US 20150225483 A1 describes administration of the Trap molecule at doses based on the patient's weight.

[0008] The present disclosure provides methods for treating a subject diagnosed with TNBC, wherein the subject has been first determined to have an increased expression level of high mobility group AT-hook 2 (HMGA2) and/or MDS1 and EVI1 complex locus protein EVI1 (MECOM) relative to a known control expression level, and then administering anti-PD-L1/TGFβ Trap protein to the subject.

SUMMARY OF THE DISCLOSURE

[0009] For an effective treatment of patients diagnosed with TNBC, the present disclosure provides a therapeutic regimen that treats TNBC in patients determined to have an increased expression level of HMGA2 or MECOM relative to a known expression level, and improves disease prognosis and overall survival of TNBC patients.

[0009a] In one aspect, the present disclosure provides use of an anti-TGFβ agent in the manufacture of a medicament for treating or managing triple negative breast cancer (TNBC) in a patient, wherein the medicament is to be administered to a patient who has been determined to have an increased level of high mobility group AT-hook 2 (HMGA2) expression relative to a known control level, and thereby treating TNBC in the patient.

[0009b] In another aspect, the present disclosure provides use of an anti-TGFβ agent in the manufacture of a medicament for achieving at least a partial response in treating or increasing survival of a triple negative breast cancer (TNBC) patient, wherein the medicament is to be administered to a patient who has been determined to have an increased level of high mobility group AT-hook 2 (HMGA2) expression relative to a known control level, and thereby achieving at least a partial response in treating TNBC in the patient.

[0009c] In yet another aspect, the present disclosure provides a method of identifying a patient suitable for treating or managing triple negative breast cancer (TNBC) in the patient with an anti-TGFβ agent, the method comprising determining the level of high mobility group AT-hook 2 (HMGA2) in a tissue sample from the patient, wherein an increased level of HMGA2 expression in the patient, relative to a known control level, identifies the patient as suitable for treating TNBC with said anti-TGFβ agent.

[0010] In one example, the present disclosure provides a method of treating or managing TNBC in a subject by administering an anti-TGFβ agent to a subject who has been determined to have an increased level of high mobility group AT-hook 2 (HMGA2) or MDS1 and EVI1 complex locus (MECOM) expression relative to a corresponding known control level, and thereby treating TNBC in the subject.

[0011] In another example, the present disclosure provides a method of achieving at least a partial response in treating or increasing survival of a triple negative breast cancer (TNBC) patient by administering an anti-TGFβ agent to a subject who has been determined to have an increased level of high mobility group AT-hook 2 (HMGA2) expression or MDS1 and EVI1 complex locus (MECOM) relative to a corresponding known control level, and thereby achieving at least a partial response in treating or increasing survival of a triple negative breast cancer (TNBC) patient.

[0012] In another example, the present disclosure provides a method of identifying a subject suitable for treating or managing TNBC in the subject with an anti-TGFβ agent, the method comprising determining the level of high mobility group AT-hook 2 (HMGA2) or MDS1 and EVI1 complex locus (MECOM) in the subject, wherein an increased level of HMGA2 or MECOM expression in the subject, relative to a corresponding known control level, identifies the subject as suitable for treating TNBC with the anti-TGFβ agent.

[0013] In certain examples, the present disclosure provides a two-step method of treating or managing TNBC in a subject, in which the first step involves identifying a subject who has an increased level of HMGA2 or MECOM expression relative to a corresponding known control level, and the second step involves administering an anti-TGFβ agent to the subject who has been determined to have an increased level of HMGA2 or MECOM, and thereby treating TNBC in the subject.

[0014] In certain examples, the present disclosure provides a two-step method of achieving at least a partial response in treating or increasing survival of a triple negative breast cancer (TNBC) patient, in which the first step involves identifying a subject who has an increased level of HMGA2 or MECOM expression relative to a corresponding known control level, and the second step involves administering an anti-TGFβ agent to the subject who has been determined to have an increased level of HMGA2 or MECOM, and thereby achieving at least a

partial response in treating or increasing survival of a triple negative breast cancer (TNBC) patient.

[0015] In certain examples, the present disclosure provides a method of identifying a subject responsive to treatment of TNBC in the subject with an anti-TGFβ agent, in which the level of HMGA2 or MECOM in the subject is determined, and wherein an increased level of HMGA2 or MECOM expression in the subject, relative to a corresponding known control level, identifies the subject as suitable for treating TNBC with the anti-TGFβ agent.

[0016] In each of the methods of the present disclosure, the HMGA2 or MECOM level of the subject is determined by analyzing a tissue sample from the patient. In certain examples, the tissue sample is a biopsy sample, blood, serum, or plasma sample.

[0017] In each of the methods of the present disclosure, the level of HMGA2 or MECOM is determined by immunochemistry or by RNA expression analysis.

[0018] In some examples, the anti-TGFβ agent is an anti-PD-L1/TGFβ Trap protein used in the treatment of TNBC patients, comprising a first polypeptide comprising: (a) at least a variable region of a heavy chain of an antibody that binds to human protein Programmed Death Ligand 1 (PD-L1); and (b) human Transforming Growth Factor β Receptor II (TGFβRII), or a fragment thereof, capable of binding Transforming Growth Factor β (TGFβ), and a second polypeptide comprising at least a variable region of a light chain of an antibody that binds PD-L1; wherein the heavy chain of the first polypeptide and the light chain of the second polypeptide, when combined, form an antigen binding site that binds PD-L1. In some examples, an anti-PD-L1/TGFβ Trap protein comprises a first polypeptide comprising the amino acid sequences of SEQ ID NOs: 35, 36, and 37, and a second polypeptide comprising the amino acid sequences of SEQ ID NOs: 38, 39, and 40. In some examples, an anti-PD-L1/TGFβ Trap protein comprises a first polypeptide comprising the amino acid sequence of SEQ ID NO: 3, and the second polypeptide comprising the amino acid sequence of SEQ ID NO: 1.

[0019] In certain examples, the subject who has been determined to have an increased level of HMGA2 or MECOM expression relative to a corresponding known control level is administered at least 1200 mg of the anti-PD-L1/TGFβ Trap protein. In certain examples, the subject who has been determined to have an increased level of HMGA2 or MECOM expression

relative to a corresponding known control level is administered at least 1800 mg of the anti-PD-L1/TGFβ Trap protein. In certain examples, the subject who has been determined to have an increased level of HMGA2 or MECOM expression relative to a corresponding known control level is administered 1800 mg to 3000 mg of the anti-PD-L1/TGFβ Trap protein. In certain examples, the subject who has been determined to have an increased level of HMGA2 or MECOM expression relative to a corresponding known control level is administered 1200 mg to 2400 mg of the anti-PD-L1/TGFβ Trap protein.

[0020] In certain examples, the subject who has been determined to have an increased level of HMGA2 or MECOM expression relative to a corresponding known control level is administered 1200 mg of the anti-PD-L1/TGFβ Trap protein. In certain examples, the subject who has been determined to have an increased level of HMGA2 or MECOM expression relative to a corresponding known control level is administered 1200 mg of the anti-PD-L1/TGFβ Trap protein, once every two weeks.

[0021] In certain examples, the subject who has been determined to have an increased level of HMGA2 or MECOM expression relative to a corresponding known control level is administered 1800 mg of the anti-PD-L1/TGFβ Trap protein, once every three weeks.

[0022] In certain examples, the subject who has been determined to have an increased level of HMGA2 or MECOM expression relative to a corresponding known control level is administered 2400 mg of the anti-PD-L1/TGFβ Trap protein. In certain examples, the subject who has been determined to have an increased level of HMGA2 or MECOM expression relative to a corresponding known control level is administered 2400 mg of the anti-PD-L1/TGFβ Trap protein, once every three weeks. In certain examples, the subject who has been determined to have an increased level of HMGA2 or MECOM expression relative to a corresponding known control level is administered 2100 mg or 3000 mg of the anti-PD-L1/TGFβ Trap protein, once every three weeks.

[0023] In certain examples, in the methods of the present disclosure, the increased HMGA2 expression is at least 2.0-fold, for example, 2.27 more than a known population mean among TNBC patients. In certain examples, in the methods of the present disclosure, the increased HMGA2 expression is at least 2-5-fold more than the known population average level of HMGA2 expression. In certain examples, in the methods of the present disclosure, the increased HMGA2 expression is at least 200% higher, at least 300% higher, at least 400%

higher, at least 500% higher, at least 600% higher, at least 700% higher, at least 800% higher, at least 900% higher, at least 1000% higher, or more than the normal level of HMGA2 expression. In certain examples, in the methods of the present disclosure, the increased HMGA2 expression in the subject is at least 19- to 35-fold more than the HMGA2 expression in a subject who is non-responsive to a treatment with an anti-TGFβ agent. In some examples, the expression level of the HMGA2 gene is measured by quantifying HMGA2 mRNA transcript normalized to house-keeping gene or genes. HMGA2 mRNA transcript and the house-keeping gene or genes are quantified by RNA quantitative methods, such as quantitative reverse transcription PCR. The house-keeping gene or genes are those that have relatively constant expression among the target population.

[0024] In certain examples, in the methods of the present disclosure, the increased MECOM expression is at least 1.5-fold more than a known population mean among TNBC patients. In certain examples, in the methods of the present disclosure, the increased MECOM expression is at least 1.5 to 4-fold more than the known population mean among TNBC patients.

[0025] In certain examples, in the methods of the present disclosure, the increased MECOM expression is at least 100%, at least 200% higher, at least 300% higher, at least 400% higher, at least 500% higher, at least 600% higher, at least 700% higher, at least 800% higher, at least 900% higher, at least 1000% higher, or more than the normal level of MECOM expression.

[0026] Determination of mRNA can be from tumor tissue or circulating tumor cells, or circulating tumor mRNA. The mRNA will be used to detect high expression of the HMGA2 or MECOM genes. High expression can be considered as tumor cells expressing a level above a certain reference level by PCR or other technologies that quantify mRNA expression.

[0027] In certain examples, in the methods of the present disclosure, the increased HMGA2 or MECOM expressions are determined via quantification of the HMGA2 and MECOM mRNA, respectively. In certain examples, in the methods of the present disclosure, HMGA2 mRNA or MECOM mRNA levels are determined via reverse transcription polymerase chain reaction (RT-qPCR) assay. In certain examples, HMGA2 mRNA or MECOM mRNA levels are determined via digital droplet PCR (ddPCR). In certain examples, in the methods of the present disclosure, the increased HMGA2 or MECOM expressions are determined via quantification of the HMGA2 and MECOM protein, respectively. In certain examples, in the methods of the present disclosure, the increased HMGA2 protein or MECOM protein levels are determined via

immunohistochemistry. In certain examples, in the methods of the present disclosure, more than 1% tumor cells (*e.g.*, more than 5%, more than 10%, more than 15%, or more than 20%) expressing HMGA2 protein in a tissue sample obtained from the TNBC subject determined the increased HMGA2 protein expression level. In certain examples, in the methods of the present disclosure, more than 1% tumor cells (*e.g.*, more than 5%, more than 10%, more than 15%, or more than 20%) expressing MECOM protein in a tissue sample obtained from the TNBC subject determined the increased MECOM protein expression level.

[0028] Other examples and details of the disclosure are presented herein below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029] **FIG. 1** is a schematic drawing of an anti-PD-L1/TGFβ Trap molecule including one anti-PD-L1 antibody fused to two extracellular domains (ECDs) of TGFβ Receptor II via a $(Gly_4Ser)_4Gly$ (SEQ ID NO: 11) linker.

[0030] **FIG. 2A** is a box plot showing results for HMGA2 from the study described in Example 1. HMGA2 expression levels are plotted against response to anti-PD-L1/TGFβ Trap

CLAIMS

WHAT IS CLAIMED IS

1. Use of an anti-TGFβ agent in the manufacture of a medicament for treating or managing triple negative breast cancer (TNBC) in a patient, wherein the medicament is to be administered to a patient who has been determined to have an increased level of high mobility group AT-hook 2 (HMGA2) expression relative to a known control level, and thereby treating TNBC in the patient.

2. Use of an anti-TGFβ agent in the manufacture of a medicament for achieving at least a partial response in treating or increasing survival of a triple negative breast cancer (TNBC) patient, wherein the medicament is to be administered to a patient who has been determined to have an increased level of high mobility group AT-hook 2 (HMGA2) expression relative to a known control level, and thereby achieving at least a partial response in treating TNBC in the patient.

3. A method of identifying a patient suitable for treating or managing triple negative breast cancer (TNBC) in the patient with an anti-TGFβ agent, the method comprising determining the level of high mobility group AT-hook 2 (HMGA2) in a tissue sample from the patient, wherein an increased level of HMGA2 expression in the patient, relative to a known control level, identifies the patient as suitable for treating TNBC with said anti-TGFβ agent.

4. The method of claim 3, wherein the tissue sample is a biopsy sample, blood, serum, or plasma sample.

5. The use of claim 1 or 2, or the method of claim 3 or 4, wherein the level of HMGA2 is determined by immunochemistry or by RNA expression analysis.

6. The use of any one of claims 1, 2, or 5, or the method of any one of claims 3 to 5, wherein the anti-TGFβ agent is an anti-PD-L1/TGFβ Trap protein comprising a first polypeptide comprising: (a) at least a variable region of a heavy chain of an antibody that binds to human protein Programmed Death Ligand 1 (PD-L1); and (b) human Transforming Growth Factor β

Receptor II (TGFβRII), or a fragment thereof, capable of binding Transforming Growth Factor β (TGFβ), and a second polypeptide comprising at least a variable region of a light chain of an antibody that binds PD-L1; wherein the heavy chain of the first polypeptide and the light chain of the second polypeptide, when combined, form an antigen binding site that binds PD-L1.

7. The use or the method of claim 6, wherein the first polypeptide comprises the amino acid sequences of SEQ ID NOs: 35, 36, and 37, and the second polypeptide comprises the amino acid sequences of SEQ ID NOs: 38, 39, and 40.

8. The use or the method of claim 6 or 7, wherein the first polypeptide comprises the amino acid sequence of SEQ ID NO: 3, and the second polypeptide comprises the amino acid sequence of SEQ ID NO: 1.

9. The use or the method of any one of claims 6 to 8, wherein the patient is to be administered:

a. at least 1200 mg of the anti-PD-L1/TGFβ Trap protein;

b. at least 1800 mg of the anti-PD-L1/TGFβ Trap protein;

c. 1200 mg to 3000 mg of the anti-PD-L1/TGFβ Trap protein;

d. 1800 mg to 3000 mg of the anti-PD-L1/TGFβ Trap protein;

e. 1800 mg to 2100 mg of the anti-PD-L1/TGFβ Trap protein;

f. 2100 mg to 2400 mg of the anti-PD-L1/TGFβ Trap protein;

g. 1200 mg of the anti-PD-L1/TGFβ Trap protein; or

h. 2400 mg of the anti-PD-L1/TGFβ Trap protein.

10. The use or the method of claim 9, wherein the patient is to be administered:

a. 1200 mg of the anti-PD-L1/TGFβ Trap protein, once every two weeks;

b. 2400 mg of the anti-PD-L1/TGFβ Trap protein, once every three weeks; or

c. 2100 mg or 3000 mg of the anti-PD-L1/TGFβ Trap protein, once every three weeks.

11. The use of claims 1, 2, or of any one of claims 5 to 10, or the method of any one of claims 3 to 10, wherein the increased level of HMGA2 expression has been determined via quantification of HMGA2 mRNA expression.

12. The use or the method of claim 11, wherein the quantification of HMGA2 mRNA expression is via PCR.

13. The use of claims 1, 2, or of any one of claims 5 to 12, or the method of any one of claims 3 to 12, wherein the increased HMGA2 expression is:

a. at least 2.27-fold more than a known population mean HMGA2 expression among TNBC patients;

b. at least 5-fold more than a known population mean HMGA2 expression among TNBC patients; and/or

c. at least 200% higher, at least 300% higher, at least 400% higher, at least 500% higher, at least 600% higher, at least 700% higher, at least 800% higher, at least 900% higher, at least 1000% higher, or more than the normal level of HMGA2 expression.

14. The use of claims 1, 2, or of any one of claims 5 to 13, or the method of any one of claims 3 to 13, wherein the increased HMGA2 expression in the patient is at least 19- to 35-fold more than the HMGA2 expression in a patient who is non-responsive to a treatment with the anti-TGFβ agent.

15. The use of claims 1, 2, or of any one of claims 5 to 14, or the method of any one of claims 3 to 14, wherein the increased HMGA2 expression has been determined by HMGA2 protein expression level.

16. The use or the method of claim 15, wherein the increased HMGA2 protein expression level has been determined via immunohistochemistry.

17. The use or the method of claim 16, wherein more than 1% tumor cells expressing HMGA2 protein in a tissue sample obtained from the TNBC patient determined the increased HMGA2 protein expression level.

18. The use or the method of any one of claims 6 to 17, wherein the anti-PD-L1/TGFβ Trap protein is to be administered by intravenous administration.

19. The use or the method of claim 18, wherein the intravenous administration is performed with a prefilled bag, a prefilled pen, or a prefilled syringe comprising a formulation comprising the protein, wherein the bag is connected to a channel comprising a tube and/or a needle.