

skin cancer. In 2015, were 3.1 million people with active disease and melanoma resulted in 59,800 deaths. Surgery can be effective for early stage melanoma, but may not be a treatment option for disease that has metastasized to distant organs. Melanomas that spread often do so to the lymph nodes in the area before spreading elsewhere. Attempts to improve survival by removing lymph nodes surgically were associated with many complications, but no overall survival benefit. Immunotherapy, chemotherapy and radiation therapy have all been used, but are often not curative, particularly for late stage melanoma. When there is distant metastasis, the cancer is generally considered incurable. The five-year survival rate of stage IV disease is 15-20%. Therefore, there is a need for improved treatments for melanoma.

[0006] All references cited herein, including patent applications, patent publications, and scientific literature, are herein incorporated by reference in their entirety, as if each individual reference were specifically and individually indicated to be incorporated by reference.

SUMMARY

[0007] ~~In one aspect, p~~Provided herein is an isolated anti-CD228 antibody, or antigen-binding fragment thereof, comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises:

- (i) a CDR-H1 comprising the amino acid sequence of SEQ ID NO:1;
- (ii) a CDR-H2 comprising the amino acid sequence of SEQ ID NO:2; and
- (iii) a CDR-H3 comprising the amino acid sequence of SEQ ID NO:3; and

wherein the light chain variable region comprises:

- (i) a CDR-L1 comprising the amino acid sequence of SEQ ID NO:4;
- (ii) a CDR-L2 comprising the amino acid sequence of SEQ ID NO:5; and
- (iii) a CDR-L3 comprising the amino acid sequence of SEQ ID NO:6. In some examples,

the antibody is humanized.

[0008] ~~In another aspect, also p~~Provided herein is a humanized anti-CD228 antibody, or antigen-binding fragment thereof, comprising a heavy chain variable region comprising an amino acid sequence at least 90% identical to SEQ ID NO: 7 provided that 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 an amino acid sequence at least 90% identical to SEQ ID NO: 8, provided that position L2 is occupied by F,

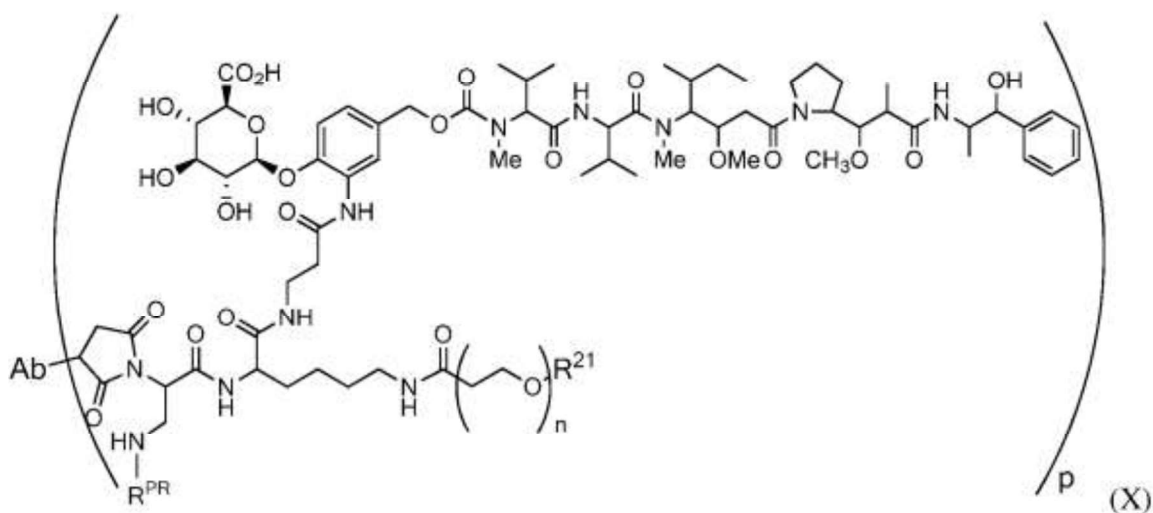
position L36 is occupied by Y and position L46 is occupied by L. In some examples, position L28 is occupied by D.

[0009] In one aspect, the present disclosure provides 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.

[0009a] ~~In yet another aspect, also p~~Provided herein is 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.

[0010] In some of any of the examples herein, 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. In some of any of the examples herein, 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. In some of any of the examples herein, the antibody or antigen-binding fragment is an antigen-binding fragment. In some of any of the examples herein, the antibody or antigen-binding fragment is a full-length antibody.

[0011] In a further aspect, also provided herein is an antibody-drug conjugate comprising the antibody or antigen-binding fragment provided herein conjugated to a cytotoxic or cytostatic agent. In some examples, the linker is a MDpr-PEG(12)-gluc linker. In some of any of the examples herein, the cytotoxic or cytostatic agent is a monomethyl auristatin. In some examples, the monomethyl auristatin is monomethyl auristatin E (MMAE). In some of any of the examples herein, 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. In some of any of the examples herein, the antibody-drug conjugate is hL49-MDpr-PEG(12)-gluc-MMAE.

[0012] In a further aspect, also provided herein is a nucleic acid(s) encoding the heavy chain variable region and/or the light chain variable region of an antibody described herein. In another aspect, also provided herein is a vector comprising a nucleic acid provided herein. In a further aspect, also provided herein is a host cell comprising a nucleic acid provided herein.

[0013] In a further aspect, also provided herein is a method of producing an anti-CD228 antibody or antigen-binding fragment provided herein, comprising culturing a host cell provided herein under a condition suitable for production of the anti-CD228 antibody or antigen-binding fragment thereof.

[0014] In a further aspect, also provided herein is a method of producing an anti-CD228 antibody-drug conjugate provided herein, comprising culturing a host cell provided herein 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.

[0014a] In a further aspect, the disclosure provides use of the antibody or antigen-binding fragment as described herein or the antibody-drug conjugate as described herein in the manufacture of a medicament for treating a cancer that expresses CD228 in a subject.

[0015] Also provided herein is a method of treating cancer in a subject, the method comprising administering to the subject an antibody or antigen-binding fragment provided herein or an antibody-drug conjugate provided herein. In some examples, 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. In some examples, the subject is a human.

[0016] In yet another aspect also provided herein is a kit comprising: (a) an antibody or antigen-binding fragment provided herein or an antibody-drug conjugate provided herein; and (b) instructions for using the antibody or antigen-binding fragment or antibody-drug conjugate according to a method provided herein.

[0017] In yet another aspect also provided herein is a pharmaceutical composition comprising an antibody or antigen-binding fragment provided herein or an antibody-drug conjugate provided herein and one or more agents selected from the group consisting of a physiologically acceptable carrier, a diluent, an excipient and an auxiliary.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0019] FIG. 1 shows an analysis of CD228 protein expression by IHC on melanoma cancer patient samples.

[0020] FIG. 2 shows an analysis of CD228 protein expression by IHC on mesothelioma cancer patient samples.

[0021] FIG. 3 shows an analysis of CD228 protein expression by IHC on colorectal cancer patient samples.

What is claimed:

~~1. An isolated anti-CD228 antibody, or antigen-binding fragment thereof, comprising a heavy chain variable region and a light chain variable region, wherein the heavy chain variable region comprises:~~

- ~~—— (i) a CDR H1 comprising the amino acid sequence of SEQ ID NO:1;~~
 - ~~—— (ii) a CDR H2 comprising the amino acid sequence of SEQ ID NO:2; and~~
 - ~~—— (iii) a CDR H3 comprising the amino acid sequence of SEQ ID NO:3; and~~
- ~~wherein the light chain variable region comprises:~~
- ~~—— (i) a CDR L1 comprising the amino acid sequence of SEQ ID NO:4;~~
 - ~~—— (ii) a CDR L2 comprising the amino acid sequence of SEQ ID NO:5; and~~
 - ~~—— (iii) a CDR L3 comprising the amino acid sequence of SEQ ID NO:6.~~

~~2. The antibody or antigen-binding fragment of claim 1, wherein the antibody is humanized.~~

~~3. A humanized anti-CD228 antibody, or antigen-binding fragment thereof, comprising a heavy chain variable region comprising an amino acid sequence at least 90% identical to SEQ ID NO: 7 provided that 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 an amino acid sequence at least 90% identical to SEQ ID NO: 8, provided that position L2 is occupied by F, position L36 is occupied by Y and position L46 is occupied by L.~~

~~4. The antibody or antigen-binding fragment of claim 3, further provided that position L28 is occupied by D.~~

15. 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 L₂.

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.

~~6. The antibody or antigen-binding fragment of any one of claims 1-5, 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.~~

27. The antibody or antigen-binding fragment of ~~any one of claims 1-5~~ 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.

38. The antibody or antigen-binding fragment of ~~any one of claims 1-5~~ 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.

49. The antibody or antigen-binding fragment of any one of claims 1-~~35~~, 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.

540. The antibody or antigen-binding fragment of any one of claims 1-~~49~~, wherein the antibody or antigen-binding fragment is an antigen-binding fragment.

611. The antibody or antigen-binding fragment of claim 510, 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.

712. The antibody or antigen-binding fragment of any one of claims 1-49, wherein the antibody or antigen-binding fragment is a full-length antibody.

813. The antibody or antigen-binding fragment of claim 712, 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.

914. The antibody or antigen-binding fragment of claim 813, wherein the heavy chain constant region is of the IgG1 isotype.

1015. The antibody or antigen-binding fragment of claim 813 or claim 914, 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.

1116. The antibody or antigen-binding fragment of claim 813 or claim 914, 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.

1217. The antibody or antigen-binding fragment of claim 813 or claim 914, 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.

1318. An antibody-drug conjugate comprising the antibody or antigen-binding fragment of any one of claims 1-1217 conjugated to a cytotoxic or cytostatic agent.

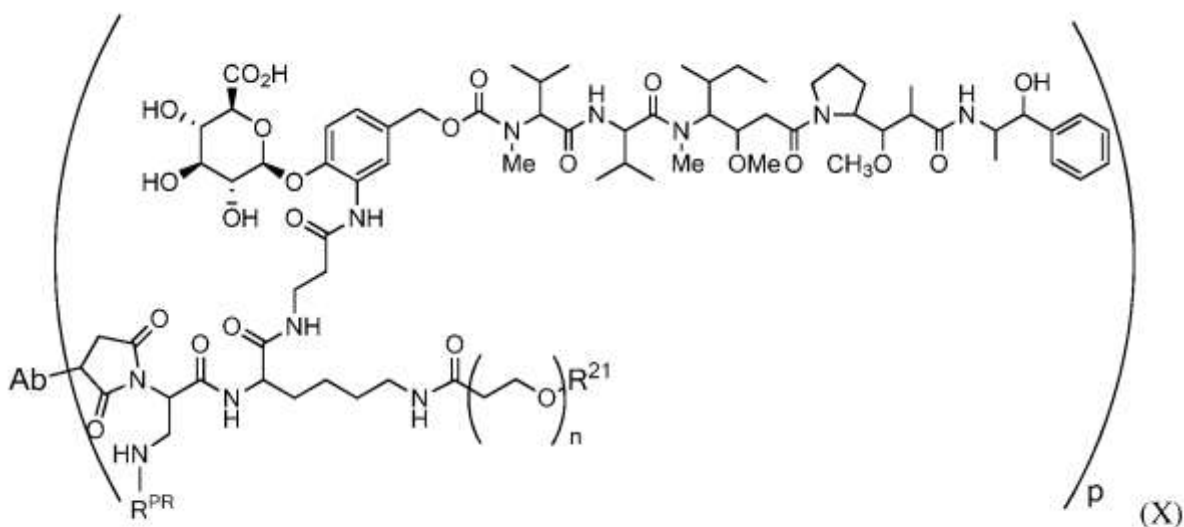
1419. The antibody-drug conjugate of claim 1318, wherein the antibody or antigen-binding fragment is conjugated to the cytotoxic or cytostatic agent via a linker.

1520. The antibody-drug conjugate of claim 1419, wherein the linker is a MDpr-PEG(12)-gluc linker.

1621. The antibody-drug conjugate of any one of claims 13-1518-20, wherein the cytotoxic or cytostatic agent is a monomethyl auristatin.

1722. The antibody-drug conjugate of claim 1621, wherein the monomethyl auristatin is monomethyl auristatin E (MMAE).

1823. The antibody-drug conjugate of claim 1722, 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.

1924. The antibody-drug conjugate of claim 1823, wherein the average value of p in a population of the antibody-drug conjugate is about 8.

2025. The antibody-drug conjugate of any one of claims 13-1918-24, wherein the antibody-drug conjugate is hL49-MDpr-PEG(12)-gluc-MMAE.

2126. A nucleic acid(s) encoding the heavy chain variable region and/or the light chain variable region as defined by any one of claims 1-1217.

2227. A vector comprising the nucleic acid of claim 2126.

2328. The vector of claim 2227, wherein the vector is an expression vector.

2429. A host cell comprising the nucleic acid(s) of claim 2126.

2530. The host cell of claim 2429, wherein the host cell is a Chinese hamster ovary (CHO) cell.

2631. A method of producing an anti-CD228 antibody or antigen-binding fragment thereof comprising culturing the host cell of claim 2429 or claim 2530 under a condition suitable for production of the anti- CD228 antibody or antigen-binding fragment thereof.

2732. The method of claim 2631, further comprising isolating the anti-CD228 antibody or antigen-binding fragment thereof produced by the host cell.

2833. A method of producing an anti- CD228 antibody-drug conjugate comprising culturing the host cell of claim 2429 or claim 2530 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.

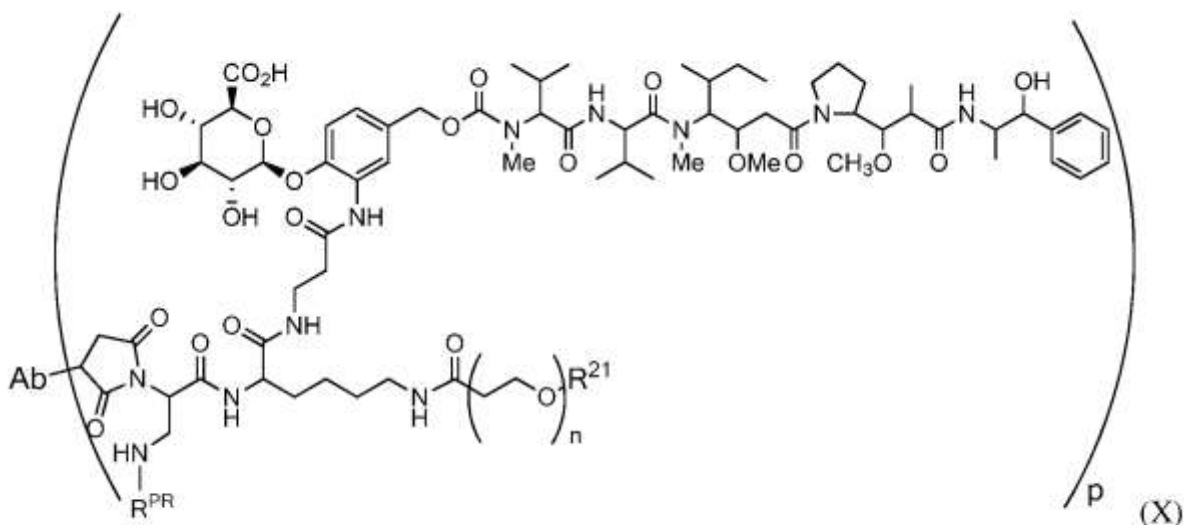
2934. The method of claim 2833, wherein the anti-CD228 antibody is conjugated to the cytotoxic or cytostatic agent via a linker.

3035. The method of claim 2934, wherein the linker is a MDpr-PEG(12)-gluc linker.

3136. The method of any one of claims 28-3033-35, wherein the cytotoxic or cytostatic agent is a monomethyl auristatin.

3237. The method of claim 3136, wherein the monomethyl auristatin is monomethyl auristatin E (MMAE).

3338. The method of claim 3237, 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, R²¹ is CH₃, and p denotes a number from 1 to 16.

3439. The method of claim 3338, wherein the average value of p in a population of the antibody-drug conjugate is about 8.

3540. The method of any one of claims 28-3433-39, wherein the antibody-drug conjugate is hL49-MDpr-PEG(12)-gluc-MMAE.

3641. Use of the antibody or antigen-binding fragment of any one of claims 1-1247 or the antibody-drug conjugate of any one of claims 18-2513-20 in the manufacture of a medicament for treating a cancer that expresses CD228 in a subject.

3742. The use of claim 3641, 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.

3843. The use of claim 3641, 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.

3944. The use of claim 3641, 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.

4045. The use of any one of claims 36-3941-44, wherein the cancer is an advanced stage cancer.

4146. The use of claim 4045, wherein the advanced stage cancer is a stage 3 or stage 4 cancer.

4247. The use of claim 4045 or claim 4146, wherein the advanced stage cancer is metastatic cancer.

4348. The use of any one of claims 41-4736-42, wherein the cancer is recurrent cancer.

4449. The use of any one of claims 36-4341-48, wherein the cancer is unresectable.

4550. The use of any one of claims 36-4441-49, wherein the subject received prior treatment with standard of care therapy for the cancer and failed the prior treatment.

4651. The use of any one of claims 46-4541-50, 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.

4752. The of claim 4651, wherein the cancer is melanoma.

4853. The of claim 4752, wherein the melanoma is cutaneous melanoma.

4954. The of claim 4853, 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.

5055. The use of claim 4954, wherein the acral lentiginous melanoma is subungual melanoma.

5156. The use of any one of claims 48-5053-55, wherein the subject received prior therapy with an inhibitor of PD-1 or PD-L1.

5257. The use of claim 5156, wherein the subject received prior therapy with an inhibitor of PD-1.

5358. The use of claim 4752, wherein the melanoma is sub-cutaneous melanoma.

5459. The use of claim 5358, wherein the sub-cutaneous melanoma is ocular melanoma or mucosal melanoma.

5560. The use of claim 4752, wherein the melanoma is non-cutaneous melanoma.

5661. The use of claim 4651, wherein the cancer is mesothelioma.

5762. The use of claim 5661, wherein the mesothelioma is selected from the group consisting of pleural mesothelioma, peritoneal mesothelioma, pericardial mesothelioma, and testicular mesothelioma.

5863. The use of claim 5762, wherein the mesothelioma is pleural mesothelioma.

5964. The use of claim 5863, wherein the subject has received prior therapy with a platinum-based therapy.

6065. The use of claim 5964, wherein the platinum-based therapy is cisplatin.

6166. The use of any one of claims 58-6063-65, wherein the subject received prior therapy with pemetrexed.

6267. The use of claim 4651, wherein the lung cancer is non-small cell lung cancer.

6368. The use of claim 6267, wherein the non-small cell lung cancer has a mutant form of epidermal growth factor receptor (EGFR).

6469. The use of claim 6267, wherein the non-small cell lung cancer has wild-type EGFR.

6570. The use of claim 6469, wherein the subject has received prior therapy with a platinum-based therapy.

6674. The use of claim 6469 or claim 6570, wherein the subject received prior therapy with an inhibitor of PD-1 or PD-L1.

6772. The use of claim 6674, wherein the subject received prior therapy with an inhibitor of PD-1.

6873. The use of claim 4651, 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.

6974. The use of claim 6873, wherein the breast cancer is HER2 negative breast cancer.

7075. The use of claim 6974, wherein the subject received one or more prior line of therapy for the HER2 negative breast cancer.

[7176](#). The use of claim [7075](#), wherein the one or more prior line of therapy comprised treatment with a taxane.

[7277](#). The use of claim [7075](#) or [claim 7176](#), wherein the subject is hormone receptor positive.

[7378](#). The use of claim [7277](#), wherein the subject received prior therapy with an inhibitor of CDK4/6.

[7479](#). The use of claim [7277](#) or [claim 7378](#), wherein the subject received prior therapy with a hormonally-directed therapy.

[7580](#). The use of claim [4651](#), 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.

[7681](#). The use of claim [7580](#), wherein the subject received two or more prior lines of therapy for the colorectal cancer.

[7782](#). The use of claim [4651](#), wherein the pancreatic cancer is an exocrine cancer or a neuroendocrine cancer.

[7883](#). The use of claim [7782](#), 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.

[7984](#). The use of claim [7883](#), wherein the pancreatic adenocarcinoma is pancreatic ductal adenocarcinoma.

[8085](#). The use of claim [7883](#) or [claim 7984](#), wherein the subject received one or more prior line of therapy for the pancreatic cancer.

8186. The use of any one of claims 36-8041-85, 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.

8287. The use of any one of claims 41-8636-81, wherein the subject is a human.

8388. A kit comprising:

(a) the antibody or antigen-binding fragment of any one of claims 1-1247 or the antibody-drug conjugate of any one of claims 13-2018-25; and

(b) instructions for using the antibody or antigen-binding fragment or antibody-drug conjugate according to the method of any one of claims 36-8241-87.

8489. A pharmaceutical composition comprising the antibody or antigen-binding fragment of any one of claims 1-1247 or the antibody-drug conjugate of any one of claims 13-2018-25 and one or more agents selected from the group consisting of a physiologically acceptable carrier, a diluent, an excipient and an auxiliary.