light chains of anti-PD-L1, and two heavy chains comprising the heavy chain of anti-PD-L1 genetically fused via a flexible glycine-serine linker to the extracellular domain of the human TGFβRII (*see* Fig. 1). This anti-PD-L1/TGFβ Trap molecule is designed to target two major mechanisms of immunosuppression in the tumor microenvironment. US patent application publication number US 20150225483 A1 describes administration of the Trap molecule at doses based on the patient's weight.

[0006] The present disclosure provides dosage regimens for targeted TGF-β inhibition with an anti-PD-L1/TGFβ Trap molecule for use in a method of treating a treatment naïve subject diagnosed with stage III NSCLC, and/or mitigating pathological conditions (*e.g.*, pulmonary fibrosis, pneumonitis) associated with concurrent cCRT.

SUMMARY OF THE DISCLOSURE

[0007] For an effective treatment of patients diagnosed with stage III NSCLC, and to counter acute and long term symptomatic lung injury due to fibrosis, the present disclosure provides a therapeutic regimen that treats stage III NSCLC, and spares as much normal lung tissue as possible from radiation-induced damage, and, thereby improves disease prognosis and overall survival of the NSCLC patients.

[0007a] 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 a treatment naïve patient diagnosed with stage III non-small cell lung cancer (NSCLC), and at risk of developing a pathological disorder of the lung associated with concomitant chemotherapy and radiotherapy (cCRT), wherein the medicament is to be administered to the patient at a first step of a dose of at least 1200 mg of said protein with concomitant cCRT, and at a second step of a dose of at least 1200 mg of said protein without concomitant cCRT to the patient, 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.

[0007b] 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 mitigating a pathological disorder associated with chemotherapy and radiotherapy (cCRT) in a treatment naïve patient diagnosed with stage III non-small cell lung cancer (NSCLC), wherein the medicament is to be administered to the patient at a first step of a dose of at least 1200 mg of said protein with concomitant chemotherapy and radiotherapy (cCRT), and at a second step of a dose of at least 1200 mg of said protein without concomitant cCRT to the patient, 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.

[0008] In one example, the present disclosure provides an anti-PD-L1/TGFβ Trap with concomitant cCRT to simultaneously target two immune suppressive pathways: PD-L1 and TGF-β, and, thereby treat stage III NSCLC, while minimizing the development of pathological conditions (*e.g.*, pulmonary fibrosis, pneumonitis) associated with concomitant radiotherapy, and increasing the time-to-onset of metastasis and/or time to distant metastasis of the stage III NSCLC in the patient.

[0009] The present disclosure provides improved dosing regimens for administration of bifunctional proteins targeting PD-L1 and TGFβ for treating stage III NSCLC, while minimizing the development of pathological conditions (*e.g.*, pulmonary fibrosis, pneumonitis) associated with concomitant radiotherapy, and increasing the time-to-onset of metastasis and/or time to distant metastasis of the stage III NSCLC in the patient. Specifically, body weight independent (BW-independent) dosing regimens and related dosage forms involving administration of at least 500 mg (*e.g.,* 1200 mg, 1800 mg, 2400 mg) of the bifunctional protein administered at various dosing frequencies can be used as an anti-tumor and anti-cancer therapeutic for treating stage III NSCLC, while minimizing the development of pathological conditions (*e.g.*, pulmonary fibrosis, pneumonitis) associated with concomitant radiotherapy, and increasing the time-to-onset of metastasis and/or time to distant metastasis of the stage III NSCLC in the patient. The BW-

independent dosing regimen ensures that all stage III NSCLC patients, irrespective of their body weight, will have adequate drug exposure at the tumor site.

[0010] 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.

[0011] In one example, the disclosure provides dosage regimens for targeted TGF-β inhibition with a bi-functional fusion protein for use in a method of treating a treatment naïve subject diagnosed with stage III non-small cell lung cancer (NSCLC), and/or mitigating a pathological condition associated with chemotherapy and radiotherapy (cCRT).

[0012] In one example, the disclosure provides dosage regimens for targeted TGF- β inhibition with a bi-functional fusion protein for use in a method of treating a stage III NSCLC that exhibits squamous or non-squamous histology, and/or mitigating a pathological condition associated with chemotherapy and radiotherapy (cCRT). In certain examples, the stage III NSCLS is unresectable.

[0013] In one example, the present disclosure provides a method of treating advanced unresectable stage III NSCLC in a patient by administering to the patient an anti-PD-L1/TGFβ Trap of the present disclosure in combination with cCRT (*e.g.*, platinum-based chemoradiation), followed by administering the anti-PD-L1/TGFβ Trap to the patient. In certain examples, the present disclosure provides a method of treating advanced unresectable stage III NSCLC in a patient by administering to the patient an anti-PD-L1/TGFβ Trap in combination with and following concurrent platinum-based chemoradiation.

[0014] In certain examples, cCRT is administered as either cisplatin/etoposide, cisplatin/pemetrexed, or carboplatin/paclitaxel concurrently with radiation (*e.g.*, radiation delivered by intensity-modulated radiation therapy).

[0015] In certain examples, the present disclosure provides a method of treating advanced unresectable stage III NSCLC, which has a non-squamous histology, in a patient by administering to the patient an anti-PD-L1/TGFβ Trap in combination with cCRT (*e.g.*, cisplatin/pemetrexed and radiation) followed by administering the anti-PD-L1/TGFβ Trap to the patient. In certain examples, the present disclosure provides a method of treating advanced unresectable stage III NSCLC in a patient by administering to the patient an anti-PD-L1/TGFβ Trap in combination with and following concurrent cisplatin/pemetrexed and radiation (*e.g.*, radiation delivered by intensity-modulated radiation therapy).

[0016] 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).

[0017] 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.

[0018] Other examples and details of the disclosure are presented herein below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] **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.

CLAIMS

1. Use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating a treatment naïve patient diagnosed with stage III non-small cell lung cancer (NSCLC), and at risk of developing a pathological disorder of the lung associated with concomitant chemotherapy and radiotherapy (cCRT), wherein the medicament is to be administered to the patient at a first step of a dose of at least 1200 mg of said protein with concomitant cCRT, and at a second step of a dose of at least 1200 mg of said protein without concomitant cCRT to the patient,

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 mitigating a pathological disorder associated with chemotherapy and radiotherapy (cCRT) in a treatment naïve patient diagnosed with stage III non-small cell lung cancer (NSCLC), wherein the medicament is to be administered to the patient at a first step of a dose of at least 1200 mg of said protein with concomitant chemotherapy and radiotherapy (cCRT), and at a second step of a dose of at least 1200 mg of said protein without concomitant cCRT to the patient,

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. The use of claim 1 or 2, wherein the use mitigates a pathological disorder of the lung associated with the cCRT at the first step and/or increases the time-to-onset of metastasis and/or time to distant metastasis of the stage III NSCLC in the patient.

4. The use of claim 3, wherein the pathological disorder is pneumonitis and/or pulmonary fibrosis.

5. The use of any one of claims 1-4, wherein the first polypeptide that comprises the amino acid sequences of SEQ ID NOs: 35, 36, and 37, and the second polypeptide that comprises the amino acid sequences of SEQ ID NOs: 38, 39, and 40.

6. The use of any one of claims 1–5, 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.

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

i. 1200 mg to 2400 mg, 1800 mg to 2400 mg, 1800 mg, or 2400 mg; and/or

ii. to be administered once every two weeks or once every three weeks.

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

i. 1200 mg, to be administered once every two weeks;

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

iii. 2400 mg, to be administered once every three weeks.

9. The use of any one of claims 1-8, wherein the stage III NSCLC exhibits squamous or non-squamous histology.

10. The use of any one of claims 1-9, wherein the stage III NSCLC exhibits PD-L1+ expression.

11. The use of any one of claims 1-9, wherein the stage III NSCLC does not exhibit PD-L1+ expression.

12. The use of any one of claims 1-11, wherein the patient has or does not have an EGFR sensitizing mutation, an anaplastic lymphoma kinase (ALK) translocation, and/or ROS1 rearrangement.

13. The use of any one of claims 1-12, 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).

14. The use of any one of claims 1-13, wherein the chemotherapy comprises cisplatin/etoposide, cisplatin/pemetrexed, and/or carboplatin/paclitaxel to be administered to the patient.

15. The use of any one of claims 1-14, wherein the chemotherapy comprises cisplatin/pemetrexed and the stage III NSCLC exhibits non-squamous histology.

16. The use of claim 14 or 15, wherein

i. cisplatin is to be intravenously administered at a dose of about 50 mg/m^2 – 80 mg/m^2;

ii. pemetrexed is to be intravenously administered at a dose of about 500 mg/m^2;

iii. etoposide is to be intravenously administered at a dose of about 50 mg/m^2;

iv. paclitaxel is to be intravenously administered at a dose of about 45 mg/m^2; and/or

v. carboplatin is to be intravenously administered based on AUC 2 over 30 minutes.

17. The use of any one of claims 1-16, wherein the radiotherapy comprises a dose of 60 - 74 Gy and/or is to be administered on days 1-5 for 6-7 weeks during the first step.

18. The use of any one of claims 1-17, wherein the protein is to be administered by intravenous administration.

19. The use of claim 18, 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.

20. The use of any one of claims 1-19, wherein the second step is to be initiated 1-42 days after completion of the first step and/or to be continued for 12-24 months.

21. The use of any one of claims 1-20, wherein the stage III non-small cell lung cancer (NSCLC) is unresectable.

22. The use of any one of claims 1-13, wherein the chemotherapy is a platinum-based chemotherapy.