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 ~~of the Invention~~

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, ~~the invention concerns~~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, ~~The invention also concerns~~there is provided 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, the ~~invention~~ disclosure concerns 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, ~~the invention concerns~~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).

~~The invention also concerns~~In one aspect, there is also provided 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.

~~Additional aspects of the invention concern~~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, ~~the invention concerns~~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; ~~and/~~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, ~~the invention concerns~~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%.

~~In another aspect, the invention concerns~~Also disclosed is 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; and/or (ii) afucosylated variant of Pertuzumab; and/or (iii) high-molecular-weight-species (HMWS) of Pertuzumab; and/or (iv) low-molecular-weight-species (LMWS) of Pertuzumab, and/or (v) Peak 1 fragment(s) of Pertuzumab, and/or (vi) Peak 2 fragment(s) of Pertuzumab.

In one aspect, there is provided 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.

~~In another aspect, the invention concerns~~Further disclosed is 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; and/or (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; 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, ~~the invention concerns~~there is provided 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 $\leq 1.0\%$.

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 Peak 1 and Peak 2 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.

Figures 3A and 3B show the amino acid sequences of Pertuzumab light chain (Fig. 3A; SEQ ID NO. 11) and heavy chain (Fig. 3B; SEQ ID No. 12). CDRs are shown in bold. Calculated molecular mass of the light chain and heavy chain are 23,526.22 Da and 49,216.56 Da (cysteines in reduced form). The carbohydrate moiety is attached to Asn 299 of the heavy chain.

Figures 4A and 4B show the amino acid sequences of Trastuzumab light chain (Fig. 4A; SEQ ID NO. 13) and heavy chain (Fig. 4B; SEQ ID NO. 14), respectively. Boundaries of the variable light and variable heavy domains are indicated by arrows.

Figures 5A and 5B depict a variant Pertuzumab light chain sequence (Fig. 5A; SEQ ID NO. 15) and a variant Pertuzumab heavy chain sequence (Fig. 5B; SEQ ID NO. 16), respectively.

Figure 6 depicts the structure of (main species) Pertuzumab including its 4 inter-chain and 12 intra-chain disulfide bonds, including the Cys23/Cys88 intra-chain disulfide bonds in each of the variable light (VL) domains. Domains depicted are: VL = variable light domain; VH = variable heavy domain; CL = light chain constant domain; CH1 = heavy chain constant domain 1; CH2 =

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. ~~A method of treating a patient with cancer comprising administering~~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 ~~to~~ a ~~cancer~~ patient with cancer.

18. The ~~method~~ 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)