

BIOLOGICAL MARKERS FOR IDENTIFYING PATIENTS FOR TREATMENT WITH VEGF ANTAGONISTS

Field of the Invention

5 The present invention is directed to methods for identifying patients that will benefit from treatment with a VEGF antagonist, *e.g.*, an anti-VEGF antibody.

Background of the Invention

Measuring expression levels of biomarkers (*e.g.*, secreted proteins in plasma) can be
10 an effective means to identify patients and patient populations that will respond to specific therapies including, *e.g.*, treatment with VEGF antagonists, such as anti-VEGF antibodies.

There is a need for effective means for determining which patients will respond to which treatment and for incorporating such determinations into effective treatment regimens for patients with VEGF antagonist therapies, whether used as single agents or combined with
15 other agents.

Summary of the Invention

The present ~~disclosure invention~~ provides methods for identifying patients who will benefit from treatment with a VEGF antagonist, such as an anti-VEGF antibody. These patients are identified based on expression levels of the genes set forth in Table 1 or 2.

20 Accordingly, in one aspect, there is ~~embodiment of the invention~~ provided a methods of determining whether a patient is likely to respond to treatment with the VEGF antagonist, the methods comprising (a) detecting expression of at least one gene set forth in Table 1 or 2 in a biological sample obtained from the patient prior to any administration of a VEGF antagonist to the patient; and (b) comparing the expression level of the at least one gene to a
25 reference expression level of the at least one gene, wherein a change in the level of expression of the at least one gene in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist.

In a ~~A further aspect, there is embodiment of the invention~~ provided a methods of optimizing therapeutic efficacy of a VEGF antagonist for a patient, the methods comprising
30 (a) detecting expression of at least one gene set forth in Table 1 or 2 in a biological sample obtained from the patient prior to any administration of a VEGF antagonist to the patient; and (b) comparing the

expression level of the at least one gene to a reference expression level of the at least one gene, wherein a change in the level of expression of the at least one gene in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist.

Referring to the aspects ~~embodiments~~ set forth above, in some ~~further~~ embodiments, the patient is in a population of patients being tested for responsiveness to a VEGF antagonist and the reference level is the median level of expression of the at least one gene in the population of patients. In some embodiments, the change in level of expression of the at least one gene in the patient sample is an increase relative to the reference level. In some
 10 embodiments, the change in level of expression of the at least one gene in the patient sample is a decrease relative to the reference level. In some embodiments, the at least one gene in the biological sample obtained from the patient is detected by measuring mRNA. In some embodiments, expression of the at least one gene in the biological sample obtained from the patient is detected by measuring plasma protein levels. In some embodiments, the biological
 15 sample is tumor tissue. In some embodiments, the methods further comprise detecting expression of at least a second, third, fourth, or more gene set forth in Table 1 or 2 in the biological sample from the patient. In some embodiments, the at least one gene is selected from the group consisting of: Alk1, CD34, CD105, CD144, Col4a1, Col4a2, Dll4, EFNB2, EGFL7, ESM1, LAMA4, NG2, Nid2, Notch1, NRP1, NRP2, RGS5, Sema3f, TSP1,
 20 VEGFR1, VEGFR2, VEGFR3, and VIM. In some embodiments, the VEGF antagonist is an anti-VEGF antibody, such as bevacizumab. In some embodiments, the patient has an angiogenic disorder. In some embodiments, the patient has cancer selected from the group consisting of: colorectal cancer, breast cancer, lung cancer, glioblastoma, and combinations thereof.

Also referring to the aspects and embodiments set forth above, the methods can further
 25 include (c) selecting a VEGF antagonist for treatment of the patient when a change in the level of expression of the at least one gene in the patient sample relative to the reference level is detected. Further, the methods can include (d) administering a VEGF antagonist (e.g., an anti-VEGF antibody, such as bevacizumab) to the patient.

In another aspect, there is ~~Another embodiment of the invention provides~~ a methods for
 30 selecting a therapy for a particular patient in a population of patients being considered for therapy, the methods comprising: (a) detecting expression of at least one gene set forth in Table 1 or 2 in a biological sample obtained from the patient prior to any administration of a VEGF antagonist

to the patient; (b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein a change in the level of expression of the at least one gene in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist, and (c) selecting a VEGF antagonist as the therapy if the patient is identified as likely to respond to treatment with a VEGF antagonist; or (d) selecting a therapy that is not a VEGF antagonist if the patient is not identified as likely to respond to treatment with a VEGF antagonist.

In some embodiments, the reference level is the median level of expression of the at least one gene in the population of patients. In some embodiments, the change in level of expression of the at least one gene in the patient sample is an increase relative to the reference level. In some embodiments, the change in level of expression of the at least one gene in the patient sample is a decrease relative to the reference level. In some embodiments, the methods further comprise detecting expression of at least a second, third, fourth, or more gene set forth in Table 1 or 2 in the biological sample from the patient. In some embodiments, the therapy of (d) is an agent selected from the group consisting of: an anti-neoplastic agent, a chemotherapeutic agent, a growth inhibitory agent, a cytotoxic agent, and combinations thereof. In some embodiments, the methods further comprise (e) administering an effective amount of a VEGF antagonist to the patient if the patient is identified as likely to respond to treatment with a VEGF antagonist. In some embodiments, the VEGF antagonist is an anti-VEGF antibody, such as bevacizumab. In some embodiments, the methods further comprising administering an effective amount of at least a second agent. In some embodiments, the second agent is selected from the group consisting of: an anti-neoplastic agent, a chemotherapeutic agent, a growth inhibitory agent, a cytotoxic agent, and combinations thereof.

In a further aspect, there is an embodiment of the invention provided a methods of identifying a biomarker for determining responsiveness to a VEGF antagonist, the methods comprising: (a) detecting the expression of a candidate biomarker in a biological sample obtained from a patient prior to administration of a VEGF antagonist to the patient; and (b) comparing the expression of the candidate biomarker to a reference expression level of the candidate biomarker, wherein a change in the level of expression of the candidate biomarker in the patient sample relative to the reference level identifies the candidate biomarker as a biomarker of a patient who will likely respond to treatment with a VEGF antagonist. In some embodiments, the reference level is the median level of expression of the at least one gene in a population of patients

being tested for the likelihood they will respond to a VEGF antagonist. In some embodiments, the reference level is the expression level of the at least one gene in a sample previously obtained from the patient. In some embodiments, the patient was previously treated with a VEGF antagonist and is currently experiencing metastasis. In some
 5 embodiments, the VEGF antagonist is an anti-VEGF antibody, such as bevacizumab. Further, the methods can further include (c) selecting a candidate biomarker having a change in level of expression relative to the reference for use as a biomarker for determining responsiveness to VEGF antagonist treatment.

In another aspect, ~~there is an embodiment, the invention provides~~ a methods for
 10 diagnosing an angiogenic disorder in a patient, the methods comprising the steps of: (a) detecting the expression level of at least one gene set forth in Table 1 or 2 or a biomarker identified according to methods such as those described above in a sample obtained from the patient prior to any administration of a VEGF antagonist to the patient; and (b) comparing the expression level of the at least one gene or biomarker to a reference level of the at least one
 15 gene; wherein a change in the level of expression of the at least one gene in the patient sample relative to the reference level identifies a patient having an angiogenic disorder. These methods can further include (c) selecting a VEGF antagonist for treatment of the patient when a change in the level of expression of the at least one gene in the patient sample relative to the reference level is detected. In addition, the methods can further include (d) administering a
 20 VEGF antagonist to the patient. In addition, the methods described herein can include a step of obtaining a sample from a patient, as described herein. Further, the methods described herein can be carried out on patients diagnosed with cancer, as described herein, to determine an optimal treatment regimen.

In any of the aspects and embodiments set forth above, the change in the level of
 25 expression of the at least one gene or biomarker in the patient sample relative to the reference level can be determined by calculating a VDV signature score (VDV_i) for the patient sample according to the algorithm:

$$VDV_i = \frac{1}{\sqrt{n}} \sum_{g=1}^n Z_{g,i}$$

in which $Z_{g=1,i}, Z_{g=2,i}, \dots, Z_{g=n,i}$ are standardized z-scores of expression values for each gene or biomarker g , from $g=1$ to $g=n$, of the sample i , and in which a VDV_i below a first defined
 30 threshold indicates a decrease relative to the reference level, and a VDV_i above a second

defined threshold indicates an increase relative to the reference level. In some embodiments, the expression values for each gene or biomarker g , from $g=1$ to $g=n$, are qRT-PCR values for each gene g , from $g=1$ to $g=n$. In some embodiments, the first defined threshold is from -4 to -0.5 (e.g., -4, -3.5, -3, -2.5, -2, -1.5, -1, or -0.5) and the second defined threshold is from 0.5 to 4 (e.g., 0.5, 1, 1.5, 2, 2.5, 3, 3.5, or 4). In some embodiments, the first defined threshold is from -4 to -1 (e.g., -4, -3.5, -3, -2.5, -2, -1.5, or -1) and the second defined threshold is from 1 to 4 (e.g., 1, 1.5, 2, 2.5, 3, 3.5, or 4). In some embodiments, the first defined threshold is from -4 to -1.5 (e.g., -4, -3.5, -3, -2.5, -2, or -1.5) and the second defined threshold is from 1.5 to 4 (e.g., 1.5, 2, 2.5, 3, 3.5, or 4). In other embodiments, the first defined threshold is from -4 to -2 (e.g., -4, -3.5, -3, -2.5, or -2) and the second defined threshold is from 2 to 4 (e.g., 2, 2.5, 3, 3.5, or 4).

In one aspect, there is provided use of a VEGF antagonist in the manufacture of a medicament for treating cancer in a patient, wherein the patient is or has been identified as being one who is likely to respond to treatment with a VEGF antagonist according to the methods as described herein.

These and other embodiments are further described by the detailed description that follows.

Brief Description of the Drawings

Fig. 1A shows histological and graphical analysis of micro-vascular density (MVD) and proliferation index determination of murine pancreatic neuro-endocrine tumor (PNET) cells at 72 hours, 7 days, and 14 days post-treatment with anti-VEGF mAb. Representative images from histological analysis of tumor vessel density via MECA-32 staining (left) and proliferative index via Ki67 (right) at various times following anti-VEGF treatment (20X magnification). Quantitation from 4-6 tumors in each case is shown in the bar graphs below as mean $\pm$ SEM. * $P < 0.05$, NS = not significant.

Fig. 1B is a graph showing the kinetics of anti-VEGF effects on tumor burden in the *RIP-T β Ag* model. Tumor burden in anti-VEGF-treated mice (red bars) is significantly lower than control-treated mice (black bars) at 21 days, but not at 14 days on the study. * $= p < 0.05$ (t-test), $n=5-8$ mice/group/time point.

Fig. 1C is a graph showing changes in VDV gene expression levels in response to treatment with a VEGF antagonist. Expression levels of genes (shown as red lines) decrease significantly relative to all genes (grey histogram). The dashed red line indicates the mean change for these selected genes. The black dashed line indicates the mean fold change for the rest of the genes.

42. A method for diagnosing an angiogenic disorder in a patient, the method comprising the steps of:

(a) detecting the expression level of at least one gene set forth in Table 1 or 2 or a biomarker identified according to the method of claim ~~34~~ 35 in a sample obtained from the patient prior to any administration of a VEGF antagonist to the patient; and

(b) comparing the expression level of the at least one gene or biomarker to a reference level of the at least one gene;

wherein a change in the level of expression of the at least one gene in the patient sample relative to the reference level identifies a patient having an angiogenic disorder.

43. The method of claim 42, further comprising (c) selecting a VEGF antagonist for treatment of said patient when a change in the level of expression of the at least one gene in the patient sample relative to the reference level is detected.

44. The method of claim 43, further comprising (d) administering a VEGF antagonist to the patient.

45. The method of any one of claims 1, 2, 21, 35, or 42, wherein the change in the level of expression of the at least one gene or biomarker in the patient sample relative to the reference level is determined by calculating a VDV signature score (VDV_i) for the patient sample according to the algorithm:

$$VDV_i = \frac{1}{\sqrt{n}} \sum_{g=1}^n Z_{g,i}$$

wherein $Z_{g=1,i}, Z_{g=2,i}, \dots, Z_{g=n,i}$ are standardized z-scores of expression values for each gene or biomarker g , from $g=1$ to $g=n$, of the sample i , and

wherein a VDV_i below a first defined threshold indicates a decrease relative to the reference level, and a VDV_i above a second defined threshold indicates an increase relative to the reference level.

46. The method of claim 45, wherein the expression values for each gene or biomarker g , from $g=1$ to $g=n$, are qRT-PCR values for each gene g , from $g=1$ to $g=n$.

47. The method of claim 45, wherein the first defined threshold is from -4 to -0.5 and the second defined threshold is from 0.5 to 4.

5 48. The method of claim 47, wherein the first defined threshold is from -4 to -1 and the second defined threshold is from 1 to 4.

49. The method of claim 48, wherein the first defined threshold is from -4 to -1.5 and the second defined threshold is from 1.5 to 4.

10 50. The method of claim 49, wherein the first defined threshold is from -4 to -2 and the second defined threshold is from 2 to 4.

15 51. Use of a VEGF antagonist in the manufacture of a medicament for treating cancer in a patient, wherein the patient is or has been identified as being one who is likely to respond to treatment with a VEGF antagonist according to the methods of any one of claims 1, 2, 21 and 42.

52. The use of claim 51, wherein said VEGF antagonist is an anti-VEGF antibody.

20 53. The use of claim 52, wherein the anti-VEGF antibody is bevacizumab.

54. The use of claim 51, wherein the medicament is to be administered with at least a second agent.

25 55. The use of claim 54, wherein the second agent is selected from the group consisting of: an anti-neoplastic agent, a chemotherapeutic agent, a growth inhibitory agent, a cytotoxic agent, and combinations thereof.