

DIAGNOSTIC METHODS AND COMPOSITIONS FOR TREATMENT OF GLIOBLASTOMA

Field of the Invention

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

Background of the Invention

10 Gliomas account for 81% of all malignant brain and CNS tumors. Glioblastoma (glioblastoma multiforme (GBM); World Health Organization (WHO) grade IV astrocytoma), in particular, accounts for 60% to 70% of malignant gliomas and remains the most aggressive subtype of glioma. It occurs mostly in adults (median age at diagnosis: 64 years) and its incidence is estimated to be 3.05/100,000 in the United States. With 1- and 5-year overall survival of 29% and 3%, respectively, the prognosis of glioblastoma remains particularly poor (Central Brain Tumor Registry of the United States (2005) (CBTRUS; <http://www.cbtrus.org>)).

15 Measuring expression levels of biomarkers can be an effective means to identify patients having glioblastomas that will respond to specific therapies including, e.g., treatment with VEGF antagonists, such as anti-VEGF antibodies.

20 There is a need for an effective means of determining which patients having glioblastomas 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 other agents.

Summary

25 The present disclosure is defined as specified in the attached set of claims. Also disclosed herein are methods for identifying patients having glioblastomas who will likely respond to treatment with a VEGF antagonist (e.g., an anti-VEGF antibody, e.g., bevacizumab). These patients are identified based on expression level of at least one of the genes set forth in Table 1, 2, or 3 below.

30 In a first example, the disclosure provides methods of determining whether a patient having a glioblastoma is likely to respond to treatment with a VEGF antagonist, the methods including: (a) detecting expression of at least one of the genes set forth in Table 1, 2, or 3 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the genes listed in Table 3 and/or at least one different gene (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, or 60 or more different genes) listed in Table 2 and/or at least one different gene (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, or 100 or more different genes) listed in Table 1) in a biological sample obtained from the patient prior to 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) informing the patient that they have an increased likelihood of being responsive to treatment with a VEGF antagonist.

21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, or 100 or more different genes) listed in Table 1) in a biological sample obtained from the patient prior to administration of a VEGF antagonist to the patient, wherein the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2; (b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 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) providing a recommendation to the patient that the anti-cancer therapy include a VEGF antagonist.

In a sixth example, the disclosure includes methods of selecting a therapy for a patient having a glioblastoma, the method including: (a) detecting expression of at least one of the genes set forth in Table 1, 2, or 3 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the genes listed in Table 3 and/or at least one different gene (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, or 60 or more different genes) listed in Table 2 and/or at least one different gene (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, or 100 or more different genes) listed in Table 1) in a biological sample obtained from the patient prior to administration of a VEGF antagonist to the patient, wherein the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2; (b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 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 therapy including a VEGF antagonist if the patient is identified as likely to respond to treatment with a VEGF antagonist and, optionally, recommending to the patient the selected therapy including a VEGF antagonist.

In one aspect, there is provided a method for selecting a therapy for a patient having a glioblastoma, the method comprising:

(a) detecting expression of at least one gene in a biological sample obtained from the patient prior to administration of a VEGF antagonist to the patient, wherein the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2;

(b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 in the patient sample relative to the reference level identifies the patient as one who has a glioblastoma of the proneural subtype and is likely to respond to treatment with a VEGF antagonist; and

(c) selecting a therapy comprising a VEGF antagonist if the patient is identified as likely to respond to treatment with a VEGF antagonist and recommending to the patient the selected therapy comprising a VEGF antagonist.

In any of the methods of the fourth, fifth, and sixth examples, the patient can be in a population of patients having glioblastomas and being tested for responsiveness to a VEGF antagonist and the reference level can be the median level of expression of the at least one gene in the population of

patients. In other examples of these methods, the reference level may be the median level of expression of the at least one gene in patients having glioblastomas and identified as not responding to VEGF antagonist treatment.

5 In a seventh example, the disclosure provides methods of determining whether a patient having a glioblastoma is likely to respond to treatment with a VEGF antagonist, the methods including: (a) detecting expression of at least one of the genes set forth in Table 1, 2, or 3 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the genes listed in Table 3 and/or at least one different gene (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, or 60 or more different genes) listed in Table 2 and/or at least one different gene (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 10 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, or 100 or more different genes) listed in Table 1) in a biological sample obtained from the patient prior to 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

comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist.

In another aspect, there is provided a method of determining whether a patient having a glioblastoma is likely to respond to treatment with a VEGF antagonist, the method comprising:

(a) detecting expression of at least one gene in a biological sample obtained from the patient prior to administration of a VEGF antagonist to the patient, wherein the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2; and

(b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 in the patient sample relative to the reference level identifies the patient as one who has a glioblastoma of the proneural subtype and is likely to respond to treatment with a VEGF antagonist.

In an eleventh example, the disclosure provides methods of optimizing therapeutic efficacy of an anti-cancer therapy for a patient having a glioblastoma, the methods including: (a) detecting expression of at least one of the genes set forth in Table 1, 2, or 3 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the genes listed in Table 3 and/or at least one different gene (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, or 60 or more different genes) listed in Table 2 and/or at least one different gene (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, or 100 or more different genes) listed in Table 1) in a biological sample obtained from the patient prior to administration of a VEGF antagonist to the patient, wherein the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2; and (b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist.

In another aspect, there is provided a method of optimizing therapeutic efficacy of an anti-cancer therapy for a patient having a glioblastoma, the method comprising:

(a) detecting expression of at least one gene in a biological sample obtained from the patient prior to administration of a VEGF antagonist to the patient, wherein the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2; and

(b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 in the patient sample relative to the reference level identifies the patient as one who has a glioblastoma of the proneural subtype and is likely to respond to treatment with a VEGF antagonist.

In a twelfth example, the disclosure includes methods of selecting a therapy for a patient having a glioblastoma, the method including: (a) detecting expression of at least one of the genes set forth in Table 1, 2, or 3 (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 of the genes listed in Table 3 and/or at least one different gene (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, or 60 or more different genes) listed in Table 2 and/or at least one different gene (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 50, 60, 70, 80, 90, or 100 or more different genes) listed in Table 1) in a biological sample obtained from the patient prior to administration of a VEGF antagonist to the patient, wherein the at least one gene is selected from the group consisting NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2; and (b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist.

In any of the methods of the tenth, eleventh, and twelfth examples, the patient can be in a population of patients having glioblastomas and being tested for responsiveness to a VEGF antagonist and the reference level can be the median level of expression of the at least one gene in the population of patients. In other examples of these methods, the reference level may be the median level of expression of the at least one gene in patients having glioblastomas and identified as not responding to VEGF antagonist treatment.

In a thirteenth example, the disclosure includes a VEGF antagonist for use in the optimization of therapeutic efficacy of an anti-cancer therapy for a patient having a glioblastoma, wherein the patient, prior to any administration of a VEGF antagonist, expresses a different level (e.g., an increased level or a

WHAT IS CLAIMED IS:

1. A method of determining whether a patient having a glioblastoma is likely to respond to treatment with a VEGF antagonist, the method comprising:

(a) detecting expression of at least one gene in a biological sample obtained from the patient prior to administration of a VEGF antagonist to the patient, wherein the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2; and

(b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 in the patient sample relative to the reference level identifies the patient as one who has a glioblastoma of the proneural subtype and is likely to respond to treatment with a VEGF antagonist.

2. A method of optimizing therapeutic efficacy of an anti-cancer therapy for a patient having a glioblastoma, the method comprising:

(a) detecting expression of at least one gene in a biological sample obtained from the patient prior to administration of a VEGF antagonist to the patient, wherein the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2; and

(b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 in the patient sample relative to the reference level identifies the patient as one who has a glioblastoma of the proneural subtype and is likely to respond to treatment with a VEGF antagonist.

3. A method for selecting a therapy for a patient having a glioblastoma, the method comprising:

(a) detecting expression of at least one gene in a biological sample obtained from the patient prior to administration of a VEGF antagonist to the patient, wherein the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2;

(b) comparing the expression level of the at least one gene to a reference expression level of the at least one gene, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and/or PFN2 in the patient sample relative

to the reference level identifies the patient as one who has a glioblastoma of the proneural subtype and is likely to respond to treatment with a VEGF antagonist; and

(c) selecting a therapy comprising a VEGF antagonist if the patient is identified as likely to respond to treatment with a VEGF antagonist and recommending to the patient the selected therapy comprising a VEGF antagonist.

4. The method of any one of claims 1-3, wherein the patient is in a population of patients having glioblastomas and 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.

5. The method of any one of claims 1-3, wherein the reference level is the median level of expression of the at least one gene in patients having glioblastomas and identified as not responding to VEGF antagonist treatment.

6. The method of any one of claims 1-5, wherein:

(i) expression of the at least one gene in the biological sample obtained from the patient is detected by measuring mRNA; and/or

(ii) expression of the at least one gene in the biological sample obtained from the patient is detected by measuring plasma protein levels.

7. The method of claim 3, wherein the therapy of (c) is an agent selected from the group consisting of an anti-neoplastic agent, a chemotherapeutic agent, a growth inhibitory agent, and a cytotoxic agent, radiotherapy, or a combination thereof.

8. The method of claim 3, wherein the therapy of (c) is temozolomide (TMZ).

9. The method of claim 1, further comprising:

(c) informing the patient that they have an increased likelihood of being responsive to treatment with a VEGF antagonist.

10. The method of claim 2, further comprising:

(c) providing a recommendation to the patient that the anti-cancer therapy comprise a VEGF antagonist.

11. The method of any one of claims 1-10, wherein the biological sample is tumor tissue.

12. The method of any one of claims 1-11, further comprising detecting expression of at least two of said genes in the biological sample from the patient.

13. The method of claim 12, further comprising detecting expression of at least three of said genes in the biological sample from the patient.

14. The method of claim 13, further comprising detecting expression of at least four of said genes in the biological sample from the patient.

15. The method of claim 14, further comprising detecting expression of at least a fourth of said genes in the biological sample from the patient.

16. The method of claim 15, further comprising detecting expression of at least a third of said genes in the biological sample from the patient.

17. The method of any one of claims 1-16, wherein the method comprises detecting expression of all of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2 in the biological sample from the patient.

18. The method of claim 17 further comprising comparing the expression level of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2 in the patient sample to a reference expression level of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2, wherein an increase in the level of expression of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2 in the patient sample relative to the reference level identifies the patient as one who has a glioblastoma of the proneural subtype and is likely to respond to treatment with a VEGF antagonist.

19. The method of any one of claims 1-18, wherein the VEGF antagonist is an anti-VEGF antibody.

20. The method of claim 19, wherein:

- (i) the anti-VEGF antibody binds the A4.6.1 epitope;
- (ii) the anti-VEGF antibody is bevacizumab; or

(iii) the 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 .

21. The method of any one of claims 1-20, wherein responsiveness to treatment with a VEGF antagonist is an increase in overall survival.

22. The method of any one of claims 1-21, further comprising detecting expression of at least one different gene listed in Table 1 or 2.

23. The method of claim 22, further comprising comparing the expression level of the at least one different gene listed in Table 1 or 2 in the patient sample to a reference expression level of the at least one different gene listed in Table 1 or 2, wherein a change in the level of expression of the at least one different gene listed in Table 1 or 2 in the patient sample relative to the reference level identifies the patient as one who has a glioblastoma of the proneural subtype and is likely to respond to treatment with a VEGF antagonist.

24. The method of claim 23, wherein the change in level of expression of the at least one different gene in the patient sample is:

- (i) an increase relative to the reference level; or
- (ii) a decrease relative to the reference level.