SUMMARY OF THE DISCLOSURE

[0009] For an effective treatment of patients diagnosed with advanced NSCLC, the present disclosure provides a therapeutic regimen that treats advanced NSCLC, and improves disease prognosis and overall survival of advanced NSCLC patients. The advanced NSCLC being treated can be either squamous or non-squamous NSCLC, and is independent of baseline PD-L1 expression levels.

[0009a] In one aspect, the present disclosure refers to use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating advanced non-small cell lung cancer (NSCLC) or inhibiting NSCLC tumor growth in a treatment naïve patient in need thereof, wherein the medicament is to be administered to the patient at a first step of a dose of at least 1800 mg of said protein with concurrent systemic chemotherapy, and at a second step of a dose of at least 1800 mg of said protein, wherein the first polypeptide comprises: (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), wherein the second polypeptide comprises at least a variable region of a light chain of an antibody that binds PD-L1, and 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.

[0009b] In another aspect, the present disclosure refers to use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating advanced non-squamous non-small cell lung cancer (NSCLC) or inhibiting non-squamous NSCLC tumor growth in a treatment naïve patient in need thereof, wherein the medicament is to be administered to the patient at a first step of a dose of at least 1800 mg of said protein with concurrent systemic chemotherapy comprising pemetrexed, and at a second step of a dose of at least 1800 mg of said protein with concurrent systemic chemotherapy consisting of pemetrexed, wherein the first polypeptide comprises: (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), wherein the second polypeptide comprises at least a variable region of a light chain of an antibody that binds PD-L1, and 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.

[0009c] In yet another aspect, the present disclosure refers to use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating advanced non-small cell lung cancer (NSCLC) or inhibiting NSCLC tumor growth in a patient indicated as having metastatic NSCLC disease progression on previous treatment with an immunotherapy in combination with chemotherapy, or on previous treatment with chemotherapy followed by treatment with an immunotherapy, or on previous treatment with an immunotherapy followed by platinum-based chemotherapy, wherein the medicament is to be administered to the patient at a first step of a dose of at least 1800 mg of said protein with concurrent systemic chemotherapy comprising docetaxel, and at a second step of a dose of at least 1800 mg of said protein, wherein the first polypeptide comprises: (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), wherein the second polypeptide comprises at least a variable region of a light chain of an antibody that binds PD-L1, and 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.

[0010] In one example, the present disclosure provides a method of treating advanced NSCLC by administering anti-PD-L1/TGFβ Trap in combination with systemic chemotherapeutic agents to induce tumor cell death, while simultaneously targeting two immune suppressive pathways: PD-L1 and TGF-β. In one example, the present disclosure provides body weight-independent dosage regimens for targeted TGF-β inhibition with a bi-functional fusion protein in combination with systemic chemotherapy agents for use in a method of treating a treatment naïve subject diagnosed with advanced NSCLC or a PDx failure metastatic NSCLC subject.

[0011] In one example, the present disclosure provides a two-step method of treating advanced NSCLC or inhibiting NSCLC tumor growth in a treatment naïve subject or a PDx failure metastatic NSCLC subject in need thereof, in which the first step involves administering to the subject a dose of at least 1200 mg (*e.g.*, 2400 mg) of anti-PD-L1/TGFβ Trap, with

concurrent systemic chemotherapy, and the second step involves administering at least 1200 mg (*e.g.*, 2400 mg) of the anti-PD-L1/TGFβ Trap. The NSCLC can be either squamous or non-squamous NSCLC. The systemic chemotherapy can be platinum-based chemotherapy, for example, including combinations of cisplatin or carboplatin with gemcitabine, docetaxel, or paclitaxel (nanoparticle albumin-bound (nab)-paclitaxel, or albumin unbound paclitaxel).

[0012] In one example, the present disclosure provides a two-step method of treating advanced non-squamous NSCLC or inhibiting non-squamous NSCLC tumor growth in a treatment-naïve subject in need thereof, in which the first step involves administering to the subject a dose of at least 1200 mg (*e.g.*, 2400 mg) of anti-PD-L1/TGFβ Trap, with concurrent systemic chemotherapy including pemetrexed, and the second step involves administering at least 1200 mg (*e.g.*, 2400 mg) of the anti-PD-L1/TGFβ Trap in combination with pemetrexed as the only chemotherapeutic agent. The systemic chemotherapy in the first step can be platinum-based chemotherapy, for example, including combinations of cisplatin or carboplatin with pemetrexed.

[0013] In one example, the present disclosure provides a two-step method of treating advanced non-squamous NSCLC or inhibiting non-squamous NSCLC tumor growth in a treatment-naïve subject in need thereof, in which the first step involves administering to the subject a dose of about 2400 mg of anti-PD-L1/TGFβ Trap, with concurrent systemic chemotherapy including pemetrexed, and the second step involves administering about 2400 mg of the anti-PD-L1/TGFβ Trap in combination with pemetrexed. The systemic chemotherapy can be platinum-based chemotherapy, for example, including combinations of cisplatin/carboplatin with pemetrexed.

[0014] In one example, the present disclosure provides a method of treating advanced non-small cell lung cancer (NSCLC) or inhibiting NSCLC tumor growth in subject indicated as having metastatic NSCLC disease progression on previous treatment with an immunotherapy in combination with chemotherapy, or on previous treatment with chemotherapy followed by treatment with an immunotherapy, or on previous treatment with an immunotherapy followed by platinum-based chemotherapy, the method comprising a first step of administering to the subject a dose of at least 1800 mg of an anti-PD-L1/ TGFβ Trap protein as provided in the present

disclosure, with concurrent systemic chemotherapy comprising docetaxel, and a second step of administering at least 1800 mg of anti-PD-L1/ TGFβ Trap protein.

[0015] In one example, the present disclosure provides a method of treating advanced non-small cell lung cancer (NSCLC) or inhibiting NSCLC tumor growth in subject indicated as having metastatic NSCLC disease progression on previous treatment with an immunotherapy in combination with chemotherapy, or on previous treatment with chemotherapy followed by treatment with an immunotherapy, or on previous treatment with an immunotherapy followed by platinum-based chemotherapy, the method comprising a first step of administering to the subject a dose of about 2400 mg of an anti-PD-L1/ TGFβ Trap protein as provided in the present disclosure, with concurrent systemic chemotherapy comprising docetaxel, and a second step of administering about 2400 mg of anti-PD-L1/ TGFβ Trap protein.

[0016] The bifunctional protein of the present disclosure (anti-PD-L1/TGFβ Trap molecule) includes a first and a second polypeptide. The first polypeptide includes: (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) (*e.g.*, a soluble fragment). The second polypeptide includes at least a variable region of a light chain of an antibody that binds PD-L1, in which 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 (*e.g.*, any of the antibodies or antibody fragments described herein). Because the bifunctional protein of the present disclosure binds to two targets, (1) PD-L1, which is largely membrane bound, and (2) TGFβ, which is soluble in blood and interstitium, the BW-independent dosing regimen requires a dose that is effective not only to inhibit PD-L1 at the tumor site but also sufficient to inhibit TGFβ.

[0017] The disclosure also features a method of promoting local depletion of TGFβ. The method includes administering a protein described above, where the protein binds TGFβ in solution, binds PD-L1 on a cell surface, and carries the bound TGFβ into the cell (*e.g.*, a cancer cell).

[0018] The disclosure also features a method of inhibiting SMAD3 phosphorylation in a cell (*e.g.*, a cancer cell or an immune cell), the method including exposing the cell in the tumor microenvironment to a protein described above.

[0019] Other examples and details of the disclosure are presented herein below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] **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.

[0021] **FIG. 2** shows a graph of a two-step ELISA demonstrating that anti-PD-L1/TGFβ Trap simultaneously binds to both PD-L1 and TGFβ.

[0022] **FIG. 3** is a graph showing anti-PD-L1/TGFβ Trap induces a dramatic increase in IL-2 levels.

[0023] **FIG. 4A** is a graph showing *in vivo* depletion of TGFβ1 in response to the anti-PD-L1/TGFβ Trap. Line graphs represent naïve, isotype control, and three different doses, as indicated in the legend. **FIG. 4B** is a graph showing *in vivo* depletion of TGFβ2 in response to the anti-PD-L1/TGFβ Trap. Line graphs represent naïve, isotype control, and three different doses, as indicated in the legend. **FIG. 4C** is a graph showing *in vivo* depletion of TGFβ3 in

CLAIMS

WHAT IS CLAIMED IS:

1. Use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating advanced non-small cell lung cancer (NSCLC) or inhibiting NSCLC tumor growth in a treatment naïve patient in need thereof, wherein the medicament is to be administered to the patient at a first step of a dose of at least 1800 mg of said protein with concurrent systemic chemotherapy, and at a second step of a dose of at least 1800 mg of said protein,

wherein the first polypeptide comprises: (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),

wherein the second polypeptide comprises at least a variable region of a light chain of an antibody that binds PD-L1, and

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.

2. Use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating advanced non-squamous non-small cell lung cancer (NSCLC) or inhibiting non-squamous NSCLC tumor growth in a treatment naïve patient in need thereof, wherein the medicament is to be administered to the patient at a first step of a dose of at least 1800 mg of said protein with concurrent systemic chemotherapy comprising pemetrexed, and at a second step of a dose of at least 1800 mg of said protein with concurrent systemic chemotherapy consisting of pemetrexed,

wherein the first polypeptide comprises: (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),

wherein the second polypeptide comprises at least a variable region of a light chain of an antibody that binds PD-L1, and

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.

3. Use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating advanced non-small cell lung cancer (NSCLC) or inhibiting NSCLC tumor growth in a patient indicated as having metastatic NSCLC disease progression on previous treatment with an immunotherapy in combination with chemotherapy, or on previous treatment with chemotherapy followed by treatment with an immunotherapy, or on previous treatment with an immunotherapy followed by platinum-based chemotherapy, wherein the medicament is to be administered to the patient at a first step of a dose of at least 1800 mg of said protein with concurrent systemic chemotherapy comprising docetaxel, and at a second step of a dose of at least 1800 mg of said protein,

wherein the first polypeptide comprises: (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),

wherein the second polypeptide comprises at least a variable region of a light chain of an antibody that binds PD-L1, and 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.

4. The use of any one of claims 1-3, 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.

5. The use of any one of claims 1-4, 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.

6. The use of any one of claims 1-5, wherein the dose is

a. 1800 mg to 3000 mg, is 2100 mg to 2400 mg, 2100 mg, 2400 mg; and/or

b. to be administered once every three weeks.

7. The use of any one of claims 1-6, wherein the dose is

a. 2100 mg, to be administered once every three weeks;

b. 2400 mg, to be administered once every three weeks; or

c. 3000 mg, to be administered once every three weeks.

8. The use of claim 1 or 2, or any one of claims 4-7, which depend from claim 1 or 2, wherein the systemic chemotherapy comprises carboplatin or cisplatin.

9. The use of claim 8, wherein the systemic chemotherapy comprises carboplatin to be administered once every three weeks.

10. The use of claim 9, wherein the carboplatin is to be administered at a dose of AUC 4 to AUC 6 IV on day 1 every 21 days for at least four cycles.

11. The use of claim 8, wherein the systemic chemotherapy comprises cisplatin.

12. The use of claim 11, wherein the cisplatin is to be administered at a dose of 75-100 mg/m^2 IV on day 1 every 21 days for at least four cycles.

13. The use of claim 1 or any one of claims 4-12, which depend from claim 1, wherein the systemic chemotherapy comprises gemcitabine or paclitaxel to be concurrently administered to the patient.

14. The use of claim 13, wherein the systemic chemotherapy comprises gemcitabine to be concurrently administered to the patient.

15. The use of claim 14, wherein the gemcitabine is to be administered at a dose of 1000-1250 mg/m^2 IV on days 1 and 8 every 21 days for at least four cycles.

16. The use of claim 13, wherein the systemic chemotherapy comprises paclitaxel to be concurrently administered to the patient.

17. The use of claim 16, wherein the paclitaxel is to be administered at a dose of 175-225 mg/m^2 IV on day 1 every 21 days for at least four cycles.

18. The use of claim 16, wherein the paclitaxel is to be administered albumin-bound 100 mg/m^2 on days 1, 8, and 15 every 21 days for at least four cycles.

19. The use of claim 1 or any one of claims 4-18, which depend from claim 1, wherein the second step further comprises a systemic chemotherapeutic agent to be administered to the patient.

20. The use of claim 2 or any one of claims 4-12, which depend from claim 2, wherein the pemetrexed is to be administered once every three weeks.

21. The use of claim 20, wherein the pemetrexed is to be administered at a dose of 500 mg/m^2 IV on day 1 every 21 days for at least four cycles.

22. The use of claim 1 or 2 or any one of claims 4-21, which depends from claim 104 or 105, wherein the NSCLC is PD-L1 positive, or the NSCLC is not PD-L1 positive.

23. The use of any one of claims 3-7, wherein the immunotherapy comprises an anti-PD-L1 or an anti-PD-L1 antibody.

24. The use of any one of claims 3-7 or 23, wherein the docetaxel is to be administered at a dose of 75-100 mg/m2 IV on day 1 every 21 days for four cycles.

25. The use of any one of claims 1, 3-19, 23, or 24, which depend from claim 1 or 3, wherein the NSCLC is squamous NSCLC or non-squamous NSCLC.

26. The use of any one of claims 1-25, wherein the treatment results in a disease response or improved survival of the patient, wherein the disease response is a complete response, a partial response, or a stable disease; and survival is progression-free survival (PFS).

27. The use of any one of claims 1-26, wherein the protein is to be administered by intravenous administration.

28. The use of claim 27, wherein the intravenous administration is to be 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.

29. The use of any one of claims 1-28, wherein the second step is to be continued for 31 or more cycles.