

What is claimed:

1. A humanized anti-CD228 antibody, or antigen-binding fragment thereof, comprising a heavy chain variable region comprising the three Kabat CDRs of SEQ ID NO: 7, wherein position H27 is occupied by D, position H30 is occupied by T, position H47 is occupied by Y, position H71 is occupied by R, and position H78 is occupied by Y, and a light chain variable region comprising the three Kabat CDRs of SEQ ID NO: 8, wherein position L2 is occupied by F, position L36 is occupied by Y and position L46 is occupied by L,

wherein the heavy chain variable region comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:7 and the light chain variable region comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO:8.

2. The antibody or antigen-binding fragment of claim 1, wherein the heavy chain variable region comprises an amino acid sequence having at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 7 and the light chain variable region comprises an amino acid sequence having at least 98% sequence identity to the amino acid sequence of SEQ ID NO: 8.

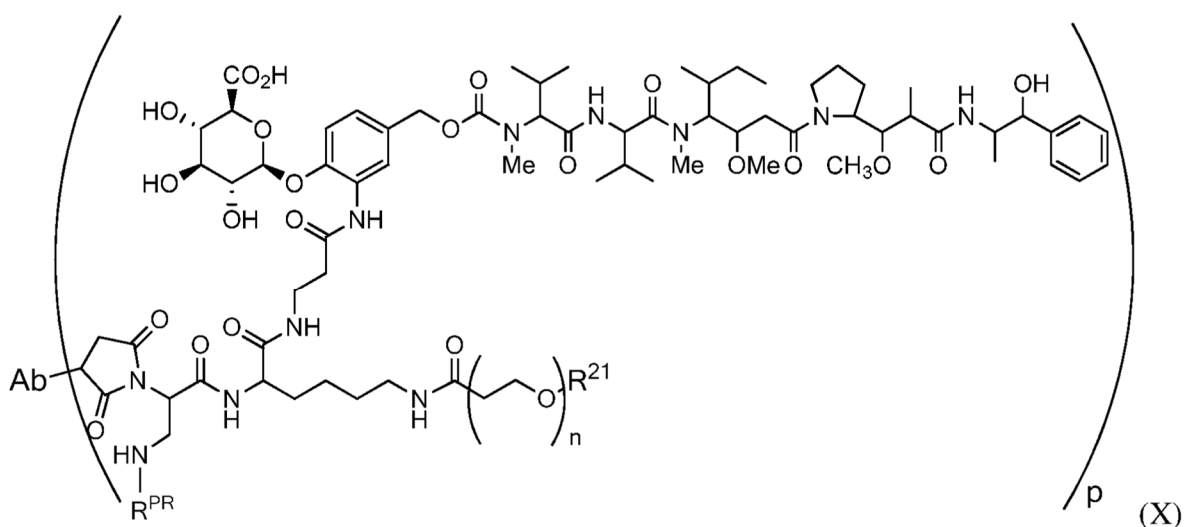
3. The antibody or antigen-binding fragment of claim 1 or claim 2, wherein the heavy chain variable region comprises an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 7 and the light chain variable region comprises an amino acid sequence having at least 99% sequence identity to the amino acid sequence of SEQ ID NO: 8.

4. The antibody or antigen-binding fragment of any one of claims 1-3, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO: 7 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:8.

5. The antibody or antigen-binding fragment of any one of claims 1-4, wherein the antibody or antigen-binding fragment is an antigen-binding fragment.

6. The antibody or antigen-binding fragment of claim 5, wherein the antigen-binding fragment is selected from the group consisting of Fab, Fab', F(ab')₂, Fab'-SH, Fv, diabody, linear antibody, and single-chain antibody fragment.
7. The antibody or antigen-binding fragment of any one of claims 1-4, wherein the antibody or antigen-binding fragment is a full-length antibody.
8. The antibody or antigen-binding fragment of claim 7, wherein the heavy chain variable region is fused to a heavy chain constant region and the light chain variable region is fused to a light chain constant region.
9. The antibody or antigen-binding fragment of claim 8, wherein the heavy chain constant region is of the IgG1 isotype.
10. The antibody or antigen-binding fragment of claim 8 or claim 9, wherein the heavy chain constant region has an amino acid sequence comprising SEQ ID NO:17 and the light chain constant region has an amino acid sequence comprising SEQ ID NO:18.
11. The antibody or antigen-binding fragment of claim 8 or claim 9, wherein the heavy chain constant region is a mutant form of a natural human constant region which has reduced binding to an Fcγ receptor relative to the natural human constant region.
12. The antibody or antigen-binding fragment of claim 8 or claim 9, wherein the heavy chain constant region has an amino acid sequence comprising SEQ ID NO:19 (S239C) and the light chain constant region has an amino acid sequence comprising SEQ ID NO:18.
13. An antibody-drug conjugate comprising the antibody or antigen-binding fragment of any one of claims 1-12 conjugated to a cytotoxic or cytostatic agent.
14. The antibody-drug conjugate of claim 13, wherein the antibody or antigen-binding fragment is conjugated to the cytotoxic or cytostatic agent via a linker.

15. The antibody-drug conjugate of claim 14, wherein the linker is a MDpr-PEG(12)-gluc linker.
16. The antibody-drug conjugate of any one of claims 13-15, wherein the cytotoxic or cytostatic agent is a monomethyl auristatin.
17. The antibody-drug conjugate of claim 16, wherein the monomethyl auristatin is monomethyl auristatin E (MMAE).
18. The antibody-drug conjugate of claim 17, wherein the linker is attached to monomethyl auristatin E forming an antibody-drug conjugate having the structure:



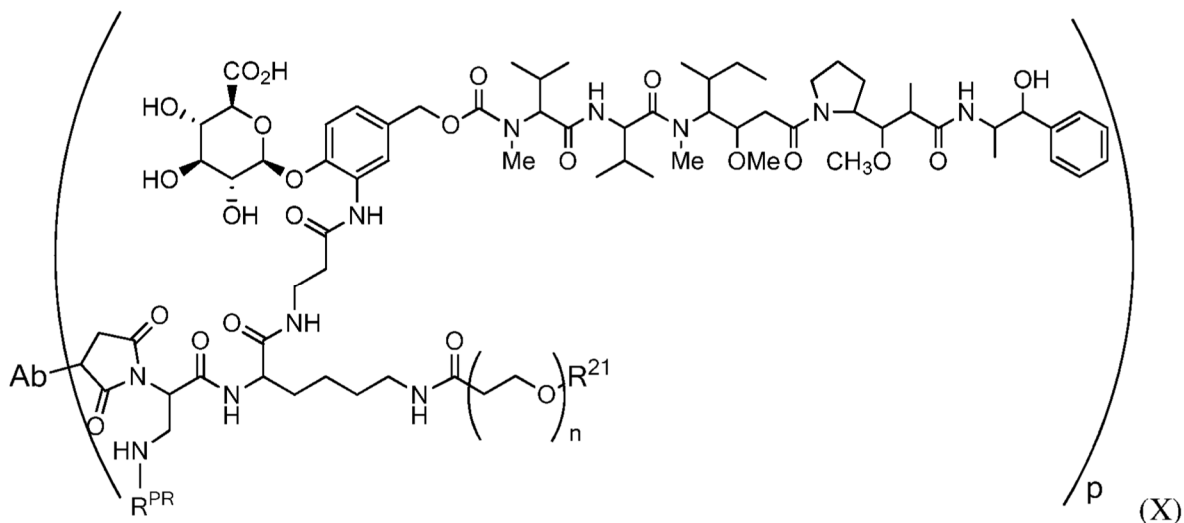
wherein Ab is the antibody hL49, n is 12, R^{PR} is hydrogen, R^{21} is CH_3 , and p denotes a number from 1 to 16.

19. The antibody-drug conjugate of claim 18, wherein the average value of p in a population of the antibody-drug conjugate is about 8.
20. The antibody-drug conjugate of any one of claims 13-19, wherein the antibody-drug conjugate is hL49-MDpr-PEG(12)-gluc-MMAE.

21. A nucleic acid(s) encoding the heavy chain variable region and the light chain variable region as defined by any one of claims 1-12.
22. A vector comprising the nucleic acid of claim 21.
23. The vector of claim 22, wherein the vector is an expression vector.
24. A host cell comprising the nucleic acid(s) of claim 21.
25. The host cell of claim 24, wherein the host cell is a Chinese hamster ovary (CHO) cell.
26. A method of producing an anti-CD228 antibody or antigen-binding fragment thereof comprising culturing the host cell of claim 24 or claim 25 under a condition suitable for production of the anti- CD228 antibody or antigen-binding fragment thereof.
27. The method of claim 26, further comprising isolating the anti-CD228 antibody or antigen-binding fragment thereof produced by the host cell.
28. A method of producing an anti- CD228 antibody-drug conjugate comprising culturing the host cell of claim 24 or claim 25 under a condition suitable for production of an anti-CD228 antibody; isolating the anti-CD228 antibody produced from the host cell; and conjugating the anti-CD228 antibody to a cytotoxic or cytostatic agent.
29. The method of claim 28, wherein the anti-CD228 antibody is conjugated to the cytotoxic or cytostatic agent via a linker.
30. The method of claim 29, wherein the linker is a MDpr-PEG(12)-gluc linker.
31. The method of any one of claims 28-30, wherein the cytotoxic or cytostatic agent is a monomethyl auristatin.

32. The method of claim 31, wherein the monomethyl auristatin is monomethyl auristatin E (MMAE).

33. The method of claim 32, wherein the linker is attached to monomethyl auristatin E forming an antibody-drug conjugate having the structure:



wherein Ab is the antibody hL49, n is 12, R^{PR} is hydrogen, R^{21} is CH_3 , and p denotes a number from 1 to 16.

34. The method of claim 33, wherein the average value of p in a population of the antibody-drug conjugate is about 8.

35. The method of any one of claims 28-34, wherein the antibody-drug conjugate is hL49-MDpr-PEG(12)-gluc-MMAE.

36. Use of the antibody or antigen-binding fragment of any one of claims 1-12 or the antibody-drug conjugate of any one of claims 13-20 in the manufacture of a medicament for treating a cancer that expresses CD228 in a subject.

37. The use of claim 36, wherein the subject has been previously treated with one or more therapeutic agents and did not respond to the treatment, wherein the one or more therapeutic agents is not the antibody, antigen-binding fragment, or antibody-drug conjugate.
38. The use of claim 36, wherein the subject has been previously treated with one or more therapeutic agents and relapsed after the treatment, wherein the one or more therapeutic agents is not the antibody, antigen-binding fragment, or antibody-drug conjugate.
39. The use of claim 36, wherein the subject has been previously treated with one or more therapeutic agents and has experienced disease progression during treatment, wherein the one or more therapeutic agents is not the antibody, antigen-binding fragment, or antibody-drug conjugate.
40. The use of any one of claims 36-39, wherein the cancer is an advanced stage cancer.
41. The use of claim 40, wherein the advanced stage cancer is a stage 3 or stage 4 cancer.
42. The use of claim 40 or claim 41, wherein the advanced stage cancer is metastatic cancer.
43. The use of any one of claims 36-42, wherein the cancer is recurrent cancer.
44. The use of any one of claims 36-43, wherein the cancer is unresectable.
45. The use of any one of claims 36-44, wherein the subject received prior treatment with standard of care therapy for the cancer and failed the prior treatment.
46. The use of any one of claims 46-45, wherein the cancer is selected from the group consisting of melanoma, pancreatic cancer, mesothelioma, colorectal cancer, lung cancer, thyroid cancer, breast cancer, cholangiocarcinoma, esophageal cancer and head and neck cancer.
47. The use of claim 46, wherein the cancer is melanoma.

48. The use of claim 47, wherein the melanoma is cutaneous melanoma.
49. The use of claim 48, wherein the cutaneous melanoma is selected from the group consisting of superficial spreading melanoma, nodular melanoma, acral lentiginous melanoma, lentigo maligna melanoma, and desmoplastic melanoma.
50. The use of claim 49, wherein the acral lentiginous melanoma is subungual melanoma.
51. The use of any one of claims 48-50, wherein the subject received prior therapy with an inhibitor of PD-1 or PD-L1.
52. The use of claim 51, wherein the subject received prior therapy with an inhibitor of PD-1.
53. The use of claim 47, wherein the melanoma is sub-cutaneous melanoma.
54. The use of claim 53, wherein the sub-cutaneous melanoma is ocular melanoma or mucosal melanoma.
55. The use of claim 47, wherein the melanoma is non-cutaneous melanoma.
56. The use of claim 46, wherein the cancer is mesothelioma.
57. The use of claim 56, wherein the mesothelioma is selected from the group consisting of pleural mesothelioma, peritoneal mesothelioma, pericardial mesothelioma, and testicular mesothelioma.
58. The use of claim 57, wherein the mesothelioma is pleural mesothelioma.
59. The use of claim 58, wherein the subject has received prior therapy with a platinum-based therapy.

60. The use of claim 59, wherein the platinum-based therapy is cisplatin.
61. The use of any one of claims 58-60, wherein the subject received prior therapy with pemetrexed.
62. The use of claim 46, wherein the lung cancer is non-small cell lung cancer.
63. The use of claim 62, wherein the non-small cell lung cancer has a mutant form of epidermal growth factor receptor (EGFR).
64. The use of claim 62, wherein the non-small cell lung cancer has wild-type EGFR.
65. The use of claim 64, wherein the subject has received prior therapy with a platinum-based therapy.
66. The use of claim 64 or claim 65, wherein the subject received prior therapy with an inhibitor of PD-1 or PD-L1.
67. The use of claim 66, wherein the subject received prior therapy with an inhibitor of PD-1.
68. The use of claim 46, wherein the breast cancer is selected from the group consisting of HER2 positive, HER2 negative, Estrogen Receptor (ER) positive, ER negative, Progesterone Receptor (PR) positive, PR negative, and triple negative breast cancer.
69. The use of claim 68, wherein the breast cancer is HER2 negative breast cancer.
70. The use of claim 69, wherein the subject received one or more prior line of therapy for the HER2 negative breast cancer.
71. The use of claim 70, wherein the one or more prior line of therapy comprised treatment with a taxane.

72. The use of claim 70 or claim 71, wherein the subject is hormone receptor positive.
73. The use of claim 72, wherein the subject received prior therapy with an inhibitor of CDK4/6.
74. The use of claim 72 or claim 73, wherein the subject received prior therapy with a hormonally-directed therapy.
75. The use of claim 46, wherein the colorectal cancer is selected from the group consisting of a colorectal adenocarcinoma, a gastrointestinal stromal tumor, a primary colorectal lymphoma, a gastrointestinal carcinoid tumor, and a leiomyosarcoma.
76. The use of claim 75, wherein the subject received two or more prior lines of therapy for the colorectal cancer.
77. The use of claim 46, wherein the pancreatic cancer is an exocrine cancer or a neuroendocrine cancer.
78. The use of claim 77, wherein the exocrine cancer is selected from the group consisting of pancreatic adenocarcinoma, acinar cell carcinoma, cystadenocarcinoma, pancreatoblastoma, adenosquamous carcinoma, signet ring carcinoma, hepatoid carcinoma, colloid carcinoma, undifferentiated carcinoma, and pancreatic mucinous cystic neoplasm.
79. The use of claim 78, wherein the pancreatic adenocarcinoma is pancreatic ductal adenocarcinoma.
80. The use of claim 78 or claim 79, wherein the subject received one or more prior line of therapy for the pancreatic cancer.
81. The use of any one of claims 36-80, wherein the antibody or antigen-binding fragment or antibody-drug conjugate is in a pharmaceutical composition comprising the antibody or antigen-binding fragment or antibody-drug conjugate and a pharmaceutically acceptable carrier.

82. The use of any one of claims 36-81, wherein the subject is a human.

83. A kit comprising:

(a) the antibody or antigen-binding fragment of any one of claims 1-12 or the antibody-drug conjugate of any one of claims 13-20; and

(b) instructions for using the antibody or antigen-binding fragment or antibody-drug conjugate according to the method of any one of claims 36-82.

84. A pharmaceutical composition comprising the antibody or antigen-binding fragment of any one of claims 1-12 or the antibody-drug conjugate of any one of claims 13-20 and one or more agents selected from the group consisting of a physiologically acceptable carrier, a diluent, an excipient and an auxiliary.