Plasma samples obtained from three Avastin trials (AVADO study (BO17708) in HER2 negative metastatic breast cancer, AVITA study (BO17706) in metastatic pancreatic cancer and AVAGAST study (BO20904) in metastatic gastric cancer) were recently analyzed for VEGFA expression levels. The sample median VEGFA concentration was prespecified as a cut point to group patients (high vs. low levels of concentration). A greater magnitude of treatment effect on progression-free survival (PFS), as measured by the hazard ratio (HR) of the treatment arm relative to the control arm, was observed for patients with high VEGF-A levels across these studies. A greater magnitude of treatment effect on overall survival (OS) was also observed for patients with high VEGFA levels in the BO17706 and BO20904 studies. The analyses suggest that plasma VEGF-A is potentially predictive of response to treatment with Avastin in these indications.

Analyses of the BO17708 study in HER2 negative metastatic breast cancer and the BO17706 study in metastatic pancreatic cancer also showed an increased duration of PFS for patients with high VEGFR2 levels in the bevacizumab treatment arm as compared to the control arm.

**Summary** ~~of the Invention~~

An investigation of the status of biomarkers related to angiogenesis and tumorigenesis revealed that the expression levels of VEGFA and VEGFR2 relative to reference levels determined in the entire biomarker patient population correlated with an improved treatment outcome in HER2 positive breast cancer patients. In particular, patients exhibiting an increased expression level of VEGFA relative to reference levels determined in the entire biomarker patient population, demonstrated a prolonged progression-free survival in response to the addition of bevacizumab to the combination chemotherapy of trastuzumab and docetaxel. Patients exhibiting a higher expression level of VEGFR2 relative to reference levels determined in the entire biomarker patient population, demonstrated a prolonged progression free survival in response to the addition of bevacizumab to the combination chemotherapy of trastuzumab and docetaxel.

The present ~~invention~~ disclosure therefore relates to a method of determining whether a patient diagnosed with HER2 positive breast cancer is sensitive to the addition of an anti-VEGF antibody to a chemotherapy regimen, by determing an expression level of VEGFA and/or VEGFR2 in a

patient sample and comparing it with reference levels.

In a first aspect, there is provided a method of determining whether a patient diagnosed with HER2 positive breast cancer is more or less suitably treated by an anti-cancer therapy comprising an anti-VEGF antibody, the method comprising:

(a) determining an expression level of VEGFA and/or VEGFR2 in a sample derived from a patient diagnosed with HER2 positive breast cancer, and

(b) identifying the patient as more or less suitably treated by an anti-cancer therapy comprising an anti-VEGF antibody based on the expression level in accordance with (a), wherein an expression level of VEGFA and/or VEGFR2 at or above a reference level indicates that the patient is more suitably treated with the anti-cancer therapy, or an expression level of VEGFA and/or VEGFR2 below a reference level indicates that the patient is less suitably treated with the anti-cancer therapy.

In a second aspect, there is provided a method of determining whether a patient diagnosed with HER2 positive breast cancer is sensitive to the addition of an anti-VEGF antibody to a chemotherapy regimen, said method comprising:

(a) determining an expression level of VEGFA and/or VEGFR2 in a sample derived from a patient diagnosed with HER2 positive breast cancer, and

(b) identifying the patient as sensitive to the addition of an anti-VEGF antibody to a chemotherapy regimen based on the expression level in accordance with (a), wherein an expression level of VEGFA and/or VEGFR2 at or above a reference level indicates that the patient is sensitive to the addition of an anti-VEGF antibody to a chemotherapy regimen.

The present ~~invention~~ disclosure also relates to a pharmaceutical composition comprising an anti-VEGF antibody, such as bevacizumab, for the treatment of a patient diagnosed with HER2 porisitve breast and having an increased expression level of VEGFA and/or VEGFR2 relative to reference levels.

In a third aspect, there is provided a pharmaceutical composition comprising an anti-VEGF antibody for the treatment of a patient diagnosed with HER2 positive breast cancer, wherein the patient has been identified as more suitably treated by an anti-cancer therapy comprising an anti-VEGF antibody in accordance with the method as defined herein.

In a fourth aspect, there is provided a pharmaceutical composition comprising an anti-VEGF antibody for the treatment of a patient diagnosed with HER2 positive breast cancer, wherein the patient has been identified as sensitive to the addition of an anti-VEGF antibody to a chemotherapy in accordance with the method as defined herein.

In a fifth aspect, there is provided a kit for carrying out the method as defined herein, comprising a set of compounds for detecting an expression level of VEGFA and/or VEGFR2, the set comprising antibodies capable of specifically binding to VEGFA and/or VEGFR2.

The present ~~invention~~ disclosure further relates to a method for improving the treatment effect of chemotherapy of a patient diagnosed with HER2 positive breast cancer by adding an anti-VEGF antibody, such as bevacizumab, based on an expression level of VEGFA and/or VEGFR2 in a patient sample.

In a sixth aspect, there is provided a method for improving the treatment effect of a chemotherapy regimen in a patient diagnosed with HER2 positive breast cancer by adding an anti-VEGF antibody to the chemotherapy regimen, the method comprising:

(a) determining an expression level of VEGFA and/or VEGFR2 in a sample derived from a patient diagnosed with HER2 positive breast cancer;

(b) identifying the patient as sensitive to the addition of an anti-VEGF antibody to a chemotherapy based on the expression level in accordance with (a), wherein an expression level of VEGFA and/or VEGFR2 at or above a reference level indicates that the patient is sensitive to the addition of an anti-VEGF antibody to a chemotherapy regimen; and

(c) formulating an effective amount of an anti-VEGF antibody in combination with an effective amount of a chemotherapy regimen for administration to the patient identified as sensitive to the addition of an anti-VEGF antibody to a chemotherapy in accordance with (b).

**Detailed Description of the Embodiments**

**1. Definitions**

The terms "administration" or "administering" as used herein mean the administration of a pharmaceutical composition, such as an angiogenesis inhibitor, to a patient in need of such treatment or medical intervention by any suitable means known in the art. Nonlimiting routes of administration include by oral, intravenous, intraperitoneal, subcutaneous, intramuscular, topical, intradermal, intranasal or intrabronchial administration (for example as effected by inhalation). Particularly preferred in context of this invention is parenteral administration, *e.g.*, intravenous administration.

The term "anti-angiogenesis agent" or "angiogenesis inhibitor" refers to a small molecular weight substance, a polynucleotide, a polypeptide, an isolated protein, a recombinant protein, an antibody, or conjugates or fusion proteins thereof, that inhibits angiogenesis, vasculogenesis, or undesirable vascular permeability, either directly or indirectly. It should be understood that the anti-angiogenesis agent includes those agents that bind and block the angiogenic activity of the angiogenic factor or its receptor. For example, an anti-angiogenesis agent is an antibody or other antagonist to an angiogenic agent as defined throughout the specification or known in the art, e.g., but are not limited to, antibodies to VEGF-A or to the VEGF-A receptor (e.g., KDR receptor or Flt-1 receptor), VEGF-trap, anti-PDGFR inhibitors such as Gleevec™ (Imatinib Mesylate). Anti-angiogensis agents also include native angiogenesis inhibitors, e.g., angiostatin, endostatin, etc. See, e.g., Klagsbrun and D'Amore, Annu. Rev. Physiol., 53:217-39 (1991); Streit and Detmar, Oncogene, 22:3172-3179 (2003) (e.g., Table 3 listing anti-angiogenic therapy in malignant melanoma); Ferrara & Alitalo, Nature Medicine 5:1359-1364 (1999); Tonini et al.,

**Claims**

1. A method of determining whether a patient diagnosed with HER2 positive breast cancer is more or less suitably treated by an anti-cancer therapy comprising an anti-VEGF antibody, the method comprising:

(a) determining an expression level of VEGFA and/or VEGFR2 in a sample derived from a patient diagnosed with HER2 positive breast cancer, and

(b) identifying the patient as more or less suitably treated by an anti-cancer therapy comprising an anti-VEGF antibody based on the expression level in accordance with (a), wherein an expression level of VEGFA and/or VEGFR2 at or above a reference level indicates that the patient is more suitably treated with the anti-cancer therapy, or an expression level of VEGFA and/or VEGFR2 below a reference level indicates that the patient is less suitably treated with the anti-cancer therapy.

2. The method of claim 1, wherein whether a patient is suitably treated by an anti-cancer therapy is determined in terms of progression-free survival.

~~3. The method of claim 1 or 2, wherein the method further comprises treating the patient with the anticancer therapy.~~

4~~3~~. The method of ~~any one of~~ claims 1~~-3~~ or 2, wherein said anti-cancer therapy comprises an anti-VEGF antibody, an anti-Her2 antibody and a taxane.

5~~4~~. A method of determining whether a patient diagnosed with HER2 positive breast cancer is sensitive to the addition of an anti-VEGF antibody to a chemotherapy regimen, said method comprising:

(a) determining an expression level of VEGFA and/or VEGFR2 in a sample derived from a patient diagnosed with HER2 positive breast cancer, and

(b) identifying the patient as sensitive to the addition of an anti-VEGF antibody to a chemotherapy regimen based on the expression level in accordance with (a), wherein an expression level of VEGFA and/or VEGFR2 at or above a reference level indicates that the patient is sensitive to the addition of an anti-VEGF antibody to a chemotherapy regimen.

6~~5~~. The method of claim 5~~4~~, wherein whether a patient is sensitive to the addition of an

anti-VEGF antibody to a chemotherapy regimen is determined in terms of progression-free survival.

~~7. The method of claim 5 or 6, wherein the method further comprises treating the patient with the anticancer therapy.~~

86. The method of ~~any one of~~ claims ~~5~~4-7 or 5, wherein said chemotherapy regimen comprises an anti-Her2 antibody and a taxane.

97. The method of any one of claims 1-86, wherein said patient is diagnosed with locally recurrent or metastatic HER2 positive breast cancer.

~~10~~8. The method of any one of claims 1-97, wherein said patient received no previous chemotherapeutic or radiation treatment.

~~11~~9. The method of any one of claims 1-~~10~~8, wherein said anti-VEGF antibody binds the A4.6.1 epitope.

~~12~~10. The method of any one of claims 1-~~11~~9, wherein said anti-VEGF antibody is bevacizumab.

~~13~~11. The method of any one of claims 1-~~12~~10, wherein said anti-VEGF antibody comprises a variable heavy chain (VH) and a variable light chain (VL), wherein said VH has an amino acid sequence of SEQ ID NO:2 and said VL has an amino acid sequence of SEQ ID NO:1.

~~14~~12. The method of any one of claims 43, 86-~~13~~11, wherein said taxane is docetaxel or paclitaxel.

~~15~~13. The method of any one of claims 43, 86-~~14~~12, wherein said taxane is docetaxel.

~~16~~14. The method of any one of claims 43, 86-~~15~~13, wherein said anti-HER2 antibody binds the 4D5 epitope.

~~17~~15. The method of any one of claims 43, ~~86-16~~14, wherein said anti-HER2 antibody is trastuzumab.

~~18~~16. The method of any one of claims 43, ~~86-17~~15, wherein said anti-HER2 antibody comprises a variable heavy chain (VH) and a variable light chain (VL), wherein said VH has an amino acid sequence of SEQ ID NO:4 and said VL has an amino acid sequence of SEQ ID NO:3.

~~19~~17. The method of any one of claims 1-~~18~~16, wherein said expression level is a protein expression level.

~~20~~18. The method of any one of claims 1-~~19~~17, wherein said sample is a blood plasma sample.

~~21~~19. The method of any one of claims 1-~~20~~18, wherein said expression level is an expression level of VEGFA.

~~22~~20. The method of any one of claims 1-~~21~~19, wherein said expression level is an expression level of VEGFR2.

~~23~~21. A pharmaceutical composition comprising an anti-VEGF antibody for the treatment of a patient diagnosed with HER2 positive breast cancer, wherein the patient has been identified as more suitably treated by an anti-cancer therapy comprising an anti-VEGF antibody in accordance with the method of any one of claims 1-43, ~~97-22~~20.

~~24~~22. A pharmaceutical composition comprising an anti-VEGF antibody for the treatment of a patient diagnosed with HER2 positive breast cancer, wherein the patient has been identified as sensitive to the addition of an anti-VEGF antibody to a chemotherapy in accordance with the method of any one of claims ~~54-22~~20.

~~25~~23. A kit for carrying out the method of any one of claims 1-~~22~~20, comprising a set of compounds for detecting an expression level of VEGFA and/or VEGFR2, the set comprising antibodies capable of specifically binding to VEGFA and/or VEGFR2.

~~26~~24. A method for improving the treatment effect of a chemotherapy regimen in a patient diagnosed with HER2 positive breast cancer by adding an anti-VEGF antibody to the chemotherapy regimen, the method comprising:

(a) determining an expression level of VEGFA and/or VEGFR2 in a sample derived from a patient diagnosed with HER2 positive breast cancer;

(b) identifying the patient as sensitive to the addition of an anti-VEGF antibody to a chemotherapy based on the expression level in accordance with (a), wherein an expression level of VEGFA and/or VEGFR2 at or above a reference level indicates that the patient is sensitive to the addition of an anti-VEGF antibody to a chemotherapy regimen; and

(c) ~~administering~~ formulating an effective amount of an anti-VEGF antibody in combination with an effective amount of a chemotherapy regimen for administration to the patient identified as sensitive to the addition of an anti-VEGF antibody to a chemotherapy in accordance with (b).

~~27~~25. The method of claim ~~26~~24, wherein a patient is sensitive to the addition of an anti-VEGF antibody to a chemotherapy regimen is determined in terms of progression-free survival.

~~28~~26. The method of claim ~~26-~~24 or ~~27~~25, wherein said anti-cancer therapy comprises an anti-Her2 antibody and a taxane.

~~29~~27. The method of any one of claims ~~26~~24-~~28~~26, wherein said patient is diagnosed with locally recurrent or metastatic HER2 positive breast cancer.

~~30~~28. The method of any one of claims ~~26~~24-~~29~~27, wherein said patient received no previous chemotherapeutic or radiation treatment.

~~31~~29. The method of any one of claims ~~26~~24-~~30~~28, wherein said anti-VEGF antibody binds the A4.6.1 epitope.

~~32~~30. The method of any one of claims ~~26~~24-~~31~~29, wherein said anti-VEGF antibody is

bevacizumab.

~~33~~31. The method of any one of claims 24~~26~~-~~32~~30, wherein said anti-VEGF antibody comprises a variable heavy chain (VH) and a variable light chain (VL), wherein said VH has an amino acid sequence of SEQ ID NO:2 and said VL has an amino acid sequence of SEQ ID NO:1.

~~34~~32. The method of any one of claims ~~28~~26-~~33~~31, wherein said taxane is docetaxel or paclitaxel.

~~35~~33. The method of any one of claims ~~28~~26-~~34~~32, wherein said taxane is docetaxel.

~~36~~34. The method of any one of claims ~~28~~26-~~35~~33, wherein said anti-HER2 antibody binds the 4D5 epitope.

~~37~~35. The method of any one of claims ~~28~~26-~~36~~34, wherein said anti-HER2 antibody is trastuzumab.

~~38~~36. The method of any one of claims ~~28~~26-~~37~~35, wherein said anti-HER2 antibody comprises a variable heavy chain (VH) and a variable light chain (VL), wherein said VH has an amino acid sequence of SEQ ID NO:4 and said VL has an amino acid sequence of SEQ ID NO:3.

~~39~~37. The method of any one of claims ~~26~~24-~~38~~36, wherein said expression level is a protein expression level.

~~40~~38. The method of any one of claims ~~26~~24-~~39~~37, wherein said sample is a blood plasma sample.

~~41~~39. The method of any one of claims ~~26~~24-~~40~~38, wherein said expression level is an expression level of VEGFA.

~~42~~40. The method of any one of claims ~~26~~24-~~41~~39, wherein said expression level is an