

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 of the Invention

25 The present invention 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.

In a second example, the disclosure also provides methods of optimizing 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; (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) providing a recommendation to the patient that the anti-cancer therapy include a VEGF antagonist.

In the methods described above, 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 the methods described above, the change in level of expression of the at least one gene in the patient sample can be an increase or a decrease relative to the reference level.

Expression of the at least one gene in the biological sample obtained from the patient can be detected by measuring, for example, mRNA levels and/or plasma protein levels.

The biological sample can be, for example, tumor tissue, such as a tumor tissue biopsy or a blood plasma sample.

These methods described herein can further include detecting expression of at least two, three, four, or more genes in a biological sample from the patient. In some examples, the methods described herein can further include detecting expression of at least a fourth or at least a third of the genes in a biological sample from the patient.

In the methods described above, the VEGF antagonist can be an anti-VEGF antibody. The anti-VEGF antibody can be, for example, an anti-VEGF antibody that binds the A4.6.1 epitope, bevacizumab, or an 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.

The methods described above can further include a step of administering a VEGF antagonist to the patient. The administered VEGF antagonist can be an anti-VEGF antibody, for example, an anti-VEGF antibody that binds the A4.6.1 epitope, bevacizumab, or an anti-VEGF antibody comprises a variable heavy chain (VH) and a variable light chain (VL), wherein the VH has an amino acid sequence of SEQ ID NO: 2 and the VL has an amino acid sequence of SEQ ID NO: 1.

The methods described above can further include carrying out therapy using (i) an agent selected from the group consisting of an anti-neoplastic agent, a chemotherapeutic agent, a growth inhibitory

agent, and a cytotoxic agent, (ii) radiotherapy, or (iii) a combination thereof. In some examples, the methods described above can further include administering a chemotherapeutic agent, such as temozolomide (TMZ), to the patient.

5 In the methods described above, responsiveness to treatment with a VEGF antagonist can be, for example, an increase in, or extension of, overall survival (OS). In some examples, responsiveness to treatment with a VEGF antagonist can be, for example, an increase in, or extension of, progression-free survival (PFS).

10 In the methods described above, a patient found to be likely to respond to treatment with a VEGF antagonist can have, for example, a glioblastoma of the proneural (PN) type (proneural subtype).

In a third 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, 15 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 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, 20 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 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.

25 In these methods, the reference level can be the median level of expression of the at least one gene in a population of patients having glioblastomas. In some examples of these methods, the reference level can 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.

30 In these methods, the change in level of expression of the at least one gene in the patient sample can be an increase or a decrease relative to the reference level. Expression of the at least one gene in the biological sample obtained from the patient can be detected by measuring, for example, mRNA levels and/or plasma protein levels. The biological sample can be, for example, tumor tissue, such as a tumor tissue biopsy or a blood plasma sample.

35 These methods described herein can further include detecting expression of at least two, three, four, or more genes in a biological sample from the patient. In some examples of these methods, further detection of the expression of at least a fourth or at least a third of the genes in a biological sample from the patient.

40 In these methods, the therapy of step (c) can be 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. In some examples, these methods further include administering a chemotherapeutic agent, such as TMZ, to the patient.

These methods can further include the step of (d): 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. The administered VEGF antagonist can be anti-VEGF antibody, for example, an anti-VEGF antibody that binds the A4.6.1 epitope, bevacizumab, or an anti-VEGF antibody comprises a VH and a VL, wherein the VH has an amino acid sequence of SEQ ID NO: 2 and the VL has an amino acid sequence of SEQ ID NO: 1. In some examples, the methods further include administering an effective amount of at least a second agent. The second agent can, for example, be 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 examples, the second agent is TMZ.

In these methods, responsiveness to treatment with a VEGF antagonist can be, for example, an increase in, or extension of, OS. In some examples of these methods, responsiveness to treatment with a VEGF antagonist can be, for example, an increase in, or extension of, PFS.

In these methods, a patient found to be likely to respond to treatment with a VEGF antagonist can have, for example, a glioblastoma of the PN type (PN subtype).

In any of the methods of the first, second, and third examples, the at least one gene can be selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2. When the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2, the change in level of expression of the at least one gene in the patient sample may be an increase relative to the reference level.

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

In a fifth 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; (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 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.

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, 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

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 some examples, the methods of the seventh example can further include informing the patient that they have an increased likelihood of being responsive to treatment with a VEGF antagonist.

In an eighth example, the disclosure also provides methods of optimizing 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; 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. In some examples, the methods of the eighth example can further include providing a recommendation to the patient that the anti-cancer therapy comprise a VEGF antagonist.

In some examples of any of the methods of the seventh and eighth 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 some instances of any of the methods of the seventh and eighth examples, the change in level of expression of the at least one gene in the patient sample can be an increase or a decrease relative to the reference level.

In some instances of any of the methods of the seventh and eighth examples, expression of the at least one gene in the biological sample obtained from the patient can be detected by measuring, for example, mRNA levels and/or plasma protein levels.

In some instances of any of the methods of the seventh and eighth examples, the biological sample can be, for example, tumor tissue, such as a tumor tissue biopsy or a blood plasma sample.

The methods of the seventh and eighth examples of the disclosure can further include detecting expression of at least two, three, four, or more genes in a biological sample from the patient. In some instances, the methods of the seventh and eighth examples of the disclosure can further include detecting expression of at least a fourth or at least a third of the genes in a biological sample from the patient.

In some instances of any of the methods of the seventh and eighth examples, the VEGF antagonist can be an anti-VEGF antibody. The anti-VEGF antibody can be, for example, an anti-VEGF antibody that binds the A4.6.1 epitope, bevacizumab, or an 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.

The methods of the seventh and eighth examples of the disclosure can further include a step of administering a VEGF antagonist to the patient. The administered VEGF antagonist can be anti-VEGF antibody, for example, an anti-VEGF antibody that binds the A4.6.1 epitope, bevacizumab, or an anti-VEGF antibody comprises a variable heavy chain (VH) and a variable light chain (VL), wherein the VH has an amino acid sequence of SEQ ID NO: 2 and the VL has an amino acid sequence of SEQ ID NO: 1.

In some instances, any of the methods of the seventh and eighth examples can further include carrying out therapy using (i) an agent selected from the group consisting of an anti-neoplastic agent, a chemotherapeutic agent, a growth inhibitory agent, and a cytotoxic agent, (ii) radiotherapy, or (iii) a combination thereof. In some examples, the methods described above can further include administering a chemotherapeutic agent, such as temozolomide (TMZ), to the patient.

In some instances of any of the methods of the seventh and eighth examples, responsiveness to treatment with a VEGF antagonist can be, for example, an increase in, or extension of, overall survival (OS). In some examples, responsiveness to treatment with a VEGF antagonist can be, for example, an increase in, or extension of, progression-free survival (PFS).

In some instances of any of the methods of the seventh and eighth examples, a patient found to be likely to respond to treatment with a VEGF antagonist can have, for example, a glioblastoma of the proneural (PN) type (proneural subtype).

In a ninth example, the disclosure features 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 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. In some instances, the methods of the ninth example can further include 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 some instances of the methods of the ninth example, the reference level can be the median level of expression of the at least one gene in a population of patients having glioblastomas. In some instances of the methods of the ninth example, the reference level can 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 some instances of the methods of the ninth example, the change in level of expression of the at least one gene in the patient sample can be an increase or a decrease relative to the reference level. Expression of the at least one gene in the biological sample obtained from the patient can be detected by measuring, for example, mRNA levels and/or plasma protein levels. The biological sample can be, for example, tumor tissue, such as a tumor tissue biopsy or a blood plasma sample.

The methods of the ninth example of the disclosure can further include detecting expression of at least two, three, four, or more genes in a biological sample from the patient. In some examples of these methods, further detection of the expression of at least a fourth or at least a third of the genes in a biological sample from the patient.

In some instances of the methods of the ninth example, the therapy of step (d) can be 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. In some examples, these methods further include administering a chemotherapeutic agent, such as TMZ, to the patient.

The methods of the ninth example of the disclosure can further include the step of (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. The administered VEGF antagonist can be an anti-VEGF antibody, for example, an anti-VEGF antibody that binds the A4.6.1 epitope, bevacizumab, or an anti-VEGF antibody comprises a VH and a VL, wherein the VH has an amino acid sequence of SEQ ID NO: 2 and the VL has an amino acid sequence of SEQ ID NO: 1.

In some instances of the methods of the ninth example, the methods further include administering an effective amount of at least a second agent. The second agent can, for example, be 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 examples, the second agent is TMZ.

In some instances of the methods of the ninth example, responsiveness to treatment with a VEGF antagonist can be, for example, an increase in, or extension of, OS. In some examples of these methods, responsiveness to treatment with a VEGF antagonist can be, for example, an increase in, or extension of, PFS.

In some instances of the methods of the ninth example, a patient found to be likely to respond to treatment with a VEGF antagonist can have, for example, a glioblastoma of the PN type (PN subtype).

In any of the methods of the seventh, eighth, and ninth examples, the at least one gene can be selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2. When the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2, the change in level of expression of the at least one gene in the patient sample may be an increase relative to the reference level.

In a tenth 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, 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 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 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

decreased level) of at least one of the genes set forth in Table 1, 2, or 3 relative to a reference expression level of the at least one gene. In some instances of the thirteenth example, the VEGF antagonist is to be administered to the patient having at least one of the genes set forth in Table 1, 2, or 3 expressed at a different level (e.g., an increased level or a decreased level) relative to the reference expression level of the at least one gene.

In a fourteenth example, the disclosure includes a VEGF antagonist for use in the treatment of a patient having a glioblastoma, wherein the patient, prior to administration of a VEGF antagonist, expresses a different level (e.g., an increased level or a decreased level) of at least one of the genes set forth in Table 1, 2, or 3.

In a fifteenth example, the disclosure includes 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 an increased level of at least one gene selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2 relative to a reference expression level of the at least one gene. In some instances of the fifteenth example, the VEGF antagonist is to be administered to the patient having at least one gene selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2 expressed at an increased level relative to the reference expression level of the at least one gene.

In a sixteenth example, the disclosure includes a VEGF antagonist for use in the treatment of a patient having a glioblastoma, wherein the patient, prior to administration of a VEGF antagonist, expresses an increased level of at least one gene selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2. In another example, the disclosure features a kit for determining whether a patient may benefit from treatment with a VEGF antagonist, the kit including: (a) polypeptides or polynucleotides capable of determining the expression level 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); and (b) instructions for use of the polypeptides or polynucleotides to determine the expression level of at least one of the genes set forth in Table 1, 2, or 3, wherein a change in the level of expression of the at least one gene relative to a reference level indicates that the patient may benefit from treatment with a VEGF antagonist.

Brief Description of the Drawings

FIGURE 1 is heatmap showing the results of the unsupervised analysis (PAM clustering) of the AvaGliO samples into k=3 groups according to the expression of the extended 108 classifiers genes. Samples assigned with a negative Silhouette width were labeled "Unclassified." Row annotation highlights the signature genes. Column annotation indicates the PAM clusters/gene expression subtype assignment for the samples.

WHAT IS CLAIMED IS:

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

(a) detecting expression of at least one of the genes set forth in Table 1, 2, or 3 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.

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

(a) detecting expression of at least one of the genes set forth in Table 1, 2, or 3 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) providing a recommendation to the patient that the anti-cancer therapy comprise a VEGF antagonist.

3. The method of claim 1 or 2, wherein the patient is in a population of patients having proneural 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.

4. The method of claim 1 or 2, wherein the reference level is the median level of expression of the at least one gene in patients having proneural glioblastomas and identified as not responding to VEGF antagonist treatment.

5. The method of any one of claims 1 to 4, wherein:

- (i) the change in level of expression of the at least one gene in the patient sample is an increase relative to the reference level; or
- (ii) the change in level of expression of the at least one gene in the patient sample is a decrease relative to the reference level.

6. The method of any one of claims 1 to 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. A method for selecting a therapy for a patient having a proneural glioblastoma, the method comprising:

(a) detecting expression of at least one of the genes set forth in Table 1, 2, or 3 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 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.

8. The method of claim 7, wherein the reference level is:

(i) the median level of expression of the at least one gene in a population of patients having proneural glioblastomas; or

(ii) the median level of expression of the at least one gene in patients having proneural glioblastomas and identified as not responding to VEGF antagonist treatment.

9. The method of claim 7 or 8, wherein:

(i) the change in level of expression of the at least one gene in the patient sample is an increase relative to the reference level; or

(ii) the change in level of expression of the at least one gene in the patient sample is a decrease relative to the reference level.

10. The method of claim 7, 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.

11. The method of claim 7, wherein the therapy of (c) is temozolomide (TMZ).

12. A method of determining whether a patient having a proneural 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;

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

13. A method of optimizing therapeutic efficacy of an anti-cancer therapy for a patient having a proneural 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 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 comprise a VEGF antagonist.

14. A method for selecting a therapy for a patient having a proneural 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 a patient who 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.

15. The method of any one of claims 12 to 14, wherein the patient is in a population of patients having proneural 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.

16. The method of any one of claims 12 to 14, wherein the reference level is the median level of expression of the at least one gene in patients having proneural glioblastomas and identified as not responding to VEGF antagonist treatment.

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

(a) detecting expression of at least one of the genes set forth in Table 1, 2, or 3 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 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.

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

(a) detecting expression of at least one of the genes set forth in Table 1, 2, or 3 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 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.

19. The method of claim 17 or 18, 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.

20. The method of claim 17 or 18, wherein the reference level is the median level of expression of the at least one gene in patients having proneural glioblastomas and identified as not responding to VEGF antagonist treatment.

21. The method of claim 17 or 18, wherein:

- (i) the change in level of expression of the at least one gene in the patient sample is an increase relative to the reference level; or
- (ii) the change in level of expression of the at least one gene in the patient sample is a decrease relative to the reference level.

22. The method of claim 17 or 18, wherein:

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

23. The method of any one of claims 1-22, wherein the biological sample is tumor tissue.

24. The method of any one of claims 1-23, further comprising detecting expression of at least two of said genes in the biological sample from the patient.

25. The method of claim 24, further comprising detecting expression of at least three of said genes in the biological sample from the patient.

26. The method of claim 25, further comprising detecting expression of at least four of said genes in the biological sample from the patient.

27. The method of claim 26, further comprising detecting expression of at least a fourth of said genes in the biological sample from the patient.

28. The method of claim 27, further comprising detecting expression of at least a third of said genes in the biological sample from the patient.

29. The method of any one of claims 1-28, wherein the VEGF antagonist is an anti-VEGF antibody.

30. The method of claim 29, 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 .

31. The method of any one of claims 1-30, wherein responsiveness to treatment with a VEGF antagonist is an increase in overall survival.

32. The method of any one of claims 1-31, wherein the at least one gene is selected from the group consisting of NCAM1, OMG, PRKCZ, GALNT13, GPR17, DNM3, FERMT1, SNAP91, ABHD6, and PFN2.

33. The method of claim 32, wherein the change in level of expression of the at least one gene in the patient sample is an increase relative to the reference level.

34. The method of claim 17, further comprising informing the patient that they have an increased likelihood of being responsive to treatment with a VEGF antagonist.

35. The method of claim 18, further comprising providing a recommendation to the patient that the anti-cancer therapy comprise a VEGF antagonist.

36. A method of determining whether a patient having a proneural 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 a patient who is likely to respond to treatment with a VEGF antagonist.

37. A method of optimizing therapeutic efficacy of an anti-cancer therapy for a patient having a proneural 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 a patient who is likely to respond to treatment with a VEGF antagonist.

38. A method for selecting a therapy for a patient having a proneural 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 a patient who 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.

39. The method of any one of claims 36-38, wherein the patient is in a population of patients having proneural 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.

40. The method of any one of claims 36-38, wherein the reference level is the median level of expression of the at least one gene in patients having proneural glioblastomas and identified as not responding to VEGF antagonist treatment.