

dose that is effective not only to inhibit PD-L1 at the tumor site but also sufficient to inhibit TGF $\beta$ .

**[0008a]** In one aspect, the present disclosure provides use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a treatment naïve patient in need thereof, wherein the medicament is to be administered to the patient at a dose of at least 500 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  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ), 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.

**[0008b]** In another aspect, the present disclosure provides use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating locally advanced or metastatic biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a patient that has failed or is intolerant to prior systemic chemotherapy, wherein the medicament is to be administered to the patient at a dose of at least 500 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  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ), 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.

**[0008c]** In another aspect, the present disclosure provides use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating locally advanced or metastatic biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a patient that has failed or is intolerant to prior systemic chemotherapy, wherein the medicament is to be administered to the patient once every two weeks at a dose of 1200 mg of said protein, wherein the first polypeptide comprises: (a) at least a variable region of a heavy chain of

an antibody comprising the amino acid sequences of SEQ ID Nos: 35, 36, and 37, which binds to human protein Programmed Death Ligand 1 (PD-L1); and (b) human Transforming Growth Factor  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ), wherein the second polypeptide comprises at least a variable region of a light chain of an antibody comprising the amino acid sequences of SEQ ID Nos: 38, 39, and 40, which binds PDL1, 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.

**[0008d]** In yet another aspect, the present disclosure provides use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a treatment naïve patient in need thereof, wherein the medicament is to be administered to the patient once every three weeks at a dose of 2400 mg of said protein, wherein the first polypeptide comprises: (a) at least a variable region of a heavy chain of an antibody comprising the amino acid sequences of SEQ ID Nos: 35, 36, and 37, which binds to human protein Programmed Death Ligand 1 (PD-L1); and (b) human Transforming Growth Factor  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ), wherein the second polypeptide comprises at least a variable region of a light chain of an antibody comprising the amino acid sequences of SEQ ID Nos: 38, 39, and 40, which binds PDL1, 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.

**[0008e]** In yet another aspect, the present disclosure provides use of an intravenous drug delivery formulation comprising 500 mg – 2400 mg of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a treatment naïve cancer patient in need thereof, wherein the medicament is to be administered 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  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ), 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.

**[0008f]** In yet another aspect, the present disclosure provides a drug delivery device for use in treating biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a treatment naïve cancer patient in need thereof, the device comprising a formulation comprising 500 mg – 2400 mg of a protein comprising a first polypeptide and a second polypeptide; 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  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ), 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.

**[0009]** In one example, the disclosure provides a method of treating biliary tract cancer (BTC) (*e.g.*, intrahepatic cholangiocarcinoma, extrahepatic cholangiocarcinoma and ampulla of Vater cancer; gallbladder cancer) or inhibiting biliary tract tumor growth in a treatment naïve patient by administering an anti-PD-L1/TGF $\beta$  Trap molecule described in the present disclosure to a patient in need. In one example, the disclosure provides treatment of biliary tract cancer (*e.g.*, advanced or metastatic biliary tract cancer) in a subject in need thereof. In one example, the present disclosure provides a method of treating BTC that exhibits positive PD-L1 expression.

**[0010]** In certain examples, the disclosure provides a method of treating biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a treatment naïve patient in need thereof by administering 1200 mg of an anti-PD-L1/TGF $\beta$  Trap molecule of the present disclosure once every two weeks to the patient. In certain other examples, the disclosure provides a method of treating biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a treatment naïve patient in need thereof by administering 2400 mg of an anti-PD-L1/TGF $\beta$  Trap molecule of the present disclosure once every three weeks to the patient.

**[0011]** In certain examples, treatment naïve subjects or patients with advanced or metastatic BTC are treated by co-administering gemcitabine and/or cisplatin with the anti-PD-L1/TGF $\beta$

Trap molecule disclosed in the present disclosure. In some examples, treatment naïve subjects or patients with advanced or metastatic BTC are treated by co-administering gemcitabine and cisplatin with the anti-PD-L1/TGFβ Trap molecule disclosed in the present disclosure.

5 [0012] In certain examples, the present disclosure describes methods of treatment in which the treatment naïve patient is administered gemcitabine and cisplatin on the same day (*e.g.*, day 1) as the protein (*e.g.*, anti-PD-L1/TGFβ Trap molecule described herein) during the treatment cycle. In certain examples, gemcitabine and cisplatin are administered on day 8 of the treatment cycle without the protein (*e.g.*, anti-PD-L1/TGFβ Trap molecule described herein). In some examples, the treatment (*e.g.*, co-administration of anti-PD-L1/TGFβ Trap with gemcitabine and 10 cisplatin on day 1 followed by administration of gemcitabine and cisplatin on day 8) is repeated (*e.g.*, 8 cycles) over a period of time (*e.g.*, 24 weeks) followed by administration of protein (*e.g.*, anti-PD-L1/TGFβ Trap molecule described herein) alone for a period of time (*e.g.*, 2 years).

[0013] 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 15 solution, binds PD-L1 on a cell surface, and carries the bound TGFβ into the cell (*e.g.*, a biliary tract cancer cell).

[0014] The disclosure also features a method of inhibiting SMAD3 phosphorylation in a cell (*e.g.*, a biliary tract cancer cell or an immune cell), the method including exposing the cell in the tumor microenvironment to a protein described above.

20 [0015] Other examples and details of the disclosure are presented herein below.

#### BRIEF DESCRIPTION OF THE DRAWINGS

[0016] 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<sub>4</sub>Ser)<sub>4</sub>Gly (SEQ ID NO: 11) linker.

25 [0017] 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.

[0018] FIG. 3 is a graph showing anti-PD-L1/TGFβ Trap induces a dramatic increase in IL-2 levels.

[0019] **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  
5 doses, as indicated in the legend. **FIG. 4C** is a graph showing *in vivo* depletion of TGFβ3 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. 4D** is a graph showing that occupancy of PD-L1 by the anti-PD-L1/TGFβ Trap supports a receptor binding model in the EMT-6 tumor system.

## CLAIMS

1. Use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a treatment naïve patient in need thereof, wherein the medicament is to be administered to the patient at a dose of at least 500 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  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ),

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. The use of claim 1, 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.

3. The use of claim 1 or 2, 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.

4. The use of any one of claims 1-3, wherein the dose is 500 mg to 2400 mg, 1200 mg to 2400 mg, 1200 mg, or 2400 mg.

5. The use of any one of claims 1-4, wherein the dose is to be administered once every two weeks or once every three weeks.

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

- a. 1200 mg, to be administered once every two weeks;
- b. 2400 mg, to be administered once every three weeks; or
- c. 2100 mg or 2400 mg, to be administered once every three weeks.

7. The use of any one of claims 1-6, wherein the BTC is locally advanced BTC and/or metastatic BTC.
8. The use of any one of claims 1-7, wherein the BTC exhibits positive PD-L1 expression.
9. The use of any one of claims 1-8, further comprising administration of gemcitabine and/or cisplatin to the patient.
10. The use of claim 9, wherein gemcitabine and cisplatin are to be administered on the same day the protein is to be administered (day 1) during the treatment cycle.
11. The use of claim 10, further comprising administration of gemcitabine and cisplatin on day 8 of the treatment cycle.
12. The use of claim 11, wherein the treatment is repeated a total of eight cycles over 24 weeks.
13. The use of claim 11, further comprising continuing treatment of the patient by administration of the protein starting at 25 weeks, without co-administration of gemcitabine and cisplatin.
14. The use of any one of claims 1-13, 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).
15. The use of any one of claims 1-14, wherein the protein is to be administered by intravenous administration.
16. The use of claim 15, 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.
17. Use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating locally advanced or metastatic biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a patient that has failed or is intolerant to prior systemic chemotherapy, wherein the medicament is to be administered to the patient at a dose of at least 500 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  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ),

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.

18. The use of claim 17, wherein the dose is

- a. 500 mg to 2400 mg, 1200 mg to 1800 mg, 1200 mg, or 1800 mg; and/or
- b. to be administered once every two weeks or once every three weeks.

19. Use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating locally advanced or metastatic biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a patient that has failed or is intolerant to prior systemic chemotherapy, wherein the medicament is to be administered to the patient once every two weeks at a dose of 1200 mg of said protein,

wherein the first polypeptide comprises: (a) at least a variable region of a heavy chain of an antibody comprising the amino acid sequences of SEQ ID Nos: 35, 36, and 37, which binds to human protein Programmed Death Ligand 1 (PD-L1); and (b) human Transforming Growth Factor  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ),

wherein the second polypeptide comprises at least a variable region of a light chain of an antibody comprising the amino acid sequences of SEQ ID Nos: 38, 39, and 40, which 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.

20. Use of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating biliary tract cancer (BTC) or inhibiting biliary tract

tumor growth in a treatment naïve patient in need thereof, wherein the medicament is to be administered to the patient once every three weeks at a dose of 2400 mg of said protein,

wherein the first polypeptide comprises: (a) at least a variable region of a heavy chain of an antibody comprising the amino acid sequences of SEQ ID Nos: 35, 36, and 37, which binds to human protein Programmed Death Ligand 1 (PD-L1); and (b) human Transforming Growth Factor  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ),

wherein the second polypeptide comprises at least a variable region of a light chain of an antibody comprising the amino acid sequences of SEQ ID Nos: 38, 39, and 40, which 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.

21. The use of claim 19 or 20, 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.

22. The use of any one of claims 19-21, further comprising administration of gemcitabine and/or cisplatin to the patient.

23. Use of an intravenous drug delivery formulation comprising 500 mg – 2400 mg of a protein comprising a first polypeptide and a second polypeptide in the manufacture of a medicament for treating biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a treatment naïve cancer patient in need thereof, wherein the medicament is to be administered 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  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ),

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.

24. A drug delivery device for use in treating biliary tract cancer (BTC) or inhibiting biliary tract tumor growth in a treatment naïve cancer patient in need thereof, the device comprising a formulation comprising 500 mg – 2400 mg of a protein comprising a first polypeptide and a second polypeptide;

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  $\beta$  Receptor II (TGF $\beta$ RII), or a fragment thereof, capable of binding Transforming Growth Factor  $\beta$  (TGF $\beta$ ),

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.