

the second immunotherapeutic agent may include “an antibody that blocks immunosuppressive functions” such as an “anti-PD- antibody”. However, no specific PD-L1/PD-1 bispecific agents were disclosed in this publication. Thus, a bispecific antibody that binds PD-L1 and PD-1 with high affinity, effectively neutralizes PD-L1 and PD-1 activation by all PDx family ligands, and/or provides superior activity relative to known therapeutics targeting the PD-1/PD-L1 pathway, or even combinations thereof, is needed as a more effective pharmacological intervention for certain cancers. Particularly, desirable are such anti-PD-L1/PD-1 bispecific antibodies that i) may more effectively treat cancers characterized as having moderate or high PD-L1 or PD-1 expression levels and ii) demonstrate *in vivo* stability, physical and chemical stability including, but not limited to, thermal stability, solubility, low self-association, and pharmacokinetic characteristics which are acceptable for development and/or use in the treatment of cancer.

## **Summary**

In one aspect, the present disclosure refers to an antibody that binds human PD-L1 (SEQ ID NO: 1) and human PD-1 (SEQ ID NO: 2) comprising:

- a) a first heavy chain (HC1) comprising a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 3;
- b) a first light chain (LC1) comprising a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 4;
- c) a second heavy chain (HC2) comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and
- d) a second light chain (LC2) comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

In another aspect, the present disclosure refers to a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding polypeptides having the amino acid sequences of SEQ ID NO: 49, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 34, wherein the cell is capable of expressing an antibody disclosed herein.

In a further aspect, the present disclosure refers to a mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding polypeptides having the amino acid sequences of SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 33, and SEQ ID NO: 34, wherein the cell is capable of expressing an antibody disclosed herein.

5        In another aspect, the present disclosure refers to a process for producing an antibody comprising cultivating the mammalian cell disclosed herein under conditions such that the antibody is expressed, and recovering the expressed antibody.

In a further aspect, the present disclosure refers to a pharmaceutical composition, comprising the antibody disclosed herein and an acceptable carrier, diluent, or excipient.

10       In another aspect, the present disclosure refers to a use of an antibody disclosed herein in the manufacture of a medicament for treating cancer.

In a further aspect, the present disclosure refers to an antibody disclosed herein for use in therapy.

15       In another aspect, the present disclosure refers to an antibody disclosed herein for use in the treatment of cancer.

In a further aspect, the present disclosure refers to an antibody disclosed herein for use in simultaneous, separate, or sequential combination with one or more antitumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), gemcitabine, topotecan, liposomal irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, epacadostat, and durvalumab, in the treatment of cancer.

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In another aspect, the present disclosure refers to a pharmaceutical composition for use in treating cancer, comprising an effective amount of the antibody disclosed herein.

**Description**

Accordingly, the present invention provides novel heterodimeric bispecific antibodies that can target PD-L1 and PD-1 simultaneously, via the pairing of two different heavy chains and two different light chains into a single IgG-like antibody.

- 5 Furthermore, the present invention provides anti-human PD-L1 and anti-human PD-1 heterodimeric bispecific antibodies that possess one or more of the following features: block all three interactions of the PD axis (PD-L1 binding to PD-1, PD-L2 binding to PD-1 and PD-L1 binding to CD80), bridge PD-L1 and PD-1 over-expressing cells, increase T cell activation and tumor cell killing due to proximity of bound T cell and tumor cell,
- 10 demonstrate significant antitumor activity at surprisingly low dosages in tumor cells with moderate to high PD ligand expression levels, and demonstrate unexpected physical and chemical stability including, but not limited to, *in vivo* stability, thermal stability, solubility, low self-association, and pharmacokinetic characteristics.

- Accordingly, the present invention provides an antibody that binds human PD-L1
- 15 (SEQ ID NO: 1) and human PD-1 (SEQ ID NO: 2) comprising:
- a) a first heavy chain (HC1) comprising a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 3;
  - b) a first light chain (LC1) comprising a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 4;

WE CLAIM:

1. An antibody that binds human PD-L1 (SEQ ID NO: 1) and human PD-1 (SEQ ID NO: 2) comprising:
  - a) a first heavy chain (HC1) comprising a heavy chain variable region (HCVR) comprising the amino acid sequence of SEQ ID NO: 3;
  - b) a first light chain (LC1) comprising a light chain variable region (LCVR) comprising the amino acid sequence of SEQ ID NO: 4;
  - c) a second heavy chain (HC2) comprising a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 5; and
  - d) a second light chain (LC2) comprising a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.
2. The antibody of claim 1 comprising:
  - a) the HC1 comprising, in order from the N-terminus to the C-terminus, the HCVR comprising the amino acid sequence of SEQ ID NO: 3, the amino acid sequence of SEQ ID NO: 9 in the CH1 domain, the amino acid sequence of SEQ ID NO: 10 in the CH2 domain, and the amino acid sequence of SEQ ID NO: 11 or SEQ ID NO: 12 in the CH3 domain;
  - b) the LC1 comprising, in order from the N-terminus to the C-terminus, the LCVR comprising the amino acid sequence of SEQ ID NO: 4 and the amino acid sequence of SEQ ID NO: 14 in the constant region;
  - c) the HC2 comprising, in order from the N-terminus to the C-terminus, the HCVR comprising the amino acid sequence of SEQ ID NO: 5, the amino acid sequence of SEQ ID NO: 13 in the CH1 domain, the amino acid sequence of SEQ ID NO: 10 in the CH2 domain, and the amino acid sequence of SEQ ID NO: 12 or the amino acid sequence of SEQ ID NO: 11 in the CH3 domain; and
  - d) the LC2 comprising, in order from N-terminus, the LCVR comprising the amino acid sequence of SEQ ID NO: 8 and the amino acid sequence of SEQ ID NO: 15 in the constant region, provided that when the amino acid sequence of SEQ ID NO: 11 is present in the CH3 domain of said HC1, the amino acid sequence of SEQ ID NO: 12 is present in the CH3 domain of said HC2; or when the amino acid sequence of SEQ ID NO: 12 is present in the CH3 domain of said HC1, the

amino acid sequence of SEQ ID NO: 11 is present in the CH3 domain of said HC2.

3. The antibody of Claim 2, wherein said HC1 comprises the amino acid sequence of SEQ ID NO: 11 in the CH3 domain, and wherein said HC2 comprises the HCVR comprising the amino acid sequence of SEQ ID NO: 6 and said HC2 comprises the amino acid sequence of SEQ ID NO: 12 in the CH3 domain.
4. The antibody of Claim 3, wherein said HC1, LC1, HC2, and LC2 comprises the amino acid sequences of SEQ ID NO: 49, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 34, respectively.
5. The antibody of Claim 3, wherein said HC1, LC1, HC2, and LC2 comprises the amino acid sequences of SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 33, and SEQ ID NO: 34, respectively.
6. A mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding polypeptides having the amino acid sequences of SEQ ID NO: 49, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO: 34, wherein the cell is capable of expressing the antibody of Claim 4.
7. A mammalian cell comprising a DNA molecule comprising a polynucleotide sequence encoding polypeptides having the amino acid sequences of SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 33, and SEQ ID NO: 34, wherein the cell is capable of expressing the antibody of Claim 5.
8. A process for producing an antibody comprising cultivating the mammalian cell of Claim 6 or 7 under conditions such that the antibody is expressed, and recovering the expressed antibody.
9. A pharmaceutical composition, comprising the antibody of any one of Claims 1-5 and an acceptable carrier, diluent, or excipient.
10. Use of an antibody of any one of Claims 1-5 in the manufacture of a medicament for treating cancer.
11. The use of Claim 10, wherein the cancer is Hodgkin's or non-Hodgkin's lymphomas, melanoma, renal cell cancer, kidney cancer, lung cancer, bladder cancer, gastric and esophageal cancer, colorectal cancer, liver cancer, hepatocellular cancer, cholangiocarcinoma, pancreatic cancer, breast cancer,

triple-negative breast cancer, ovarian cancer, endometrial cancer, prostate cancer, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), mesothelioma, squamous cancer of head neck cancer (SCCHN), soft tissue sarcoma, or glioblastoma multiforme.

- 5    12.    The use of Claim 11, wherein the lung cancer is NSCLC, small cell lung cancer, or mesothelioma.
13.    The use of any one of Claims 10-12, wherein one or more antitumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, 10    eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), gemcitabine, topotecan, liposomal irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, 15    epacadostat, and durvalumab is to be further administered simultaneously, separately, or sequentially.
14.    The antibody of any one of Claims 1-5 for use in therapy.
15.    The antibody of any one of Claims 1-5 for use in the treatment of cancer.
16.    The antibody for use of Claim 15, wherein the cancer is Hodgkin's or non- 20    Hodgkin's lymphomas, melanoma, renal cell cancer, kidney cancer, lung cancer, bladder cancer, gastric and esophageal cancer, colorectal cancer, liver cancer, hepatocellular cancer, cholangiocarcinoma, pancreatic cancer, breast cancer, triple-negative breast cancer, ovarian cancer, endometrial cancer, prostate cancer, small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), 25    mesothelioma, squamous cancer of head neck cancer (SCCHN), soft tissue sarcoma, or glioblastoma multiforme.
17.    The antibody for use of Claim 16, wherein the cancer is non-small cell lung cancer, small cell lung cancer, or mesothelioma.
18.    The antibody of any one of Claims 1-5 for use in simultaneous, separate, or 30    sequential combination with one or more antitumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal doxorubicin,

- docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin), FOLFIRI (leucovorin, fluorouracil, and irinotecan), gemcitabine, topotecan, liposomal
- 5 irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, epacadostat, and durvalumab, in the treatment of cancer.
19. A pharmaceutical composition for use in treating cancer, comprising an effective amount of the antibody of any one of Claims 1-5.
- 10 20. The composition for use of Claim 19, wherein the cancer is Hodgkin's or non-Hodgkin's lymphomas, melanoma, renal cell cancer, kidney cancer, lung cancer, bladder cancer, gastric and esophageal cancer, colorectal cancer, liver cancer, hepatocellular cancer, cholangiocarcinoma, pancreatic cancer, breast cancer, triple-negative breast cancer, ovarian cancer, endometrial cancer, prostate cancer,
- 15 small cell lung cancer (SCLC), non-small cell lung cancer (NSCLC), mesothelioma, squamous cancer of head neck cancer (SCCHN), soft tissue sarcoma, or glioblastoma multiforme.
21. The composition for use of Claim 20, wherein the cancer is NSCLC, small cell lung cancer, or mesothelioma.
- 20 22. The composition of any one of Claims 19-22, wherein one or more antitumor agents selected from the group consisting of cisplatin, carboplatin, dacarbazine, liposomal doxorubicin, docetaxel, cyclophosphamide and doxorubicin, navelbine, eribulin, paclitaxel, paclitaxel protein-bound particles for injectable suspension, ixabepilone, capecitabine, FOLFOX (leucovorin, fluorouracil, and oxaliplatin),
- 25 FOLFIRI (leucovorin, fluorouracil, and irinotecan), gemcitabine, topotecan, liposomal irinotecan, pemetrexed, cetuximab, nivolumab, ipilimumab, pidilizumab, pembrolizumab, tremelimumab, urelumab, lirilumab, atezolizumab, epacadostat, and durvalumab is to be administered simultaneously, separately, or in sequential combination.