

SUMMARY

[0005] Provided herein are anti-PD-L1 antibodies and PD-L1-directed antibody-drug conjugates (ADCs). In particular, provided herein are PD-L1-directed camptothecin ADCs and MMAE ADCs. Also provided herein are methods of using anti-PD-L1-directed antibodies and ADCs to treat PD-L1-expressing disorders. Preferred anti-PD-L1 antibodies exhibit a binding affinity to the human PD-L1 protein that is between 3 nM and 300 nM. Other preferred anti-PD-L1 antibodies comprise heavy chain CDR sequences of SEQ ID NOs: 3-5 and light chain CDR sequences of SEQ ID NOs: 6-8, wherein the antibodies comprise one or more amino acid substitutions within one or more of the CDRs. Other preferred anti-PD-L1 antibodies comprise heavy chain CDR sequences of SEQ ID NOs: 13-15 and light chain CDR sequences of SEQ ID NOs: 16-18.

[0006] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind to the human Programmed Death-Ligand 1 (PD-L1) protein, where the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 300 nM. In one aspect, the present disclosure provides an antibody or antigen-binding fragment thereof that specifically binds to the human PD-L1 protein, wherein the antibody comprises heavy chain CDR sequences of SEQ ID NOs: 13-15 and light chain CDR sequences of SEQ ID NOs: 16-18. In some examples, the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.

[0007] In some examples, the antibody further exhibits a total internalization that is higher than the total internalization of Ab1. In some examples, the total internalization is between a 9% and 155% increase of AUC over the AUC of Ab1. In some examples, the total internalization is determined by a FabFluor internalization assay.

[0008] In some examples, the antibody further exhibits an x50 that is lower than the x50 of Ab1. In some examples, the antibody is conjugated to monomethyl auristatin E (MMAE), and wherein the x50 is between 3 ng/mL and 20 ng/mL in an MDA-MB-231 cell line.

[0009] In some examples, the antibody is conjugated to camptothecin, and where the x50 is between 15 ng/mL and 55 ng/mL in an MDA-MB-231 cell line.

[0010] In some examples, the antibody includes heavy chain CDR sequences of SEQ ID NOs: 13-15 and light chain CDR sequences of SEQ ID NOs: 16-18.

[0011] In some examples, the antibody includes heavy chain CDR sequences of SEQ ID NOs: 3-5 and light chain CDR sequences of SEQ ID NOs: 6-8, wherein the antibody includes one or more amino acid substitutions within one or more of the CDRs.

[0012] In some examples, the antibody includes a heavy chain variable region sequence having at least 80% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 80% sequence identity to SEQ ID NO: 12. In some examples, the antibody includes a heavy chain variable region sequence having at least 90% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 90% sequence identity to SEQ ID NO: 12. In some examples, the antibody includes a heavy chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 12. In some examples, the antibody includes a heavy chain variable region sequence of SEQ ID NO: 11 and a light chain variable region sequence of SEQ ID NO: 12.

[0013] In some examples, the antibody includes a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.

[0014] In some examples, the fragment is a Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, or single-chain antibody fragment.

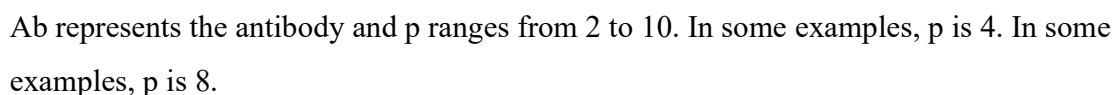
[0015] In some examples, the antibody contains L234A and L235A mutations in the heavy chain of the antibody.

[0016] In some examples, the heavy chain constant region is of the IgG1 isotype.

[0017] In some examples, the antibody is a humanized or chimeric antibody.

[0018] In some examples, the antibody is conjugated to a cytotoxic agent via a linker.

[0019] In some examples, the antibody is conjugated to monomethyl auristatin E (MMAE). In some examples, the antibody is conjugated to MMAE via an enzyme-cleavable linker unit. In some examples, the enzyme-cleavable linker unit includes a Val-Cit linker. In some examples, the antibody is conjugated to MMAE via a linker forming an antibody-drug conjugate having the structure:

[illegible]

represents the antibody and p ranges from 2 to 10. In some examples, p is 4. In some examples, p is 8.

[0021] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind to the human PD-L1 protein, wherein the antibody includes heavy chain CDR sequences of SEQ ID NOs: 3-5 and light chain CDR sequences of SEQ ID NOs: 6-8, wherein the antibody includes one or more amino acid substitutions within one or more of the CDRs.

[0022] In some examples, the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 300 nM. In some examples, the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.

[0023] In some examples, the antibody further exhibits a total internalization that is higher than the total internalization of Ab1. In some examples, the total internalization is between a 9% and 155% increase of AUC over the AUC of Ab1. In some examples, the total internalization is determined by a FabFluor internalization assay.

[0024] In some examples, the antibody further exhibits an x50 that is higher than the x50 of Ab1.

[0025] In some examples, the antibody is conjugated to monomethyl auristatin E (MMAE), and where the x50 is between 3 ng/mL and 20 ng/mL in an MDA-MB-231 cell line.

[0026] In some examples, the antibody is conjugated to camptothecin, and where the x50 is between 15 ng/mL and 55 ng/mL in an MDA-MB-231 cell line.

[0027] In some examples, the antibody includes heavy chain CDR sequences of SEQ ID NOs: 13-15 and light chain CDR sequences of SEQ ID NOs: 16-18. In some examples, the antibody includes a heavy chain variable region sequence having at least 80% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 80% sequence identity to SEQ ID NO: 12. In some examples, the antibody includes a heavy chain variable region sequence having at least 90% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 90% sequence identity to SEQ ID NO: 12. In some examples, the antibody includes a heavy chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 12. In some examples, the antibody includes a heavy chain variable region sequence of SEQ ID NO: 11 and a light chain variable region sequence of SEQ ID NO: 12.

[0028] In some examples, the antibody includes a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.

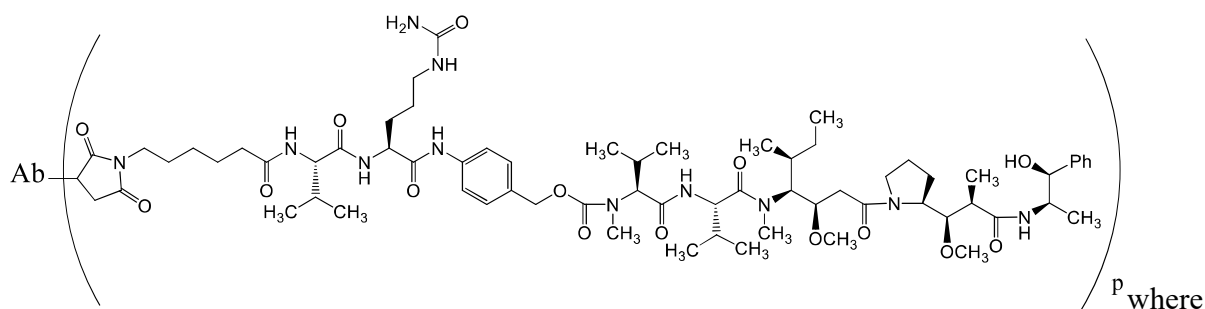
[0029] In some examples, the fragment is a Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, or single-chain antibody fragment.

[0030] In some examples, the antibody contains L234A and L235A mutations in the heavy chain of the antibody.

[0031] In some examples, the heavy chain constant region is of the IgG1 isotype.

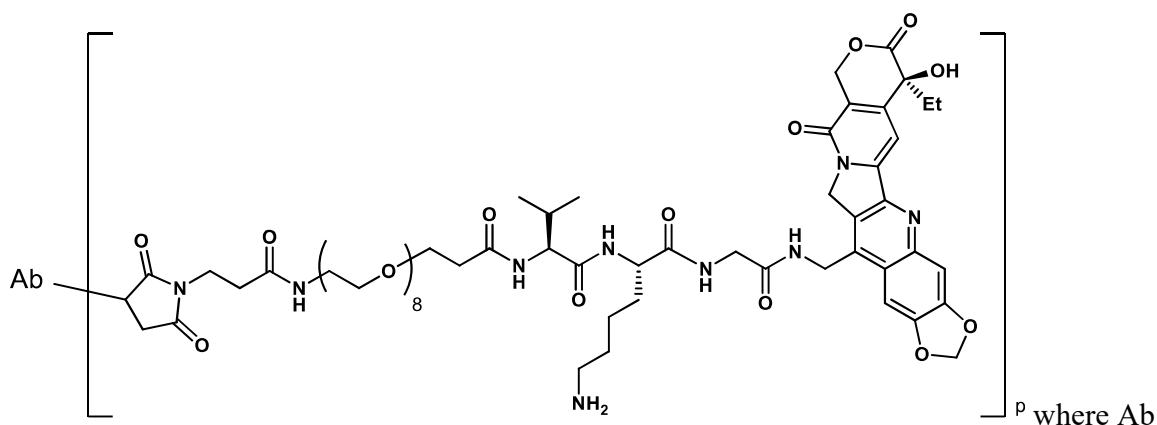
[0032] In some examples, the antibody is a humanized or chimeric antibody.

[0033] In some examples, the antibody is conjugated to a cytotoxic agent via a linker. In some examples, the antibody is conjugated to monomethyl auristatin E (MMAE). In some examples, the antibody is conjugated to MMAE via an enzyme-cleavable linker unit. In some examples, the enzyme-cleavable linker unit includes a Val-Cit linker. In some examples, the antibody is conjugated to MMAE via a linker forming an antibody-drug conjugate having the structure:



Ab represents the antibody and p ranges from 2 to 10. In some examples, p is 4. In some examples, p is 8.

[0034] In some examples, the antibody is conjugated to camptothecin. In some examples, the antibody is conjugated to camptothecin via an enzyme-cleavable linker unit. In some examples, the enzyme-cleavable linker unit includes a Val-Lys-Gly linker. In some examples, the antibody is conjugated to camptothecin via a linker forming an antibody-drug conjugate having the structure:



represents the antibody and p ranges from 2 to 10. In some examples, p is 4. In some examples, p is 8.

[0035] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind to the human PD-L1 protein, where the antibody includes heavy chain CDR sequences of SEQ ID NOs: 13-15 and light chain CDR sequences of SEQ ID NOs: 16-18.

[0036] In some examples, the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 300 nM. In some examples, the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.

[0037] In some examples, the antibody further exhibits a total internalization that is higher than the total internalization of Ab1. In some examples, the total internalization is between a 9% and 155% increase of AUC over the AUC of Ab1. In some examples, the total internalization is determined by a FabFluor internalization assay.

[0038] In some examples, the antibody further exhibits an x50 that is higher than the x50 of Ab1.

[0039] In some examples, the antibody is conjugated to monomethyl auristatin E (MMAE), and where the x50 is between 3 ng/mL and 20 ng/mL in an MDA-MB-231 cell line.

[0040] In some examples, the antibody is conjugated to camptothecin, and where the x50 is between 15 ng/mL and 55 ng/mL in an MDA-MB-231 cell line.

[0041] In some examples, the antibody includes a heavy chain variable region sequence having at least 80% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 80% sequence identity to SEQ ID NO: 12. In some examples, the antibody includes a heavy chain variable region sequence having at least 90% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 90% sequence identity to SEQ ID NO: 12. In some examples, the antibody includes a heavy chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 12. In some examples, the antibody includes a heavy chain variable region sequence of SEQ ID NO: 11 and a light chain variable region sequence of SEQ ID NO: 12.

[0042] In some examples, the antibody includes a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.

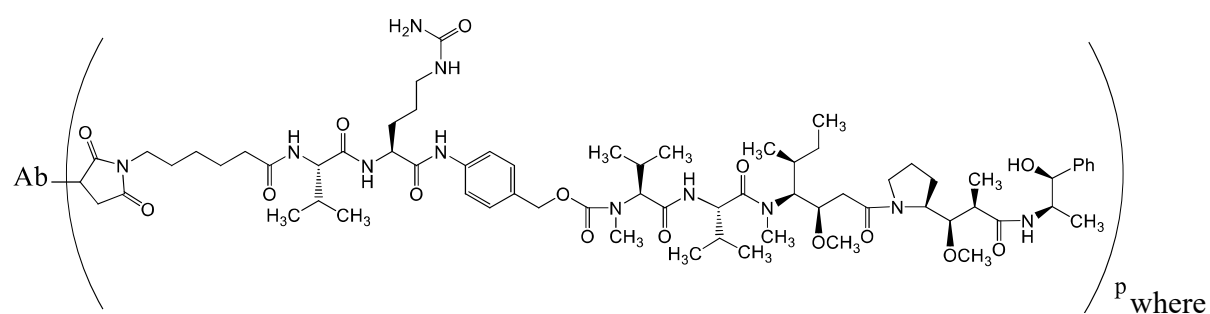
[0043] In some examples, the fragment is a Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, or single-chain antibody fragment.

[0044] In some examples, the antibody contains L234A and L235A mutations in the heavy chain of the antibody.

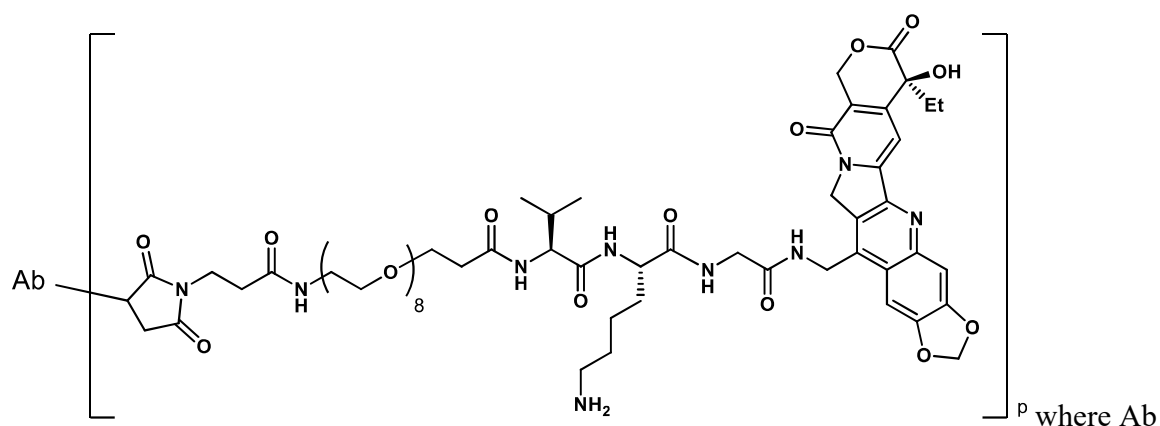
[0045] In some examples, the heavy chain constant region is of the IgG1 isotype.

[0046] In some examples, the antibody is a humanized or chimeric antibody.

[0047] In some examples, the antibody is conjugated to a cytotoxic agent via a linker. In some examples, the antibody is conjugated to monomethyl auristatin E (MMAE). In some examples, the antibody is conjugated to MMAE via an enzyme-cleavable linker unit. In some examples, the enzyme-cleavable linker unit includes a Val-Cit linker. In some examples, the antibody is conjugated to MMAE via a linker forming an antibody-drug conjugate having the structure:

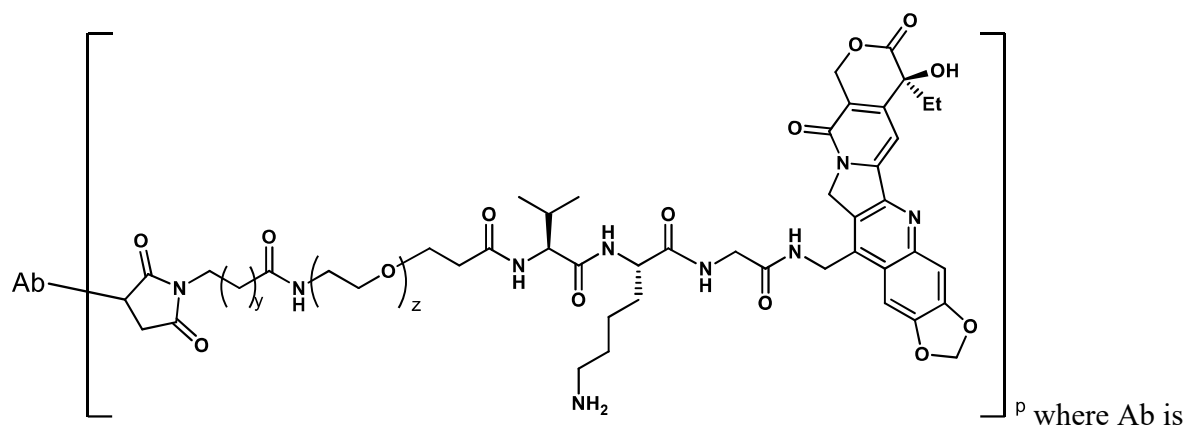


[0048] In some examples, the antibody is conjugated to camptothecin. In some examples, the antibody is conjugated to camptothecin via an enzyme-cleavable linker unit. In some examples, the enzyme-cleavable linker unit includes a Val-Lys-Gly linker. In some examples, the antibody is conjugated to camptothecin via a linker forming an antibody-drug conjugate having the structure:



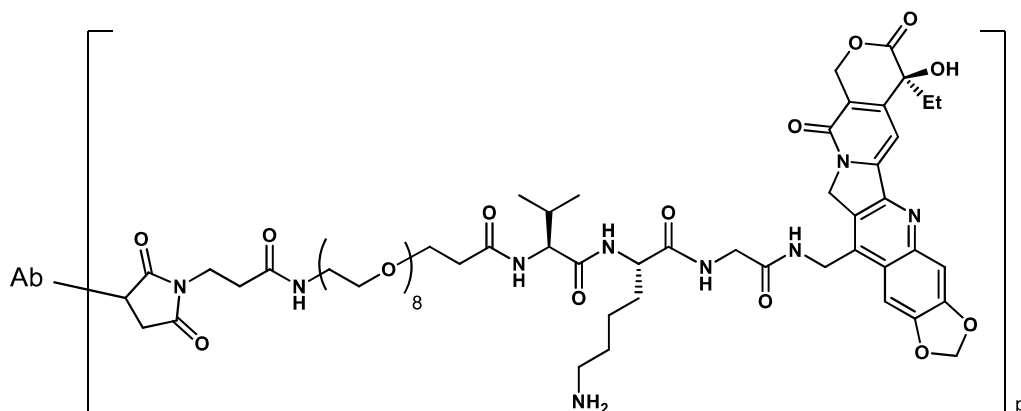
represents the antibody and p ranges from 2 to 10. In some examples, p is 4. In some examples, p is 8.

[0049] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind to the human PD-L1 protein, where the antibody is conjugated to camptothecin forming an antibody-drug conjugate, where the antibody-drug conjugate has the structure:



an anti-PD-L1 antibody; y is 1, 2, 3, or 4, or is 1 or 4; and z is an integer from 2 to 12, or is 2, 4, 8, or 12; and p is 1-16.

[0050] In some examples, the antibody-drug conjugate has the structure:



[0051] In some examples, p ranges from 2 to 10.

[0052] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind to the human Programmed Death-Ligand 1 (PD-L1) protein, wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is greater than that of Ab1. In some examples, the antibody exhibits a binding affinity that is greater than 2.7 nM.

[0053] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind to the human Programmed Death-Ligand 1 (PD-L1) protein, wherein the antibody exhibits a k_{assoc} to the human PD-L1 protein that is less than that of Ab1. In some examples, the antibody exhibits a k_{assoc} to the human PD-L1 protein that is less than $5 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$.

[0054] Also provided herein are antibodies or antigen-binding fragments thereof that specifically bind to the human Programmed Death-Ligand 1 (PD-L1) protein, wherein the antibody exhibits a k_{dissoc} to the human PD-L1 protein that is greater than that of Ab1. In some examples, the antibody exhibits a k_{dissoc} to the human PD-L1 protein that is greater than $2 \times 10^3 \text{ s}^{-1}$.

[0055] Also provided herein are antibody-drug conjugates including an antibody or antigen-binding fragment thereof that specifically binds to the human PD-L1 protein, where the antibody includes heavy chain CDR sequences of SEQ ID NOs: 3-5 and light chain CDR sequences of SEQ ID NOs: 6-8, where the antibody includes one or more amino acid substitutions within one or more of the CDRs, and where the antibody exhibits a binding affinity to the human PD-L1 protein that is between 5 nM and 15 nM, and where the antibody is conjugated to MMAE.

[0056] Also provided herein are antibody-drug conjugates including an antibody or antigen-binding fragment thereof that specifically binds to the human PD-L1 protein, where the antibody includes heavy chain CDR sequences of SEQ ID NOs: 3-5 and light chain CDR sequences of SEQ ID NOs: 6-8, where the antibody includes one or more amino acid substitutions within one or more of the CDRs, and where the antibody exhibits a binding affinity to the human PD-L1 protein that is between 5 nM and 15 nM, and where the antibody is conjugated to camptothecin.

[0057] In another aspect, the present disclosure provides a pharmaceutical composition that include a therapeutically effective amount of the antibodies described herein and a pharmaceutically acceptable excipient.

[0058] In another aspect, the present disclosure provides the antibody as described herein for use in therapy. In yet another aspect, the present disclosure provides a use of the antibody as described herein in the manufacture of a medicament for treating cancer in a subject. Also provided herein are methods of treating cancer in a subject, including administering to the subject any of the antibodies described herein. In some examples, the subject is a human subject. In some examples, the cancer is melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck cancer, triple negative breast cancer (TNBC), ovarian cancer, urothelial cancer, hepatocellular carcinoma (HCC), gastric cancer, or cervical cancer.

[0059] In a further aspect, the present disclosure provides a nucleic acid encoding any of the antibodies described herein.

[0060] In a further aspect, the present disclosure provides a vector including any of the nucleic acids described herein.

[0061] In a further aspect, the present disclosure provides a host cell described herein that include any of the nucleic acids described herein. In some examples, the host cell is a Chinese hamster ovary (CHO) cell.

[0062] In a further aspect, the present disclosure provides a method of producing an antibody or antigen-binding fragment thereof that specifically binds to the human PD-L1 protein, including culturing any of the host cells described herein under a condition suitable for production of the antibody.

[0063] In a further aspect, the present disclosure provides a method of producing an antibody drug-conjugate that specifically binds to the human PD-L1 protein, including culturing any of the host cells described herein under a condition suitable for production of the antibody; and conjugating the antibody to a cytotoxic agent. In some examples, the cytotoxic agent is MMAE or camptothecin.

CLAIMS

1. An antibody or antigen-binding fragment thereof that specifically binds to the human PD-L1 protein, wherein the antibody comprises heavy chain CDR sequences of SEQ ID NOs: 13-15 and light chain CDR sequences of SEQ ID NOs: 16-18.
2. The antibody of claim 1, wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 300 nM.
3. The antibody of claim 1 or 2, wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.
4. The antibody of any of claims 1-3, wherein the antibody further exhibits a total internalization that is higher than the total internalization of Ab1.
5. The antibody of claim 4, wherein the total internalization is between a 9% and 155% increase of AUC over the AUC of Ab1.
6. The antibody of claim 5, wherein the total internalization is determined by a FabFluor internalization assay.
7. The antibody of any of claims 1-6, wherein the antibody further exhibits an x50 that is higher than the x50 of Ab1.
8. The antibody of claim 7, wherein the antibody is conjugated to monomethyl auristatin E (MMAE), and wherein the x50 is between 3 ng/mL and 20 ng/mL in an MDA-MB-231 cell line.
9. The antibody of claim 7, wherein the antibody is conjugated to camptothecin, and wherein the x50 is between 15 ng/mL and 55 ng/mL in an MDA-MB-231 cell line.
10. The antibody of any of claims 1-9, wherein the antibody comprises a heavy chain variable region sequence having at least 80% sequence identity to SEQ ID NO: 11

and a light chain variable region sequence having at least 80% sequence identity to SEQ ID NO: 12.

11. The antibody of any of claims 1-10, wherein the antibody comprises a heavy chain variable region sequence having at least 90% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 90% sequence identity to SEQ ID NO: 12.

12. The antibody of any of claims 1-11, wherein the antibody comprises a heavy chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 11 and a light chain variable region sequence having at least 95% sequence identity to SEQ ID NO: 12.

13. The antibody of any of claims 1-12, wherein the antibody comprises a heavy chain variable region sequence of SEQ ID NO: 11 and a light chain variable region sequence of SEQ ID NO: 12.

14. The antibody of any of claims 1-13, wherein the antibody comprises a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.

15. The antibody of any of claims 1-14, wherein the fragment is a Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, or single-chain antibody fragment.

16. The antibody of any of claims 1-15, wherein the antibody contains L234A and L235A mutations in the heavy chain of the antibody.

17. The antibody of any of claims 1-16, wherein the heavy chain constant region is of the IgG1 isotype.

18. The antibody of any of claims 1-17, wherein the antibody is a humanized or chimeric antibody.

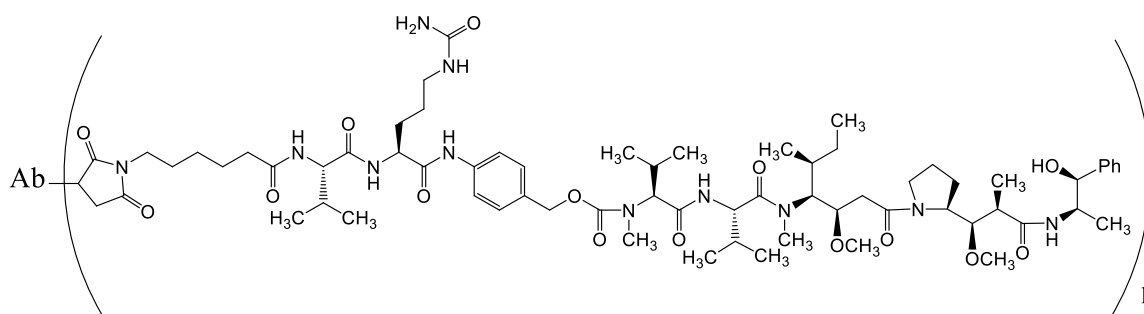
19. The antibody of any of claims 1-18, wherein the antibody is conjugated to a cytotoxic agent via a linker.

20. The antibody of claim 19, wherein the antibody is conjugated to monomethyl auristatin E (MMAE).

21. The antibody of claim 20, wherein the antibody is conjugated to MMAE via an enzyme-cleavable linker unit.

22. The antibody of claim 21, wherein the enzyme-cleavable linker unit comprises a Val-Cit linker.

23. The antibody of any one of claims 19-22, wherein the antibody is conjugated to MMAE via a linker forming an antibody-drug conjugate having the structure:



wherein Ab represents the antibody and p ranges from 2 to 10.

24. The antibody of claim 23, wherein p is 4.

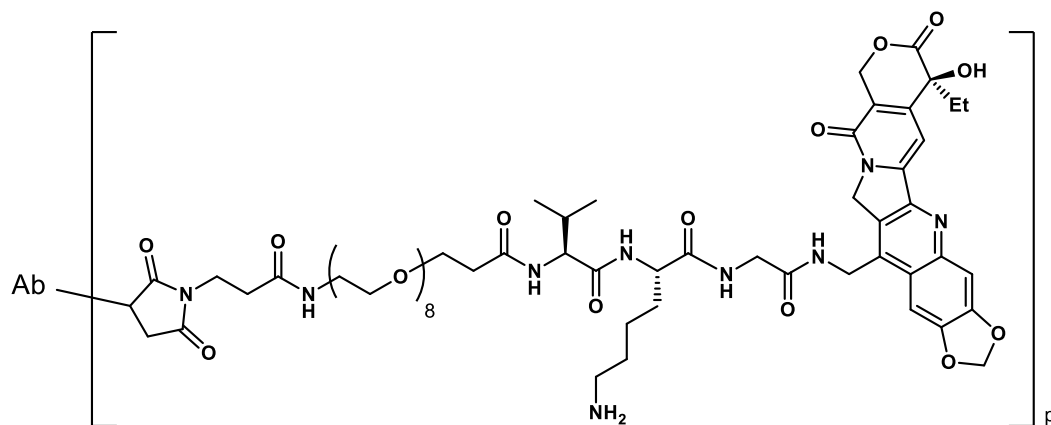
25. The antibody of claim 23, wherein p is 8.

26. The antibody of claim 19, wherein the antibody is conjugated to camptothecin.

27. The antibody of claim 26, wherein the antibody is conjugated to camptothecin via an enzyme-cleavable linker unit.

28. The antibody of claim 27, wherein the enzyme-cleavable linker unit comprises a Val-Lys-Gly linker.

29. The antibody of any one of claims 26-28, wherein the antibody is conjugated to camptothecin via a linker forming an antibody-drug conjugate having the structure:



wherein Ab represents the antibody and p ranges from 2 to 10.

30. The antibody of claim 29, wherein p is 4.
31. The antibody of claim 29, wherein p is 8.
32. A pharmaceutical composition that comprises a therapeutically effective amount of the antibody of any of claims 1-31 and a pharmaceutically acceptable excipient.
33. The antibody of any of claims 1-31 for use in therapy.
34. Use of the antibody of any of claims 1-31 in the manufacture of a medicament for treating cancer in a subject.
35. The use of claim 34, wherein the subject is a human subject.
36. The use of claim 34 or 35, wherein the cancer is melanoma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), head and neck cancer, triple negative breast cancer (TNBC), ovarian cancer, urothelial cancer, hepatocellular carcinoma (HCC), gastric cancer, or cervical cancer.
37. A nucleic acid encoding the antibody as defined by any one of claims 1-31.
38. A vector comprising the nucleic acid of claim 37.
39. A host cell comprising the nucleic acid of claim 38.

40. The host cell of claim 39, wherein the host cell is a Chinese hamster ovary (CHO) cell.

41. A method of producing an antibody or antigen-binding fragment thereof that specifically binds to the human PD-L1 protein, comprising culturing the host cell of claim 39 or 40 under a condition suitable for production of the antibody.

42. A method of producing an antibody drug-conjugate that specifically binds to the human PD-L1 protein, comprising culturing the host cell of claim 39 or 40 under a condition suitable for production of the antibody; and conjugating the antibody to a cytotoxic agent.

43. The method of claim 42, wherein the cytotoxic agent is MMAE or camptothecin.