[0028] Thus, despite prior advances, a need remains for improved compositions, and methods for treating cancers expressing HER2/neu, and particularly metastatic breast cancer and cancers expressing low levels of HER2/neu. The present invention is directed to such compositions and to methods for their use in the treatment of HER2/neu-positive breast cancer and other cancers expressing HER2/neu.

Summary:

[0029] This disclosure relates to a pharmaceutical composition that comprises a first molecule that specifically binds to HER2/neu and a second molecule that specifically binds to a cell-surface receptor that is involved in regulating an immune checkpoint (or the ligand thereof). The disclosure particularly relates to the embodiment wherein the second molecule binds to PD-1. The disclosure also relates to the use of such pharmaceutical compositions to treat cancer and other diseases.

[0030] In detail, the disclosure provides a method of treating a cancer comprising administering to a subject in need thereof, an antibody that specifically binds HER2/neu and a molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint.

[0031] The disclosure particularly concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu is a "Variant Chimeric 4D5 Antibody" comprising a light chain variable domain having the amino acid sequence of **SEQ ID NO:4** and a heavy chain having an amino acid sequence selected from the group consisting of **SEQ ID NO:9**, **SEQ ID NO:11**, and **SEQ ID NO:13**.

[0031a] In one aspect, there is provided a Variant Chimeric 4D5 Antibody in combination with a molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint, for use in the treatment of cancer in a subject in need thereof, wherein said Variant Chimeric 4D5 Antibody comprises a light chain variable domain having the amino acid sequence of **SEQ ID NO:4,** and a heavy chain having an amino acid sequence selected from the group consisting of **SEQ ID NO:9**, **SEQ ID NO:11**, and **SEQ ID NO:13**.

[0031b] In another aspect, there is provided use of a Variant Chimeric 4D5 Antibody in combination with a molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint, in the manufacture of a medicament for the treatment of cancer in a subject in need thereof, wherein said Variant Chimeric 4D5 Antibody comprises a light chain variable domain having the amino acid sequence of **SEQ**

ID NO:4, and a heavy chain having an amino acid sequence selected from the group consisting of **SEQ ID NO:9, SEQ ID NO:11,** and **SEQ ID NO:13.**

[0032] The disclosure particularly concerns the embodiment of such methods wherein the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint is an anti-PD-1 antibody, or an antigen-binding fragment thereof.

[0033] The disclosure further concerns embodiments of such methods wherein the anti-PD-1 antibody, or antigen-binding fragment thereof:

(a) competes for PD-1 binding with nivolumab, pembrolizumab, pidilizumab, antibody EH12.2H7, antibody hPD-1 mAb 2, antibody hPD-1 mAb 7, antibody hPD-1 mAb 9, antibody hPD-1 mAb 15, or with another anti-PD-1 antibody selected from **Table 1**; or

(b) has the three heavy chain CDRs and the three light chain CDRs of nivolumab, pembrolizumab, pidilizumab, antibody EH12.2H7, antibody hPD-1 mAb 2, antibody hPD-1 mAb 7, antibody hPD-1 mAb 9, antibody hPD-1 mAb 15, or of another anti-PD-1 antibody selected from **Table 1**; or

(c) has the heavy chain variable domain and the light chain variable domain of nivolumab, pembrolizumab, pidilizumab, antibody EH12.2H7, antibody hPD-1 mAb 2, antibody hPD-1 mAb 7, antibody hPD-1 mAb 9, antibody hPD-1 mAb 15, or of another anti-PD-1 antibody selected from **Table 1.**

[0034] The disclosure further concerns embodiments of such methods wherein the anti-PD-1 antibody, or antigen-binding fragment thereof comprises an Fc Region. The disclosure further concerns the embodiments of such methods wherein the Fc Region comprises one or more amino acid modifications that reduce the affinity of the variant Fc Region for FcγRIIIa (CD16A) and/or reduces ADCC activity. The disclosure further concerns the embodiments of such methods, wherein the modifications comprise the substitution of L234A; L235A; or L234A and L235A.

[0035] The disclosure further concerns embodiments of such methods wherein the anti-PD-1 antibody is nivolumab, pembrolizumab, pidilizumab, antibody EH12.2H7, antibody hPD-1 mAb 2, antibody hPD-1 mAb 7, antibody hPD-1 mAb 9, antibody hPD-1 mAb 15, or another anti-PD-1 antibody selected from **Table 1.** The disclosure further concerns embodiments of such methods wherein the antigen-binding fragment of the anti-PD-1 antibody is an antigen-binding fragment of nivolumab, pembrolizumab, pidilizumab, antibody EH12.2H7, antibody hPD-1 mAb 2, antibody hPD-1 mAb 7, antibody hPD-1 mAb 9, antibody hPD-1 mAb 15, or of another anti-PD-1 antibody selected from **Table 1.**

[0036] The disclosure additionally concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu (particularly a Variant Chimeric 4D5 Antibody) is administered at a dosage of approximately 6-18 mg/kg body weight every three weeks and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint (particularly an anti-PD-1 antibody) is administered at a fixed dosage of approximately 200 mg every three weeks. The disclosure also concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu (particularly a Variant Chimeric 4D5 Antibody) is administered at a dosage of approximately 6-18 mg/kg body weight every three weeks and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint (particularly an anti-PD-1 antibody) is administered at a dosage of approximately 1-10 mg/kg body weight every three weeks. The disclosure further concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu (particularly a Variant Chimeric 4D5 Antibody) is administered at a dosage of approximately 6 mg/kg body weight, approximately 10 mg/kg body weight, approximately 15 mg/kg body weight, or approximately 18 mg/kg body weight every three weeks. The disclosure further concerns embodiments of such methods wherein the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint (particularly an anti-PD-1 antibody) is administered at a dosage of approximately 1 mg/kg body weight, approximately 2 mg/kg body weight, approximately 3 mg/kg body weight, or approximately 10 mg/kg body weight.

[0037] The disclosure additionally concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu (particularly a Variant Chimeric 4D5 Antibody) and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint (particularly an anti-PD-1 antibody) are administered concurrently to the subject in a single pharmaceutical composition.

[0038] The disclosure additionally concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu (particularly a Variant Chimeric 4D5 Antibody) and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint (particularly an anti-PD-1 antibody) are administered concurrently in separate compositions such that both compositions are administered within a 24-hour period.

[0039] The disclosure additionally concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu (particularly a Variant Chimeric 4D5 Antibody) and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint (particularly an anti-PD-1 antibody) are administered sequentially to the subject in separate pharmaceutical compositions, particularly wherein second administered composition is administered at least 24 hours, or more, after the administration of the first administered composition.

[0040] The disclosure particularly concerns embodiments of such methods wherein the cancer is a HER2/neu-expressing cancer. The disclosure further concerns embodiments of such methods wherein the cancer is breast cancer, gastric cancer, prostate cancer, uterine cancer, ovarian cancer, colon cancer, endometrial cancer, adrenal carcinoma, non-small cell lung cancer, head and neck cancer, laryngeal cancer, liver cancer, renal cancer, glioblastoma, or pancreatic cancer.

[0041] The disclosure additionally concerns embodiments of such methods further comprising the step of administering a third therapeutic agent, particularly wherein the third therapeutic agent is an anti-angiogenic agent, an anti-neoplastic agent, a chemotherapeutic agent, or a cytotoxic agent.

[0042] The disclosure further concerns embodiments of such methods wherein the third therapeutic agent is administered concurrently with, or separately from, the antibody that specifically binds HER2/neu (particularly a Variant Chimeric 4D5 Antibody) and/or the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint (particularly an anti-PD-1 antibody).

[0043] The disclosure further concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu is margetuximab and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is the anti-PD-1 antibody pembrolizumab.

[0044] The disclosure further concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu is margetuximab and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is the anti-PD-1 antibody nivolumab.

[0045] The disclosure further concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu is margetuximab and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is the anti-PD-1 antibody pidilizumab.

[0046] The disclosure further concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu is margetuximab and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is the anti-PD-1 antibody EH12.2H7.

[0047] The disclosure further concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu is margetuximab and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is the anti-PD-1 antibody hPD-1 mAb 2.

[0048] The disclosure further concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu is margetuximab and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is the anti-PD-1 antibody hPD-1 mAb 7.

[0049] The disclosure further concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu is margetuximab and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is the anti-PD-1 antibody hPD-1 mAb 9.

[0050] The disclosure further concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu is margetuximab and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is the anti-PD-1 antibody hPD-1 mAb 15.

[0051] The disclosure further concerns embodiments of such methods wherein the antibody that specifically binds HER2/neu is margetuximab and the molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is an anti-PD-1 antibody selected from **Table 1**.

[0052] Additional advantages and features of the present disclosure will be apparent from the following detailed description, drawings and examples, which illustrate preferred embodiments of the invention.

What is claimed is:

Claim 1. A Variant Chimeric 4D5 Antibody in combination with a molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint, for use in the treatment of cancer in a subject in need thereof, wherein said Variant Chimeric 4D5 Antibody comprises a light chain variable domain having the amino acid sequence of **SEQ ID NO:4,** and a heavy chain having an amino acid sequence selected from the group consisting of **SEQ ID NO:9, SEQ ID NO:11,** and **SEQ ID NO:13.**

Claim 2. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of claim 1, wherein said molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is an anti-PD-1 antibody, or an antigen-binding fragment thereof.

Claim 3. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of claim 2, wherein said anti-PD-1 antibody, or antigen-binding fragment thereof:

(a) competes for PD-1 binding with nivolumab, pembrolizumab, pidilizumab, antibody EH12.2H7, antibody hPD-1 mAb 2, antibody hPD-1 mAb 7, antibody hPD-1 mAb 9, antibody hPD-1 mAb 15, or an anti-PD-1 antibody selected from Table 1; or

(b) has the three heavy chain CDRs and the three light chain CDRs of nivolumab, pembrolizumab, pidilizumab, antibody EH12.2H7, antibody hPD-1 mAb 2, antibody hPD-1 mAb 7, antibody hPD-1 mAb 9, antibody hPD-1 mAb 15, or an anti-PD-1 antibody selected from Table 1; or

(c) has the heavy chain variable domain and the light chain variable domain of nivolumab, pembrolizumab, pidilizumab, antibody EH12.2H7, antibody hPD-1 mAb 2, antibody hPD-1 mAb 7, antibody hPD-1 mAb 9, antibody hPD-1 mAb 15, or an anti-PD-1 antibody selected from Table 1.

Claim 4. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of claim 2 or 3, wherein said anti-PD-1 antibody or antigen-binding fragment thereof comprises an Fc Region.

Claim 5. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of claim 4, wherein said anti-PD-1 antibody or antigen-binding fragment thereof, comprises a variant Fc Region having at least one modification in the Fc Region that reduces ADCC activity.

Claim 6. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-5, wherein said anti-PD-1 antibody is nivolumab, pembrolizumab, pidilizumab, antibody EH12.2H7, antibody hPD-1 mAb 2, antibody hPD-1 mAb 7, antibody hPD-1 mAb 9, antibody hPD-1 mAb 15, or an anti-PD-1 antibody selected from Table 1.

Claim 7. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-6, wherein:

(a) said Variant Chimeric 4D5 Antibody is for administration at a dosage of approximately 6-18 mg/kg and said anti-PD-1 antibody is for administration at a fixed dosage of approximately 100-500 mg; or

(b) said Variant Chimeric 4D5 Antibody is for administration at a dosage of approximately 6-18 mg/kg and said anti-PD-1 antibody is for administration at a dosage of approximately 1-10 mg/kg.

Claim 8. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-7, wherein said Variant Chimeric 4D5 Antibody is for administration at a dosage of approximately 6-18 mg/kg every three weeks and

said anti-PD-1 antibody is for administration at a fixed dosage of approximately 200 mg every three weeks.

Claim 9. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-7, wherein said Variant Chimeric 4D5 Antibody is for administration at a dosage of approximately 6-18 mg/kg every three weeks and said anti-PD-1 antibody is for administration at a dosage of approximately 1-10 mg/kg every three weeks.

Claim 10. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 7-9, wherein said Variant Chimeric 4D5 Antibody is for administration at a dosage selected from 6 mg/kg, 10 mg/kg, 15 mg/kg and 18 mg/kg every three weeks.

Claim 11. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 7, 9 or 10, wherein said anti-PD-1 is for administration at a dosage selected from 1 mg/kg, 2 mg/kg, 3 mg/kg and 10 mg/kg every three weeks.

Claim 12. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 1-11, wherein said Variant Chimeric 4D5 Antibody and said molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint are for concurrent administration in a single pharmaceutical composition.

Claim 13. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 1-11, wherein said Variant Chimeric 4D5 Antibody and said molecule that specifically binds a cell-surface receptor, or ligand thereof, that

regulates an immune checkpoint are for concurrent administration in separate pharmaceutical compositions within a 24-hour period.

Claim 14. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 1-11, wherein one of said Variant Chimeric 4D5 Antibody and said molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint is for administration at least 24 hours, or more, after the administration of the other of said Variant Chimeric 4D5 Antibody and said molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint.

Claim 15. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 1 to 14, wherein said cancer is a cancer in which HER2/neu is expressed.

Claim 16. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of claim 15, wherein said cancer is a breast cancer, gastric cancer, prostate cancer, uterine cancer, ovarian cancer, colon cancer, endometrial cancer, adrenal carcinoma, non-small cell lung cancer, head and neck cancer, laryngeal cancer, liver cancer, renal cancer, glioblastoma, or pancreatic cancer.

Claim 17. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 1 to 16, for use in further combination with a third therapeutic agent selected from the group consisting of an anti-angiogenic agent, an anti-neoplastic agent, a chemotherapeutic agent, and a cytotoxic agent.

Claim 18. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of claim 17, wherein the third therapeutic agent is for concurrent administration with

said Variant Chimeric 4D5 Antibody and/or said molecule that specifically binds a cell-surface receptor, or ligand thereof, that regulates an immune checkpoint.

Claim 19. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of claim 17, wherein the third therapeutic agent is for administration separately from said Variant Chimeric 4D5 Antibody and/or said molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint.

Claim 20. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 1-19, wherein said Variant Chimeric 4D5 Antibody is margetuximab.

Claim 21. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-20, wherein said anti-PD-1 antibody is pembrolizumab.

Claim 22. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-20, wherein said anti-PD-1 antibody is nivolumab.

Claim 23. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-20, wherein said anti-PD-1 antibody is pidilizumab.

Claim 24. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-20, wherein said anti-PD-1 antibody is antibody EH12.2H7.

Claim 25. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-20, wherein said anti-PD-1 antibody is antibody hPD-1 mAb 2.

Claim 26. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-20, wherein said anti-PD-1 antibody is antibody hPD-1 mAb 7.

Claim 27. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-20, wherein said anti-PD-1 antibody is antibody hPD-1 mAb 9.

Claim 28. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-20, wherein said anti-PD-1 antibody is antibody hPD-1 mAb 15.

Claim 29. The Variant Chimeric 4D5 Antibody and the molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint of any one of claims 2-20, wherein said anti-PD-1 antibody is selected from Table 1.

Claim 30. Use of a Variant Chimeric 4D5 Antibody in combination with a molecule that specifically binds a cell-surface receptor, or a ligand thereof, that regulates an immune checkpoint, in the manufacture of a medicament for the treatment of cancer in a subject in need thereof, wherein said Variant Chimeric 4D5 Antibody comprises a light chain variable domain having the amino acid sequence of **SEQ ID NO:4,** and a heavy chain having an amino acid sequence selected from the group consisting of **SEQ ID NO:9, SEQ ID NO:11,** and **SEQ ID NO:13.**