

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 embodimentexamples, the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.

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

[0008] In some embodimentexamples, the antibody further exhibits an x50 that is lower than the x50 of Ab1. In some embodimentexamples, 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 embodimentexamples, 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 ~~embodiment~~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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), the antibody includes a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.

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

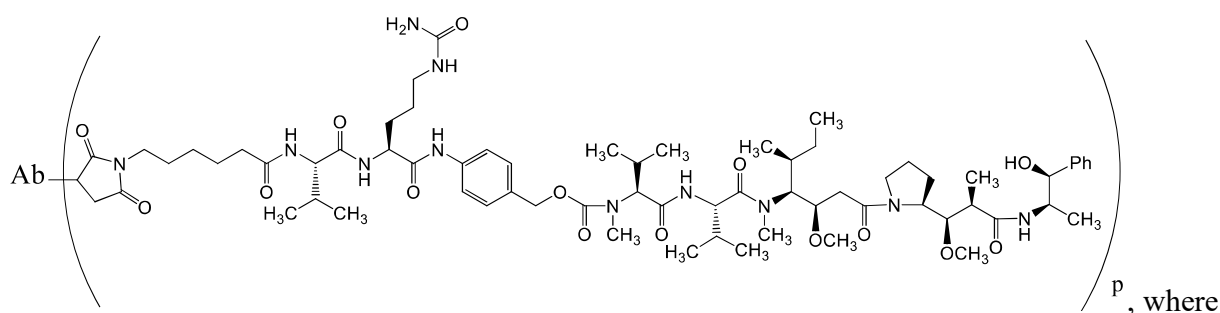
[0015] In some [embodimentexamples](#), the antibody contains L234A and L235A mutations in the heavy chain of the antibody.

[0016] In some [embodimentexamples](#), the heavy chain constant region is of the IgG1 isotype.

[0017] In some [embodimentexamples](#), the antibody is a humanized or chimeric antibody.

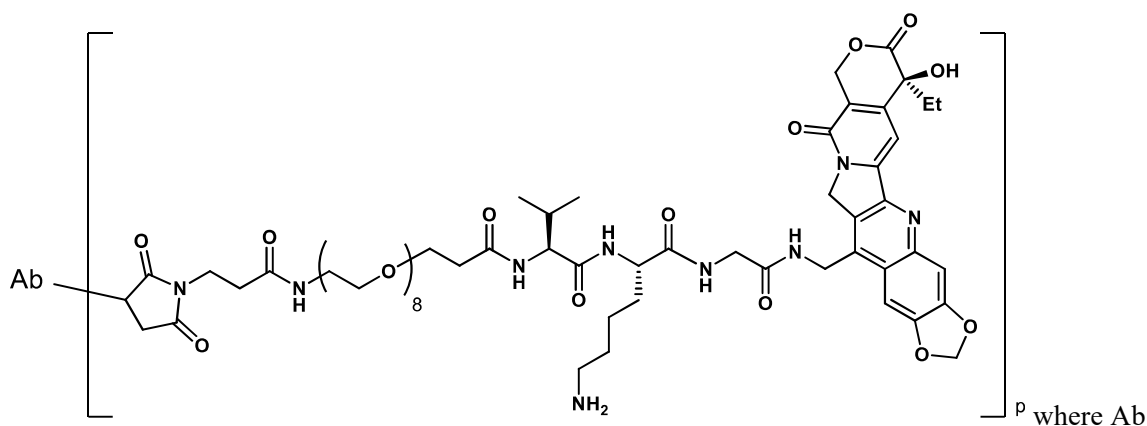
[0018] In some [embodimentexamples](#), the antibody is conjugated to a cytotoxic agent via a linker.

[0019] In some [embodimentexamples](#), the antibody is conjugated to monomethyl auristatin E (MMAE). In some [embodimentexamples](#), the antibody is conjugated to MMAE via an enzyme-cleavable linker unit. In some [embodimentexamples](#), the enzyme-cleavable linker unit includes a Val-Cit linker. In some [embodimentexamples](#), 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 [embodimentexamples](#), p is 4. In some [embodimentexamples](#), p is 8.

[0020] In some [embodimentexamples](#), the antibody is conjugated to camptothecin. In some [embodimentexamples](#), the antibody is conjugated to camptothecin via an enzyme-cleavable linker unit. In some [embodimentexamples](#), the enzyme-cleavable linker unit includes a Val-Lys-Gly linker. In some [embodimentexamples](#), 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 [embodimentexamples](#), p is 4. In some [embodimentexamples](#), 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 [embodimentexamples](#), the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 300 nM. In some [embodimentexamples](#), the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.

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

[0024] In some [embodimentexamples](#), the antibody further exhibits an x50 that is higher than the x50 of Ab1.

[0025] In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), the antibody includes a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.

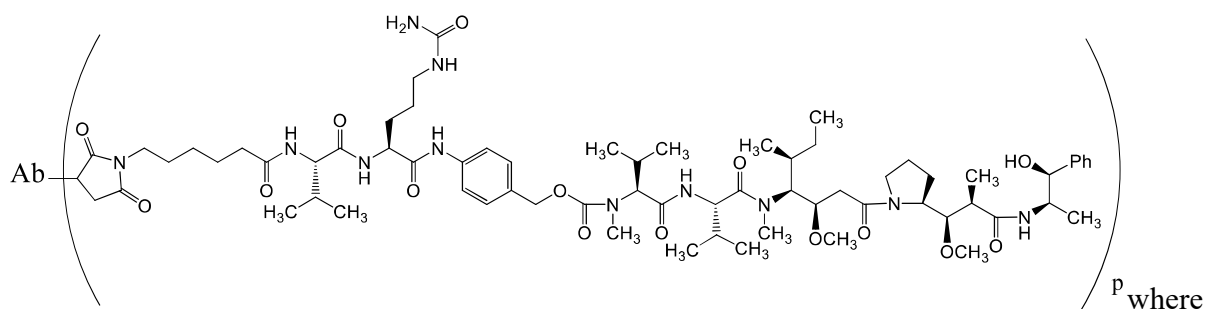
[0029] In some [embodimentexamples](#), the fragment is a Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, or single-chain antibody fragment.

[0030] In some [embodimentexamples](#), the antibody contains L234A and L235A mutations in the heavy chain of the antibody.

[0031] In some [embodimentexamples](#), the heavy chain constant region is of the IgG1 isotype.

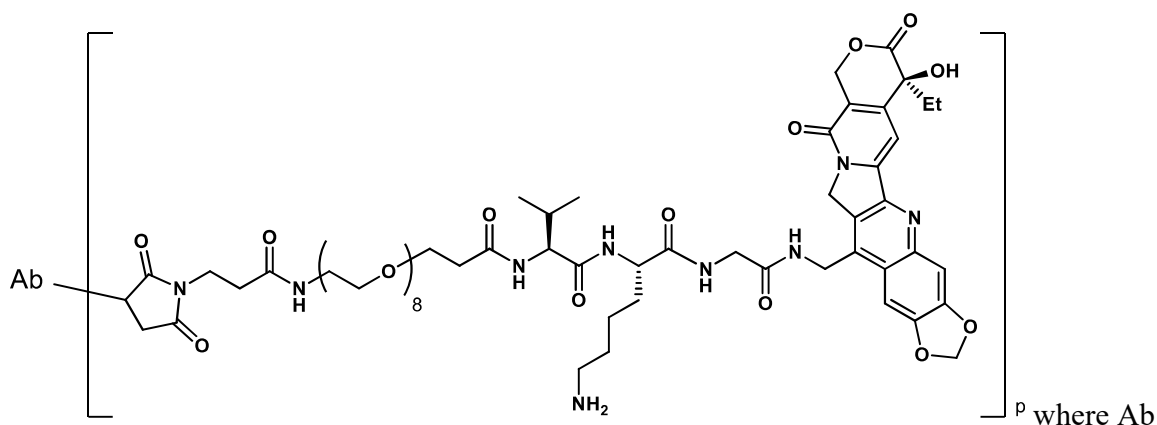
[0032] In some [embodimentexamples](#), the antibody is a humanized or chimeric antibody.

[0033] In some [embodimentexamples](#), the antibody is conjugated to a cytotoxic agent via a linker. In some [embodimentexamples](#), the antibody is conjugated to monomethyl auristatin E (MMAE). In some [embodimentexamples](#), the antibody is conjugated to MMAE via an enzyme-cleavable linker unit. In some [embodimentexamples](#), the enzyme-cleavable linker unit includes a Val-Cit linker. In some [embodimentexamples](#), 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 [embodimentexamples](#), p is 4. In some [embodimentexamples](#), p is 8.

[0034] In some [embodimentexamples](#), the antibody is conjugated to camptothecin. In some [embodimentexamples](#), the antibody is conjugated to camptothecin via an enzyme-cleavable linker unit. In some [embodimentexamples](#), the enzyme-cleavable linker unit includes a Val-Lys-Gly linker. In some [embodimentexamples](#), 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 [embodimentexamples](#), p is 4. In some [embodimentexamples](#), 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 [embodimentexamples](#), the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 300 nM. In some [embodimentexamples](#), the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.

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

[0038] In some [embodimentexamples](#), the antibody further exhibits an x50 that is higher than the x50 of Ab1.

[0039] In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexample](#), 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 [embodimentexamples](#), the antibody includes a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.

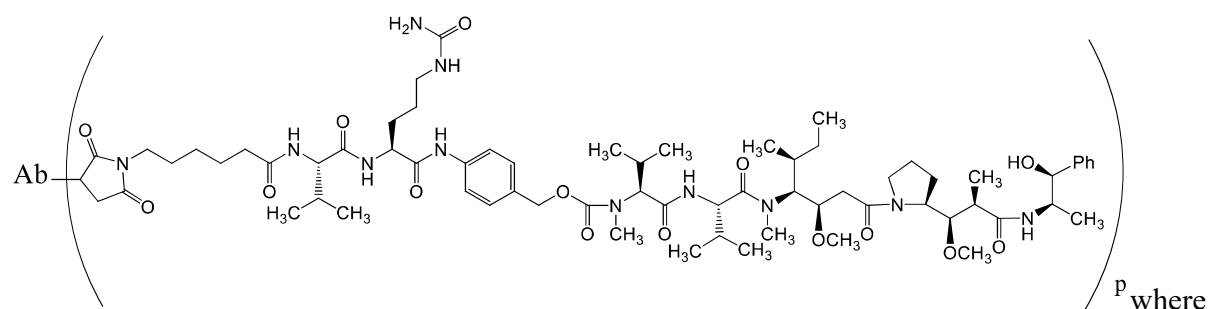
[0043] In some [embodimentexamples](#), the fragment is a Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, or single-chain antibody fragment.

[0044] In some [embodimentexamples](#), the antibody contains L234A and L235A mutations in the heavy chain of the antibody.

[0045] In some [embodimentexamples](#), the heavy chain constant region is of the IgG1 isotype.

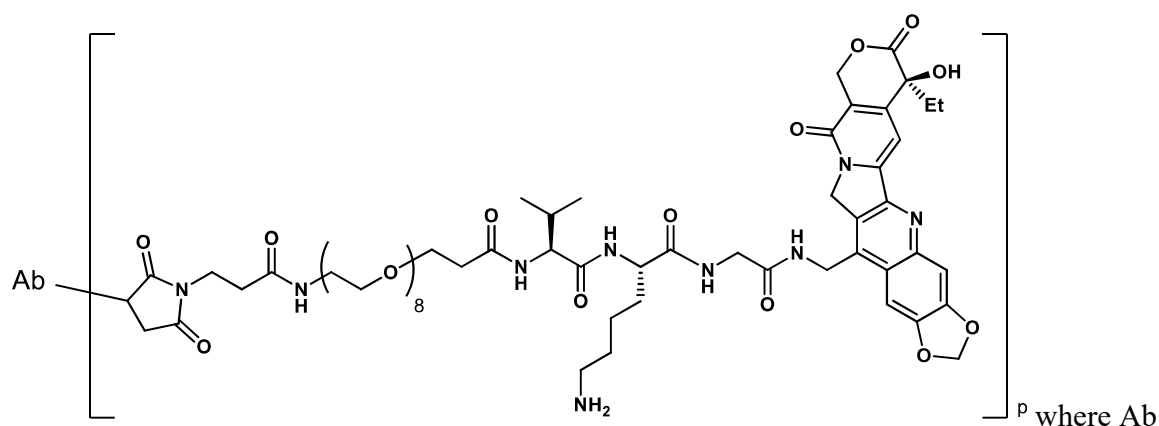
[0046] In some [embodimentexamples](#), the antibody is a humanized or chimeric antibody.

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



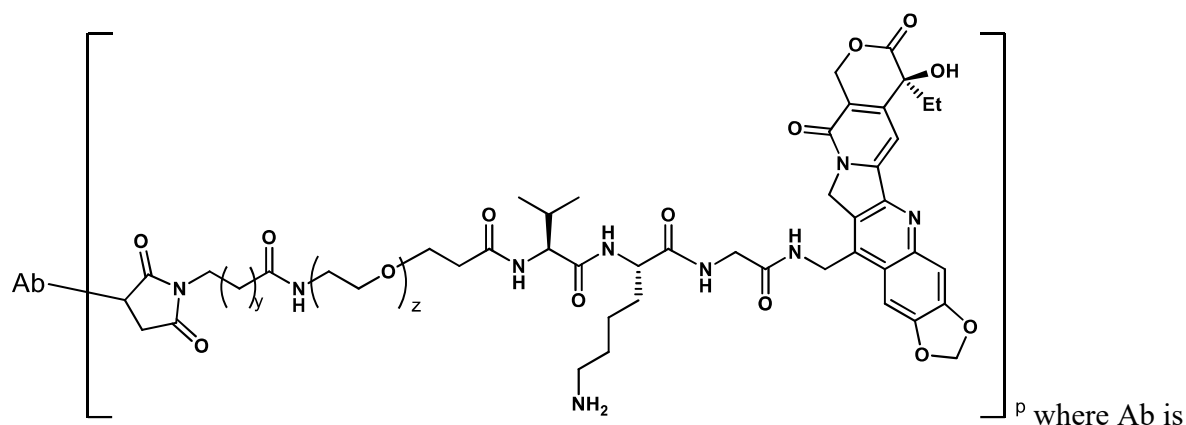
Ab represents the antibody and p ranges from 2 to 10. In some [embodimentexamples](#), p is 4. In some [embodimentexamples](#), p is 8.

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



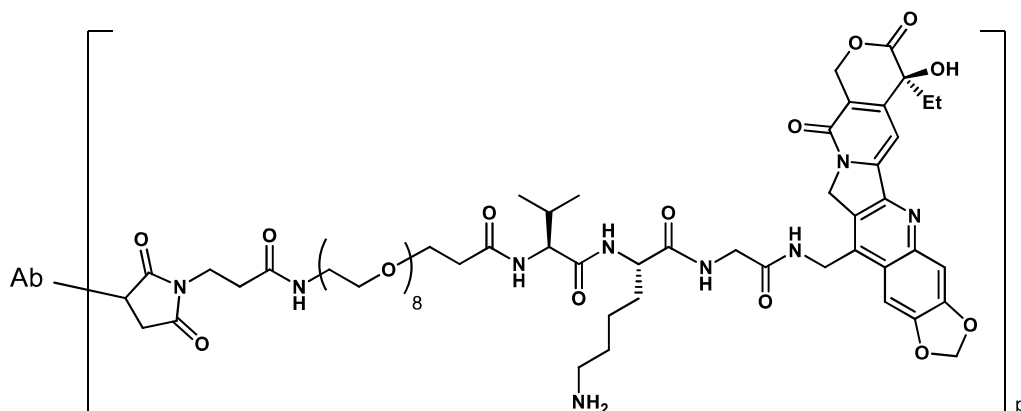
represents the antibody and p ranges from 2 to 10. In some [embodimentexamples](#), p is 4. In some [embodimentexamples](#), p is 8.

[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 [embodimentexamples](#), the antibody-drug conjugate has the structure:



[0051] In some [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexamples](#), 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 [embodimentexample](#)s, 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 ~~Also provided herein are~~ pharmaceutical compositions 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 ~~embodiment~~examples, the subject is a human subject. In some ~~embodiment~~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 ~~Also provided herein are~~ nucleic acid encoding any of the antibodies described herein.

[0060] In a further aspect, the present disclosure provides a ~~Also provided herein are~~ vectors including any of the nucleic acids described herein.

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

[0062] In a further aspect, the present disclosure provides a ~~Also provided herein are~~ methods 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 ~~Also provided herein are~~ methods 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 ~~embodiment~~examples, the cytotoxic agent is MMAE or camptothecin.

CLAIMS

1. ~~An antibody or antigen-binding fragment thereof that specifically binds 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 between 3 and 300 nM.~~
2. ~~The antibody of claim 1, wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.~~
3. ~~The antibody of claim 1 or claim 2, wherein the antibody further exhibits a total internalization that is higher than the total internalization of Ab1.~~
4. ~~The antibody of claim 3, wherein the total internalization is between a 9% and 155% increase of AUC over the AUC of Ab1.~~
5. ~~The antibody of claim 3, wherein the total internalization is determined by a FabFluor internalization assay.~~
6. ~~The antibody of any of claims 1-5, wherein the antibody further exhibits an x50 that is lower than the x50 of Ab1.~~
7. ~~The antibody of claim 6, 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.~~
8. ~~The antibody of claim 6, 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.~~
9. ~~The antibody of any of claims 1-8, 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.~~
10. ~~The antibody of any of claims 1-8, wherein the antibody comprises heavy chain CDR sequences of SEQ ID NOs: 3-5 and light chain CDR sequences of SEQ ID NOs: 6-8,~~

~~wherein the antibody comprises one or more amino acid substitutions within one or more of the CDRs;~~

~~11. — The antibody of any of claims 1-10, 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;~~

~~12. — The antibody of any of claims 1-11, 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;~~

~~13. — The antibody of any of claims 1-12, 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;~~

~~14. — The antibody of any of claims 1-13, 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;~~

~~15. — The antibody of any of claims 1-14, wherein the antibody comprises a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10;~~

~~16. — The antibody of any of claims 1-15, wherein the fragment is a Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, or single chain antibody fragment;~~

~~17. — The antibody of any of claims 1-16, wherein the antibody contains L234A and L235A mutations in the heavy chain of the antibody;~~

~~18. — The antibody of any of claims 1-17, wherein the heavy chain constant region is of the IgG1 isotype;~~

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

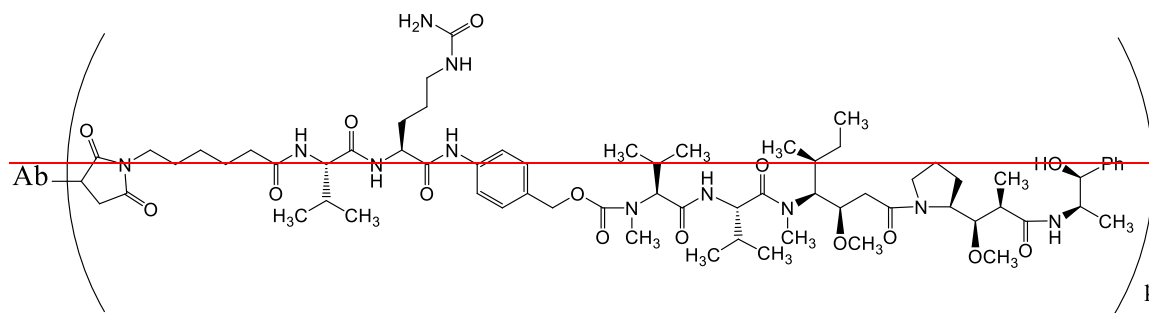
20. —The antibody of any of claims 1-19, wherein the antibody is conjugated to a cytotoxic agent via a linker.

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

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

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

24. —The antibody of any one of claims 20-23, 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.

25. —The antibody of claim 24, wherein p is 4.

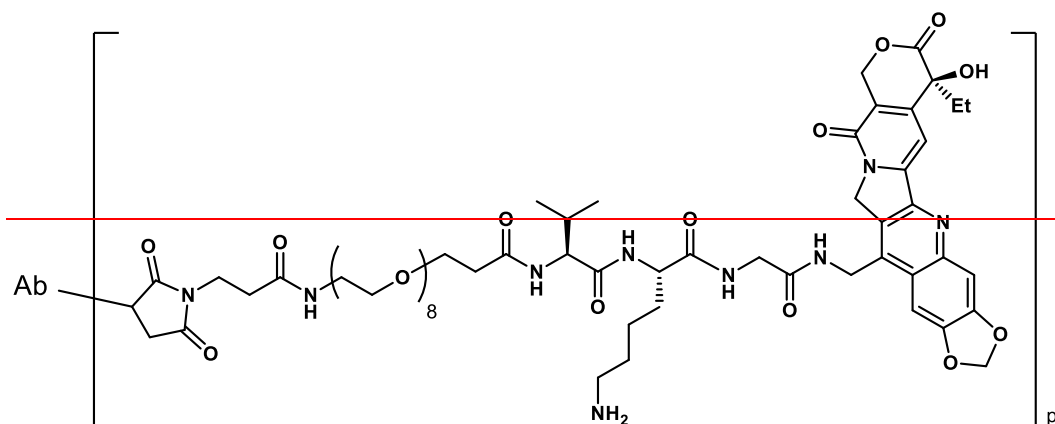
26. —The antibody of claim 24, wherein p is 8.

27. —The antibody of claim 20, wherein the antibody is conjugated to camptothecin.

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

~~29. The antibody of claim 28, wherein the enzyme-cleavable linker unit comprises a Val-Lys-Gly linker.~~

~~30. The antibody of any one of claims 27-29, 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.~~

~~31. The antibody of claim 30, wherein p is 4.~~

~~32. The antibody of claim 30, wherein p is 8.~~

~~33. A pharmaceutical composition that comprises a therapeutically effective amount of the antibody of any of claims 1-32 and a pharmaceutically acceptable excipient.~~

~~34. A method of treating cancer in a subject, comprising administering to the subject the antibody of any of claims 1-33.~~

~~35. The method of claim 34, wherein the subject is a human subject.~~

~~36. The method of claim 34 or claim 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.~~

- ~~33. 37. — A nucleic acid encoding the antibody as defined by any one of claims 1–~~
- ~~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.~~
- ~~44. — 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: 3–5 and light chain CDR sequences of SEQ ID NOs: 6–8, wherein the antibody comprises one or more amino acid substitutions within one or more of the CDRs.~~
- ~~45. — The antibody of claim 44, wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 300 nM.~~
- ~~46. — The antibody of claim 44 or 45, wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.~~
- ~~47. — The antibody of any of claims 44–46, wherein the antibody further exhibits a total internalization that is higher than the total internalization of Ab1.~~

~~48.—The antibody of claim 47, wherein the total internalization is between a 9% and 155% increase of AUC over the AUC of Ab1.~~

~~49.—The antibody of claim 48, wherein the total internalization is determined by a FabFluor internalization assay.~~

~~50.—The antibody of any of claims 44-49, wherein the antibody further exhibits an x50 that is higher than the x50 of Ab1.~~

~~51.—The antibody of claim 50, 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.~~

~~52.—The antibody of claim 50, 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.~~

~~53.—The antibody of any of claims 44-52, 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.~~

~~54.—The antibody of any of claims 44-53, 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.~~

~~55.—The antibody of any of claims 44-54, 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.~~

~~56.—The antibody of any of claims 44-55, 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.~~

~~57. The antibody of any of claims 44-56, 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.~~

~~58. The antibody of any of claims 44-57, wherein the antibody comprises a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.~~

~~59. The antibody of any of claims 44-58, wherein the fragment is a Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, or single-chain antibody fragment.~~

~~60. The antibody of any of claims 44-59, wherein the antibody contains L234A and L235A mutations in the heavy chain of the antibody.~~

~~61. The antibody of any of claims 44-60, wherein the heavy chain constant region is of the IgG1 isotype.~~

~~62. The antibody of any of claims 44-61, wherein the antibody is a humanized or chimeric antibody.~~

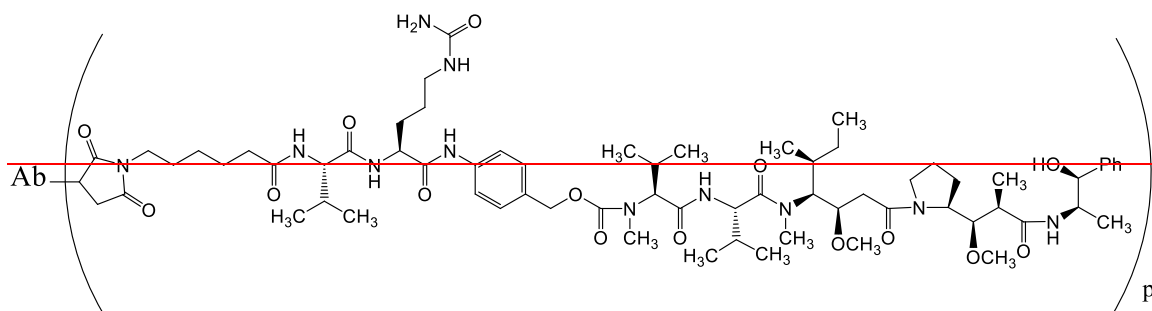
~~63. The antibody of any of claims 44-62, wherein the antibody is conjugated to a cytotoxic agent via a linker.~~

~~64. The antibody of claim 63, wherein the antibody is conjugated to monomethyl auristatin E (MMAE).~~

~~65. The antibody of claim 64, wherein the antibody is conjugated to MMAE via an enzyme-cleavable linker unit.~~

~~66. The antibody of claim 65, wherein the enzyme-cleavable linker unit comprises a Val-Cit linker.~~

~~67. The antibody of any one of claims 63-66, 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.

68. The antibody of claim 67, wherein p is 4.

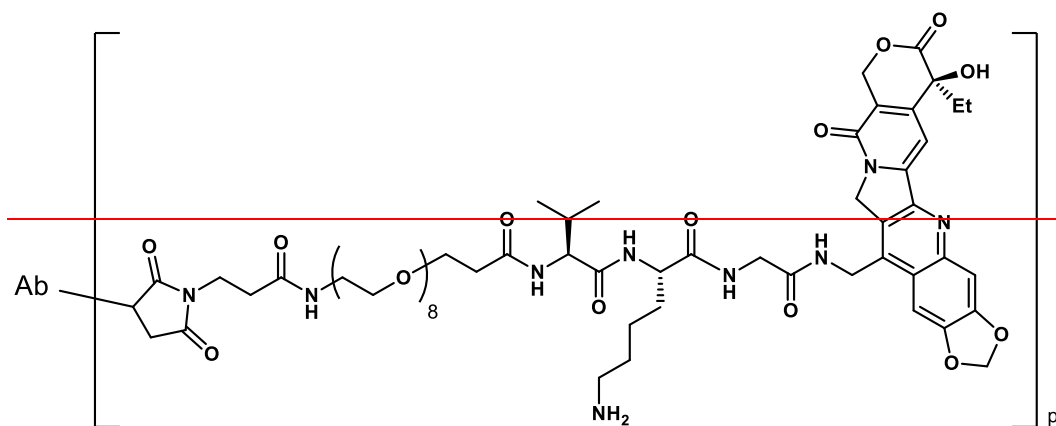
69. The antibody of claim 67, wherein p is 8.

70. The antibody of claim 63, wherein the antibody is conjugated to camptothecin.

71. The antibody of claim 70, wherein the antibody is conjugated to camptothecin via an enzyme-cleavable linker unit.

72. The antibody of claim 71, wherein the enzyme-cleavable linker unit comprises a Val-Lys-Gly linker.

73. The antibody of any one of claims 70-72, 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.

~~74. The antibody of claim 73, wherein p is 4.~~

~~75. The antibody of claim 73, wherein p is 8.~~

~~76. A pharmaceutical composition that comprises a therapeutically effective amount of the antibody of any of claims 44-75 and a pharmaceutically acceptable excipient.~~

~~77. A method of treating cancer in a subject, comprising administering to the subject the antibody of any of claims 44-76.~~

~~78. The method of claim 77, wherein the subject is a human subject.~~

~~79. The method of claim 77 or claim 78, 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.~~

~~80. A nucleic acid encoding the antibody as defined by any one of claims 44-76.~~

~~81. A vector comprising the nucleic acid of claim 80.~~

~~82. A host cell comprising the nucleic acid of claim 81.~~

~~83. The host cell of claim 82, wherein the host cell is a Chinese hamster ovary (CHO) cell.~~

~~84. 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 82 or 83 under a condition suitable for production of the antibody.~~

~~85. A method of producing an antibody drug conjugate that specifically binds to the human PD-L1 protein, comprising culturing the host cell of claim 82 or 83 under a condition suitable for production of the antibody; and conjugating the antibody to a cytotoxic agent.~~

~~86. The method of claim 85, wherein the cytotoxic agent is MMAE or camptothecin.~~

871. 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.

882. The antibody of claim 871, wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 300 nM.

893. The antibody of claim 871 or 882, wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.

904. The antibody of any of claims 871-893, wherein the antibody further exhibits a total internalization that is higher than the total internalization of Ab1.

915. The antibody of claim 904, wherein the total internalization is between a 9% and 155% increase of AUC over the AUC of Ab1.

926. The antibody of claim 915, wherein the total internalization is determined by a FabFluor internalization assay.

937. The antibody of any of claims 871-926, wherein the antibody further exhibits an x50 that is higher than the x50 of Ab1.

948. The antibody of claim 937, 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.

959. The antibody of claim 937, 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.

9610. The antibody of any of claims 871-959, 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.

9711. The antibody of any of claims 871-9610, 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.

9812. The antibody of any of claims 871-9711, 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.

9913. The antibody of any of claims 871-9812, 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.

10014. The antibody of any of claims 871-9913, wherein the antibody comprises a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.

10115. The antibody of any of claims 871-10014, wherein the fragment is a Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, or single-chain antibody fragment.

10216. The antibody of any of claims 871-10115, wherein the antibody contains L234A and L235A mutations in the heavy chain of the antibody.

10317. The antibody of any of claims 871-10216, wherein the heavy chain constant region is of the IgG1 isotype.

10418. The antibody of any of claims 871-10317, wherein the antibody is a humanized or chimeric antibody.

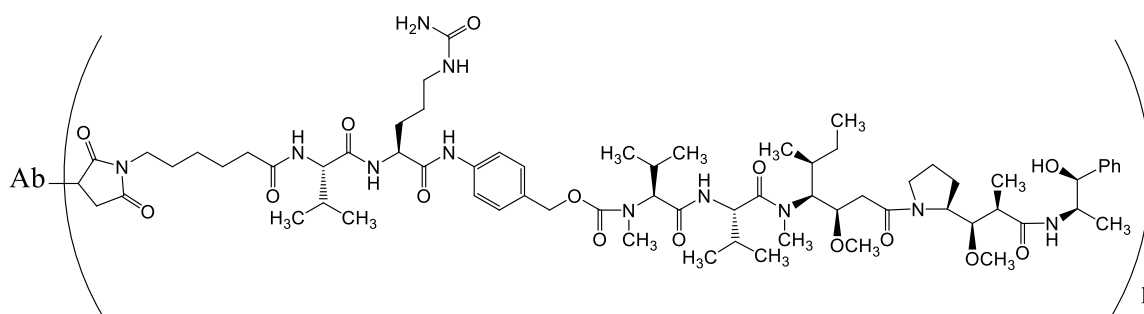
10519. The antibody of any of claims 871-10418, wherein the antibody is conjugated to a cytotoxic agent via a linker.

10620. The antibody of claim **10519**, wherein the antibody is conjugated to monomethyl auristatin E (MMAE).

10721. The antibody of claim **10620**, wherein the antibody is conjugated to MMAE via an enzyme-cleavable linker unit.

10822. The antibody of claim **10721**, wherein the enzyme-cleavable linker unit comprises a Val-Cit linker.

10923. The antibody of any one of claims **10519-10822**, 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.

11024. The antibody of claim **10923**, wherein p is 4.

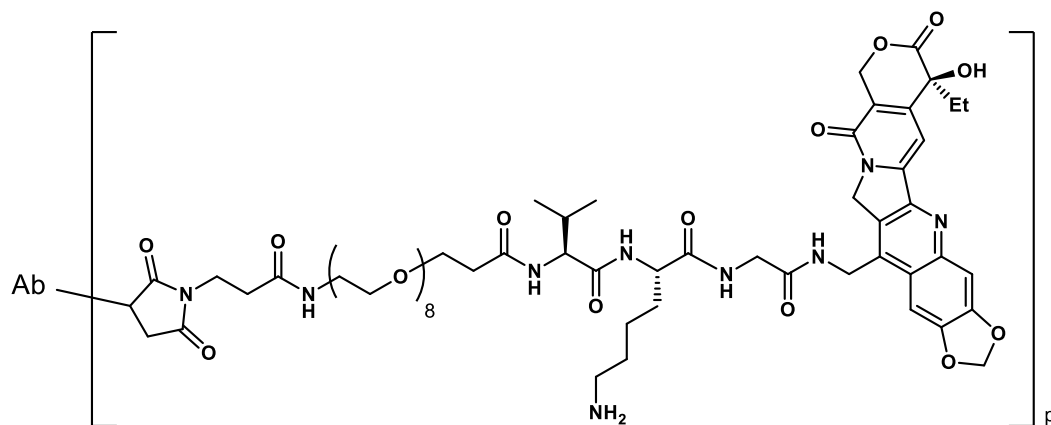
11125. The antibody of claim **10923**, wherein p is 8.

11226. The antibody of claim **10519**, wherein the antibody is conjugated to camptothecin.

11327. The antibody of claim **11226**, wherein the antibody is conjugated to camptothecin via an enzyme-cleavable linker unit.

11428. The antibody of claim **11327**, wherein the enzyme-cleavable linker unit comprises a Val-Lys-Gly linker.

11529. The antibody of any one of claims **11226-11428**, 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.

11630. The antibody of claim 11529, wherein p is 4.

11731. The antibody of claim 11529, wherein p is 8.

11832. A pharmaceutical composition that comprises a therapeutically effective amount of the antibody of any of claims 871-11731 and a pharmaceutically acceptable excipient.

11933. ~~A method of treating cancer in a subject, comprising administering to the subject~~ The antibody of any of claims 871-11831 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.

12035. The ~~method-use~~ of claim 11934, wherein the subject is a human subject.

12136. The ~~method-use~~ of claim 11934 or 12035, 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.

12237. A nucleic acid encoding the antibody as defined by any one of claims 871-11831.

~~12338~~. A vector comprising the nucleic acid of claim ~~12237~~.

~~12439~~. A host cell comprising the nucleic acid of claim ~~12338~~.

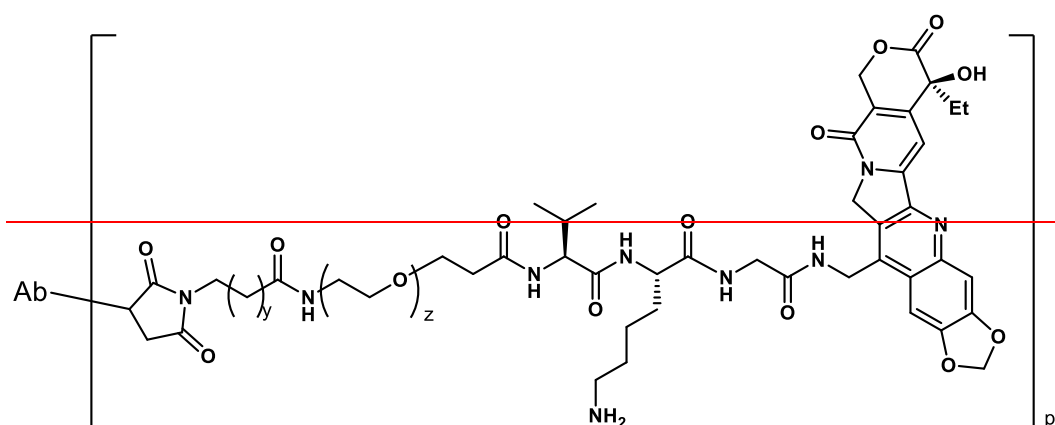
~~12540~~. The host cell of claim ~~12439~~, wherein the host cell is a Chinese hamster ovary (CHO) cell.

~~12641~~. 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 ~~12439~~ or ~~12540~~ under a condition suitable for production of the antibody.

~~12742~~. A method of producing an antibody drug-conjugate that specifically binds to the human PD-L1 protein, comprising culturing the host cell of claim ~~12439~~ or ~~12540~~ under a condition suitable for production of the antibody; and conjugating the antibody to a cytotoxic agent.

~~12843~~. The method of claim ~~12742~~, wherein the cytotoxic agent is MMAE or camptothecin.

~~129. — An antibody or antigen-binding fragment thereof that specifically binds to the human PD-L1 protein, wherein the antibody is conjugated to camptothecin forming an antibody-drug conjugate, wherein the antibody-drug conjugate has the structure:~~



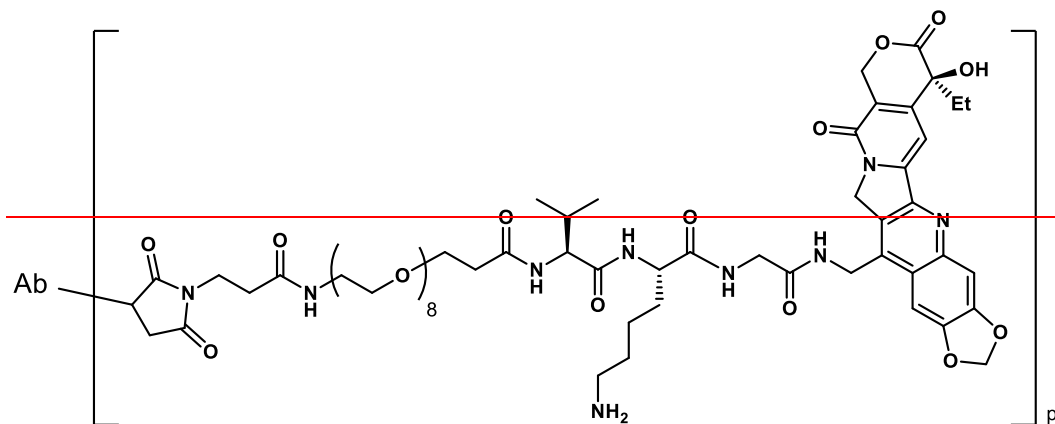
~~wherein~~

~~Ab is 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.

130. The antibody of claim 129, wherein the antibody drug conjugate has the structure:



131. The antibody of claim 129 or 130, wherein p ranges from 2 to 10.

132. An antibody or antigen-binding fragment thereof that specifically binds 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.

133. The antibody of claim 132, wherein the antibody exhibits a binding affinity that is greater than 2.7 nM.

134. An antibody or antigen-binding fragment thereof that specifically binds 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.

135. The antibody of claim 134, wherein the antibody exhibits a k_{assoc} to the human PD-L1 protein that is less than $5 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$.

136. An antibody or antigen-binding fragment thereof that specifically binds 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.

~~137.—The antibody of claim 136, wherein the antibody exhibits a k_{dissoc} to the human PD-L1 protein that is greater than $2 \times 10^3 \text{ s}^{-1}$.~~

~~138.—An antibody drug conjugate comprising 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: 3-5 and light chain CDR sequences of SEQ ID NOs: 6-8, wherein the antibody comprises one or more amino acid substitutions within one or more of the CDRs, and wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 5 nM and 15 nM, and wherein the antibody is conjugated to MMAE.~~

~~139.—An antibody drug conjugate comprising 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: 3-5 and light chain CDR sequences of SEQ ID NOs: 6-8, wherein the antibody comprises one or more amino acid substitutions within one or more of the CDRs, and wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 5 nM and 15 nM, and wherein the antibody is conjugated to camptothecin.~~