

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

10 Measuring expression levels of biomarkers (*e.g.*, secreted proteins in plasma) can be 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

 The present disclosure 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 provided a method of determining whether a patient is likely to respond to treatment with a VEGF antagonist, the method comprising (a) detecting expression of at least three genes 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 three genes to a reference expression
25 level of the at least three genes, wherein a change in the level of expression of the at least three genes in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist, wherein the VEGF antagonist is an anti-VEGF antibody and the change in the level of expression of the at least three genes in the patient sample is an increase relative to the reference level.

30 In a further aspect, there is provided a method of optimizing therapeutic efficacy of a VEGF antagonist for a patient, the method comprising: (a) detecting expression of at least three genes 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 three genes to a reference expression level of the at least three genes, wherein a change in the level of expression of the at least three genes in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist, wherein the VEGF antagonist is an anti-VEGF antibody and the change in the level of expression of the at least three genes in the patient sample is an increase relative to the reference level.

Referring to the aspects set forth above, in some 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 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 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, 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 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 provided a method for selecting a therapy for a particular patient in a population of patients being considered for therapy, the method comprising: (a) detecting expression of at least three genes 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 three genes to a reference expression level of the at least three genes, wherein a change in the level of expression of the at least three genes in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist, wherein the change in the level of expression of the at least three genes in the patient sample is an increase relative to the reference level, 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, wherein the VEGF antagonist is an anti-VEGF antibody.

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 provided~~Disclosed is a method of identifying a biomarker for determining responsiveness to a VEGF antagonist, the method 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, wherein the VEGF antagonist is an anti-VEGF antibody and the change in the level of expression of the candidate biomarker in the patient sample is an increase relative to the reference level,

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 provided~~Disclosed is a method for diagnosing an angiogenic
 10 disorder in a patient, the method comprising the steps of: (a) detecting the expression level of at least three genes 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; (b) comparing the expression level of the at least three genes or biomarker to a reference level of the at least three genes or biomarker,
 15 wherein a change in the level of expression of the at least three genes or biomarker in the patient sample relative to the reference level identifies a patient having an angiogenic disorder; and (c) selecting an VEGF antagonist for treatment of said patient when a change in the level of expression of the at least three genes or biomarker in the patient sample relative to the reference level is detected, wherein the VEGF antagonist is an anti-VEGF antibody;
 20 wherein the change in the level of expression of the at least three genes 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

25 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.

In addition, the method can further include (d) administering a VEGF antagonist to the patient. In addition, the methods described herein can include a step of obtaining a sample
 30 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.

WHAT IS CLAIMED IS:

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

5 (a) detecting expression of at least three genes 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 three genes to a reference expression level of the at least three genes,

10 wherein a change in the level of expression of the at least three genes in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist, wherein the VEGF antagonist is an anti-VEGF antibody and the change in the level of expression of the at least three genes in the patient sample is an increase relative to the reference level.

15 2. A method of optimizing therapeutic efficacy of a VEGF antagonist for a patient, the method comprising:

(a) detecting expression of at least three genes 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

20 (b) comparing the expression level of the at least three genes to a reference expression level of the at least three genes,

wherein a change in the level of expression of the at least three genes in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist, wherein the VEGF antagonist is an anti-VEGF antibody and the change in the level of expression of the at least three genes in the patient sample is an increase relative to the reference level.

3. The method of claim 1 or 2, wherein 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 three genes in the population of patients.

4. The method of claim 1 or 2, wherein expression of the at least three genes in the biological sample obtained from the patient is detected by measuring mRNA.

5. The method of claim 1 or 2, wherein expression of the at least three genes in the biological sample obtained from the patient is detected by measuring plasma protein levels.

6. The method of claim 1 or 2, wherein the biological sample is tumor tissue.

7. The method of claim 1 or 2, further comprising detecting expression of at least a fourth gene set forth in Table 1 or 2 in the biological sample from the patient.

8. The method of claim 1 or 2, wherein the at least three genes is selected from the group consisting of: Alk1, CD34, CD105, CD144, Col4a1, Col4a2, Dll4, EFNB2, EGFL7, ESM1, LAMA4, NG2, Nid2, Notch1, NRP1, NRP2, RGS3, Sema3f, TSP1, VEGFR1, VEGFR2, VEGFR3, and VIM.

9. The method of claim 1 or 2, wherein the anti-VEGF antibody is bevacizumab.

10. The method of claim 1 or 2, wherein the patient has an angiogenic disorder.

11. The method of claim 10, wherein the patient has cancer selected from the group consisting of: colorectal cancer, breast cancer, lung cancer, glioblastoma, and combinations thereof.

12. The method of claim 1 or 2, further comprising (c) selecting an anti-VEGF antibody for treatment of said patient when a change in the level of expression of the at least three genes in the patient sample relative to the reference level is detected.

13. The method of claim 12, wherein the anti-VEGF antibody is bevacizumab.

14. A method for selecting a therapy for a particular patient in a population of patients being considered for therapy, the method comprising:

(a) detecting expression of at least three genes 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 three genes to a reference expression level of the at least three genes,

5 wherein a change in the level of expression of the at least three genes in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist, wherein the change in the level of expression of the at least three genes in the patient sample is an increase relative to the reference level, and

(c) selecting a VEGF antagonist as the therapy if the patient is identified as likely to
10 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,
wherein the VEGF antagonist is an anti-VEGF antibody.

15 15. The method of claim 14, wherein the reference level is the median level of expression of the at least three genes in the population of patients.

16. The method of claim 14, further comprising detecting expression of at least a
20 fourth gene set forth in Table 1 or 2 in the biological sample from the patient.

17. The method of claim 14, 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, a cytotoxic agent, and combinations thereof.

25 18. The method of claim 14, wherein the anti-VEGF antibody is bevacizumab.

19. The method of claim 14, wherein 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.

30 20. A method of identifying a biomarker for determining responsiveness to a VEGF antagonist, the method 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, wherein the VEGF antagonist is an anti-VEGF antibody and the change in the level of expression of the candidate biomarker in the patient sample is an increase relative to the reference level,

wherein the change in the level of expression of the 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 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.

21. The method of claim 20, wherein the reference level is the median level of expression of the candidate biomarker in a population of patients being tested for the likelihood they will respond to a VEGF antagonist.

22. The method of claim 20, wherein the reference level is the expression level of the candidate biomarker in a sample previously obtained from the patient.

23. The method of claim 22, wherein the patient was previously treated with a VEGF antagonist and is currently experiencing metastasis.

24. The method of claim 20, wherein the anti-VEGF antibody is bevacizumab.

25. The method of claim 20, further comprising (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.

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

(a) detecting the expression level of at least three genes set forth in Table 1 or 2 or a biomarker identified according to the method of claim 20 in a 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 three genes or biomarker to a reference level of the at least three genes or biomarker, wherein a change in the level of expression of the

at least three genes or biomarker in the patient sample relative to the reference level identifies a patient having an angiogenic disorder; and

(c) selecting an VEGF antagonist for treatment of said patient when a change in the level of expression of the at least three genes or biomarker in the patient sample relative to the reference level is detected, wherein the VEGF antagonist is an anti-VEGF antibody,

wherein the change in the level of expression of the at least three genes 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.

27. The method of any one of claims 1, 2, or 14, wherein the change in the level of expression of the at least three genes 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.

28. The method of claim 27, 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 $g=n$.

29. The method of claim 27, wherein the first defined threshold is from -4 to -0.5 and the second defined threshold is from 0.5 to 4.

30. The method of claim 29, wherein the first defined threshold is from -4 to -1 and the second defined threshold is from 1 to 4.

31. The method of claim 30, wherein the first defined threshold is from -4 to -1.5 and the second defined threshold is from 1.5 to 4.

32. The method of claim 31, wherein the first defined threshold is from -4 to -2 and the second defined threshold is from 2 to 4.

33. Use of an anti-VEGF antibody 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 an anti-VEGF antibody according to the methods of any one of claims 1, 2, and 14.

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34. The use of claim 33, wherein the anti-VEGF antibody is bevacizumab.

35. The use of claim 33, wherein the medicament is to be administered with at least a second agent.

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36. The use of claim 35, 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.

WHAT IS CLAIMED IS:

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

5 (a) detecting expression of at least three genes set forth in Table 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 three genes to a reference expression level of the at least three genes,

10 wherein a change in the level of expression of the at least three genes in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist, wherein the VEGF antagonist is an anti-VEGF antibody and the change in the level of expression of the at least three genes in the patient sample is an increase relative to the reference level.

15 2. A method of optimizing therapeutic efficacy of a VEGF antagonist for a patient, the method comprising:

(a) detecting expression of at least three genes set forth in Table 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 three genes to a reference expression
20 level of the at least three genes,

25 wherein a change in the level of expression of the at least three genes in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist, wherein the VEGF antagonist is an anti-VEGF antibody and the change in the level of expression of the at least three genes in the patient sample is an increase relative to the reference level.

3. The method of claim 1 or 2, wherein 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 three genes in the population of patients.

30 4. The method of claim 1 or 2, wherein expression of the at least three genes in the biological sample obtained from the patient is detected by measuring mRNA.

5. The method of claim 1 or 2, wherein expression of the at least three genes in the biological sample obtained from the patient is detected by measuring plasma protein levels.

6. The method of claim 1 or 2, wherein the biological sample is tumor tissue.

7. The method of claim 1 or 2, further comprising detecting expression of at least a fourth gene set forth in Table 2 in the biological sample from the patient.

8. The method of claim 1 or 2, wherein the anti-VEGF antibody is bevacizumab.

9. The method of claim 1 or 2, wherein the patient has an angiogenic disorder.

10. The method of claim 9, wherein the patient has cancer selected from the group consisting of: colorectal cancer, breast cancer, lung cancer, glioblastoma, and combinations thereof.

11. The method of claim 1 or 2, further comprising (c) selecting an anti-VEGF antibody for treatment of said patient when an increase in the level of expression of the at least three genes in the patient sample relative to the reference level is detected.

12. The method of claim 11, wherein the anti-VEGF antibody is bevacizumab.

13. A method for selecting a therapy for a particular patient in a population of patients being considered for therapy, the method comprising:

(a) detecting expression of at least three genes set forth in Table 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 three genes to a reference expression level of the at least three genes,

wherein a change in the level of expression of the at least three genes in the patient sample relative to the reference level identifies a patient who is likely to respond to treatment with a VEGF antagonist, wherein the change in the level of expression of the at least three genes in the patient sample is an increase relative to the reference level, 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,

wherein the VEGF antagonist is an anti-VEGF antibody.

14. The method of claim 13, wherein the reference level is the median level of expression of the at least three genes in the population of patients.

15. The method of claim 13, further comprising detecting expression of at least a fourth gene set forth in Table 2 in the biological sample from the patient.

16. The method of claim 13, 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, a cytotoxic agent, and combinations thereof.

17. The method of claim 13, wherein the anti-VEGF antibody is bevacizumab.

18. The method of claim 13, wherein 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.

19. The method of any one of claims 1, 2, and 13, wherein the change in the level of expression of the at least three genes 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 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.

20. The method of claim 19, wherein the expression values for each gene g , from $g=1$ to $g=n$, are qRT-PCR values for each gene g , from $g=1$ to $g=n$.

5 21. The method of claim 19, wherein the first defined threshold is from -4 to -0.5 and the second defined threshold is from 0.5 to 4.

10 22. The method of claim 21, wherein the first defined threshold is from -4 to -1 and the second defined threshold is from 1 to 4.

15 23. The method of claim 22, wherein the first defined threshold is from -4 to -1.5 and the second defined threshold is from 1.5 to 4.

24. The method of claim 23, wherein the first defined threshold is from -4 to -2 and the second defined threshold is from 2 to 4.

20 25. Use of an anti-VEGF antibody 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 an anti-VEGF antibody according to the methods of any one of claims 1, 2, and 13.

25 26. The use of claim 25, wherein the anti-VEGF antibody is bevacizumab.

27. The use of claim 25, wherein the medicament is to be administered with at least a second agent.

30 28. The use of claim 27, 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.