unpaired cysteines were found to have no significant impact on binding of the antibody to CD20, and mAb A with unpaired cysteines was fully active in a potency assay (complement-dependent cytotoxicity, CDC, assay).

WO 2009/009523 (Kao et al.) discloses prevention of inter-chain disulfide bond reduction during recombinant production of the ocreclizumab (rhuMAb 2H7) antibody which binds CD20.

Harris, R. *Dev. Biol.* (Basel, Switzerland) 122: 117-127 (2005) disclosed unpaired cysteines (Cys22 and Cys96) in the variable heavy (VH) doman of omalizumab, a humanized anti-IgE antibody. The unpaired cysteine form had significantly lower potency.

Summary

The experimental data herein concerns variant forms of Pertuzumab, including an unpaired cysteine variant, afucosylated variant, low-molecular-weight-species (LMWS), and high-molecular-weight-species (HMWS). Means for identifying, characterizing, and quantifying these variants are valuable in the manufacture and quality control methods for the Pertuzumab drug composition.

Thus, in a first aspect, there is provided a composition comprising Pertuzumab and unpaired cysteine variant thereof, wherein the unpaired cysteine variant comprises Cys23/Cys88 unpaired cysteines in one or both variable light domains of Pertuzumab. The unpaired cysteine variant includes a heterodimer variant (comprising Cys23/Cys88 unpaired cysteines in only one variable light domain of Pertuzumab) and/or a homodimer variant (comprising Cys23/Cys88 unpaired cysteines in both variable light domains of Pertuzumab).

The composition optionally further comprises one or more additional variants of Pertuzumab such as afucosylated variant, low-molecular-weight-species (LMWS) variant, high-molecular-weight-species (HMWS) variant, glycated variant, disulfide reduced variant, non-reducible variant, deamidated variant, sialylated variant,VHS-variant, C-terminal lysine variant, methionine-oxidized variant, G1 glycosylation variant, G2 glycosylation variant, and non-glycosylated heavy chain variant.

~~In another aspect, there is provided~~Disclosed is a composition comprising Pertuzumab and an afucosylated variant of Pertuzumab, wherein the amount of the afucosylated variant is from 0.9 to 4.1% of the composition. In one ~~embodiment~~aspect, ~~the disclosure concerns~~there is provided a composition comprising Pertuzumab and an afucosylated variant of Pertuzumab, wherein the amount of the afucosylated variant is greater than 2% of the composition. According to this embodiment, the amount of the afucosylated variant is greater than that reported in US Patent No. 7,560,111, US Patent No. 7,879,325, and US Patent No. 8,241,630 (Kao et al.).

In an additional aspect, there is provided a composition comprising a mixture of Pertuzumab, low-molecular-weight species (LMWS) of Pertuzumab, and high-molecular-weight-species (HMWS) of Pertuzumab, wherein the amount of LMWS is ≤ 1.6% and the amount of HMWS is ≤ 1.7% as determined by size exclusion high performance liquid chromatography (SE-HPLC).

In one aspect, there is also provided a composition comprising a mixture of Pertuzumab, Pertuzumab fragment(s) which are of a size smaller than Pertuzumab light chain (LC)~~Peak 1~~, and Pertuzumab fragment(s) which are of a size larger than Pertuzumab light chain (LC) and smaller than Pertuzumab non-glycosylated heavy chain (NGHC)~~Peak 2~~, wherein the amount of Pertuzumab fragment(s) which are of a size smaller than Pertuzumab light chain (LC)~~Peak 1~~ is ≤ 0.5% and the amount of Pertuzumab fragment(s) which are of a size larger than Pertuzumab light chain (LC) and smaller than Pertuzumab non-glycosylated heavy chain (NGHC)~~Peak 2~~ is ≤ 1.0% as measured by reduced capilliary electrophoresis sodium dodecyl sulphate (R-CE-SDS) assay.

Also disclosed are pharmaceutical compositions, articles of manufacture, and methods of treating a cancer patient using or comprising the compositions herein.

In one aspect, there is provided a pharmaceutical composition comprising the composition as described above and one or more pharmaceutically acceptable excipients.

In one aspect, there is provided an article of manufacture comprising a container with the pharmaceutical composition as described above therein, and a package insert with prescribing information instructing the user thereof to use the pharmaceutical composition to treat a cancer patient.

In one aspect, there is provided use of the composition as described above or the pharmaceutical composition as described above in the manufacture of a medicament for treating a patient with cancer.

In an additional aspect, there is provided a method for evaluating a Pertuzumab composition comprising: (1) measuring the amount of unpaired cysteine variant in the composition, wherein the unpaired cysteine variant comprises Cys23/Cys88 unpaired cysteines in one or both variable light domains of Pertuzumab; and/or (2) measuring the amount of afucosylated Pertuzumab in the composition; or (3) measuring the amount of low-molecular-weight-species (LMWS) or high-molecular-weight-species (HMWS) of Pertuzumab in the composition.

In yet an additional aspect, there is provided a method for evaluating the biological activity of a Pertuzumab composition comprising measuring the amount of afucosylated Pertuzumab variant in the composition to determine the antibody-dependent cell-mediated cytotoxicity (ADCC) activity of the composition, and confirming the amount of afucosylated Pertuzumab is ~~in the range from about 0.9 to about 4.1%~~greater than 2% of the composition.

Pertuzumab; and/or (c) afucosylated variant of Pertuzumab; and/or (d) high-molecular-weight-species (HMWS) of Pertuzumab; and/or (e) low-molecular-weight-species (LMWS) of Pertuzumab; and/or (f) Peak 1 fragment(s) of Pertuzumab, and/or (g) Peak 2 fragment(s) of Pertuzumab.

In one aspect, there is provided an isolated variant of Pertuzumab, wherein the isolated variant comprises: (a) an unpaired cysteine variant of Pertuzumab, wherein the variant is a heterodimer variant comprising Cys23/Cys88 unpaired cysteines in only one variable light domain of Pertuzumab; (b) an unpaired cysteine variant of Pertuzumab, wherein the variant is a homodimer variant comprising Cys23/Cys88 unpaired cysteines in both variable light domains of Pertuzumab; (c) afucosylated variant of Pertuzumab; (d) high-molecular-weight-species (HMWS) of Pertuzumab; or (e) low-molecular-weight-species (LMWS) of Pertuzumab.

In an additional aspect, there is provided a method for evaluating fragmentation of a Pertuzumab composition comprising measuring the amount of Pertuzumab fragment(s) which are of a size smaller than Pertuzumab light chain (LC)~~Peak 1~~ and Pertuzumab fragment(s) which are of a size larger than Pertuzumab light chain (LC) and smaller than Pertuzumab non-glycosylated heavy chain (NGHC)~~Peak 2~~ in the composition by reduced capilliary electrophoresis sodium dodecyl sulphate (R-CE-SDS) assay and confirming the amount of Pertuzumab fragment(s) which are of a size smaller than Pertuzumab light chain (LC)~~Peak 1~~ is $\leq$ 5% and the amount of Pertuzumab fragment(s) which are of a size larger than Pertuzumab light chain (LC) and smaller than Pertuzumab non-glycosylated heavy chain (NGHC)~~Peak 2~~ is $\leq$ 1.0%.

In one aspect, there is provided a method for making a composition comprising: (1) producing a composition comprising Pertuzumab and one or more fragments thereof, and (2) subjecting the composition so-produced to an analytical assay to evaluate the amount of Pertuzumab fragment(s) which are of a size smaller than Pertuzumab light chain (LC)~~Peak 1~~ and Pertuzumab fragment(s) which are of a size larger than Pertuzumab light chain (LC) andsmaller than Pertuzumab non-glycosylated heavy chain (NGHC)~~Peak 2~~ as measured by reduced capilliary electrophoresis sodium dodecyl sulphate (R-CE-SDS) assay in the composition.

Brief Description of the Drawings

Figure 1 provides a schematic of the HER2 protein structure, and amino acid sequences for Subdomains I-IV (SEQ ID Nos.1-4, respectively) of the extracellular domain thereof.

Figures 2A and 2B depict alignments of the amino acid sequences of the variable light (VL) (Fig. 2A) and variable heavy (VH) (Fig. 2B) domains of murine monoclonal antibody 2C4 (SEQ ID Nos. 5 and 6, respectively); VL and VH domains of variant 574/Pertuzumab (SEQ ID Nos. 7 and 8, respectively), and human VL and VH consensus frameworks (hum κ1, light kappa subgroup I; humIII, heavy subgroup III) (SEQ ID Nos. 9 and 10, respectively). Asterisks identify differences between variable domains of Pertuzumab and murine monoclonal antibody 2C4 or between variable domains of Pertuzumab and the human framework. Complementarity Determining Regions (CDRs) are in brackets.

WHAT IS CLAIMED IS:

1. A composition comprising Pertuzumab and unpaired cysteine variant thereof, wherein the unpaired cysteine variant comprises Cys23/Cys88 unpaired cysteines in one or both variable light domains of Pertuzumab.

2. The composition of claim 1, wherein the unpaired cysteine variant is a heterodimer variant comprising Cys23/Cys88 unpaired cysteines in only one variable light domain of Pertuzumab.

3. The composition of claim 1, wherein the unpaired cysteine variant is a homodimer variant comprising Cys23/Cys88 unpaired cysteines in both variable light domains of Pertuzumab.

4. The composition of any one of the preceding claims, wherein the Pertuzumab and the unpaired cysteine variant each comprise the variable light and variable heavy amino acid sequences in SEQ ID NOs. 7 and 8, respectively.

5. The composition of any one of the preceding claims, wherein the Pertuzumab and the unpaired cysteine variant each comprise the light chain amino acid sequence in SEQ ID No. 11 or 15 and the heavy chain amino acid sequence in SEQ ID No. 12 or 16.

6. The composition of any one of the preceding claims, further comprising one or more additional variants of Pertuzumab, wherein the additional variants are selected from the group consisting of: afucosylated variant, low-molecular-weight-species (LMWS), high-molecular-weight-species (HMWS), glycated variant, disulfide reduced variant, non-reducible variant, deamidated variant, sialylated variant,VHS-variant, C-terminal lysine variant, methionine-oxidized variant, G1 glycosylation variant, G2 glycosylation variant, and non-glycosylated heavy chain variant.

7. The composition of any one of the preceding claims, wherein the amount of the unpaired cysteine variant in the composition is ≤ about 25% as determined by Fab hydrophobic interaction chromatography (HIC).

8. The composition of claim 3, wherein the amount of the homodimer variant in the composition is ≤ 4.9% as determined by hydrophobic interaction chromatography (HIC) of intact antibody.

9. The composition of claim 2, wherein the amount of the heterodimer variant in the composition is from about 13% to about 18% as determined by hydrophobic interaction chromatography (HIC) of intact antibody.

10. The composition of any one of claims 1 to 9, which has been subjected to an analytical assay to confirm that the amount of the unpaired cysteine variant in the composition is ≤ about 25% as determined by Fab hydrophobic interaction chromatography (HIC).

11. A composition comprising Pertuzumab and an afucosylated variant of Pertuzumab, wherein the amount of the afucosylated variant is from 0.9 to 4.1% of the composition.

12. The composition of claim 11, wherein the amount of the afucosylated variant is greater than 2% of the composition.

13. A composition comprising a mixture of Pertuzumab, low-molecular-weight species (LMWS) of Pertuzumab, and high-molecular-weight-species (HMWS) of Pertuzumab, wherein the amount of LMWS is ≤ 1.6% and the amount of HMWS is ≤ 1.7% as determined by size exclusion high performance liquid chromatography (SE-HPLC).

14. A composition comprising a mixture of Pertuzumab, Peak 1, and Peak 2, wherein the amount of Peak 1 is ≤ 0.5% and the amount of Peak 2 is ≤ 1.0% as measured by reduced capilliary electrophoresis sodium dodecyl sulphate (R-CE-SDS) assay.

15. A pharmaceutical composition comprising the composition of any one of claims 1 to 14 and one or more pharmaceutically acceptable excipients.

16. An article of manufacture comprising a container with the pharmaceutical composition of claim 15 therein, and a package insert with prescribing information instructing the user thereof to use the pharmaceutical composition to treat a cancer patient.

17. Use of the composition of any one of claims 1 to 14 or the pharmaceutical composition of claim 15 in the manufacture of a medicament for treating a patient with cancer.

18. The use of claim 17, wherein the cancer is breast cancer, gastric cancer, ovarian cancer, HER2-positive cancer, or low HER3 cancer.

19. A method for evaluating a Pertuzumab composition comprising: (1) measuring the amount of unpaired cysteine variant in the composition, wherein the unpaired cysteine variant comprises Cys23/Cys88 unpaired cysteines in one or both variable light domains of Pertuzumab; (2) measuring the amount of afucosylated Pertuzumab in the composition; or (3)

measuring the amount of low-molecular-weight-species (LMWS) or high-molecular-weight-species (HMWS) of Pertuzumab in the composition.

20. The method of claim 19, comprising two or three of (1), (2), and (3).

21. A method for evaluating the biological activity of a Pertuzumab composition comprising measuring the amount of afucosylated Pertuzumab variant in the composition to determine the antibody-dependent cell-mediated cytotoxicity (ADCC) activity of the composition, and confirming the amount of afucosylated Pertuzumab is in the range from about 0.9% to about 4.1%.

22. The method of claim 21, comprising measuring the amount of afucosylated Pertuzumab using capillary electrophoresis-laser-induced fluorescence (CE-LIF).

23. A method for making a composition comprising: (1) producing a composition comprising Pertuzumab and one or more variants thereof, and (2) subjecting the composition so-produced to an analytical assay to evaluate the amount of the variant(s) therein, wherein the variant(s) comprise: (i) unpaired cysteine variant comprising Cys23/Cys88 unpaired cysteines in one or both variable light domains of Pertuzumab; (ii) afucosylated variant of Pertuzumab; (iii) high-molecular-weight-species (HMWS) of Pertuzumab; or (iv) low-molecular-weight-species (LMWS) of Pertuzumab.

24. The method of claim 23, wherein (1) comprises expressing Pertuzumab and the variant(s) from a recombinant Chinese Hamster Ovary (CHO) at manufacturing scale and purifying the composition.

25. An isolated variant of Pertuzumab, wherein the isolated variant comprises: (a) an unpaired cysteine variant of Pertuzumab, wherein the variant is a heterodimer variant comprising Cys23/Cys88 unpaired cysteines in only one variable light domain of Pertuzumab; (b) an unpaired cysteine variant of Pertuzumab, wherein the variant is a homodimer variant comprising Cys23/Cys88 unpaired cysteines in both variable light domains of Pertuzumab; (c) afucosylated variant of Pertuzumab; (d) high-molecular-weight-species (HMWS) of Pertuzumab; or (e) low-molecular-weight-species (LMWS) of Pertuzumab.

26. A method for evaluating fragmentation of a Pertuzumab composition comprising measuring the amount of Peak 1 and Peak 2 in the composition by reduced capilliary electrophoresis sodium dodecyl sulphate (R-CE-SDS) assay and confirming the amount of Peak 1 is $\leq 5\%$ and the amount of Peak 2 is $\leq 1.0\%$.

27. A method for making a composition comprising: (1) producing a composition comprising Pertuzumab and one or more fragments thereof, and (2) subjecting the composition so-produced to an analytical assay to evaluate the amount of Peak 1 and Peak 2 in the composition.

WHAT IS CLAIMED IS:

1. A composition comprising Pertuzumab and unpaired cysteine variant thereof, wherein the unpaired cysteine variant comprises Cys23/Cys88 unpaired cysteines in one or both variable light domains of Pertuzumab.

2. The composition of claim 1, wherein the unpaired cysteine variant is a heterodimer variant comprising Cys23/Cys88 unpaired cysteines in only one variable light domain of Pertuzumab, optionally wherein the amount of the heterodimer variant in the composition is from about 13% to about 18% as determined by hydrophobic interaction chromatography (HIC) of intact antibody.

3. The composition of claim 1, wherein the unpaired cysteine variant is a homodimer variant comprising Cys23/Cys88 unpaired cysteines in both variable light domains of Pertuzumab, optionally wherein the amount of the homodimer variant in the composition is $\leq$ 4.9% as determined by hydrophobic interaction chromatography (HIC) of intact antibody.

4. The composition of any one of the preceding claims, wherein the Pertuzumab and the unpaired cysteine variant each comprise the variable light and variable heavy amino acid sequences in SEQ ID NOs. 7 and 8, respectively.

5. The composition of any one of the preceding claims, wherein the Pertuzumab and the unpaired cysteine variant each comprise the light chain amino acid sequence in SEQ ID No. 11 or 15 and the heavy chain amino acid sequence in SEQ ID No. 12 or 16.

6. The composition of any one of the preceding claims, further comprising one or more additional variants of Pertuzumab, wherein the additional variants are selected from the group consisting of: afucosylated variant, low-molecular-weight-species (LMWS), high-molecular-weight-species (HMWS), glycated variant, disulfide reduced variant, non-reducible variant, deamidated variant, sialylated variant,VHS-variant, C-terminal lysine variant, methionine-oxidized variant, G1 glycosylation variant, G2 glycosylation variant, and non-glycosylated heavy chain variant.

7. The composition of any one of the preceding claims, wherein the amount of the unpaired cysteine variant in the composition is $\leq$ about 25% as determined by Fab hydrophobic interaction chromatography (HIC).

8. A composition comprising Pertuzumab and an afucosylated variant of Pertuzumab, wherein the amount of the afucosylated variant is greater than 2% of the composition.

9. A composition comprising a mixture of Pertuzumab, low-molecular-weight species (LMWS) of Pertuzumab, and high-molecular-weight-species (HMWS) of Pertuzumab, wherein the amount of LMWS is $\leq 1.6\%$ and the amount of HMWS is $\leq 1.7\%$ as determined by size exclusion high performance liquid chromatography (SE-HPLC).

10. A composition comprising a mixture of Pertuzumab, Pertuzumab fragment(s) which are of a size smaller than Pertuzumab light chain (LC), and Pertuzumab fragment(s) which are of a size larger than Pertuzumab light chain (LC) and smaller than Pertuzumab non-glycosylated heavy chain (NGHC), wherein the amount of Pertuzumab fragment(s) which are of a size smaller than Pertuzumab light chain (LC) is $\leq 0.5\%$ and the amount of Pertuzumab fragment(s) which are of a size larger than Pertuzumab light chain (LC) and smaller than Pertuzumab non-glycosylated heavy chain (NGHC) is $\leq 1.0\%$ as measured by reduced capilliary electrophoresis sodium dodecyl sulphate (R-CE-SDS) assay.

11. A pharmaceutical composition comprising the composition of any one of claims 1 to 10 and one or more pharmaceutically acceptable excipients.

12. An article of manufacture comprising a container with the pharmaceutical composition of claim 11 therein, and a package insert with prescribing information instructing the user thereof to use the pharmaceutical composition to treat a cancer patient.

13. Use of the composition of any one of claims 1 to 10 or the pharmaceutical composition of claim 11 in the manufacture of a medicament for treating a patient with cancer.

14. The use of claim 13, wherein the cancer is breast cancer, gastric cancer, ovarian cancer, HER2-positive cancer, or low HER3 cancer.

15. A method for evaluating a Pertuzumab composition comprising: (1) measuring the amount of unpaired cysteine variant in the composition, wherein the unpaired cysteine variant comprises Cys23/Cys88 unpaired cysteines in one or both variable light domains of Pertuzumab; (2) measuring the amount of afucosylated Pertuzumab in the composition; or (3) measuring the amount of low-molecular-weight-species (LMWS) or high-molecular-weight-species (HMWS) of Pertuzumab in the composition.

16. The method of claim 15, comprising two or three of (1), (2), and (3).

17. A method for evaluating the biological activity of a Pertuzumab composition comprising measuring the amount of afucosylated Pertuzumab variant in the composition to determine the antibody-dependent cell-mediated cytotoxicity (ADCC) activity of the composition, and confirming the amount of afucosylated Pertuzumab is greater than 2% of the composition.

18. The method of claim 17, comprising measuring the amount of afucosylated Pertuzumab is measured using capillary electrophoresis-laser-induced fluorescence (CE-LIF).

19. A method for making a composition comprising: (1) producing a composition comprising Pertuzumab and one or more variants thereof, and (2) subjecting the composition so-produced to an analytical assay to evaluate the amount of the variant(s) therein, wherein the variant(s) comprise: (i) unpaired cysteine variant comprising Cys23/Cys88 unpaired cysteines in one or both variable light domains of Pertuzumab; (ii) afucosylated variant of Pertuzumab; (iii) high-molecular-weight-species (HMWS) of Pertuzumab; or (iv) low-molecular-weight-species (LMWS) of Pertuzumab.

20. The method of claim 19, wherein (1) comprises expressing Pertuzumab and the variant(s) from a recombinant Chinese Hamster Ovary (CHO) at manufacturing scale and purifying the composition.

21. An isolated variant of Pertuzumab, wherein the isolated variant comprises: (a) an unpaired cysteine variant of Pertuzumab, wherein the variant is a heterodimer variant comprising Cys23/Cys88 unpaired cysteines in only one variable light domain of Pertuzumab; (b) an unpaired cysteine variant of Pertuzumab, wherein the variant is a homodimer variant comprising Cys23/Cys88 unpaired cysteines in both variable light domains of Pertuzumab; (c) afucosylated variant of Pertuzumab; (d) high-molecular-weight-species (HMWS) of Pertuzumab; or (e) low-molecular-weight-species (LMWS) of Pertuzumab.

22. A method for evaluating fragmentation of a Pertuzumab composition comprising measuring the amount of Pertuzumab fragment(s) which are of a size smaller than Pertuzumab light chain (LC) and Pertuzumab fragment(s) which are of a size larger than Pertuzumab light chain (LC) and smaller than Pertuzumab non-glycosylated heavy chain (NGHC) in the composition by reduced capilliary electrophoresis sodium dodecyl sulphate (R-CE-SDS) assay and confirming the amount of Pertuzumab fragment(s) which are of a size smaller than Pertuzumab light chain (LC) is $\leq 5\%$ and the amount of Pertuzumab fragment(s) which are of a size larger than Pertuzumab light chain (LC) and smaller than Pertuzumab non-glycosylated heavy chain (NGHC) is $\leq 1.0\%$.

23. A method for making a composition comprising: (1) producing a composition comprising Pertuzumab and one or more fragments thereof, and (2) subjecting the composition so-produced to an analytical assay to evaluate the amount of Pertuzumab fragment(s) which are of a size smaller than Pertuzumab light chain (LC) and Pertuzumab fragment(s) which are of a size larger than Pertuzumab light chain (LC) andsmaller than Pertuzumab non-glycosylated

heavy chain (NGHC) as measured by reduced capilliary electrophoresis sodium dodecyl sulphate (R-CE-SDS) assay in the composition.