150 mg/ml, between 75 mg/ml and 150 mg/ml, between 100 mg/ml and 150 mg/ml, between 125 mg/ml and 150 mg/ml, between 25 mg/ml and 125 mg/ml, between 50 mg/ml and 125 mg/ml, between 75 mg/ml and 125 mg/ml, between 100 mg/ml and 125 mg/ml, between 25 mg/ml and 100 mg/ml, between 50 mg/ml and 100 mg/ml, between 75 mg/ml and 100 mg/ml, between 25 mg/ml and 75 mg/ml, between 50 mg/ml and 75 mg/ml, or between 25 mg/ml and 50 mg/ml.

28. The formulation of any one of claims 25 to 27, further comprising an excipient selected from the group consisting of buffering agent, sugar, salt, surfactant, amino acid, polyol, chelating agent, emulsifier, and preservative.

29. The formulation of claim 28, wherein:
(a) the buffering agent is selected from the group consisting of histidine, citrate, phosphate, glycine, and acetate;
(b) the sugar is selected from the group consisting of trehalose, sucrose, mannitol, maltose, raffinose, and sorbitol; and/or
(c) the surfactant is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 80, and polysorbate 80.

30. Use of the formulation of any one of claims 25 to 29 in the manufacture of a medicament for treating a subject having a disease or disorder in which TNFα activity is detrimental, wherein a therapeutically effective amount of the formulation is to be administered to the subject, thereby treating the TNFα-associated disease or disorder.

31. The use according to claim 30, wherein the disease or disorder in which TNFα activity is detrimental is selected from the group consisting of rheumatoid arthritis, juvenile idiopathic arthritic, psoriatic arthritis, ankylosing spondylitis, Crohn's Disease, and plaque psoriasis.

32. A method for producing the composition of any one of claims 18 to 24, comprising culturing cells expressing said human anti-TNFα antibodies in a culture media.

33. The method of claim 32, further comprising supplementing the media with one or more amino acids during cell culture, optionally wherein the one or more amino acids is selected from the group consisting of arginine, lysine, histidine, ornithine, and combinations thereof.

34. The method of claim 33, wherein the one or more amino acids is increased to a concentration of between about 0.025 and 20 g/L, or between about 0.05 and 15 g/L, or between about 0.1 and 14 g/L, or between about 0.2 and 13 g/L, or between about 0.25 and 12 g/L, or between about 0.5 and 11 g/L, or between about 1 and 10 g/L, or between about 1.5 and 9.5 g/L, or between about 2 and 9 g/L, or between about 2.5 and 8.5 g/L, or between about 3 and 8 g/L, or between about 3.5 and 7.5 g/L, or between about 4 and 7 g/L, or between about 4.5 and 6.5 g/L, or between about 5 and 6 g/L.

35. The method of claim 32, further comprising supplementing the media with zinc during cell culture.

36. The method of any one of claims 32 to 35, wherein the cultured cells are mammalian cells, optionally wherein the mammalian cells are selected from the group consisting of CHO cells, NS0 myeloma cells, COS cells, and SP2 cells.