

ANTI-PD-L1 ANTIBODIES AND DIAGNOSTIC USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of the filing date of U.S. Provisional Application No.

5 62/023,741, filed on July 11, 2014, the disclosure of which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

10 The present invention relates to anti-programmed death-ligand 1 (PD-L1) antibodies and methods of using the same.

BACKGROUND

Programmed death-ligand 1 (PD-L1) is a protein that has been implicated in the suppression of immune system responses during chronic infections, pregnancy, tissue allografts, autoimmune diseases, and cancer. PD-L1 regulates the immune response by binding to an inhibitory receptor, known as programmed death 1 (PD-1), which is expressed on the surface of T-cells, B-cells, and monocytes. PD-L1 negatively regulates T-cell function also through interaction with another receptor, B7.1 (also known as B7-1 or CD80). Formation of the PD-L1/PD-1 and PD-L1/B7.1 complexes negatively regulates T-cell receptor signaling, resulting in the subsequent downregulation of T cell activation and suppression of anti-tumor immune activity. PD-L1 is overexpressed in many cancers, including a wide variety of solid tumors, such as bladder, breast, colon, lung, melanoma, ovarian, salivary, stomach, and thyroid tumors. PD-L1 overexpression in tumor cells may advance tumor invasion and is often associated with poor prognosis.

25 Given the role of PD-L1 in cancer development and immune system regulation, additional tools to detect the presence of PD-L1, for example for diagnosis and/or patient selection, are desirable.

SUMMARY

The present invention relates to anti-programmed death-ligand 1 (PD-L1) antibodies and methods of using the same.

30 In one aspect, the invention features an isolated antibody that specifically binds to PD-L1, wherein the antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 17; or (c) a VH sequence as in (a) and a VL sequence as in (b). In some embodiments, the antibody comprises the following hypervariable regions (HVRs): (a) an HVR-H1 comprising the amino acid sequence of SNGLT (SEQ ID NO: 2); (b) an HVR-H2 comprising the amino acid sequence of TINKDASAYYASWAKG (SEQ ID NO: 3); and (c) an HVR-H3 comprising the amino acid sequence of IAFKTGTSI (SEQ ID NO: 4). In some embodiments, the antibody further comprises the following heavy chain variable domain framework regions (FRs): (a) FR-H1 comprising the amino acid sequence of QSLEESGGRLVKPDETLTITCTVSGIDLS (SEQ ID NO: 5); (b) FR-H2 comprising the amino acid sequence of WVRQAPGEGLEWIG (SEQ ID NO: 6); (c) FR-H3 comprising the amino acid sequence of RLTISKPSSTKVLDLKITSPTTEDTATYFCGR (SEQ ID NO: 7); and

GVPSRFKSGSGTQFTLTISGVQCDDAATYYC (SEQ ID NO: 14); and (h) FR-L4 comprising the amino acid sequence of FGGGTEVVVR (SEQ ID NO: 15). In some embodiments, the antibody comprises a VH sequence of SEQ ID NO: 16 and a VL sequence of SEQ ID NO: 17.

In another embodiment, there is provided an isolated antibody that competes for binding to PD-L1 with any one of the preceding antibodies.

In another embodiment, there is provided an isolated antibody that binds to the same epitope as any one of the preceding antibodies.

In some embodiments, any one of the preceding antibodies can be a monoclonal antibody. In some embodiments, the monoclonal antibody can be a rabbit monoclonal antibody.

In some embodiments, any one of the preceding antibodies can be an IgG antibody (e.g., an IgG1 antibody).

In some embodiments, any one of the preceding antibodies can be an antibody fragment that specifically binds PD-L1. In some embodiments, the antibody fragment is selected from the group consisting of Fab, single chain variable fragment (scFv), Fv, Fab', Fab'-SH, F(ab')₂, and diabody.

In another aspect, the invention features an immunoconjugate comprising any one of the preceding antibodies.

In another aspect, the invention features an isolated nucleic acid that encodes any of the antibodies described herein. The disclosure features a vector (e.g., an expression vector) comprising the nucleic acid for expressing the antibody. The disclosure also features host cells comprising the preceding nucleic acids and/or vectors. In one aspect, the invention features a vector comprising the preceding nucleic acid. In another aspect, the invention features a host cell comprising the preceding vector.

In some aspects, any one of the preceding antibodies can be for use in detecting the presence or expression level of PD-L1 in a biological sample. In some embodiments, the detecting is by immunohistochemistry (IHC), immunofluorescence (IF), flow cytometry, ELISA, or immunoblot. In some embodiments, the detecting is by IHC. In some embodiments, the sample comprises a fixed tissue. In some embodiments, the fixed tissue is a formalin-fixed paraffin-embedded (FFPE) tissue. In some embodiments, the sample is from a subject having, or at risk of, a cancer or an immune dysfunction. In some embodiments, the immune dysfunction is a T-cell dysfunctional disorder. In some embodiments, the T-cell dysfunctional disorder is an unresolved acute infection, chronic infection, or tumor immunity.

A further aspect of the invention is a method of detecting the presence or expression level of PD-L1 in a biological sample comprising contacting the biological sample with any one of the preceding antibodies and detecting the presence of the bound antibody. In some embodiments, the detecting is by IHC, IF, flow cytometry, ELISA, or immunoblot. In some embodiments, the detecting is by IHC. In some embodiments, the sample comprises a fixed tissue. In some embodiments, the fixed tissue is a FFPE tissue. In some embodiments, the sample is from a subject having, or at risk of, a cancer or an immune dysfunction. In some embodiments, the immune dysfunction is a T-cell dysfunctional disorder. In some embodiments, the T-cell dysfunctional disorder is an unresolved acute infection, chronic infection, or tumor immunity. In some embodiments, the sample is from a subject having a cancer. In some embodiments, the presence or expression level of PD-L1 in the sample indicates that the subject is likely to respond to treatment with an anti-cancer therapy. In some embodiments, the presence or expression

level of PD-L1 in the sample indicates that the subject is more likely to respond to treatment with an anti-cancer therapy. In some embodiments, the presence or expression level of PD-L1 in the sample indicates the likelihood that the subject will exhibit benefit from treatment with an anti-cancer therapy. In some embodiments, the method further comprises selecting an anti-cancer therapy for the subject based on the presence or expression level of PD-L1 in the sample. In some embodiments, the method further comprises administering a therapeutically effective amount of an anti-cancer therapy to the subject.

In one aspect, the invention features use of a therapeutically effective amount of an anti-cancer therapy comprising a PD-1 axis binding antagonist in the manufacture of a medicament for treating a subject having a cancer, wherein the subject is one who has been selected based on the presence or expression of PD-L1 in a biological sample from the subject according to any of the preceding methods.

In some embodiments, the cancer is selected from the group consisting of non-small cell lung cancer, squamous cell cancer, small-cell lung cancer, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia, and head and neck cancer. In some embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the NSCLC is adenocarcinoma of the lung or squamous carcinoma of the lung. In some embodiments, the sample is a tumor sample. In some embodiments, the tumor sample comprises tumor-infiltrating immune cells, tumor cells, stromal cells, or any combination thereof. In some embodiments, the tumor sample has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 1% or more of the tumor sample by area. In some embodiments, the tumor sample has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 5% or more of the tumor sample by area. In some embodiments, the tumor sample has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 10% or more of the tumor sample by area. In some embodiments, the tumor sample has a detectable expression level of PD-L1 in about 1% or more of the tumor cells in the tumor sample. In some embodiments, the tumor sample has a detectable expression level of PD-L1 in about 5% or more of the tumor cells in the tumor sample. In some embodiments, the tumor sample has a detectable expression level of PD-L1 in about 10% or more of the tumor cells in the tumor sample. In some embodiments, the anti-cancer therapy comprises a PD-1 axis binding antagonist. In some embodiments, the PD-1 axis binding antagonist is selected from the group consisting of a PD-L1 binding antagonist, a PD-1 binding antagonist, and a PD-L2 binding antagonist. In some embodiments, the PD-1 axis binding antagonist is a PD-L1 binding antagonist. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to one or more of its ligand binding partners. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1. In some embodiments, the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1. In some embodiments, the PD-L1 binding antagonist is an antibody. In some embodiments, the antibody is selected from the group consisting of: YW243.55.S70, MPDL3280A (atezolizumab), MDX-1105, MEDI4736 (durvalumab), and MSB0010718C (avelumab). In some embodiments, the PD-1 axis binding antagonist is a PD-1 binding antagonist. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to one or more of its ligand binding partners. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD L1. In some embodiments, the PD-1 binding antagonist inhibits the binding of PD-1 to PD L2. In some

WHAT IS CLAIMED IS:

1. An isolated antibody that specifically binds to PD-L1, wherein the antibody comprises (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 16; (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 17; or (c) a VH sequence as in (a) and a VL sequence as in (b).

2. The antibody of claim 1, wherein the antibody comprises the following hypervariable regions (HVRs):

- (a) an HVR-H1 comprising the amino acid sequence of SNGLT (SEQ ID NO: 2);
 - (b) an HVR-H2 comprising the amino acid sequence of TINKDASAYYASWAKG (SEQ ID NO: 3);
- and
- (c) an HVR-H3 comprising the amino acid sequence of IAFKTGTSL (SEQ ID NO: 4).

3. The antibody of claim 2, further comprising the following heavy chain variable domain framework regions (FRs):

- (a) FR-H1 comprising the amino acid sequence of QSLEESGGRLVKPDETLTITCTVSGIDLS (SEQ ID NO: 5);
- (b) FR-H2 comprising the amino acid sequence of WVRQAPGEGLEWIG (SEQ ID NO: 6);
- (c) FR-H3 comprising the amino acid sequence of RLTISKPSSTKVLDKITSPTTEDTATYFCGR (SEQ ID NO: 7); and
- (d) FR-H4 comprising the amino acid sequence of WPGTLVTVSS (SEQ ID NO: 8).

4. The antibody of claim 2 or 3, further comprising the following HVRs:

- (a) an HVR-L1 comprising the amino acid sequence of QASESVYSNNYLS (SEQ ID NO: 9);
- (b) an HVR-L2 comprising the amino acid sequence of LASTLAS (SEQ ID NO: 10); and
- (c) an HVR-L3 comprising the amino acid sequence of IGGKSSSTDGNA (SEQ ID NO: 11).

5. The antibody of claim 4, further comprising the following light chain variable domain FRs:

- (a) FR-L1 comprising the amino acid sequence of AIVMTQTPSPVSAVGGTVTINC (SEQ ID NO: 12);
- (b) FR-L2 comprising the amino acid sequence of WFQQKPGQPPKLLIY (SEQ ID NO: 13);
- (c) FR-L3 comprising the amino acid sequence of
GVPSRFKSGSGTQFTLTISGVQCDDAATYYC
(SEQ ID NO: 14); and
- (d) FR-L4 comprising the amino acid sequence of FGGGTEVVVR (SEQ ID NO: 15)

6. The antibody of claim 1, wherein the antibody comprises the following HVRs:

- (a) an HVR-L1 comprising the amino acid sequence of QASESVYSNNYLS (SEQ ID NO: 9);
- (b) an HVR-L2 comprising the amino acid sequence of LASTLAS (SEQ ID NO: 10); and
- (c) an HVR-L3 comprising the amino acid sequence of IGGKSSSTDGNA (SEQ ID NO: 11).

- 7 The antibody of claim 6, further comprising the following light chain variable domain FRs:
 - (a) FR-L1 comprising the amino acid sequence of AIVMTQTPSPVSAAVGGTVTINC (SEQ ID NO: 12);
 - (b) FR-L2 comprising the amino acid sequence of WFQQKPGQPPKLLIY (SEQ ID NO: 13);
 - (c) FR-L3 comprising the amino acid sequence of GVPSRFKSGSGTQFTLTISGVQCDDAATYYC (SEQ ID NO: 14); and
 - (d) FR-L4 comprising the amino acid sequence of FGGGTEVVVR (SEQ ID NO: 15).
8. The antibody of claim 1, comprising a VH sequence of SEQ ID NO: 16.
9. The antibody of claim 1, comprising a VL sequence of SEQ ID NO: 17.
10. An isolated antibody that specifically binds PD-L1, wherein the antibody comprises the following HVRs:
 - (a) an HVR-H1 comprising the amino acid sequence of SNGLT (SEQ ID NO: 2);
 - (b) an HVR-H2 comprising the amino acid sequence of TINKDASAYYASWAKG (SEQ ID NO: 3);
 - (c) an HVR-H3 comprising the amino acid sequence of IAFKTGTSL (SEQ ID NO: 4);
 - (d) an HVR-L1 comprising the amino acid sequence of QASESVYSNNYLS (SEQ ID NO: 9);
 - (e) an HVR-L2 comprising the amino acid sequence of LASTLAS (SEQ ID NO: 10); and
 - (f) an HVR-L3 comprising the amino acid sequence of IGGKSSSTDGNA (SEQ ID NO: 11).
11. The antibody of claim 10, wherein the antibody further comprises the following heavy chain variable domain and light chain variable domain FRs:
 - (a) FR-H1 comprising the amino acid sequence of QSLEESGGRLVKPDETLTITCTVSGIDLS (SEQ ID NO: 5);
 - (b) FR-H2 comprising the amino acid sequence of WVRQAPGEGLEWIG (SEQ ID NO: 6);
 - (c) FR-H3 comprising the amino acid sequence of RLTISKPSSTKVLDKITSPTTEDTATYFCGR (SEQ ID NO: 7);
 - (d) FR-H4 comprising the amino acid sequence of WPGGTLVTVSS (SEQ ID NO: 8);
 - (e) FR-L1 comprising the amino acid sequence of AIVMTQTPSPVSAAVGGTVTINC (SEQ ID NO: 12);
 - (f) FR-L2 comprising the amino acid sequence of WFQQKPGQPPKLLIY (SEQ ID NO: 13);
 - (g) FR-L3 comprising the amino acid sequence of GVPSRFKSGSGTQFTLTISGVQCDDAATYYC (SEQ ID NO: 14); and
 - (h) FR-L4 comprising the amino acid sequence of FGGGTEVVVR (SEQ ID NO: 15).

12. The antibody of claim 10 or 11, wherein the antibody comprises a VH sequence of SEQ ID NO: 16 and a VL sequence of SEQ ID NO: 17.
13. The antibody of any one of claims 1-12, wherein the antibody is a monoclonal antibody.
14. The antibody of claim 13, wherein the monoclonal antibody is a rabbit monoclonal antibody.
15. The antibody of any one of claims 1-12, wherein the antibody is an IgG antibody.
16. The antibody of any one of claims 1-12, wherein the antibody is an antibody fragment that specifically binds PD-L1.
17. The antibody of claim 16, wherein the antibody fragment is selected from the group consisting of Fab, single chain variable fragment (scFv), Fv, Fab', Fab'-SH, F(ab')₂, and diabody.
18. An isolated nucleic acid encoding an isolated antibody of any one of claims 1-17.
19. A vector comprising the nucleic acid of claim 18.
20. A host cell comprising the vector of claim 19.
21. An immunoconjugate comprising the antibody of any one of claims 1-17.
22. The antibody of any one of claims 1-17 for use in detecting the presence or expression level of PD-L1 in a biological sample.
23. The antibody of claim 22, wherein the detecting is by immunohistochemistry (IHC), immunofluorescence (IF), flow cytometry, Enzyme-Linked Immunosorbant Assay (ELISA), or immunoblotting.
24. The antibody of claim 23, wherein the detecting is by IHC.
25. The antibody of any one of claims 22-24, wherein the sample comprises a fixed tissue.

26. The antibody of claim 25, wherein the fixed tissue is a formalin-fixed paraffin-embedded (FFPE) tissue.

27. The antibody of any one of claims 22-26, wherein the sample is from a subject having, or at risk of, a cancer or an immune dysfunction, wherein the immune dysfunction is an unresolved acute infection, chronic infection, or an autoimmune disease.

28. A method of detecting the presence or expression level of PD-L1 in a biological sample comprising contacting the biological sample with the antibody of any one of claims 1-17 and detecting the presence of the bound antibody.

29. The method of claim 28, wherein the detecting is by IHC, IF, flow cytometry, ELISA, or immunoblotting.

30. The method of claim 29, wherein the detecting is by IHC.

31. The method of any one of claims 28-30, wherein the sample comprises a fixed tissue.

32. The method of claim 31, wherein the fixed tissue is a FFPE tissue.

33. The method of any one of claims 28-32, wherein the sample is from a subject having, or at risk of, a cancer or an immune dysfunction, wherein the immune dysfunction is an unresolved acute infection, chronic infection, or an autoimmune disease.

34. The method of claim 33, wherein the sample is from a subject having a cancer.

35. The method of any one of claims 28-34, wherein the presence or expression level of PD-L1 in the sample indicates that the subject is likely to respond to treatment with an anti-cancer therapy comprising a PD-1 axis binding antagonist.

36. The method of any one of claims 28-34, wherein the presence or expression level of PD-L1 in the sample indicates that the subject is more likely to respond to treatment with an anti-cancer therapy comprising a PD-1 axis binding antagonist.

37. The method of any one of claims 28-34, wherein the presence or expression level of PD-L1 in the sample indicates the likelihood that the subject will exhibit benefit from treatment with an anti-cancer therapy comprising a PD-1 axis binding antagonist.

38. The method of any one of claims 34-37, wherein the method further comprises selecting an anti-cancer therapy comprising a PD-1 axis binding antagonist for the subject based on the presence or expression level of PD-L1 in the sample.

39. Use of a therapeutically effective amount of an anti-cancer therapy comprising a PD-1 axis binding antagonist in the manufacture of a medicament for treating a subject having a cancer, wherein the subject is one who has been selected based on the presence or expression of PD-L1 in a biological sample from the subject according to the method of any one of claims 34-38.

40. The method or use of any one of claims 33-39, wherein the cancer is selected from the group consisting of non-small cell lung cancer, squamous cell cancer, small-cell lung cancer, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia, and head and neck cancer.

41. The method or use of claim 40, wherein the cancer is non-small cell lung cancer (NSCLC).

42. The method or use of claim 41, wherein the NSCLC is adenocarcinoma of the lung or squamous carcinoma of the lung.

43. The method or use of any one of claims 33-42, wherein the sample is a tumor sample.

44. The method or use of claim 43, wherein the tumor sample comprises tumor-infiltrating immune cells, tumor cells, stromal cells, or any combination thereof.

45. The method or use of claim 44, wherein the tumor sample has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 1% or more of the tumor sample by area.

46. The method or use of claim 45, wherein the tumor sample has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 5% or more of the tumor sample by area.

47. The method or use of claim 46, wherein the tumor sample has a detectable expression level of PD-L1 in tumor-infiltrating immune cells that comprise about 10% or more of the tumor sample by area.

48. The method or use of any one of claims 43-47, wherein the tumor sample has a detectable expression level of PD-L1 in about 1% or more of the tumor cells in the tumor sample.

49. The method or use of claim 48, wherein the tumor sample has a detectable expression level of PD-L1 in about 5% or more of the tumor cells in the tumor sample.

50. The method or use of claim 49, wherein the tumor sample has a detectable expression level of PD-L1 in about 10% or more of the tumor cells in the tumor sample.

51. The method or use of any one of claims 35-50, wherein the PD-1 axis binding antagonist is selected from the group consisting of a PD-L1 binding antagonist, a PD-1 binding antagonist, and a PD-L2 binding antagonist.

52. The method or use of claim 51, wherein the PD-1 axis binding antagonist is a PD-L1 binding antagonist.

53. The method or use of claim 52, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to one or more of its ligand binding partners.

54. The method or use of claim 53, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to PD-1.

55. The method or use of claim 53, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to B7-1.

56. The method or use of any one of claims 53-55, wherein the PD-L1 binding antagonist inhibits the binding of PD-L1 to both PD-1 and B7-1.

57. The method or use of any one of claims 52-56, wherein the PD-L1 binding antagonist is an antibody.

58. The method or use of claim 57, wherein the antibody is selected from the group consisting of: YW243.55.S70, MPDL3280A (atezolizumab), MDX-1105, and MEDI4736 (durvalumab), and MSB0010718C (avelumab).

59. The method or use of claim 51, wherein the PD-1 axis binding antagonist is a PD-1 binding antagonist.

60. The method or use of claim 59, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to one or more of its ligand binding partners.

61. The method or use of claim 60, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L1.

62. The method or use of claim 60, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to PD-L2.

63. The method or use of any one of claims 60-62, wherein the PD-1 binding antagonist inhibits the binding of PD-1 to both PD-L1 and PD-L2.

64. The method or use of any one of claims 59-63, wherein the PD-1 binding antagonist is an antibody.

65. The method or use of claim 64, wherein the antibody is selected from the group consisting of: MDX 1106 (nivolumab), MK-3475 (pembrolizumab), MEDI-0680 (AMP-514), PDR001, REGN2810, and BGB-108.

66. The method or use of any one of claims 59-63, wherein the PD-1 binding antagonist is an Fc-fusion protein.

67. The method or use of claim 66, wherein the Fc-fusion protein is AMP-224.

68. The use of any one of claims 39-67, wherein the medicament is to be administered to the patient with an effective amount of a second therapeutic agent.

69. The use of claim 68, wherein the second therapeutic agent is selected from the group consisting of a cytotoxic agent, a growth-inhibitory agent, a radiation therapy agent, an anti-angiogenic agent, and combinations thereof.

70. The method or use of any one of claims 28-69, wherein the subject is a human.