

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.

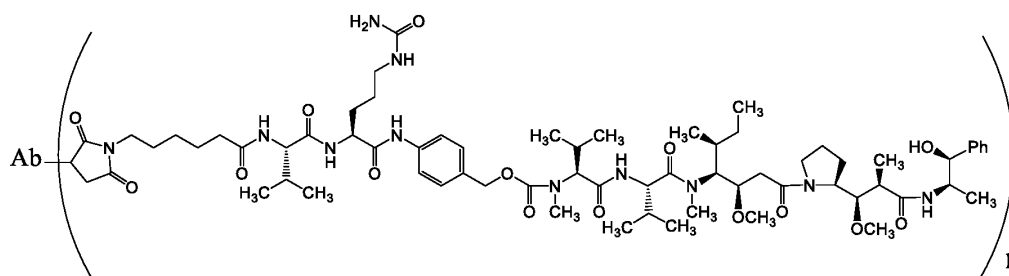
15. The antibody of any of claims 1-14, wherein the antibody comprises a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.

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:

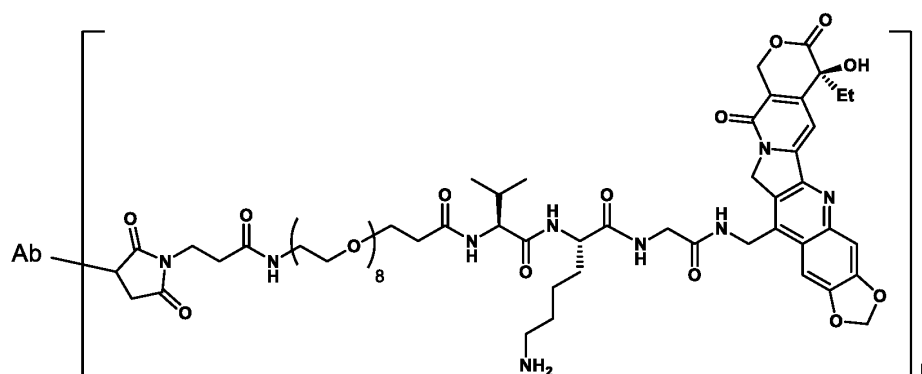


wherein Ab represents the antibody and p ranges from 2 to 10.

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.

37. A nucleic acid encoding the antibody as defined by any one of claims 1-33.
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.

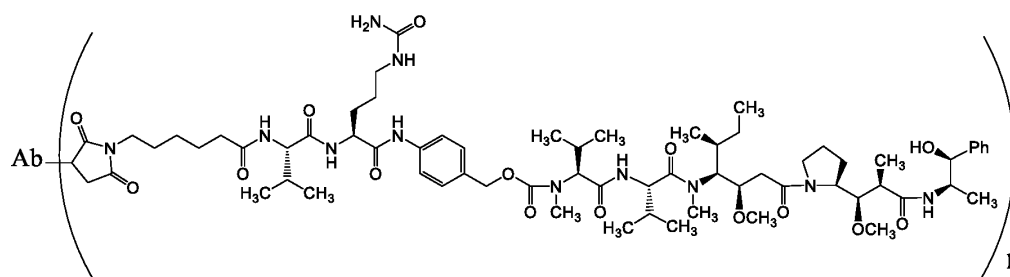
63. The antibody of any of claims 44-62, wherein the antibody is conjugated to a cytotoxic agent via a linker.

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.

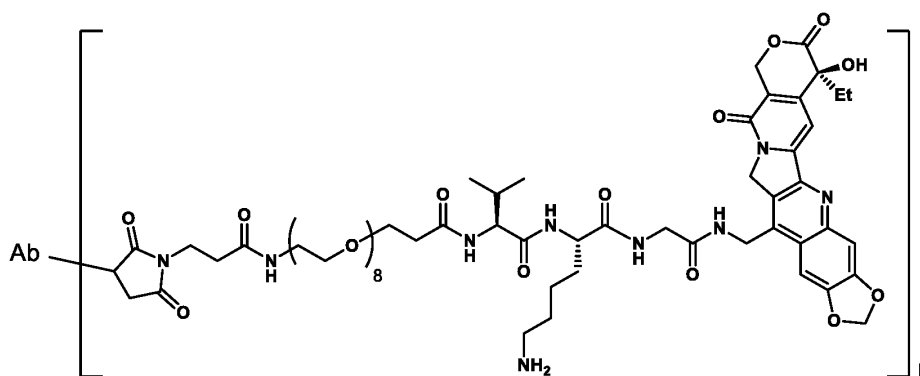
66. The antibody of claim 65, wherein the enzyme-cleavable linker unit comprises a Val-Cit linker.

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.
87. 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.
88. The antibody of claim 87, wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 300 nM.
89. The antibody of claim 87 or 88, wherein the antibody exhibits a binding affinity to the human PD-L1 protein that is between 3 and 15 nM.
90. The antibody of any of claims 87-89, wherein the antibody further exhibits a total internalization that is higher than the total internalization of Ab1.
91. The antibody of claim 90, wherein the total internalization is between a 9% and 155% increase of AUC over the AUC of Ab1.
92. The antibody of claim 91, wherein the total internalization is determined by a FabFluor internalization assay.
93. The antibody of any of claims 87-92, wherein the antibody further exhibits an x50 that is higher than the x50 of Ab1.
94. The antibody of claim 93, 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.
95. The antibody of claim 93, 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.
96. The antibody of any of claims 87-95, 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.

97. The antibody of any of claims 87-96, 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.

98. The antibody of any of claims 87-97, 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.

99. The antibody of any of claims 87-98, 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.

100. The antibody of any of claims 87-99, wherein the antibody comprises a light chain of SEQ ID NO: 9 and a heavy chain of SEQ ID NO: 10.

101. The antibody of any of claims 87-100, wherein the fragment is a Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, or single-chain antibody fragment.

102. The antibody of any of claims 87-101, wherein the antibody contains L234A and L235A mutations in the heavy chain of the antibody.

103. The antibody of any of claims 87-102, wherein the heavy chain constant region is of the IgG1 isotype.

104. The antibody of any of claims 87-103, wherein the antibody is a humanized or chimeric antibody.

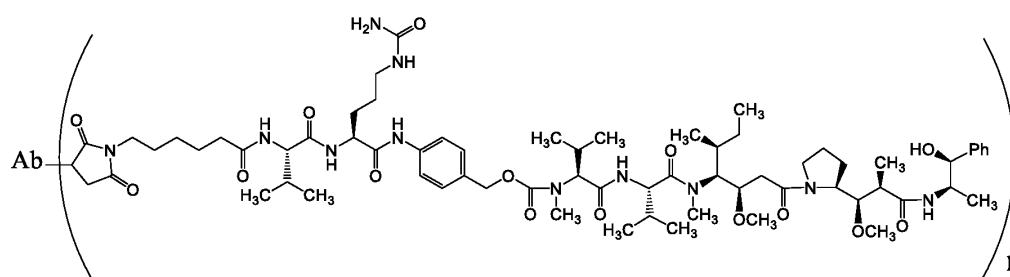
105. The antibody of any of claims 87-104, wherein the antibody is conjugated to a cytotoxic agent via a linker.

106. The antibody of claim 105, wherein the antibody is conjugated to monomethyl auristatin E (MMAE).

107. The antibody of claim 106, wherein the antibody is conjugated to MMAE via an enzyme-cleavable linker unit.

108. The antibody of claim 107, wherein the enzyme-cleavable linker unit comprises a Val-Cit linker.

109. The antibody of any one of claims 105-108, 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.

110. The antibody of claim 109, wherein p is 4.

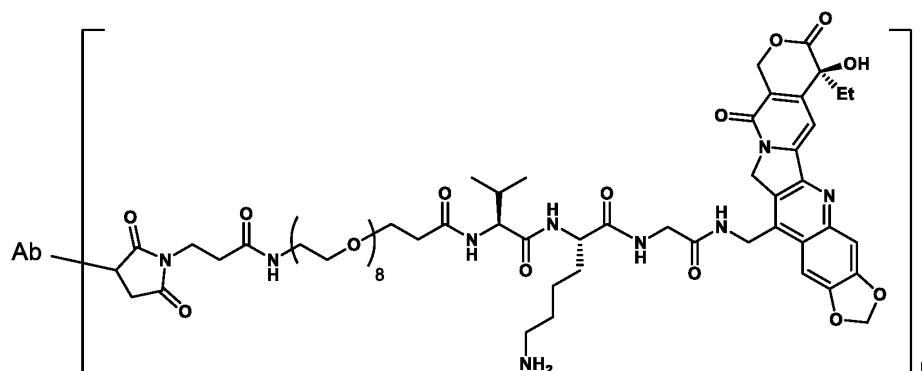
111. The antibody of claim 109, wherein p is 8.

112. The antibody of claim 105, wherein the antibody is conjugated to camptothecin.

113. The antibody of claim 112, wherein the antibody is conjugated to camptothecin via an enzyme-cleavable linker unit.

114. The antibody of claim 113, wherein the enzyme-cleavable linker unit comprises a Val-Lys-Gly linker.

115. The antibody of any one of claims 112-114, 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.

116. The antibody of claim 115, wherein p is 4.

117. The antibody of claim 115, wherein p is 8.

118. A pharmaceutical composition that comprises a therapeutically effective amount of the antibody of any of claims 87-117 and a pharmaceutically acceptable excipient.

119. A method of treating cancer in a subject, comprising administering to the subject the antibody of any of claims 87-118.

120. The method of claim 119, wherein the subject is a human subject.

121. The method of claim 119 or 120, 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.

122. A nucleic acid encoding the antibody as defined by any one of claims 87-118.

123. A vector comprising the nucleic acid of claim 122.

124. A host cell comprising the nucleic acid of claim 123.

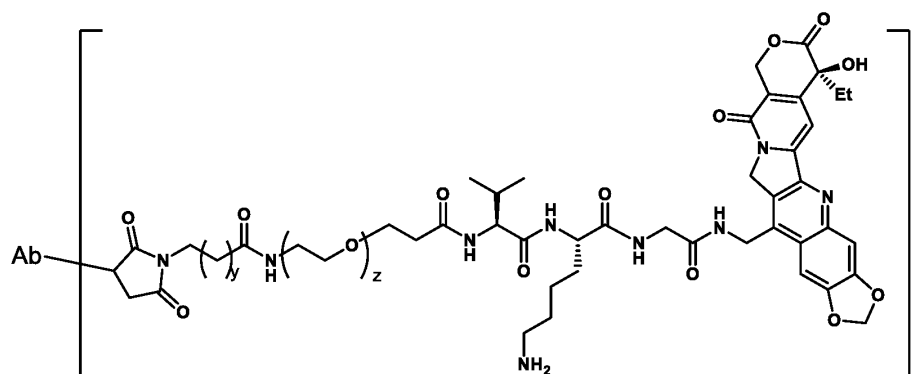
125. The host cell of claim 124, wherein the host cell is a Chinese hamster ovary (CHO) cell.

126. 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 124 or 125 under a condition suitable for production of the antibody.

127. A method of producing an antibody drug-conjugate that specifically binds to the human PD-L1 protein, comprising culturing the host cell of claim 124 or 125 under a condition suitable for production of the antibody; and conjugating the antibody to a cytotoxic agent.

128. The method of claim 127, 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

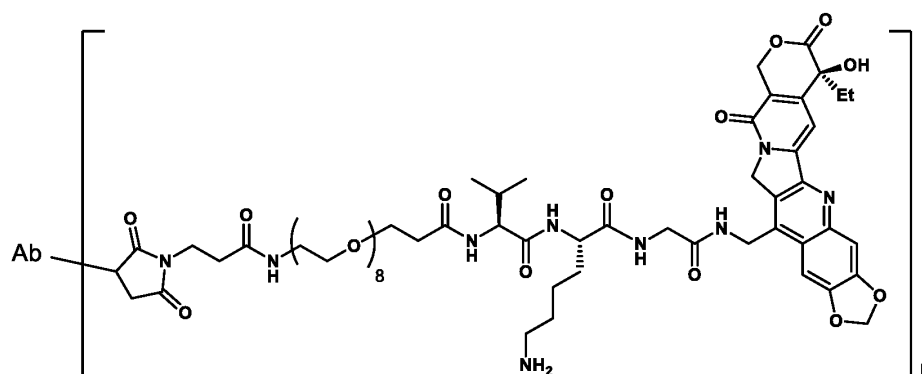
Ab is an anti-PD-L1 antibody;

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.