5T4 ANTIBODY DRUG CONJUGATES AND METHODS OF THEIR USE

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application Nos. 63/599,497, filed November 15, 2023, and 63/608,755, filed December 11, 2023, the disclosure of each of which is incorporated by reference herein in its entirety.

2. SEQUENCE LISTING

[0002] This application contains an electronic Sequence Listing which has been submitted in XML file format with this application, the entire content of which is incorporated by reference herein in its entirety. The Sequence Listing XML file submitted with this application is entitled "14529-168-228_SEQ_LISTING.xml", was created on November 12, 2024, and is 146,919 bytes in size.

3. FIELD

[0003] The present disclosure relates generally to a 5T4 antibody drug conjugate and methods of its use in treating cancer.

4. BACKGROUND

[0004] 5T4, also known as Wnt-Activated Inhibitory Factor 1 or WAIF1, is an N-glycosylated transmembrane 72 kDa glycoprotein containing eight leucine-rich repeats. 5T4 is often referred to as an oncofetal antigen due to its expression in fetal trophoblast (where it was first discovered) or as trophoblast glycoprotein (TPBG). 5T4 is found in various tumors. Although its confined expression would appear to give 5T4 the potential to be a target in cancer therapy, therapeutic success with antibodies, ADCs, and vaccines targeting 5T4 has not yet been achieved.

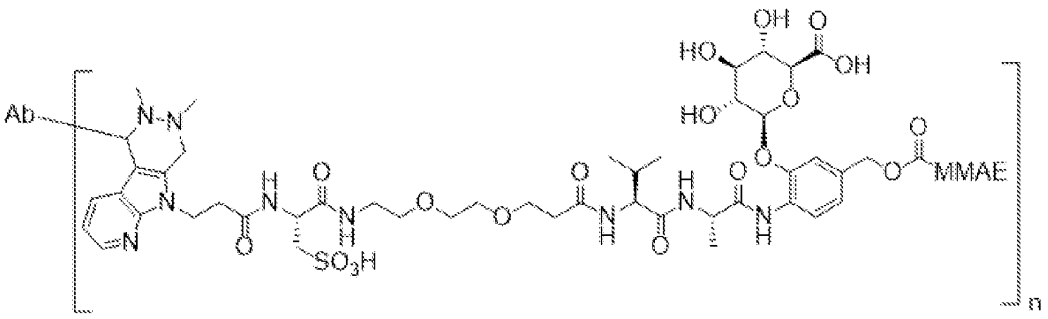
[0005] There remains a need in the art for ADCs that can target 5T4 to treat, prevent, or alleviate 5T4-mediated diseases, disorders, or conditions, such as those involving tumor cells expressing 5T4.

5. SUMMARY

[0006] Provided is a method for treating cancer in a subject. The method comprises administering an ADC to the subject. The cancer is any one or more of breast cancer, endometrial cancer (EC), head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer. In some embodiments, the cancer is triple-negative breast cancer (TNBC). In some embodiments, the cancer is hormone-receptor positive breast cancer (HR+

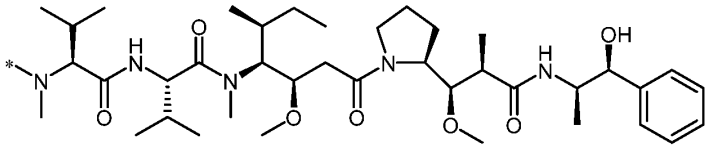
BC). In some embodiments, the cancer is pancreatic ductal adenocarcinoma (PDAC). In some embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the cancer is head and neck squamous cell carcinoma (HNSCC). In some embodiments, the cancer is endometrial cancer (EC).

[0007] In some embodiments, the ADC is represented by **Formula (I')**:



(I'),

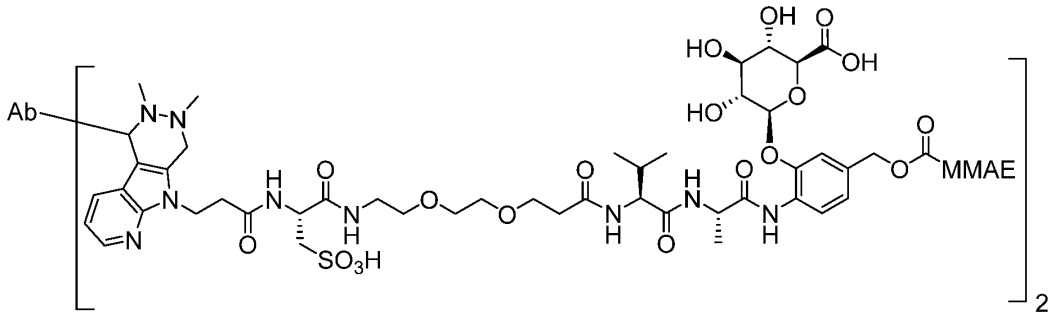
wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; wherein the Ab further comprises one or more *fGly'*, wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I')** is attached; wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



(III),

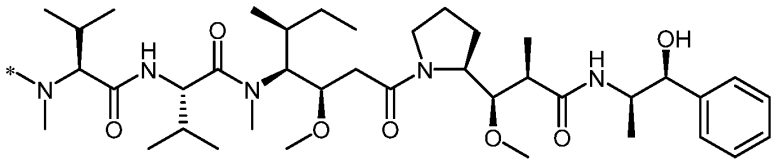
wherein * indicates the point of attachment in **Formula (I')**; and wherein n is an integer. In further embodiments, n is an integer selected from any one of 1 to 10. In yet further embodiments, n is 2.

[0008] In some embodiments, the ADC is represented by **Formula (I):**



(I),

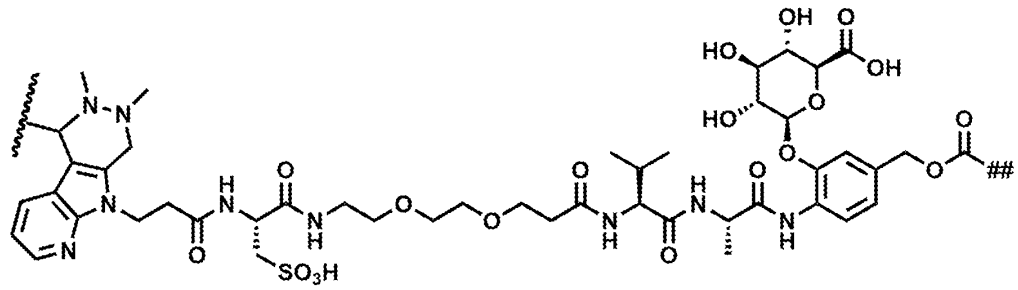
wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; wherein the Ab further comprises one or more *fGly'*, wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



(III),

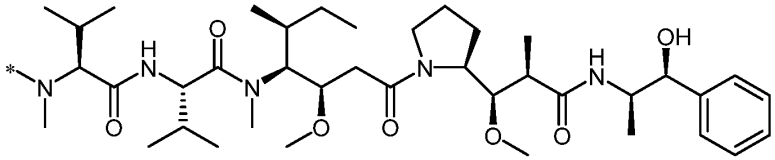
wherein * indicates the point of attachment in **Formula (I)** and wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I)** is attached.

[0009] In some embodiments, the ADC comprises an antibody (Ab) conjugated to a monomethyl auristatin E (MMAE) by a linker (**L-1**):



(L-1),

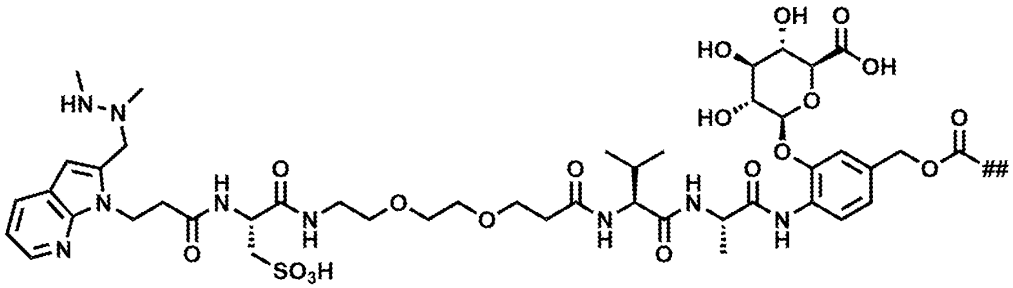
wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE; wherein the MMAE is represented by **Formula (III)**:



(III),

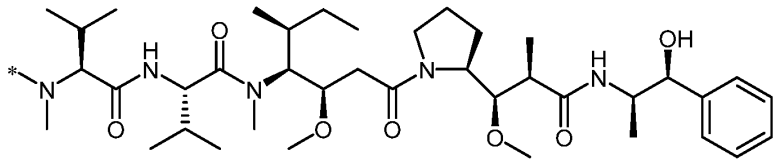
wherein * indicates the point of attachment to (**L-1**); wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; and wherein the Ab further comprises one or more *fGly'* and wherein *fGly'* is a formylglycine amino acid residue to which linker (**L-1**) is attached. In further embodiments, one Ab is conjugated to one to ten MMAEs and each MMAE is conjugated to Ab by one linker (**L-1**). In yet further embodiments, one Ab is conjugated to two MMAEs and each MMAE is conjugated to Ab by one linker (**L-1**).

[0010] In some embodiments, the ADC is produced by conjugating an antibody (Ab) to a linker-drug comprising a pre-conjugate linker **(L-1a')** and a monomethyl auristatin E (MMAE):



(L-1a'),

wherein the ## indicates the point of attachment to the MMAE; wherein the MMAE is represented by **Formula (III)**:



(**III**),

wherein * indicates the point of attachment to (**L-1a'**); wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; and wherein the Ab further comprises one or more *fGly'* and wherein *fGly'* is a formylglycine amino acid residue to which linker (**L-1a'**) is attached after conjugating the Ab and the linker-drug. In further embodiments, one Ab is conjugated to one to ten linker-drugs. In yet further embodiments, one Ab is conjugated to two linker-drugs.

[0011] In any of the embodiments as disclosed herein, for example, in the ADCs represented by **Formula (I')** or **Formula (I)**, or the ADCs comprising **(L-1)**, or the ADCs produced by conjugating **(L-1a')**, the Ab comprises one to ten *fGly'*. In further embodiments, the Ab comprises two *fGly'*.

[0012] In any of the embodiments as disclosed herein, for example, in the ADCs represented by **Formula (I')** or **Formula (I)**, or the ADCs comprising **(L-1)**, or the ADCs produced by conjugating **(L-1a')**, the Ab comprises a motif of X^1(*fGly'*)$X^2Z^2X^3Z^3$ (SEQ ID NO: 67), wherein: Z^2 is either a proline (P) or alanine (A) residue; Z^3 is a basic amino acid residue or an aliphatic amino acid residue; X^1 is present or absent and, when present, is any amino acid residue, with the proviso that when the sequence of SEQ ID NO: 67 is at the N-terminus of the antibody Ab, X^1 is present; and X^2 and X^3 independently is any amino acid residue. In some embodiments, the basic amino acid residue is selected from the group consisting of: arginine (R), lysine (K), and histidine (H). Additionally or alternatively, the aliphatic amino acid residue is selected from the group consisting of: alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), and proline (P). In some embodiments, the motif of SEQ ID NO: 67 is selected from the group consisting of: L(*fGly'*)TPSR (SEQ ID NO: 1),

M(*fGly'*)TPSR (SEQ ID NO: 45), V(*fGly'*)TPSR (SEQ ID NO: 46), L(*fGly'*)SPSR (SEQ ID NO: 47), L(*fGly'*)APSR (SEQ ID NO: 48), L(*fGly'*)VPSR (SEQ ID NO: 49), L(*fGly'*)GPSR (SEQ ID NO: 50), I(*fGly'*)TPAR (SEQ ID NO: 51), L(*fGly'*)TPSK (SEQ ID NO: 52), M(*fGly'*)TPSK (SEQ ID NO: 53), V(*fGly'*)TPSK (SEQ ID NO: 54), L(*fGly'*)SPSK (SEQ ID NO: 55), L(*fGly'*)APSK (SEQ ID NO: 56), L(*fGly'*)VPSK (SEQ ID NO: 57), L(*fGly'*)GPSK (SEQ ID NO: 58), L(*fGly'*)TPSA (SEQ ID NO: 59), I(*fGly'*)TPAA (SEQ ID NO: 60), M(*fGly'*)TPSA (SEQ ID NO: 61), V(*fGly'*)TPSA (SEQ ID NO: 62), L(*fGly'*)SPSA (SEQ ID NO: 63), L(*fGly'*)APSA (SEQ ID NO: 64), L(*fGly'*)VPSA (SEQ ID NO: 65), and L(*fGly'*)GPSA (SEQ ID NO: 66).

[0013] In any of the embodiments as disclosed herein, for example, in the ADCs represented by **Formula (I')** or **Formula (I)**, or the ADCs comprising **(L-1)**, or the ADCs produced by conjugating **(L-1a')**, the Ab comprises two heavy chains and two light chains, and wherein each of the heavy chains comprises a motif of SEQ ID NO: 67. In any of the embodiments as disclosed herein, for example, in the ADCs represented by **Formula (I')** or **Formula (I)**, or the ADCs comprising **(L-1)**, or the ADCs produced by conjugating **(L-1a')**, the Ab comprises an amino acid sequence of L(*fGly'*)TPSR (SEQ ID NO: 1). In further embodiments, the Ab comprises two heavy chains and two light chains, and wherein each of the heavy chains comprises a motif of L(*fGly'*)TPSR (SEQ ID NO: 1). In some embodiments, the *fGly'* of the motif is located in the heavy chain at a position corresponding to the 453rd amino acid residue of SEQ ID NO: 2.

[0014] In any of the embodiments as disclosed herein, for example, in the ADCs represented by **Formula (I')** or **Formula (I)**, or the ADCs comprising **(L-1)**, or the ADCs produced by conjugating **(L-1a')**, the Ab is a 5T4 antibody or antigen binding fragment thereof as disclosed herein.

[0015] In some embodiments, the Ab comprises HCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 19, 24, 25, and 30; HCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 20, 26, 31, and 36; HCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 21, 27, and 32; LCDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22, 28, and 33; LCDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 23, and 34; and LCDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 29, and 35.

[0016] In some embodiments, HCDR1 comprises the amino acid sequence of SEQ ID NO: 13, HCDR2 comprises the amino acid sequence of SEQ ID NO: 14, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18. In some embodiments, HCDR1 comprises the amino acid sequence of SEQ ID NO: 19, HCDR2 comprises the amino acid sequence of SEQ ID NO: 20, HCDR3 comprises the amino acid sequence of SEQ ID NO: 21, LCDR1 comprises the amino acid sequence of SEQ ID NO: 22, LCDR2 comprises the amino acid sequence of SEQ ID NO: 23, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18. In some embodiments, HCDR1 comprises the amino acid sequence of SEQ ID NO: 24, HCDR2 comprises the amino acid sequence of SEQ ID NO: 14, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18. In some embodiments, HCDR1 comprises the amino acid sequence of SEQ ID NO: 25, HCDR2 comprises the amino acid sequence of SEQ ID NO: 26, HCDR3 comprises the amino acid sequence of SEQ ID NO: 27, LCDR1 comprises the amino acid sequence of SEQ ID NO: 28, LCDR2 comprises the amino acid sequence of SEQ ID NO: 23, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 29. In some embodiments, HCDR1 comprises the amino acid sequence of SEQ ID NO: 30, HCDR2 comprises the amino acid sequence of SEQ ID NO: 31, HCDR3 comprises the amino acid sequence of SEQ ID NO: 32, LCDR1 comprises the amino acid sequence of SEQ ID NO: 33, LCDR2 comprises the amino acid sequence of SEQ ID NO: 34, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 35. In some embodiments, HCDR1 comprises the amino acid sequence of SEQ ID NO: 13, HCDR2 comprises the amino acid sequence of SEQ ID NO: 36, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18.

[0017] In some embodiments, the Ab comprises a VH comprising the amino acid sequence of SEQ ID NO: 11 and a VL comprising the amino acid sequence of SEQ ID NO: 12. In further embodiments, the Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 2 and a light chain comprising the amino acid sequence of SEQ ID NO: 3.

[0018] In some embodiments, a method of administering an ADC as disclosed herein is combined with administrating to the subject means for inhibiting interaction between PD-1 and PD-L1, such as a PD-L1/PD-1 inhibitor. One example of such means for inhibiting interaction between PD-1 and PD-L1 is pembrolizumab. In some embodiments, the method as disclosed herein further comprises administrating to the subject means for inhibiting interaction between PD-1 and PD-L1. In some embodiments, the ADC is administered concurrently with the means for inhibiting interaction between PD-1 and PD-L1. In some embodiments, the ADC is administered after the means for inhibiting interaction between PD-1 and PD-L1. In some embodiments, the ADC is administered before the means for inhibiting interaction between PD-1 and PD-L1.

[0019] In some embodiments, a method of administering an ADC as disclosed herein is combined with administrating to the subject a PD-L1/PD-1 inhibitor. In some embodiments, the method as disclosed herein further comprises administrating to the subject a PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered concurrently with the PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered after the PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered before the PD-L1/PD-1 inhibitor. Additionally or alternatively, in some embodiments, the PD-L1/PD-1 inhibitor is an antibody or an antigen binding fragment thereof comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 of pembrolizumab. In further embodiments, the PD-L1/PD-1 inhibitor is pembrolizumab. In yet further embodiments, the PD-L1/PD-1 inhibitor is administered to the subject at a dose of about 200 mg. Additionally or alternatively, in some embodiments, the PD-L1/PD-1 inhibitor is administered intravenously. Additionally or alternatively, in some embodiments, the PD-L1/PD-1 inhibitor is administered once every 3 weeks (q3w).

[0020] In any of the embodiments as disclosed herein, for example, in the ADCs represented by **Formula (I')** or **Formula (I)**, or the ADCs comprising **(L-1)**, or the ADCs produced by conjugating **(L-1a')**, the ADC is administered intravenously. Additionally or alternatively, in some embodiments, the ADC is administered once every 3 weeks (q3w).

[0021] In some embodiments, wherein the subject is human.

6. BRIEF DESCRIPTION OF THE DRAWINGS

[0022] **FIG. 1** provides a method for synthesizing the ADC as disclosed herein (such as **5T4-ADC-1**) according to **Example 1**.

[0023] **FIG. 2** depicts a schematic of the BOIN design.

[0024] **FIG. 3** depicts a scheme depicting the BOIN design for the Dose Escalation Stage described in the Examples.

[0025] **FIGs. 4A-4B** show *in vitro* cytotoxic activities of **5T4-ADC-1** in MCF-7 (**FIG. 4A**) and MDA-MB-468 (**FIG. 4B**) breast cancer cell lines, as further illustrated in **Example 3**. **5T4-ADC-1** mediated *in vitro* cytotoxicity was observed in both tumor cell lines that were used for *in vivo* xenograft studies. In both cell lines, **5T4-ADC-1** demonstrated improved potency and similar maximal inhibition compared with the positive control (5T4-targeting mafodotin ADC).

[0026] **FIGs. 5A-5E** show *in vivo* tumor growth inhibition and survival curves following IV administration of **5T4-ADC-1** in the MCF-7 (**FIGs. 5A-5D**) and MDA-MB-468 (**FIG. 5E**) murine breast cancer xenograft models, as further illustrated in **Example 3**. Dose response study with either a single dose (**FIG. 5A**) or once per week for two weeks (QWx2) dosing (**FIG. 5B**) of **5T4-ADC-1** was performed. Survival curves for single dose (**FIG. 5C**) and QWx2 dose (**FIG. 5D**) studies were plotted. **5T4-ADC-1** led to dose-related tumor volume suppression and longer survival (right-shifted curves) compared with vehicle following either dosing regimen. As shown in **FIG. 5E**, **5T4-ADC-1** produced maximal and durable suppression starting at 20 days post dose. Survival curves were not generated for this study as only 2/8 animals in the vehicle reached the prespecified survival outcome. SEM, standard error of the mean.

[0027] **FIGs. 6A-6B** show combinatorial efficacy for **5T4-ADC-1** and anti-PD-1 checkpoint inhibitor in a mouse syngeneic MC38-h5T4 model, as further illustrated in **Example 3**. Combination of **5T4-ADC-1** and anti-PD-1 reagent enhanced anti-tumor activity and improved survival compared with either agent administered alone. BIW, twice-weekly; *i.v.*, intravenous; PD-1, programmed cell death protein 1; SEM, standard error of the mean.

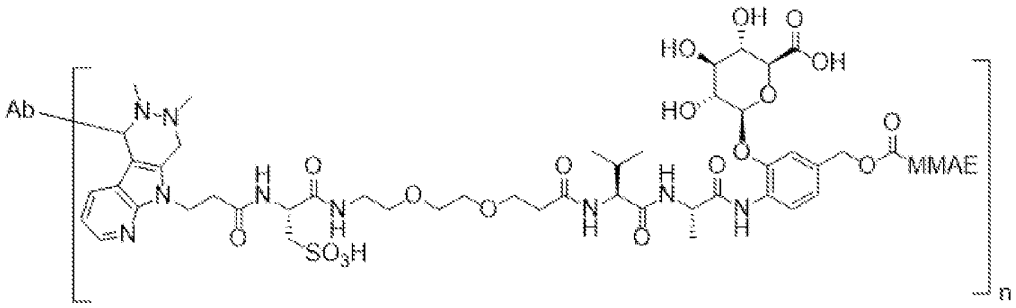
[0028] **FIGs. 7A-7C** show *in vivo* tumor growth inhibition following single dose IV administration of **5T4-ADC-1** (10 mg/kg) in murine PDX models of HNSCC (**FIG. 7A**), NSCLC (**FIG. 7B**), and breast cancer (non-TNBC) (**FIG. 7C**), as further illustrated in **Example 3**. IV, intravenous; NSCLC, non-small cell lung cancer; SEM, standard error of the mean; HNSCC, squamous cell cancer of the head and neck; TGI, tumor growth inhibition.

[0029] **FIGs. 8A-8B** plot 5T4 expression of patient tumor tissue samples (**FIG. 8A**, HScores; **FIG. 8B**, percentages of 5T4 positive tumor cells), as further illustrated in **Example 4**.

7. DETAILED DESCRIPTION

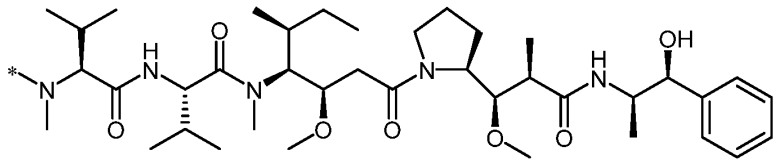
[0030] In one aspect, provided herein is a method for treating a cancer in a subject, the method comprising administering an ADC to the subject at a therapeutically effective dose, wherein the ADC is represented by **Formula (I') or Formula (I).**

[0031] In some embodiments, the ADC is represented by **Formula (I')**:



(I'),

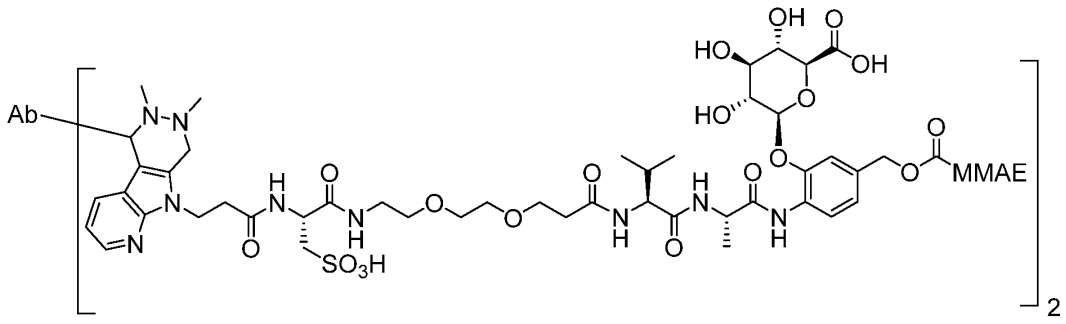
wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; wherein the Ab further comprises one or more *fGly'*, wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I')** is attached; wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



(III),

wherein * indicates the point of attachment in **Formula (I')**; and wherein n is an integer. In further embodiments, n is an integer selected from any one of 1 to 10. In yet further embodiments, n is 2.

[0032] In some embodiments, the ADC is represented by **Formula (I)** and comprises an Ab conjugated to a monomethyl auristatin E (MMAE) by a linker (**L-1**):



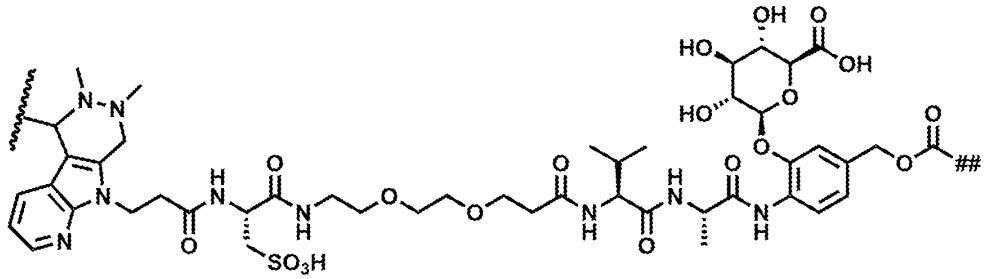
(I),

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which a linker-drug comprising (**L-1**) is attached, and wherein **mAbA15** comprises:

a) a heavy chain comprising an amino acid sequence of:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSY
TEYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL
(*fGly'*)TPSRGS (SEQ ID NO: 2); and

b) a light chain comprising an amino acid sequence of:
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGV
PSRFSGSRSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3);

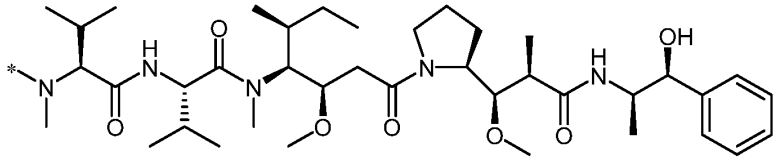
wherein **L-1** is:



(**L-1**),

wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE; and

wherein the MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment to (**L-1**).

[0033] In some embodiments, the ADC is represented by **Formula (I):**

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; wherein the Ab further comprises one or more *fGly'*, wherein the MMAE is monomethyl auristatin E and represented by **Formula (III).**

[0034] In certain embodiments, the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, or bladder cancer. In certain embodiments, the cancer is pancreatic adenocarcinoma (PDAC, also referred to herein as pancreatic ductal adenocarcinoma). In certain embodiments, the cancer is lung adenocarcinoma or squamous cell lung carcinoma. In certain embodiments, the cancer is non-small cell lung cancer (NSCLC). In certain embodiments, the cancer is head and neck cancer. In certain embodiments, the cancer is head

and neck squamous cell carcinoma (HNSCC). In certain embodiments, the cancer is renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer.

[0035] In certain embodiments, the ADC is administered to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg. In certain embodiments, the ADC is administered to the subject at a dose of about 0.3 mg/kg to about 6.0 mg/kg, or about 0.3 mg/kg to about 2.0 mg/kg. In certain embodiments, the ADC is administered to the subject at a dose of about 0.3 mg/kg, about 0.6 mg/kg, about 1.0 mg/kg, about 1.5 mg/kg, or about 2.0 mg/kg.

[0036] In certain embodiments, the Ab comprises two heavy chains (HCs) and two light chains (LCs), and wherein each of the HCs comprises one motif of L(*fGly'*)TPSR (SEQ ID NO: 1).

[0037] In certain embodiments, the ADC is administered to the subject on the first day of a treatment cycle for one or more treatment cycles. In certain embodiments, each treatment cycle is 3 weeks ± 3 days. In certain embodiments, the ADC is administered to the subject once every three weeks (q3w). In certain embodiments, the ADC is administered to the subject intravenously.

[0038] In certain embodiments, the method comprises administering to the subject a PD-L1/PD-1 inhibitor. In certain embodiments, the PD-L1/PD-1 inhibitor is administered to the subject once every three weeks (q3w). In certain embodiments, the PD-L1/PD-1 inhibitor is administered to the subject intravenously. In certain embodiments, the PD-L1/PD-1 inhibitor is pembrolizumab. In certain embodiments, the PD-L1/PD-1 inhibitor is administered to the subject at a dose of about 200 mg.

[0039] In certain embodiments, the subject is human.

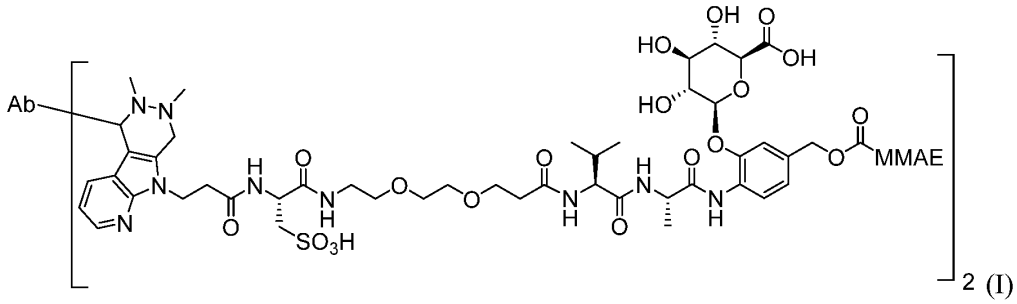
[0040] In another aspect, provided herein is a method of treating a cancer for two or more treatment cycles in a subject experiencing an adverse event when administered an ADC, the method comprising:

(i) administering the ADC to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg in a first treatment cycle;

(ii) delaying a further administration of the ADC to the subject upon the subject having an adverse event after the first treatment cycle and before the second treatment cycle; and

(iii) resuming the administration of the ADC in a second treatment cycle,

wherein the ADC is represented by **Formula (I)** and comprises an Ab conjugated to a monomethyl auristatin E (MMAE) by a linker (**L-1**):

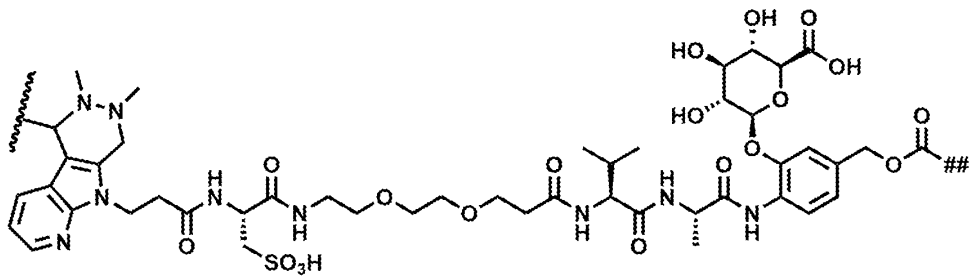


(I)

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which a linker-drug comprising (**L-1**) is attached, and wherein **mAbA15** comprises:

a) a heavy chain comprising an amino acid sequence of:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSY
TEYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL
(*fGly'*)TPSRGS (SEQ ID NO: 2); and

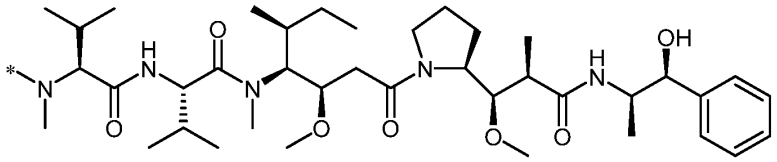
b) a light chain comprising an amino acid sequence of:
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGV
PSRFSGSRSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3);
wherein (**L-1**) is:



(**L-1**),

wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE; and

wherein the MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment to (**L-1**).

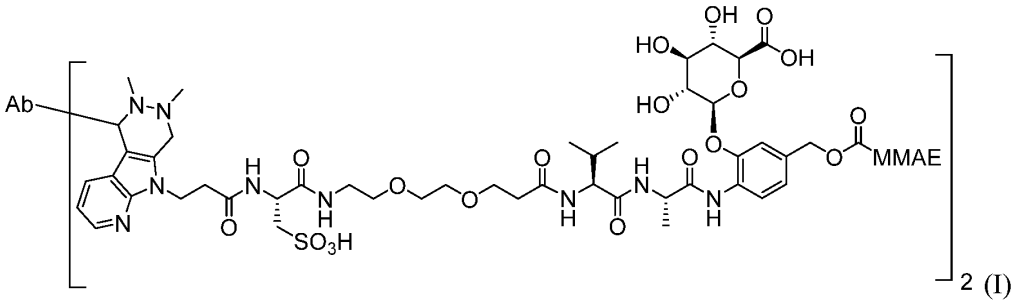
[0041] In certain embodiments, the administration is resumed in step (iii) once the adverse event is alleviated or recovered. In certain embodiments, the administration of the second treatment cycle is at the dose of the first treatment cycle. In certain embodiments, the administration of the second treatment cycle is at a dose lower than that of the first treatment cycle.

[0042] In a further aspect, provided herein is a method of treating a cancer for two or more treatment cycles in a subject experiencing an adverse event when administered an ADC, the method comprising:

i) administering the ADC to the subject at a first dose of about 0.1 mg/kg to about 10.0 mg/kg in a first treatment cycle;

ii) in a second treatment cycle, administering the ADC to the subject at a second dose which is lower than the first dose upon the subject having an adverse event,

wherein the ADC is represented by **Formula (I)** and comprises an Ab conjugated to a monomethyl auristatin E (MMAE) by a:

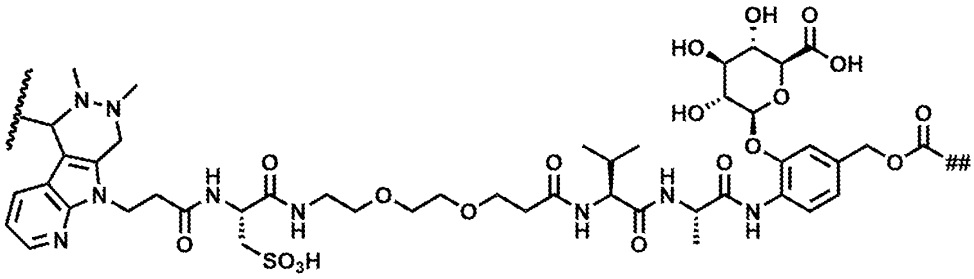


(I)

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which a linker-drug comprising (**L-1**) is attached, and wherein **mAbA15** comprises:

a) a heavy chain comprising an amino acid sequence of:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSY
TEYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWG
QGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTS
GVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDK
THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI
SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL
(*fGly'*)TPSRGS (SEQ ID NO: 2); and

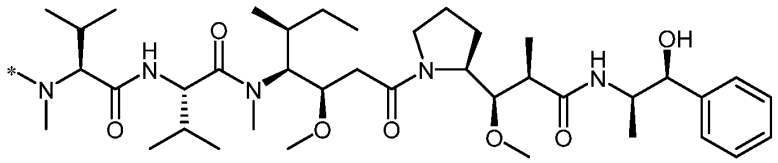
b) a light chain comprising an amino acid sequence of:
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGV
PSRFSGSRSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3);
wherein (**L-1**) is:



(**L-1**),

wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE; and

wherein the MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment to (**L-1**).

[0043] In certain embodiments, the subject is administered at the second dose in step (ii) once the adverse event is alleviated or recovered. In certain embodiments, the adverse event is selected from the group consisting of Grade 3 thrombocytopenia with clinically significant bleeding, Grade 4 thrombocytopenia, Grade 3 or higher neutropenia with a measured fever of at least 38.3°C, Grade 3 or higher neutropenia with a sustained fever of at least 38°C for at least an hour, Grade 4 neutropenia for greater than 7 days, Grade 4 anemia, alanine transaminase or aspartate transaminase levels that are greater than 3 times the upper limit of normal and a total bilirubin level that is greater than 2 times the upper limit of normal in the absence of biliary obstruction or other explanation for liver abnormality (such as viral hepatitis, alcohol or autoimmune hepatitis, Gilbert Syndrome, or treatment with other hepatotoxic drug), and a Grade 5 adverse event.

7.1 Definitions

[0044] Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person skilled in the art of the present disclosure. As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

[0045] In some embodiments, chemical structures are disclosed with a corresponding chemical name. In case of conflict, the chemical structure controls the meaning, rather than the name.

[0046] Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context otherwise, as used herein, the terms “a,” “an,” and “the” are understood to be singular or plural.

[0047] The terms “about” and “approximately” mean within 20%, within 15%, within 10%, within 9%, within 8%, within 7%, within 6%, within 5%, within 4%, within 3%, within 2%, within 1%, or less variation of a given value or range.

[0048] In some embodiments, the terms “first,” “second,” “third,” “fourth” and similar in a component name are used to distinguish and identify more than one component sharing certain identity in their names. For example, “first antibody” and “second antibody” are used to distinguish two antibodies.

[0049] It is understood that wherever embodiments are described herein with the term “comprising” otherwise analogous embodiments described in terms of “consisting of” and/or “consisting essentially of” are also provided. It is also understood that wherever embodiments are described herein with the phrase “consisting essentially of” otherwise analogous embodiments described in terms of “consisting of” are also provided.

[0050] The term “between” as used in a phrase as such “between A and B” or “between A-B” refers to a range including both A and B or any subranges thereof.

[0051] As used herein, a range of A to B includes both A and B or any numbers or subranges thereof.

[0052] The term “and/or” as used in a phrase such as “A and/or B” herein is intended to include both A and B; A or B; A (alone); and B (alone). Likewise, the term “and/or” as used in a phrase such as “A, B, and/or C” is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

[0053] The term “optional” or “optionally” means that the subsequently described circumstance may or may not occur, so that the description includes instances wherein the circumstance occurs, and the instances wherein the circumstance does not occur.

[0054] The term “patient” and “subject” are used interchangeably and refer to human and non-human subjects, especially mammalian subjects. In one embodiment, the patient or subject is a human.

[0055] Dosing of the 5T4 ADCs as disclosed herein are provided in units of milligrams of ADC per kilogram of subject weight (mg/kg). However, dosing may also be specified having a unit of milligrams of ADC per square meters of body surface (mg/m^2). One of skill in the art will readily be able to convert a dose in mg/kg to a dose in mg/m^2 using the K_m ratio for

humans (1.62 m^2 for a 60 kg human or 37 kg/m^2). That is, a dose in mg/kg can be converted to a dose in mg/m^2 by multiplying by 37 kg/m^2. In a human subject, a dose of about 0.3 mg/kg is equal to a dose of about 11 mg/m^2. A dose of 5 mg/kg ADC is equal to a dose of 185 mg/m^2 in a human.

[0056] The term "treating" or "treatment" as used herein means the treating or treatment of a disease or medical condition in a subject, such as a mammal (particularly a human) that includes: (a) preventing the disease or medical condition from occurring, such as, prophylactic treatment of a subject; (b) ameliorating the disease or medical condition, such as, eliminating or causing regression of the disease or medical condition in a subject; (c) suppressing the disease or medical condition, for example by, slowing or arresting the development of the disease or medical condition in a subject; or (d) alleviating a symptom of the disease or medical condition in a subject. In some embodiments, the term "treating," or "treatment" excludes a prophylactic treatment. In some embodiments, the term "treating" or "treatment" as it relates to the treating or treatment of cancer may include reversing tumor progression (*e.g.*, reducing tumor size), slowing tumor progression, or halting tumor progression. In one embodiment, tumor progression is determined by the response assessment criteria selected for the clinical trial.

[0057] The term "tumor," in any embodiment herein, refers to any neoplastic cell growth or proliferation, whether malignant or benign, and to all pre-cancerous and cancerous cells and tissues.

[0058] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Cancers that may be treated according to the methods disclosed herein include, but are not limited to, breast cancer (*e.g.*, triple-negative breast cancer (TNBC) or hormone-receptor positive breast cancer (HR+ BC)), endometrial cancer, head and neck cancer (*e.g.*, head and neck squamous cell carcinoma (HNSCC)), pancreatic cancer (*e.g.*, pancreatic adenocarcinoma (PDAC, also referred to herein as pancreatic ductal adenocarcinoma)), lung cancer (*e.g.*, non-small cell lung cancer (NSCLC)), prostate cancer, cervical cancer, or bladder cancer. In certain embodiments, the cancer is lung adenocarcinoma or squamous cell lung carcinoma. In certain embodiments, the NSCLC is characterized by a mutation selected from the group consisting of an EGFR sensitizing mutation, an ALK rearrangement, a ROS1 rearrangement, a BRAF V600E mutation, a MET Exon 14 skipping mutation, and a RET rearrangement. In certain embodiments, the cancer is renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer. In some embodiments, the cancer is a genitourinary cancer. In some

embodiments, the cancer is a gastrointestinal cancer. In some embodiments, the cancer is a gynecological cancer. Additionally or alternatively, the cancer is previously untreatable. Additionally or alternatively, the cancer is recurrent. Additionally or alternatively, the cancer is metastatic. Additionally or alternatively, the cancer is advanced, for example locally advanced. Additionally or alternatively, the cancer is inoperable.

[0059] As used herein, "EGFR sensitizing mutation" includes Exon 19 deletions and Exon 21 point mutations. Exon 19 Deletions destabilize the inactive form of EGFR, causing it to remain in the active conformation. These deletions account for around 45% of all EGFR mutations. The most common frame shift deletion is delE756-A750, followed by delL747_P753insS and delL747_A750insP. Exon 21 Point Mutations lock EGFR in a constitutively active conformation and account for 40% of EGFR mutations. The most common exon 21 mutation is L858R.

[0060] As used herein, "ALK rearrangement" or "alk-positive" includes any rearrangement of the ALK gene with another gene that results in a fusion gene. The most comment ALK rearrangement is rearrangement of the EML4 gene and the ALK (anaplastic lymphoma kinase) gene that results in a fusion oncogene EML4-ALK.

[0061] As used herein, "ROS1 rearrangement", refers to the fusion of the ROS1 gene with a part of another gene.

[0062] As used herein, "BRAF V600E mutation," refers to a mutation of the BRAF gene from a valine to a glutamic acid at amino acid 600.

[0063] As used herein, "MET Exon 14 skipping mutation" refers to a presence of a splice site mutation that results in a loss of transcription of exon 14 of the MET gene.

[0064] As used herein "RET rearrangement," refers to the fusion of the RET gene with a part of another gene.

[0065] The term "therapeutically effective amount" as used herein refers to the amount of an antibody or ADC described herein that is sufficient to reduce and/or ameliorate the severity and/or duration of a given disease, disorder, or condition, and/or a symptom related thereto. A therapeutically effective amount of an agent, including a therapeutic agent, can be an amount necessary for (i) reduction or amelioration of the advancement or progression of a given disease, disorder, or condition, (ii) reduction or amelioration of the recurrence, development or onset of a given disease, disorder or conditions, and/or (iii) to improve or enhance the therapeutic effect of another therapy (*e.g.,* a therapy other than the administration of an antibody or ADC described herein). A "therapeutically effective amount" of a substance/molecule/agent of the present disclosure (*e.g.,* a 5T4 antibody or ADC) can vary

based on a number of factors such as the disease state, age, sex, and weight of the individual, and the ability of the substance/molecule/agent, to elicit a desired response in the individual. A therapeutically effective amount encompasses an amount in which any toxic or detrimental effects of the substance/molecule/agent are outweighed by the therapeutically beneficial effects. In some embodiments, the term "therapeutically effective amount" refers to an amount of an antibody or other agent (*e.g.,* or drug) effective to "treat" a disease, disorder, or condition, in a subject or mammal.

[0066] The term "pharmaceutically acceptable" as used herein means being approved by a regulatory agency of the federal or a state government, or listed in the U.S. Pharmacopeia, European Pharmacopeia, or other generally recognized Pharmacopeia for use in animals, and more particularly in humans.

[0067] "Excipients" include carriers, excipients, preservatives, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed and can be included, for example, to affect stability, bulk up formulations, or to confer a therapeutic enhancement on the active ingredient in the final dosage form (*e.g.*, facilitating absorption, reducing viscosity, enhancing solubility). An "excipient" can be an organic or inorganic ingredient, natural or synthetic with which the active ingredient is combined to facilitate the use of the active ingredient, *e.g.*, the administration of the active ingredient to a subject. Examples of excipients include buffers such as phosphate, citrate, and other organic acids; antioxidants such as ascorbic acid; low molecular weight (*e.g.*, less than about ten amino acid residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates such as glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™. The term "excipient" can also refer to a diluent, adjuvant (*e.g.*, Freund's adjuvant (complete or incomplete)), excipient, or vehicle with which the therapeutic is administered. Such excipients can be sterile liquids, such as water and oils, such as those of petroleum, animal, vegetable, or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil, and the like. Water is an exemplary excipient when a composition (*e.g.*, a pharmaceutical composition) is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid excipients, particularly for injectable solutions. Suitable excipients (*e.g.*, pharmaceutical excipients) include starch, glucose,

lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol, and the like. The composition, in any embodiment, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. Compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations, and the like. Oral compositions, such as formulations, can include standard excipients such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, and the like. Examples of suitable excipients are described in Remington: The Science and Practice of Pharmacy (2020) (Elsevier Science, Amsterdam, Netherlands), including pharmaceutical compounds, can contain an effective amount or therapeutically effective amount of a 5T4 ADC, for example, in isolated or purified form, together with a suitable amount of excipient to provide the form for proper administration to the subject. The formulation should suit the mode of administration.

[0068] The term "5T4" refers to a polypeptide ("polypeptide" and "protein" are used interchangeably herein) or any native 5T4 from any vertebrate source, including mammals such as primates (*e.g.*, humans, cynomolgus monkey (cyno)), dogs, and rodents (*e.g.*, mice and rats), unless otherwise indicated. 5T4, also known as "5T4 Oncofetal Trophoblast Glycoprotein" or "Wnt-Activated Inhibitory Factor 1" or "5T4 Oncotrophoblast Glycoprotein" or "5T4 Oncofetal Antigen" or "WAIF1" or "M6P1" or "5T4-AG" or "5T4AG" or "TPBG" is a glycoprotein encoded by the TPBG gene. The term 5T4 encompasses "full-length," 5T4, as well as any form of 5T4 or any fragment thereof that results from processing in the cell. In some embodiments, the 5T4 comprises a signal sequence. In some embodiments, the 5T4 does not include a signal sequence. In some embodiments, the term 5T4 refers to a fragment of the full-length 5T4, which comprises a 5T4 extracellular domain. The term 5T4 also encompasses naturally occurring variants of 5T4, such as SNP variants, splice variants and allelic variants. The full-length amino acid sequence of human 5T4 is provided below (exemplary signal sequence = italic text; exemplary extracellular domain = underlined text):

*MPGGCSRGPA AGDGRLRLAR LALVLLGWVS S*SSPTSSASS FSSSAPFLAS AVSAQPPLPD QCPALCECSE AARTVKCVNR NLTEVPTDLP AYVRNLFLTG NQLAVLPAGA FARRPPLAEL AALNLSGSRL DEVRAGAFEH LPSLRQLDLS HNPLADLSPF AFSGSNASVS APSPLVELIL NHIVPPEDER QNRSFEGMVV AALLAGRALQ GLRRLELASN HFLYLPRDVL AQLPSLRHLD LSNNSLVSLT YVSFRNLTHL ESLHLEDNAL KVLHNGTLAE LQGLPHIRVF LDNNPWVCDC

HMADMVTWLK ETEVVQGKDR LTCAYPEKMR NRVLLELNSA DLDCDPILPP SLQTSYVFLG IVLALIGAIF LLVLYLNRKG IKKWMHNIRD ACRDHMEGYH YRYEINADPR LTNLSSNSDV (SEQ ID NO: 4).

[0069] The full-length amino acid sequence of cynomolgus monkey (cyno) 5T4 is provided below (exemplary signal sequence = italic text; exemplary extracellular domain = underlined text):

*MPGGCSRGPA AGDGRLRLAR LALVLLGWVS S*SSSTSSASS SSSSAPFLAS AASAQPPLPD QCPALCECSE AARTVKCVNR NLTEVPTDLP LYVRNLFLTG NQLAVLPAGA FARRPPLAEL AALNLSGSRL DEVRGGAFEH LPSLRQLDLS HNPLAYLSPF AFSGSNASIS APSPLVELIL NHIVPPDDKR QNRSFEGMVA AALVAGRALQ GLHLLELASN HFLYLPRDVL AQLPSLRYLD LSNNSLVSLT YVSFRNLTHL ESLHLEDNAL KVLHNGTLAE LQGLPHVRVF LDNNPWVCDC HMADMVTWLK QTGVVQGKDR LTCAFPEKMR NRVLLELNSA DLDCDPILPP SLQTSYVFLG IVLALIGAIF LLVLYLNRKG IKKWMHNIRD ACRDHMEGYH YRYEINADPR LTNLSSNSDV (SEQ ID NO: 5).

[0070] The full-length amino acid sequence of mouse 5T4 is provided below (exemplary signal sequence = italic text; exemplary extracellular domain = underlined text):

*MPGAGSRGPS AGDGRLRLAR LALVLLGWVS A*SAPSSSVPS SSTSPAAFLA SGSAQPPPAE RCPAACECSE AARTVKCVNR NLLEVPADLP PYVRNLFLTG NQMTVLPAGA FARQPPLADL EALNLSGNHL KEVCAGAFEH LPGLRRLDLS HNPLTNLSAF AFAGSNASVS APSPLEELIL NHIVPPEDQR QNGSFEGMVA FEGMVAAALR SGLALRGLTR LELASNHFLF LPRDLLAQLP SLRYLDLRNN SLVSLTYASF RNLTHLESLH LEDNALKVLH NSTLAEWHGL AHVKVFLDNN PWVCDCYMAD MVAWLKETEV VPDKARLTCA FPEKMRNRGL LDLNSSDLDC DAVLPQSLQT SYVFLGIVLA LIGAIFLLVL YLNRKGIKKW MHNIRDACRD HMEGYHYRYE INADPRLTNL SSNSDV (SEQ ID NO: 6).

[0071] The terms “constant region” and “constant domain” are used interchangeably herein, are well-known antibody terms of art, and refer to an antibody portion, for example, a carboxyl terminal portion of a light and/or heavy chain which is not directly involved in binding of an antibody to an antigen, but which can exhibit various effector functions, such as interaction with an Fc receptor. The term includes the portion of an immunoglobulin molecule having a generally more conserved amino acid sequence relative to an immunoglobulin variable region.

[0072] The terms “antibody,” “immunoglobulin,” or “Ig” are used interchangeably herein and are used in the broadest sense and specifically covers, for example polyclonal antibodies, monoclonal antibodies (including agonist, antagonist, neutralizing antibodies, full length monoclonal antibodies), antibody compositions with polyepitopic or monoepitopic

specificity, recombinantly produced antibodies, single domain antibodies, monospecific antibodies, multispecific antibodies (including bispecific antibodies), synthetic antibodies, chimeric antibodies, humanized antibodies, or human versions of antibodies having full length heavy and/or light chains. Antibodies also include single antibody domains as well as antibody fragments (and/or polypeptides that comprise antibody fragments) that retain 5T4 binding characteristics (also referred to herein as antigen binding fragment). Non-limiting examples of antibody fragments include antigen-binding regions and/or effector regions of the antibody, *e.g.*, Fab, Fab', $F(ab')_2$, Fv, scFv, $(scFv)_2$, single-chain antibody molecule, dual variable domain antibody, single variable domain antibody, linear antibody, V region, a multispecific antibody formed from antibody fragments, $F(ab)_2$, Fd, Fc, diabody, di-diabody, disulfide-linked Fvs (dsFv), single-domain antibody (*e.g.,* nanobody) or other fragments (*e.g.*, fragments consisting of the variable regions of the heavy and light chains that are non-covalently coupled). In general terms, a variable (V) region can be any suitable arrangement of immunoglobulin heavy (VH) and/or light (VL) variable regions. For example, antibodies also include tetrameric antibodies comprising two heavy chain and two light chain molecules, an antibody light chain monomer, and an antibody heavy chain monomer. Thus, for example, the V region can be dimeric and contain VH-VH, VH-VL, or VL-VL dimers that bind 5T4. In any embodiment, a VH region and a VL region can be covalently coupled either directly or through a linker to form a single-chain Fv (scFv). For ease of reference, scFv proteins are referred to herein as included in the category "antibody fragments." Another form of an antibody fragment is a peptide comprising one or more complementarity determining regions (CDRs) of an antibody. CDRs (also termed "minimal recognition units" or "hypervariable regions") can be obtained by constructing polynucleotides that encode one or more CDRs of interest. Such polynucleotides are prepared, for example, by using the polymerase chain reaction to synthesize the variable region using mRNA of antibody-producing cells as a template (see, for example, Larrick et al., Methods: A Companion to Methods in Enzymology, 2:106 (1991); Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in Monoclonal Antibodies Production, Engineering and Clinical Application, Ritter et al. (eds.), page 166, Cambridge University Press (1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in Monoclonal Antibodies: Principles and Applications, Birch et al., (eds.), page 137, Wiley-Liss, Inc. (1995)). Antibody fragments can be incorporated, for example, into single domain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, variable regions of new antigen receptors (v-NAR), and bis-single-chain Fv regions (see, *e.g.*, Hollinger and Hudson, Nature

Biotechnology, 23(9):1126-1136, 2005). In some embodiments, antibodies comprising a VH and/or VL contain a light chain and/or a heavy chain constant region, such as one or more constant regions, including one or more IgG1, IgG2, IgG3 and/or IgG4 constant regions. In some embodiments, antibodies can include epitope-binding fragments of any of the above. The antibodies described herein can be of any class (*e.g.*, IgG, IgE, IgM, IgD, and IgA) or any subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) of immunoglobulin molecule.

[0073] The terms "variable region" and "variable domain" are used interchangeably to refer to a portion of the light and heavy chains of an antibody that are generally located at the amino-terminal of the light and heavy chain, has a length of about 120 to 130 amino acids in the heavy chain, about 100 to 110 amino acids in the light chain, and is used in the binding and specificity of each antibody for its antigen. The variable region of the heavy chain is referred to herein as "VH." The variable region of the light chain is referred to herein as "VL." The term "variable" refers to the fact that certain segments of the variable regions differ extensively in sequence among antibodies. The V region mediates antigen binding and defines specificity of a particular antibody for its antigen. However, the variability is not evenly distributed across the 110-amino acid span of the variable regions. Instead, the V regions consist of less variable (*e.g.,* relatively invariant) stretches called framework regions (FRs) of about 15-30 amino acids separated by shorter regions of greater variability (*e.g.,* extreme variability) called "hypervariable regions" or alternatively called "complementarity determining regions." The variable regions of heavy and light chains each comprise four frameworks (FR1, FR2, FR3 and FR4), largely adopting a β sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β sheet structure. The hypervariable regions in each chain are held together in proximity by the frameworks and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see, *e.g.,* Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, (1991)). The constant regions are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC). The variable regions differ extensively in sequence between different antibodies. The variability in sequence is concentrated in the CDRs while the less variable portions in the variable region are referred to as framework regions (FR). The CDRs of the light and heavy chains are primarily responsible for the interaction of the antibody with antigen. In specific embodiments, the variable region is a human variable region.

[0074] The term "heavy chain" when used in reference to an antibody refers to a polypeptide chain of about 50-70 kDa, wherein the amino-terminal portion includes a variable region of about 120 to 130 or more amino acids, and a carboxy-terminal portion includes one or more constant regions. The "heavy chain" can refer to any distinct types, *e.g.*, for example, alpha (α), delta (δ), epsilon (ε), gamma (γ) and mu (μ), based on the amino acid sequence of the constant region, which give rise to IgA, IgD, IgE, IgG and IgM classes of antibodies, respectively, including subclasses of IgG, *e.g.*, IgG1, IgG2, IgG3 and IgG4.

[0075] As used herein, the term "light chain" when used in reference to an antibody can refer to a polypeptide chain of about 25 kDa, wherein the amino-terminal portion includes a variable region of about 100 to about 110 or more amino acids, and a carboxy-terminal portion includes a constant region. The approximate length of a light chain is 211 to 217 amino acids. There are two distinct types, *e.g.*, kappa (κ) or lambda (λ) based on the amino acid sequence of the constant regions. Light chain amino acid sequences are well known in the art.

[0076] The terms "identical" or percent "identity" in the context of two or more nucleic acids or polypeptides, refer to two or more sequences or subsequences that are the same or have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned (introducing gaps, if necessary) for maximum correspondence, not considering any conservative amino acid substitutions as part of the sequence identity. The percent identity can be measured using sequence comparison software or algorithms or by visual inspection. Various algorithms and software that can be used to obtain alignments of amino acid or nucleotide sequences are well known in the art. These include, but are not limited to, basic local alignment search tool (BLAST), ALIGN, MegAlign, BestFit, GCG Wisconsin Package, and variants thereof. In some embodiments, two nucleic acids or polypeptides are substantially identical, meaning they have at least 70%, at least 75%, at least 80%, at least 85%, or at least 90%, and in some embodiments, at least 95%, 96%, 97%, 98%, or 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using a sequence comparison algorithm or by visual inspection. In some embodiments, identity exists over a region of the amino acid sequences that is at least about ten residues, at least about 20 residues, at least about 40-60 residues, at least about 60-80 residues in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 residues, such as at least about 80-100 residues, and in some embodiments, the sequences are substantially identical over the full-length of the sequences being compared, such as the coding region of a target protein or an antibody. In

some embodiments, identity exists over a region of the nucleotide sequences that is at least about ten bases, at least about 20 bases, at least about 40-60 bases, at least about 60-80 bases in length or any integral value there between. In some embodiments, identity exists over a longer region than 60-80 bases, such as at least about 80-1000 bases or more, and in some embodiments, the sequences are substantially identical over the full length of the sequences being compared, such as a nucleotide sequence encoding a protein of interest.

[0077] A "conservative amino acid substitution" is one in which one amino acid residue is replaced with another amino acid residue having a side chain with similar chemical characteristics. Families of amino acid residues having similar side chains have been generally defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). For example, substitution of a phenylalanine for a tyrosine is a conservative substitution. Generally, conservative substitutions in the sequences of the polypeptides, soluble proteins, and/or antibodies of the disclosure do not abrogate the binding of the polypeptide, soluble protein, or antibody containing the amino acid sequence, to the target binding site. Methods of identifying amino acid conservative substitutions that do not eliminate binding are well known in the art.

[0078] The term "polypeptide" refers to a polymer of amino acids of any length. The polymer can be linear or branched, it can comprise modified amino acids, and it can include (*e.g.,* be substituted with) non-amino acids. The terms also encompass an amino acid polymer that has been modified naturally or by intervention; for example, disulfide bond formation, glycosylation, lipidation, acetylation, phosphorylation, or any other manipulation or modification, such as linkage to or conjugation with (directly or indirectly) a moiety such as a labeling component or a drug (*e.g.*, toxin). Also included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids), as well as other modifications known in the art. It is understood that, because the polypeptides of this disclosure can be based upon antibodies or other members of the immunoglobulin superfamily, in some embodiments, the polypeptides can occur as single chains or dimers of single chains.

[0079] As used herein, a first amino acid residue in a first peptide corresponding to a second amino acid residue in a second peptide refers to the first amino acid residue aligned to

the second amino acid residue in a sequence alignment between the first peptide and the second peptide. Alignment methods are available to one of skilled in the art, such as BLAST as disclosed herein and/or Clustal Omega.

[0080] As used herein, an "antigen" is a moiety or molecule that contains an epitope to which an antibody can bind. As such, an antigen can be bound by an antibody. In some embodiments, the antigen to which an antibody described herein binds is 5T4 (*e.g.*, human 5T4), or a fragment thereof, such as a fragment that comprises one or more regions of 5T4.

[0081] As used herein, an "epitope" is a term in the art and refers to a localized region of an antigen to which an antibody can bind. An epitope can be a linear epitope or a conformational, non-linear, or discontinuous epitope. In the case of a polypeptide antigen, for example, an epitope can be contiguous amino acids of the polypeptide (a "linear" epitope), or an epitope can comprise amino acids from two or more non-contiguous regions of the polypeptide (a "conformational," "non-linear" or "discontinuous" epitope), *e.g.*, human 5T4. It will be appreciated by one of skill in the art that, in general, a linear epitope may or may not be dependent on secondary, tertiary, or quaternary structure. For example, in some embodiments, an antibody binds to a group of amino acids regardless of whether they are folded in a natural three-dimensional protein structure. In other embodiments, an antibody requires amino acid residues making up the epitope to exhibit a particular conformation (*e.g.*, bend, twist, turn or fold) in order to recognize and bind the epitope.

[0082] An antibody binds "an epitope," "essentially the same epitope," or "the same epitope" as a reference antibody. The most widely used and rapid methods for determining whether two antibodies bind to identical, overlapping, or adjacent epitopes in a three-dimensional space are competition assays, which can be configured in a number of different formats, for example, using either labeled antigen or labeled antibody. In some assays, the antigen is immobilized on a 96-well plate, or expressed on a cell surface, and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured using radioactive, fluorescent or enzyme labels.

[0083] The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain, including, for example, native sequence Fc regions, recombinant Fc regions, and variant Fc regions. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is often defined to stretch from an amino acid residue at position Cys226 (of the EU numbering system) or from Pro230 (of the EU numbering system) to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 of the EU numbering system) of the Fc region can be

removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. An exemplary Fc region sequence is provided below (CH2 domain = bold text; CH3 domain = underlined text): CPPCP**APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK**GQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 7).

[0084] A "functional Fc region" possesses an "effector function" of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.,* B cell receptor; BCR), and the like. Such effector functions generally require the Fc region to be combined with a binding region or binding domain (*e.g.,* an antibody variable region or domain) and can be assessed using various assays as disclosed.

[0085] A "native sequence Fc region" comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature, and not manipulated, modified, and/or changed (*e.g.,* isolated, purified, selected, including, or combining with other sequences such as variable region sequences) by a human. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region; as well as naturally occurring variants thereof.

[0086] A "variant Fc region" comprises an amino acid sequence that differs from that of a native sequence Fc region by virtue of at least one amino acid modification, (*e.g.,* substituting, addition, or deletion) preferably one or more amino acid substitution(s). In some embodiments, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, for example, from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region described herein can possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, or at least about 90% homology therewith, for example, at least about 95% homology therewith. The variant Fc region herein described herein can have a loss of effector function (*e.g.*, silent Fc). An exemplary variant Fc region ("silent Fc") sequence is provided below (CH2 domain = bold text with amino acid changes underlined; CH3 domain = underlined text):

CPPCP**APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALKAPIEKTISKAK**GQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO: 8).

[0087] The terms "antigen binding fragment," "antigen binding domain," "antigen binding region," and similar terms refer to a portion of an antibody that comprises amino acid residues that interact with an antigen and confer on the binding fragment or region its specificity and affinity for the antigen (*e.g.*, the CDRs). "Antigen binding fragment" as used herein includes "antibody fragment," which comprises a portion of an antibody including one or more CDRs, such as the antigen binding or variable region of the antibody.

[0088] Antibodies described herein include, but are not limited to, synthetic antibodies, monoclonal antibodies, recombinantly produced antibodies, multispecific antibodies (*e.g.*, including bispecific antibodies), human antibodies, humanized antibodies, chimeric antibodies, intrabodies, single-chain Fvs (scFv) (*e.g.*, including monospecific, bispecific, and the like), camelized antibodies, Fab fragments, F(ab') fragments, disulfide-linked Fvs (sdFv), anti-idiotypic (anti-Id) antibodies, and epitope-binding fragments of any of the above.

[0089] In some embodiments, antibodies described herein include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, such as molecules that contain one or more antigen binding sites that bind to 5T4.

[0090] A 5T4 antibody, as described herein, can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA or IgY), any class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2), or any subclass (*e.g.*, IgG2a or IgG2b) of immunoglobulin molecule. In some embodiments, a 5T4 antibody, as described herein, is an IgG antibody (*e.g.*, human IgG), or a class (*e.g.*, human IgG1, IgG2, IgG3, or IgG4) or a subclass thereof. In some embodiments, a 5T4 antibody is an IgG1 antibody. Additionally or alternatively, a 5T4 antibody is a kappa antibody.

[0091] In some embodiments, a 5T4 antibody is a 4-chain antibody unit comprising two heavy (H) chain / light (L) chain pairs, wherein the amino acid sequences of the H chains are identical and the amino acid sequences of the L chains are identical. In some embodiments, the H and/or L chains comprise constant regions, for example, human constant regions. In some embodiments, the L chain constant region of a 5T4 antibody is a kappa or lambda light chain constant region, for example, a human kappa or lambda light chain constant region. In some embodiments, the L chain constant region of a 5T4 antibody is a kappa light chain constant region, for example, a human kappa light chain constant region. Additionally or alternatively, in some embodiments, the H chain constant region of a 5T4 antibody comprises

a gamma heavy chain constant region, for example, a human gamma heavy chain constant region. In some embodiments, a 5T4 antibody comprises an IgG constant region, for example, human IgG constant regions (*e.g.,* IgG1, IgG2, IgG3, and/or IgG4 constant regions). In some embodiments, a 5T4 antibody comprises an IgG1 constant region. In some embodiments, a 5T4 antibody comprises an IgG1 heavy chain constant region and a kappa light chain constant region.

[0092] As used herein, "5T4 antibody" and "antibody that binds to 5T4" are used interchangeably and refer to an antibody that preferentially binds to 5T4. An antibody or fragment thereof can preferentially bind to 5T4, such as human 5T4, which means that the antibody or fragment thereof binds to 5T4, such as human 5T4, with greater affinity than it binds to an unrelated control protein. For example, the antibody or fragment thereof can specifically recognize and bind to 5T4 or a portion thereof. "Specific binding" means that the 5T4 antibody or fragment thereof binds to 5T4 with an affinity that is at least 5, 10, 15, 20, 25, 50, 100, 250, 500, 1000, or 10,000 times greater than the affinity for an unrelated control protein (*e.g.*, hen egg white lysozyme). In some embodiments, the 5T4 antibody or fragment thereof can bind 5T4 substantially exclusively (*e.g.*, is able to distinguish 5T4 from other known polypeptides, for example, by virtue of measurable differences in binding affinity). In some embodiments, a 5T4 antibody can react with 5T4 sequences other than human 5T4 sequences (*e.g.,* cynomolgus 5T4 sequences).

[0093] The terms "hypervariable region," "HVR," "HV," "complementarity determining region," and "CDR" when used herein refer to the regions of an antibody variable region that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions: three in the VH (H1 or VH CDR1 or HCDR1, H2 or VH CDR2 or HCDR2, and H3 or VH CDR3 or HCDR3), and three in the VL (L1 or VL CDR1 or LCDR1, L2 or VL CDR2 or LCDR2, and L3 or VL CDR3 or LCDR3). Several hypervariable region delineations are in use and are encompassed herein. The Kabat CDRs are based on sequence variability and are the most used (see, *e.g.,* Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Chothia refers instead to the location of the structural loops (see, *e.g.,* Chothia and Lesk, J. Mol. Biol. 196:901-917 (1987)). The end of the Chothia CDR-H1 loop when numbered using the Kabat numbering convention varies between H32 and H34 depending on the length of the loop (this is because the Kabat numbering scheme places the insertions at H35A and H35B; if neither 35A nor 35B is present, the loop ends at 32; if only 35A is present, the loop ends at 33; if both 35A and 35B are present, the loop ends at 34).

The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops and are used by Oxford Molecular's AbM antibody modeling software (see, *e.g.,* Martin, in Antibody Engineering, Vol. 2, Chapter 3, Springer Verlag). The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these hypervariable regions or CDRs are noted below.

[0094] A universal numbering system has been developed and widely adopted, ImMunoGeneTics (IMGT®) Information System (Lefranc et al., Dev. Comp. Immunol. 27(1):55-77 (2003)). IMGT® is an integrated information system specializing in immunoglobulins (IG), T cell receptors (TR) and major histocompatibility complex (MHC) of human and other vertebrates. Herein, the CDRs are referred to in terms of both the amino acid sequence and the location within the light or heavy chain. As the "location" of the CDRs within the structure of the immunoglobulin variable region is conserved between species and present in structures called loops, by using numbering systems that align variable region sequences of structural features, CDR and framework residues are readily identified. This information can be used in grafting and replacement of CDR residues from immunoglobulins of one species into an acceptor framework from, typically, a human antibody. An additional numbering system (AHon) has been developed by Honegger and Plückthun, *J. Mol. Biol.* 309: 657-670 (2001). Correspondence between the numbering system, including, for example, the Kabat numbering and the IMGT® unique numbering system, is well known to one skilled in the art (see, *e.g.,* Kabat, *supra*; Chothia and Lesk, *supra*; Martin, *supra*; Lefranc et al., *supra*) and is also illustrated below. Various systems known in the art or described herein represent different ways of delineating CDRs, and when they are used to define the same antibody, they are often considered equivalent. An exemplary system, shown herein, combines Kabat and Chothia.

	Exemplary	**IMGT®**	**Kabat**	**AbM**	**Chothia**	**Contact**
V_H CDR1	26-35	27-38	31-35	26-35	26-32	30-35
V_H CDR2	50-65	56-65	50-65	50-58	53-55	47-58
V_H CDR3	95-102	105-117	95-102	95-102	96-101	93-101
V_L CDR1	24-34	27-38	24-34	24-34	26-32	30-36
V_L CDR2	50-56	56-65	50-56	50-56	50-52	46-55
V_L CDR3	89-97	105-117	89-97	89-97	91-96	89-96

[0095] Hypervariable regions can comprise "extended hypervariable regions" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the VL and 26-35 or 26-35A (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the VH. As used

herein, the terms "hypervariable region," "HVR," "HV," "complementarity determining region," or "CDR" are used interchangeably.

[0096] As used herein, "chemotherapeutic" refers to drugs that are cytotoxic drugs that can destroy or inhibit the growth and division of malignant cells in the treatment of cancer.

[0097] As used herein, "PD-L1 inhibitor," "PD-1 inhibitor," "PD-1/PD-L1 inhibitor," and "PD-L1/PD-1 inhibitor" are used interchangeably and refer to a group of checkpoint inhibitor anticancer drugs that block the activity of PD-1 and PD-L1 immune checkpoint proteins present on the surface of cells. Non-limiting examples of PD-L1/PD-1 inhibitors include nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab, cemiplimab, dostarlimab, and retifanlimab. See, for example US8354509B2 which is incorporated herein by reference in its entirety.

[0098] Pembrolizumab is a humanized monoclonal immunoglobulin G4 (IgG4) kappa antibody specific for PD-1 cell surface membrane receptor that has demonstrated the ability to generate increased antitumor immune responses and improvements in survival of cancer patients. In some embodiments, Pembrolizumab comprises or consists of two heavy chains and two light chains. In further embodiments, each of the heavy chains of Pembrolizumab comprises or consists of an amino acid sequence as set forth below: QVQLVQSGVEVKKPGASVKVSCKASGYTFTNYYMYWVRQAPGQGLEWMGGINPSNGGTNFNE KFKNRVTLTTDSSTTTAYMELKSLQFDDTAVYYCARRDYRFDMGFDYWGQGTTVTVSSASTK GPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLS SVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLH QDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHN HYTQKSLSLSLGK (SEQ ID NO: 9). In yet further embodiments, each of the light chains of Pembrolizumab comprises or consists of an amino acid sequence as set forth below: EIVLTQSPATLSLSPGERATLSCRASKGVSTSGYSYLHWYQQKPGQAPRLLIYLASYLESGV PARFSGSGSGTDFTLTISSLEPEDFAVYYCQHSRDLPLTFGGGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLS KADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 10). In some embodiments, Pembrolizumab is described in any one or more of the following: KEGG Entry: D10574, CAS: 1374853-91-4, or PubChem: 254741536, PubChem 377483934, and DrugBank Accession Number: DB09037, each of which is incorporated herein by reference in its entirety.

[0099] As used herein, the term "cytopenia" means a deficiency in the number of blood cells and/or a deficiency in the number of a given type of blood cell. Cytopenia includes anemia, leukopenia, neutropenia, thrombocytopenia, and pancytopenia.

[00100] As used herein, the term "neutropenia" means a deficiency in the number of circulating neutrophils, for example, an absolute neutrophil count (ANC) less than 1,500 cells/µl (Grades 2 or higher). As used herein, the following grades of neutropenia are: Grade 0: ANC ≥ 2,000 cells/µL; Grade 1: ANC ≥ 1,500 cells/µL to < 2,000 cells/µL; Grade 2: ANC ≥ 1,000 cells/µL to < 1,500 cells/µL; Grade 3: ANC ≥ 500 cells/µL to < 1,000/µL; Grade 4: ANC < 500 cells/µL. *See*, *e.g.*, Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, November 2017, National Institutes of Health, National Cancer Institute, incorporated herein by reference in its entirety. It will be understood by those skilled in the art that Grade 0 neutropenia indicates the absence of neutropenia, that is, where there is no deficiency in the number of circulating neutrophils.

[00101] As used herein, the term "thrombocytopenia" means a deficiency in the number of circulating platelets, for example, a platelet count of less than 100,000 cells/µL. As used herein, the following grades of thrombocytopenia are: Grade 0: platelet count ≥ 150,000 cells/µL; Grade 1: platelet count ≥ 75,000 cells/µL to < 150,000 cells/µL; Grade 2: platelet count ≥ 50,000 cells/µL to < 75,000 cells/µL; Grade 3: platelet count ≥ 25,000 cells/µL to < 50,000/µL; Grade 4: platelet count < 25,000 cells/µL. *See*, *e.g.*, Common Terminology Criteria for Adverse Events (CTCAE), Version 5.0, November 2017, National Institutes of Health, National Cancer Institute. Grade 1 thrombocytopenia may also be categorized as Grade 1A: platelet count ≥ 100,000 cells/µL to < 150,000 cells/µL; and Grade 1B: platelet count ≥ 75,000 cells/µL to < 100,000 cells/µL. It will be understood by those skilled in the art that Grade 0 thrombocytopenia indicates the absence of thrombocytopenia, that is, where there is no deficiency in the number of circulating platelets.

[00102] As used herein, the term "absolute neutrophil count (ANC)" means the total number of neutrophils in the white blood cell count. Those skilled in the art will know how to calculate ANC. As an example, ANC may be calculated as follows: ANC = WBC (cells/µL) x [percent (PMNs + bands) ÷ 100]; where "WBC" is the white blood cell count, "PMNs" is the percent of neutrophils, and "bands" are young neutrophils.

[00103] The disclosure can be understood more fully by reference to the following detailed description and illustrative examples, which are intended to exemplify non-limiting embodiments.

7.2 5T4 ANTIBODIES

[00104] 5T4 ADCs described herein comprise a drug and 5T4 antibody conjugated thereto. In some embodiments, a 5T4 antibody refers to an antibody, specifically binding to 5T4, such as a 5T4 protein, a 5T4 polypeptide, a 5T4 polypeptide fragment, a 5T4 peptide, or a 5T4 epitope. In some embodiments, the 5T4 antibody is a human or humanized antibody (*e.g.*, comprising human constant regions) that binds to 5T4. In some embodiments, a 5T4 antibody can bind to 5T4 which is expressed on the surface of a mammalian (*e.g.*, human) cell, including a 5T4-expressing tumor cell. In some embodiments, a 5T4 antibody binds a 5T4 extracellular epitope expressed on a cell such as a tumor cell (*e.g.*, an extracellular 5T4 epitope). In some embodiments, 5T4 is a human 5T4. An exemplary amino acid sequence of human 5T4 is described herein.

[00105] In some embodiments, the 5T4 antibody competes for binding to 5T4 with a reference 5T4 antibody that comprises a VH region, VL region, HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and/or LCDR3 of any one of the antibodies described herein, such as an amino acid sequence of a VH region, VL region, HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and/or LCDR3 as described in **Table 1**. Accordingly, in some embodiments, the 5T4 antibody competes for binding to 5T4 with a reference 5T4 antibody that comprises one, two, and/or three VH CDRs and/or one, two, and/or three VL CDRs of the antibody designated as **mAbA15**. In some embodiments, a 5T4 ADC comprises a drug conjugated (directly or indirectly) to a 5T4 antibody that competes for binding to 5T4 with a reference 5T4 antibody that comprises one, two, and/or three VH CDRs and one, two, and/or three VL CDRs from the antibody designated as **mAbA15**. In some embodiments, the 5T4 antibody competes for binding to 5T4 with a reference 5T4 antibody that comprises a VH region and VL region of the antibody designated as **mAbA15**. In some embodiments, the 5T4 antibody competes for binding to 5T4 with a reference 5T4 antibody that comprises: a VH region comprising the amino acid sequence of SEQ ID NO: 11 and a VL region comprising the amino acid sequence of SEQ ID NO: 12.

[00106] In some embodiments, the 5T4 antibody comprises a VH region, VL region, HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and/or LCDR3 of any one of the antibodies described herein, such as an amino acid sequence of a VH region, VL region, HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and/or LCDR3 as described in **Table 1**. Accordingly, in some embodiments, the 5T4 antibody comprises one, two, and/or three heavy chain CDRs and/or one, two, and/or three light chain CDRs from the antibody designated as **mAbA15**. In

some embodiments, the 5T4 antibody comprises one, two, and/or three heavy chain CDRs and one, two, and/or three light chain CDRs from the antibody designated as **mAbA15**.

[00107] In some embodiments, the 5T4 antibody comprises a VH region, which comprises one or more (such as one, two, or three) of VH CDR1, VH CDR2, and VH CDR3 as described herein, such as in **Table 1**; and/or a VL region, which comprises one or more (such as one, two, or three) of VL CDR1, VL CDR2, and VL CDR3 as described herein, such as in **Table 1**.

[00108] The antibody designated as **mAbA15** comprises a VH comprising an amino acid sequence of SEQ ID NO: 11 and a VL comprising an amino acid sequence of SEQ ID NO: 12. In further embodiments, the antibody designated as **mAbA15** comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 2 and a light chain comprising an amino acid sequence of SEQ ID NO: 3.

Table 1: Antibody mAbA15

		Exemplary	IMGT®	Kabat	Chothia	Contact	AbM
VH CDR Seq.	HCDR1	GFTFSSYYIH (SEQ ID NO: 13)	GFTFSSYY (SEQ ID NO: 19)	SYYIH (SEQ ID NO: 24)	GFTFSSY (SEQ ID NO: 25)	SSYYIH (SEQ ID NO: 30)	GFTFSSYYIH (SEQ ID NO: 13)
	HCDR2	YITSTGSYTEYAD SVKG (SEQ ID NO: 14)	ITSTGSYT (SEQ ID NO: 20)	YITSTGSYTEYAD SVKG (SEQ ID NO: 14)	STGS (SEQ ID NO: 26)	WVAYITSTGSYTE (SEQ ID NO: 31)	YITSTGSYTE (SEQ ID NO: 36)
	HCDR3	YDRSTRYSGLDY (SEQ ID NO: 15)	ARYDRSTRYSGLDY (SEQ ID NO: 21)	YDRSTRYSGLDY (SEQ ID NO: 15)	DRSTRYSGLD (SEQ ID NO: 27)	ARYDRSTRYSGLD (SEQ ID NO: 32)	YDRSTRYSGLDY (SEQ ID NO: 15)
VL CDR Seq.	LCDR1	RASQSVSSAVA (SEQ ID NO: 16)	QSVSSA (SEQ ID NO: 22)	RASQSVSSAVA (SEQ ID NO: 16)	SQSVSSA (SEQ ID NO: 28)	SSAVAWY (SEQ ID NO: 33)	RASQSVSSAVA (SEQ ID NO: 16)
	LCDR2	SASSLYS (SEQ ID NO: 17)	SAS (SEQ ID NO: 23)	SASSLYS (SEQ ID NO: 17)	SAS (SEQ ID NO: 23)	LLIYSASSLY (SEQ ID NO: 34)	SASSLYS (SEQ ID NO: 17)
	LCDR3	QQAYATPVT (SEQ ID NO: 18)	QQAYATPVT (SEQ ID NO: 18)	QQAYATPVT (SEQ ID NO: 18)	AYATPV (SEQ ID NO: 29)	QQAYATPV (SEQ ID NO: 35)	QQAYATPVT (SEQ ID NO: 18)
VH Amino Acid Sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVKGRFTISADTSKNTAYLQMNSLR AEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSS (SEQ ID NO: 11)							
VL Amino Acid Sequence: DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ AYATPVTFGQGTKVEIK (SEQ ID NO: 12)							

[00109] In some embodiments, the 5T4 antibody comprises a VH region. In some embodiments, the 5T4 antibody comprises a VL region. In some embodiments, the 5T4 antibody has a combination of (i) a VH region; and (ii) a VL region.

[00110] In some embodiments, the 5T4 antibody comprises a heavy chain having a combination of (i) a VH as described herein, such as in **Table 1**, and (ii) one or more heavy chain constant regions (*e.g.*, CH1, hinge, CH2, and CH3). An exemplary IgG heavy chain can comprise any VH amino acid sequence as described herein and the following CH1, hinge, CH2, and CH3 amino acid sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK (SEQ ID NO: 37).

Another exemplary IgG heavy chain can comprise any VH amino acid sequence as described herein and the following CH1, hinge, CH2, and CH3 amino acid sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD
WLNGKEYKCKVSNKALKAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDI
AVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK (SEQ ID NO: 38).

[00111] In some embodiments, a 5T4 antibody comprises a light chain having a combination of (i) a VL region as described herein, such as in **Table 1**; and (ii) a light chain constant region (CL). An exemplary light chain (*e.g.*, for pairing with an IgG heavy chain) can comprise any VL amino acid sequence described herein and the following CL amino acid sequence:

RTVAAPSVFIFPPSDSQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 39).

[00112] In some embodiments, the 5T4 antibody comprises (a) a heavy chain having a combination of (i) a VH as described herein, such as in **Table 1**, and (ii) one or more heavy chain constant regions (*e.g.*, CH1, hinge, CH2, and CH3); and (b) a light chain having a combination of (i) a VL as described herein, such as in **Table 1**, and (ii) a light chain constant region in an IgG format (CL).

[00113] In some embodiments, an antibody that binds to 5T4 comprises a heavy chain having an amino acid sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSLCTP SRGS (SEQ ID NO: 40), and a light chain having an amino acid sequence of DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3).

[00114] In some embodiments, the antibody that binds to 5T4 comprises a heavy chain which has been modified to include a formyl glycine residue and therefore have the amino acid sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(*fGly*)TPSRGS (SEQ ID NO: 41), wherein *f(Gly)* is the formylglycine residue. In further embodiments, the antibody that binds to 5T4 further comprises a light chain having an amino acid sequence of DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3).

[00115] In some embodiments, a 5T4 ADC comprises a heavy chain having an amino acid sequence of EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK

GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL
(*fGly'*)TPSRGS (SEQ ID NO: 2), wherein (*fGly'*) is an amino acid (formylglycine) conjugated to (such as by having its side chain replaced by) a linker-drug as disclosed herein. In further embodiments, the antibody that binds to 5T4 further comprises a light chain having an amino acid sequence of
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3).

[00116] As described herein, to produce the conjugate, the polypeptide containing the *fGly* residue may be conjugated to a drug or active agent by reaction of the *fGly* with a reactive moiety (*e.g.*, hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl coupling moiety, as described herein) of a linker attached to the drug or active agent to produce an *fGly'*-containing sulfatase motif. As used herein, the term *fGly'* refers to the amino acid residue of the sulfatase motif that is coupled to the drug or active agent through a linker as described herein.

[00117] In some embodiments, a 5T4 ADC comprises a heavy chain having an amino acid sequence of
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL*n*TP
SRGS (SEQ ID NO: 42), wherein *n* is an amino acid (formylglycine) conjugated to (such as by having its side chain replaced by) a linker-drug as disclosed herein. In some embodiments, the amino acid *n* refers to *fGly'*. In further embodiments, the antibody that binds to 5T4 further comprises a light chain having an amino acid sequence of
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS

RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3).

[00118] In some embodiments, a 5T4 antibody comprises one or more CDRs (*e.g.*, one, two, three, four, five, or six CDRs), for example, an HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and/or LCDR3 as described in **Table 1**.

[00119] In some embodiments, a 5T4 antibody comprises one or more CDRs (*e.g.*, one, two, or three VH CDRs), for example, an HCDR1, HCDR2, HCDR3, as described in **Table 1**. In some embodiments, a 5T4 antibody comprises one or more CDRs (*e.g.*, one, two, or three VL CDRs), for example, an LCDR1, LCDR2, and/or LCDR3, as described in **Table 1**. In some embodiments, a 5T4 antibody comprises one or more CDRs (*e.g.*, one, two, or three VH CDRs), for example, an HCDR1, HCDR2, HCDR3, as described in **Table 1,** and one or more CDRs (*e.g.*, one, two, or three VL CDRs), for example, an LCDR1, LCDR2, and/or LCDR3, as described in **Table 1**.

[00120] In some embodiments, a 5T4 antibody comprises a heavy chain variable region (VH) comprising: (1) an HCDR1 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 13, (ii) SEQ ID NO: 19, (iii) SEQ ID NO: 24, (iv) SEQ ID NO: 25, and (v) SEQ ID NO: 30; (2) an HCDR2 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 14, (ii) SEQ ID NO: 20, (iii) SEQ ID NO: 26, (iv) SEQ ID NO: 31, and (v) SEQ ID NO: 36; and (3) an HCDR3 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 15, (ii) SEQ ID NO: 21, (iii) SEQ ID NO: 27, and (iv) SEQ ID NO: 32; and/or a VL region comprising: (1) a VCDR1 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 16, (ii) SEQ ID NO: 22, (iii) SEQ ID NO: 28, and (iv) SEQ ID NO: 33; (2) an LCDR2 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 17, (ii) SEQ ID NO: 23, and (iii) SEQ ID NO: 34; and (3) an LCDR3 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 18, (ii) SEQ ID NO: 29, and (iii) SEQ ID NO: 35.

[00121] In some embodiments, a 5T4 antibody comprises a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 13, (ii) SEQ ID NO: 19, (iii) SEQ ID NO: 24, (iv) SEQ ID NO: 25, and (v) SEQ ID NO: 30; (2) an HCDR2 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 14, (ii) SEQ ID NO: 20, (iii) SEQ ID NO: 26, (iv) SEQ ID NO: 31, and (v) SEQ ID NO: 36; and (3) an HCDR3 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 15, (ii) SEQ ID NO: 21, (iii) SEQ ID NO: 27, and (iv) SEQ ID NO: 32.

[00122] In some embodiments, a 5T4 antibody comprises a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 16, (ii) SEQ ID NO: 22, (iii) SEQ ID NO: 28, and (iv) SEQ ID NO: 33; (2) an LCDR2 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 17, (ii) SEQ ID NO: 23, and (iii) SEQ ID NO: 34; and (3) an LCDR3 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 18, (ii) SEQ ID NO: 29, and (iii) SEQ ID NO: 35.

[00123] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises all three heavy chain CDRs and/or all three light chain CDRs from the antibody designated as **mAbA15** that comprises a VH amino acid sequence of SEQ ID NO: 11 and a VL amino acid sequence of SEQ ID NO: 12. In some embodiments, a 5T4 ADC comprises a 5T4 antibody, wherein the 5T4 antibody comprises all three heavy chain CDRs and/or all three light chain CDRs from the antibody designated as **mAbA15**.

[00124] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises: (a) a VH region comprising an HCDR1, an HCDR2, and an HCDR3 amino acid sequence as described herein, such as in **Table 1**; and/or (b) a VL region comprising an LCDR1, an LCDR2, and an LCDR3 amino acid sequence as described herein, such as in **Table 1**. In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4 wherein the antibody comprises: a VH region comprising an HCDR1, an HCDR2, and an HCDR3 amino acid sequence as described herein, such as in **Table 1**. In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises a VL region comprising an LCDR1, an LCDR2, and an LCDR3 amino acid sequence as described herein, such as in **Table 1**.

[00125] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises a VH region and/or VL region described herein, and wherein the VH and/or VL comprises human framework sequences. In some embodiments, the VH region and/or VL region comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and/or a framework 4 (FR4) sequence, such as a human FR1, a human FR2, a human FR3 and/or a human FR4.

[00126] In some embodiments, CDRs of a 5T4 antibody can be determined by the Kabat system (Kabat et al. (1971) Ann. NY Acad. Sci. 190:382-391 and, Kabat et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242).

[00127] In some embodiments, CDRs of a 5T4 antibody can be determined by the Chothia system, which will be referred to herein as the "Chothia CDRs" (see, *e.g.*, Chothia and Lesk,

1987, J. Mol. Biol., 196:901-917; Al-Lazikani et al., 1997, J. Mol. Biol., 273:927-948; Chothia et al., 1992, J. Mol. Biol., 227:799-817; Tramontano A. et al., 1990, J. Mol. Biol. 215(1):175-82; and U.S. Patent No. 7,709,226).

[00128] In some embodiments, CDRs of a 5T4 antibody can be determined by the ImMunoGeneTics (IMGT®) system, for example, as described in Lefranc, M.-P., 1999, The Immunologist, 7:132-136 and Lefranc, M.-P. et al., 1999, Nucleic Acids Res., 27:209-212 ("IMGT® CDRs").

[00129] In some embodiments, CDRs of a 5T4 antibody can be determined by the AbM system, which will be referred to herein as the "AbM CDRs," for example as described in MacCallum et al., 1996, J. Mol. Biol., 262:732-745. See also, *e.g.*, Martin, A., "Protein Sequence and Structure Analysis of Antibody Variable Domains," in Antibody Engineering, Kontermann and Dübel, eds., Chapter 31, pp. 422-439, Springer-Verlag, Berlin (2001).

[00130] In some embodiments, CDRs of a 5T4 antibody can be determined by the Contact system, which will be referred to herein as the "Contact CDRs" (*see, e.g.*, MacCallum RM et al., 1996, J Mol Biol 5: 732-745). The Contact CDRs are based on an analysis of the available complex crystal structures.

[00131] In some embodiments, the position of one or more (*e.g.*, one, two, three) CDRs along the VH (*e.g.,* CDR1, CDR2, or CDR3) and/or VL (*e.g.,* CDR1, CDR2, or CDR3) region of a 5T4 antibody can vary by one, two, three, four, five, or six amino acid positions provided that binding to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.,* substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). For example, in some embodiments, the position defining a CDR of **Table 1** can vary by shifting the N-terminal and/or C-terminal boundary of the CDR by one, two, three, four, five, or six amino acids, relative to the current CDR position, provided that binding to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.,* substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). Additionally or alternatively, in some embodiments, the length of one or more (*e.g.*, one, two, three) CDRs along the VH (*e.g.,* CDR1, CDR2, or CDR3) and/or VL (*e.g.,* CDR1, CDR2, or CDR3) region of a 5T4 antibody (*e.g.*, a human 5T4 antibody) described herein can vary (*e.g.,* be shorter or longer) by one, two, three, four, five, or more amino acids, provided that binding to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.,* substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). For example, in some embodiments, a VH and/or VL CDR1, CDR2, and/or CDR3 can be one, two, three, four, five or more amino acids shorter than one or more of the CDRs described by SEQ ID NOs:13-36, provided that binding to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.,* substantially

maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). In other embodiments, a VH and/or VL CDR1, CDR2, and/or CDR3 can be one, two, three, four, five or more amino acids longer than one or more of the CDRs described by SEQ ID NOs: 13-36, provided that binding to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). Additionally or alternatively, in some embodiments, the amino terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 can be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs described by SEQ ID NOs: 13-36, provided that binding to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.,* substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). Additionally or alternatively, in some embodiments, the carboxy terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 can be extended by one, two, three, four, five or more amino acids compared to one or more of the CDRs described by SEQ ID NOs: 13-36, provided that binding to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). Additionally or alternatively, in some embodiments, the amino terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 can be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described by SEQ ID NOs: 13-36, provided that binding to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.,* substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). In some embodiments, the carboxy terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 can be shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described by SEQ ID NOs: 13-36, provided that binding to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.,* substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). Any method known in the art can be used to ascertain whether binding to 5T4 (*e.g.,* human 5T4) is maintained, such as those disclosed in WO2023220620 and WO2023220626, each of which is enclosed herein by reference in its entirety.

7.3 ANTIBODY DRUG CONJUGATES AND CONJUGATION METHODS

[00132] In some embodiments, the antibody can be modified before conjugation to the linker-drug. Modification of the antibody can produce a modified antibody that contains one or more reactive groups suitable for conjugation to the linker-drug.

[00133] In some embodiments, the antibody can be modified at one or more amino acid residues to provide one or more reactive groups suitable for conjugation to the linker-drug. For

example, carbonyl groups introduced into a polypeptide can be selectively reacted with α-nucleophiles, such as aminooxy- and hydrazide-bearing compounds. Chemistries selective for carbonyl functional groups on a protein with enhanced kinetics, site selectivity and conjugate stability can result in improved bioconjugates.

[00134] In some embodiments, the antibody can be modified to include a reactive aldehyde group (*e.g.*, a reactive aldehyde). A reactive aldehyde can be included in an "aldehyde tag" or "ald-tag," which is meant to refer to an amino acid sequence derived from a sulfatase motif that has been converted by action of a formylglycine generating enzyme (FGE) to contain a 2-formylglycine residue (referred to herein as "*fGly*"). The *fGly* residue generated by an FGE is also referred to in the literature as a "formylglycine." Stated differently, the term "aldehyde tag" is used herein to refer to an amino acid sequence comprising a "converted" sulfatase motif (*e.g.*, a sulfatase motif in which the cysteine or the serine residue has been converted to *fGly* by action of an FGE, *e.g.*, L(*fGly*)TPSR (SEQ ID NO: 43)). A converted sulfatase motif can be derived from an amino acid sequence comprising an "unconverted" sulfatase motif (*e.g.*, a sulfatase motif in which the cysteine or serine residues has not been converted to *fGly* by an FGE, but is capable of being converted, *e.g.*, an unconverted sulfatase motif with the sequence: L(C/S)TPSR (SEQ ID NO: 44)). "Conversion," used in the context of action of a formylglycine generating enzyme (FGE) on a sulfatase motif, refers to biochemical modification of a cysteine or serine residue in a sulfatase motif to a formylglycine (*fGly*) residue (*e.g.*, Cys to *fGly*, or Ser to *fGly*). As used herein, an aldehyde-tagged antibody refers to an antibody where a cysteine or serine residue has been modified to form a formylglycine residue. Additional aspects of aldehyde tags and uses thereof in site-specific protein modification are described in U.S. 7,985,783, U.S. 8,729,232, WO2008036350, and WO2009120611, the disclosures of which are each incorporated herein by reference. Accordingly, the *fGly* residue conjugated to the linker or linker-drug as disclosed herein is referred to herein as *fGly'*.

[00135] Conversion of a polypeptide to include *fGly* can be accomplished by cell-based (*in vivo*) or cell-free methods (*in vitro*). Similarly, modification of a polypeptide to produce a polypeptide suitable for conjugation (*e.g.*, modification to produce a polypeptide containing a reactive group suitable for conjugation) can be accomplished by cell-based (*in vivo*) or cell-free methods (*in vitro*).

[00136] The amino acid sequence of an antibody can be modified to include a sulfatase motif that contains a serine or cysteine residue that is capable of being converted (oxidized) to an *fGly* residue by action of an FGE either *in vivo* (*e.g.*, at the time of translation of an aldehyde tag-containing protein in a cell) or *in vitro* (*e.g.*, by contacting an aldehyde tag-containing protein

with an FGE in a cell-free system). Such sulfatase motifs may also be referred to herein as an FGE-modification site. A 5T4 antibody used to generate a 5T4 ADC includes at least an Ig constant region, *e.g.*, an Ig heavy chain constant region (*e.g.*, at least a CH1 domain; at least a CH1 and a CH2 domain; a CH1, a CH2, and a CH3 domain; or a CH1, a CH2, a CH3, and a CH4 domain), or an Ig light chain constant region.

[00137] A sulfatase motif can be introduced into any convenient site in the antibody. As noted herein, in some embodiments, the extent of modification of the native amino acid sequence of the target polypeptide is minimized, to minimize the number of amino acid residues that are inserted, deleted, substituted (replaced), and/or added (*e.g.*, to the N- or C-terminus). Minimizing the extent of amino acid sequence modification of the target antibody can minimize the impact such modifications can have upon antibody function and/or structure.

[00138] In some embodiments, an aldehyde-tagged antibody comprises an aldehyde-tagged Ig heavy chain constant region (*e.g.*, at least a CH1 domain; at least a CH1 and a CH2 domain; a CH1, a CH2, and a CH3 domain; or a CH1, a CH2, a CH3, and a CH4 domain). The aldehyde-tagged Ig heavy chain constant region can include heavy chain constant region sequences of an IgA, IgM, IgD, IgE, IgG1, IgG2, IgG3, or IgG4 isotype heavy chain or any allotypic variant of same, *e.g.*, human heavy chain constant region sequences or mouse heavy chain constant region sequences, a hybrid heavy chain constant region, a synthetic heavy chain constant region, or a consensus heavy chain constant region sequence, and the like, modified to include at least one sulfatase motif that can be modified by an FGE to generate an *fGly*-modified Ig polypeptide. See, *e.g.*, WO2012097333 and WO2018169953, each of which is incorporated herein by reference in its entirety. Allotypic variants of Ig heavy chains are known in the art. See, *e.g.*, Jefferis and Lefranc (2009) *MAbs* 1:4.

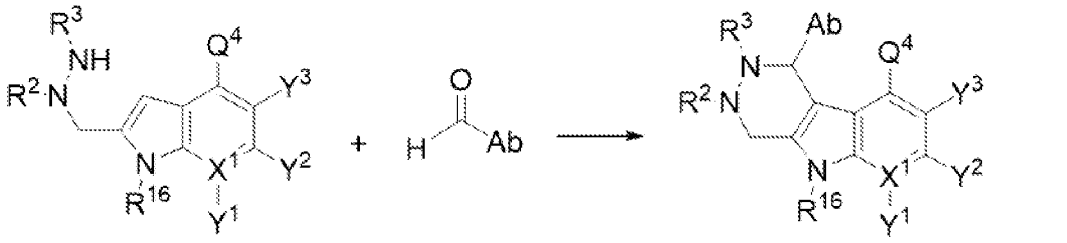
[00139] In some embodiments, an aldehyde-tagged antibody comprises an aldehyde-tagged Ig light chain constant region. The aldehyde-tagged Ig light chain constant region can include constant region sequences of a kappa light chain, a lambda light chain, *e.g.*, kappa or lambda light chain constant regions, a hybrid light chain constant region, a synthetic light chain constant region, or a consensus light chain constant region sequence, and the like, modified to include at least one sulfatase motif that can be modified by an FGE to generate an *fGly*-modified antibody. See, *e.g.*, WO2012097333 and WO2017189432, each of which is incorporated herein by reference in its entirety.

[00140] Alternatively, isolated, unmodified polypeptides can be isolated following recombinant production in a host cell lacking a suitable enzyme or by synthetic production. The isolated polypeptide can then be contacted with a suitable enzyme under conditions to provide for

the desired modification of the polypeptide to include *fGly*. The polypeptide can be unfolded by methods known in the art (*e.g.*, using heat, adjustment of pH, chaotropic agents, (*e.g.*, urea, and the like), organic solvents (*e.g.*, hydrocarbons: octane, benzene, chloroform), and the like) and the denatured protein contacted with a suitable enzyme. The modified polypeptide can then be refolded under suitable conditions.

[00141] In some embodiments, the modified antibody containing the *fGly* residue can be conjugated to a linker-drug by reaction of the *fGly* with a coupling moiety on the linker-drug, such as a hydrazinyl-pyrrolo coupling moiety as described herein. For example, an *fGly*-containing antibody can be isolated from a production source (*e.g.*, recombinant host cell production, synthetic production), and contacted with a reactive partner-containing drug or other moiety (*e.g.*, detectable label) under conditions suitable to provide for conjugation of the drug or other moiety to the 5T4 antibody. For example, the reactive partner-containing drug or other moiety can include a conjugating moiety (*e.g.*, a hydrazinyl-pyrrolo coupling moiety as described herein). The hydrazinyl-pyrrolo-containing drug can be reacted with the antibody to produce a 5T4 ADC, as described herein. See, *e.g.*, WO2014078566 and WO2015081282, each of which is incorporated herein by reference in its entirety.

[00142] A general scheme for coupling an antibody to a pyridazine-pyrrolo coupling moiety is shown below.



azaHIPS antibody with *fGly* residue pyridazine-pyrrolo coupling moiety

[00143] A hydrazinyl-pyrrolo coupling moiety, which can interchangeably be referred to herein as an aza-hydrazino-*iso*-Pictet-Spengler (azaHIPS) coupling moiety, upon conjugation to a formyl-glycine, forms a pyridazine-pyrrolo coupling moiety, such as that shown above. A 5T4 antibody can include a 2-formylglycine residue (*fGly*) that is reacted with azaHIPS coupling moiety, thus conjugating the two together. To generate a 5T4 ADC, a drug can be coupled directly or indirectly (*e.g.*, through a linker moiety) to the azaHIPS moiety at any location of the azaHIPs moiety (*e.g.*, R^{16}, Y^1, Y^2, Y^3, or Q^4). R^2 and R^3 can each independently be any desired substituent, such as, but not limited to, hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino,

carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl.

[00144] A minimal sulfatase motif of an aldehyde tag is usually 5 or 6 amino acid residues in length, usually no more than 6 amino acid residues in length. Sulfatase motifs provided in an Ig polypeptide are at least 5 or 6 amino acid residues, and can be, for example, from 5 to 16, 6-16, 5-15, 6-15, 5-14, 6-14, 5-13, 6-13, 5-12, 6-12, 5-11, 6-11, 5-10, 6-10, 5-9, 6-9, 5-8, or 6-8 amino acid residues in length, so as to define a sulfatase motif of less than 16, 15, 14, 13, 12, 11, 10, 9, 8, 7 or 6 amino acid residues in length.

[00145] In certain embodiments, 5T4 antibodies of interest include those where one or more amino acid residues, such as 2 or more, or 3 or more, or 4 or more, or 5 or more, or 6 or more, or 7 or more, or 8 or more, or 9 or more, or 10 or more, or 11 or more, or 12 or more, or 13 or more, or 14 or more, or 15 or more, or 16 or more, or 17 or more, or 18 or more, or 19 or more, or 20 or more amino acid residues have been inserted, deleted, substituted (replaced) relative to the native amino acid sequence to provide for a sequence of a sulfatase motif in the 5T4 antibody. In certain embodiments, the 5T4 antibody includes a modification (insertion, addition, deletion, and/or substitution/replacement) of less than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3 or 2 amino acid residues of the amino acid sequence relative to the native amino acid sequence of the 5T4 antibody (such as a light chain of SEQ ID NO: 3 and/or a heavy chain comprising a VH of SEQ ID NO: 11 and a Fc region). Where an amino acid sequence native to the 5T4 antibody contains one or more residues of the desired sulfatase motif, the total number of modifications of residues can be reduced, *e.g.*, by site-specification modification (insertion, addition, deletion, substitution/replacement) of amino acid residues flanking the native amino acid residues to provide a sequence of the desired sulfatase motif. In certain embodiments, the extent of modification of the native amino acid sequence of the target antibody is minimized, so as to minimize the number of amino acid residues that are inserted, deleted, substituted (replaced), or added (*e.g.*, to the N- or C-terminus). Minimizing the extent of amino acid sequence modification of the target antibody may minimize the impact such modifications may have upon antibody function and/or structure.

[00146] It should be noted that while aldehyde tags of particular interest are those comprising at least a minimal sulfatase motif (also referred to a "consensus sulfatase motif"), it will be readily appreciated that longer aldehyde tags are both contemplated and encompassed by the present disclosure and can find use in the compositions and methods of the present disclosure.

Aldehyde tags can thus comprise a minimal sulfatase motif of 5 or 6 residues or can be longer and comprise a minimal sulfatase motif which can be flanked at the N- and/or C-terminal sides of the motif by additional amino acid residues. Aldehyde tags of, for example, 5 or 6 amino acid residues are contemplated, as well as longer amino acid sequences of more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acid residues.

[00147] An aldehyde tag can be present at or near the C-terminus of an Ig heavy chain; *e.g.*, an aldehyde tag can be present within 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids of the C-terminus of a native, wild-type Ig heavy chain. An aldehyde tag can be present within a CH1 domain of an Ig heavy chain. An aldehyde tag can be present within a CH2 domain of an Ig heavy chain. An aldehyde tag can be present within a CH3 domain of an Ig heavy chain. An aldehyde tag can be present in an Ig light chain constant region, *e.g.*, in a kappa light chain constant region or a lambda light chain constant region.

[00148] In some embodiments, the 5T4 antibody as disclosed herein comprises a sulfatase motif.

[00149] In certain embodiments, the sulfatase motif used may be described by:

$X^1Z^1X^2Z^2X^3Z^3$ (SEQ ID NO: 68)

wherein:

Z^1 is cysteine or serine (which can also be represented by (C/S));

Z^2 is either a proline or alanine residue (which can also be represented by (P/A));

Z^3 is a basic amino acid (*e.g.*, arginine (R), and may be lysine (K) or histidine (H), *e.g.*, lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), *e.g.*, A, G, L, V, or I;

X^1 is present or absent and, when present, can be any amino acid, *e.g.*, an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (*e.g.*, other than an aromatic amino acid or a charged amino acid), *e.g.*, L, M, V, S or T, *e.g.*, L, M, S or V, with the proviso that when the sulfatase motif is at the N-terminus of the target 5T4 antibody, X^1 is present; and

X^2 and X^3 independently can be any amino acid, though usually an aliphatic amino acid, a polar, uncharged amino acid, or a sulfur containing amino acid (*e.g.*, other than an aromatic amino acid or a charged amino acid), *e.g.*, S, T, A, V, G or C, *e.g.*, S, T, A, V or G.

[00150] The amino acid sequence of an antibody heavy and/or light chain can be modified to provide a sequence of at least 5 amino acids of $X^1Z^1X^2Z^2X^3Z^3$ (SEQ ID NO: 68)

wherein:

Z^1 is cysteine or serine;

Z^2 is a proline or alanine residue;

Z^3 is an aliphatic amino acid or a basic amino acid;

X^1 is present or absent and, when present, is any amino acid, with the proviso that when the heterologous sulfatase motif is at an N-terminus of the 5T4 antibody, X^1 is present;

X^2 and X^3 are each independently any amino acid.

[00151] The sulfatase motif is generally selected so as to be capable of conversion by a selected FGE, *e.g.*, an FGE present in a host cell in which the aldehyde-tagged antibody is expressed or an FGE which is to be contacted with the aldehyde-tagged antibody in a cell-free *in vitro* method.

[00152] For example, where the FGE is a eukaryotic FGE (*e.g.*, a mammalian FGE, including a human FGE), the sulfatase motif can be of:

$X^1CX^2PX^3Z^3$ (SEQ ID NO: 69)

wherein:

X^1 may be present or absent and, when present, can be any amino acid, *e.g.*, an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (*e.g.*, other than an aromatic amino acid or a charged amino acid), *e.g.*, L, M, S or V, with the proviso that when the sulfatase motif is at the N-terminus of the target 5T4 antibody, X^1 is present;

X^2 and X^3 independently can be any amino acid, *e.g.*, an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (*e.g.*, other than an aromatic amino acid or a charged amino acid), *e.g.*, S, T, A, V, G, or C, *e.g.*, S, T, A, V or G; and Z^3 is a basic amino acid (*e.g.*, arginine (R), and may be lysine (K) or histidine (H), *e.g.*, lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), *e.g.*, A, G, L, V, or I.

[00153] Specific examples of sulfatase motifs include LCTPSR (SEQ ID NO: 70), MCTPSR (SEQ ID NO: 71), VCTPSR (SEQ ID NO: 72), LCSPSR (SEQ ID NO: 73), LCAPSR (SEQ ID NO: 74), LCVPSR (SEQ ID NO: 75), LCGPSR (SEQ ID NO: 76), ICTPAR (SEQ ID NO: 77), LCTPSK (SEQ ID NO: 78), MCTPSK (SEQ ID NO: 79), VCTPSK (SEQ ID NO: 80), LCSPSK (SEQ ID NO: 81), LCAPSK (SEQ ID NO: 82), LCVPSK (SEQ ID NO: 83), LCGPSK (SEQ ID NO: 84), LCTPSA (SEQ ID NO: 85), ICTPAA (SEQ ID NO: 86), MCTPSA (SEQ ID NO: 87), VCTPSA (SEQ ID NO: 88), LCSPSA (SEQ ID NO: 89), LCAPSA (SEQ ID NO: 90), LCVPSA (SEQ ID NO: 91), and LCGPSA (SEQ ID NO: 92).

[00154] In some embodiments, the 5T4 antibody prior to conjugating to a linker or a linker-drug as disclosed herein as disclosed herein comprises a *fGly*-containing sulfatase motif.

[00155] Upon action of FGE on the antibody heavy and/or light chain, the serine or the cysteine in the sulfatase motif is modified to *fGly*. Thus, the *fGly*-containing sulfatase motif can be o:

X^1(*fGly*)$X^2Z^2X^3Z^3$ (SEQ ID NO: 93)

wherein:

fGly is the formylglycine residue;

Z^2 is either a proline or alanine residue (which can also be represented by (P/A));

Z^3 is a basic amino acid (*e.g.*, arginine (R), and may be lysine (K) or histidine (H), usually lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), *e.g.*, A, G, L, V, or I;

X^1 may be present or absent and, when present, can be any amino acid, *e.g.*, an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (*e.g.*, other than an aromatic amino acid or a charged amino acid), *e.g.*, L, M, V, S or T, *e.g.*, L, M or V, with the proviso that when the sulfatase motif is at the N-terminus of the target 5T4 antibody, X^1 is present; and

X^2 and X^3 independently can be any amino acid, *e.g.*, an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (*e.g.*, other than an aromatic amino acid or a charged amino acid), *e.g.*, S, T, A, V, G or C, *e.g.*, S, T, A, V or G.

[00156] Specific examples of *fGly*-containing sulfatase motifs include L(*fGly*)TPSR (SEQ ID NO: 94), M(*fGly*)TPSR (SEQ ID NO: 95), V(*fGly*)TPSR (SEQ ID NO: 96), L(*fGly*)SPSR (SEQ ID NO: 97), L(*fGly*)APSR (SEQ ID NO: 98), L(*fGly*)VPSR (SEQ ID NO: 99), L(*fGly*)GPSR (SEQ ID NO: 100), I(*fGly*)TPAR (SEQ ID NO: 101), L(*fGly*)TPSK (SEQ ID NO: 102), M(*fGly*)TPSK (SEQ ID NO: 103), V(*fGly*)TPSK (SEQ ID NO: 104), L(*fGly*)SPSK (SEQ ID NO: 105), L(*fGly*)APSK (SEQ ID NO: 106), L(*fGly*)VPSK (SEQ ID NO: 107), L(*fGly*)GPSK (SEQ ID NO: 108), L(*fGly*)TPSA (SEQ ID NO: 109), I(*fGly*)TPAA (SEQ ID NO: 110), M(*fGly*)TPSA (SEQ ID NO: 111), V(*fGly*)TPSA (SEQ ID NO: 112), L(*fGly*)SPSA (SEQ ID NO: 113), L(*fGly*)APSA (SEQ ID NO: 114), L(*fGly*)VPSA (SEQ ID NO: 115), and L(*fGly*)GPSA (SEQ ID NO: 116).

[00157] In some embodiments, the 5T4 antibody as disclosed herein comprises a *fGly*'-containing sulfatase motif. In some embodiments, the 5T4 antibody as disclosed herein comprises at least one *fGly*'-containing sulfatase motif.

[00158] As described above, to produce the conjugate, the 5T4 antibody containing the *fGly* residue may be conjugated to a drug or active agent by reaction of the *fGly* with a reactive moiety (*e.g.*, a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety, as described

above) of a linker attached to the drug or active agent to produce an *fGly*'-containing sulfatase motif. As used herein, the term *fGly*' refers to the amino acid residue of the sulfatase motif that is coupled to the drug or active agent through a linker (*e.g.*, a branched linker) as described herein. Thus, the *fGly*'-containing sulfatase motif can be of:

X^1(*fGly*')$X^2Z^2X^3Z^3$ (SEQ ID NO: 67)

wherein:

fGly' is the amino acid residue coupled to the drug or active agent through a linker (*e.g.*, a branched linker) as described herein;

Z^2 is either a proline or alanine residue (which can also be represented by (P/A));

Z^3 is a basic amino acid (*e.g.*, arginine (R), and may be lysine (K) or histidine (H), usually lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), *e.g.*, A, G, L, V, or I;

X^1 may be present or absent and, when present, can be any amino acid, *e.g.*, an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (*e.g.*, other than an aromatic amino acid or a charged amino acid), *e.g.*, L, M, V, S or T, *e.g.*, L, M or V, with the proviso that when the sulfatase motif is at the N-terminus of the target 5T4 antibody, X^1 is present; and

X^2 and X^3 independently can be any amino acid, *e.g.*, an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (*e.g.*, other than an aromatic amino acid or a charged amino acid), *e.g.*, S, T, A, V, G or C, *e.g.*, S, T, A, V or G.

[00159] Specific examples of *fGly*'-containing sulfatase motifs include L(*fGly*')TPSR (SEQ ID NO: 1), M(*fGly*')TPSR (SEQ ID NO: 45), V(*fGly*')TPSR (SEQ ID NO: 46), L(*fGly*')SPSR (SEQ ID NO: 47), L(*fGly*')APSR (SEQ ID NO: 48), L(*fGly*')VPSR (SEQ ID NO: 49), L(*fGly*')GPSR (SEQ ID NO: 50), I(*fGly*')TPAR (SEQ ID NO: 51), L(*fGly*')TPSK (SEQ ID NO: 52), M(*fGly*')TPSK (SEQ ID NO: 53), V(*fGly*')TPSK (SEQ ID NO: 54), L(*fGly*')SPSK (SEQ ID NO: 55), L(*fGly*')APSK (SEQ ID NO: 56), L(*fGly*')VPSK (SEQ ID NO: 57), L(*fGly*')GPSK (SEQ ID NO: 58), L(*fGly*')TPSA (SEQ ID NO: 59), I(*fGly*')TPAA (SEQ ID NO: 60), M(*fGly*')TPSA (SEQ ID NO: 61), V(*fGly*')TPSA (SEQ ID NO: 62), L(*fGly*')SPSA (SEQ ID NO: 63), L(*fGly*')APSA (SEQ ID NO: 64), L(*fGly*')VPSA (SEQ ID NO: 65), and L(*fGly*')GPSA (SEQ ID NO: 66).

[00160] As noted above, the amino acid sequence of an antibody is modified to include a sulfatase motif that contains a serine or cysteine residue that is capable of being converted (oxidized) to an *fGly* residue by action of an FGE either *in vivo* (*e.g.*, at the time of translation of an aldehyde tag-containing protein in a cell) or *in vitro* (*e.g.*, by contacting an aldehyde tag-

containing protein with an FGE in a cell-free system). The antibody used to generate a conjugate of the present disclosure include at least an Ig constant region, *e.g.*, an Ig heavy chain constant region (*e.g.*, at least a CH1 domain; at least a CH1 and a CH2 domain; a CH1, a CH2, and a CH3 domain; or a CH1, a CH2, a CH3, and a CH4 domain), or an Ig light chain constant region. Such Ig antibodies are referred to herein as "target Ig polypeptides" or "target antibodies."

[00161] The site in an antibody into which a sulfatase motif is introduced can be any convenient site. As noted above, in some instances, the extent of modification of the native amino acid sequence of the target polypeptide is minimized, so as to minimize the number of amino acid residues that are inserted, deleted, substituted (replaced), and/or added (*e.g.*, to the N- or C-terminus). Minimizing the extent of amino acid sequence modification of the target antibody may minimize the impact such modifications may have upon antibody function and/or structure.

[00162] An antibody heavy chain constant region can include Ig constant regions of any heavy chain isotype, non-naturally occurring Ig heavy chain constant regions (including consensus Ig heavy chain constant regions). An Ig constant region amino acid sequence can be modified to include an aldehyde tag, where the aldehyde tag is present in or adjacent a solvent-accessible loop region of the Ig constant region. An Ig constant region amino acid sequence can be modified by insertion and/or substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 amino acids, or more than 16 amino acids, to provide an amino acid sequence of a sulfatase motif as described above.

[00163] In some cases, an aldehyde-tagged antibody comprises an aldehyde-tagged Ig heavy chain constant region (*e.g.*, at least a CH1 domain; at least a CH1 and a CH2 domain; a CH1, a CH2, and a CH3 domain; or a CH1, a CH2, a CH3, and a CH4 domain). The aldehyde-tagged Ig heavy chain constant region can include heavy chain constant region sequences of an IgA, IgM, IgD, IgE, IgG1, IgG2, IgG3, or IgG4 isotype heavy chain or any allotypic variant of same, *e.g.*, human heavy chain constant region sequences or mouse heavy chain constant region sequences, a hybrid heavy chain constant region, a synthetic heavy chain constant region, or a consensus heavy chain constant region sequence, etc., modified to include at least one sulfatase motif that can be modified by an FGE to generate an *fGly*-modified Ig polypeptide. Allotypic variants of Ig heavy chains are known in the art. See, *e.g.*, Jefferis and Lefranc (2009) *MAbs* 1:4.

[00164] In some cases, an aldehyde-tagged antibody comprises an aldehyde-tagged Ig light chain constant region. The aldehyde-tagged Ig light chain constant region can include constant region sequences of a kappa light chain, a lambda light chain, *e.g.*, human kappa or lambda light chain constant regions, a hybrid light chain constant region, a synthetic light chain constant

region, or a consensus light chain constant region sequence, etc., modified to include at least one sulfatase motif that can be modified by an FGE to generate an *fGly*-modified antibody. Exemplary constant regions include human gamma 1 and gamma 3 regions. With the exception of the sulfatase motif, a constant region may have a wild-type amino acid sequence, or it may have an amino acid sequence that is at least 70% identical (*e.g.*, at least 80%, at least 90% or at least 95% identical) to a wild-type amino acid sequence.

[00165] In some embodiments the sulfatase motif is at a position other than, or in addition to, the C-terminus of the Ig polypeptide heavy chain. As noted above, an isolated aldehyde-tagged antibody can comprise a heavy chain constant region amino acid sequence modified to include a sulfatase motif as described above, where the sulfatase motif is in or adjacent to a surface-accessible loop region of the antibody heavy chain constant region.

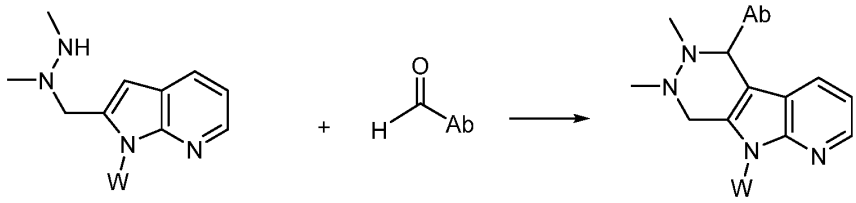
[00166] A sulfatase motif can be provided within or adjacent to one or more of these amino acid sequences of such modification sites of an Ig heavy chain. For example, an Ig heavy chain polypeptide amino acid sequence can be modified (*e.g.*, where the modification includes one or more amino acid residue insertions, deletions, and/or substitutions) at one or more of these amino acid sequences to provide a sulfatase motif adjacent and N-terminal and/or adjacent and C-terminal to these modification sites. Alternatively, or in addition, an Ig heavy chain polypeptide amino acid sequence can be modified (*e.g.*, where the modification includes one or more amino acid residue insertions, deletions, and/or substitutions) at one or more of these amino acid sequences to provide a sulfatase motif between any two residues of the Ig heavy chain modifications sites. In some embodiments, an Ig heavy chain polypeptide amino acid sequence may be modified to include two motifs, which may be adjacent to one another, or which may be separated by one, two, three, four or more (*e.g.*, from about 1 to about 25, from about 25 to about 50, or from about 50 to about 100, or more, amino acids. Alternatively, or in addition, where a native amino acid sequence provides for one or more amino acid residues of a sulfatase motif sequence, selected amino acid residues of the modification sites of an Ig heavy chain polypeptide amino acid sequence can be modified (*e.g.*, where the modification includes one or more amino acid residue insertions, deletions, and/or substitutions) so as to provide a sulfatase motif at the modification site.

[00167] An antibody used in an antibody-drug conjugate of the present disclosure can have any of a variety of antigen-binding specificities, including but not limited to, *e.g.*, an antigen present on a cancer cell; an antigen present on an autoimmune cell; an antigen present on a pathogenic microorganism; an antigen present on a virus-infected cell (*e.g.*, a human immunodeficiency virus-infected cell); an antigen present on a diseased cell; and the like. For example, an antibody

conjugate can bind an antigen, where the antigen is present on the surface of the cell. An antibody conjugate of the present disclosure can bind antigen with a suitable binding affinity, *e.g.*, from 5 x 10^{-6} M to 10^{-7} M, from 10^{-7} M to 5 x 10^{-7} M, from 5 x 10^{-7} M to 10^{-8} M, from 10^{-8} M to 5 x 10^{-8} M, from 5 x 10^{-8} M to 10^{-9} M, or a binding affinity greater than 10^{-9} M.

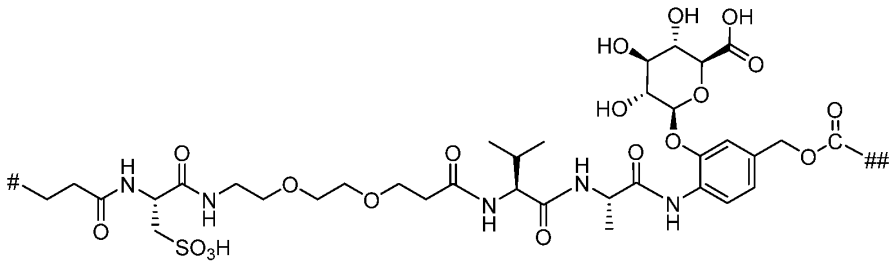
[00168] As non-limiting examples, a subject antibody conjugate can bind an antigen present on a cancer cell (*e.g.*, a tumor-specific antigen; an antigen that is over-expressed on a cancer cell; etc.), and the conjugated moiety can be a drug, such as a cytotoxic compound (*e.g.*, a cytotoxic small molecule, a cytotoxic synthetic peptide, etc.). For example, a subject antibody conjugate can be specific for an antigen on a cancer cell, where the conjugated moiety is a drug, such as a cytotoxic compound (*e.g.*, a cytotoxic small molecule, a cytotoxic synthetic peptide, etc.).

[00169] The modified 5T4 antibody containing the *fGly* residue can be conjugated to a linker-drug comprising an aza-hydrazino-iso-Pictet-Spengler (azaHIPS) coupling moiety, *e.g.*, such as in linker (**L-1**), as shown below in **SCHEME 1**:



SCHEME 1

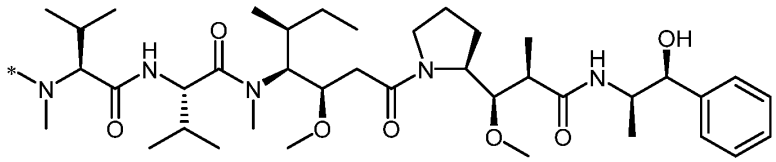
wherein W is Formula (**L-2**):



(**L-2**),

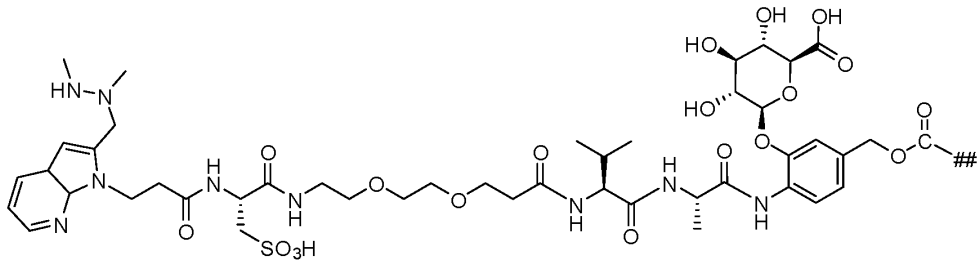
wherein # represents the point of conjugation to azaHIPS moiety and ## indicates the point of attachment to the MMAE; and

wherein the MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment to (**L-2**).

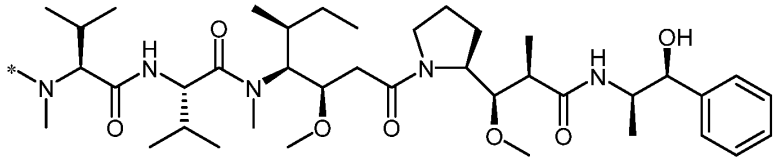
[00170] More particularly, one or more *fGly* modifications on the 5T4 antibody may be reacted with the azaHIPS moiety on a linker-drug comprising pre-conjugate linker (**L-1a**), shown below, to generate a 5T4 antibody comprising one more conjugated linker-drugs comprising linker (**L-1**):



(**L-1a**),

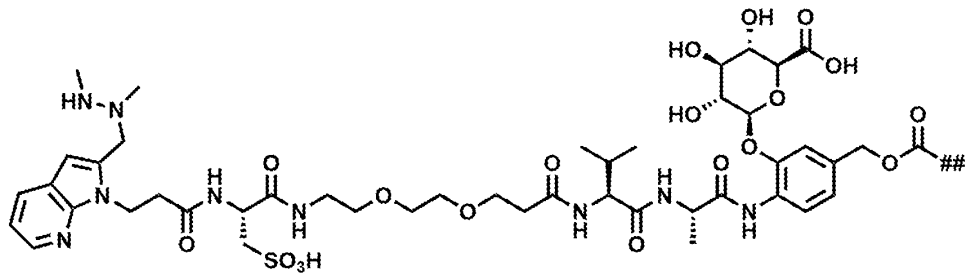
wherein ## indicates the point of attachment to the MMAE; and

wherein the MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment to (**L-1a**).

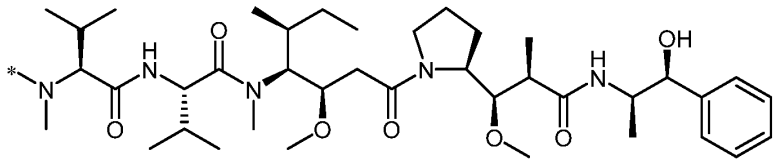
[00171] More particularly, one or more *fGly* modifications on the 5T4 antibody may be reacted with the azaHIPS moiety on a linker-drug comprising pre-conjugate linker (**L-1a'**), shown below, to generate a 5T4 antibody comprising one more conjugated linker-drugs comprising linker (**L-1**):



(**L-1a'**),

wherein ## indicates the point of attachment to the MMAE; and

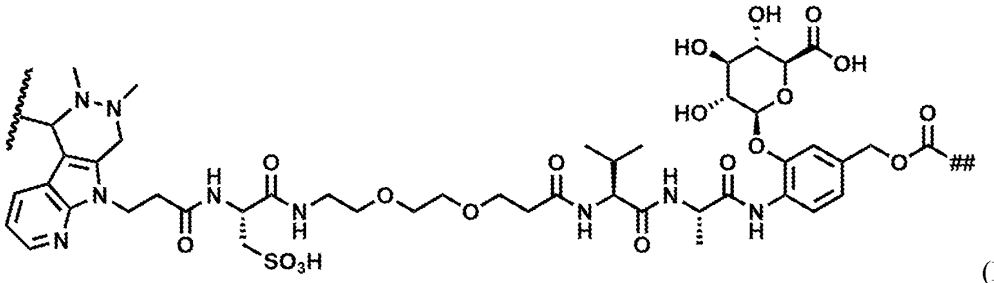
wherein the MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment to (**L-1a'**).

[00172] In some embodiments, (**L-1a'**) refers to (2*S*,3*S*,4*S*,5*R*,6*S*)-6-(5-((5*S*,8*S*,11*S*,12*R*)-11-((*S*)-sec-butyl)-12-(2-((*S*)-2-((1*R*,2*R*)-3-(((1*S*,2*R*)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-2-oxoethyl)-5,8-diisopropyl-4,10-dimethyl-3,6,9-trioxo-2,13-dioxa-4,7,10-triazatetradecyl)-2-((2*S*,5*S*,18*R*)-22-(2-((1,2-dimethylhydrazineyl)methyl)-1H-pyrrolo[2,3-b]pyridin-1-yl)-5-isopropyl-2-methyl-4,7,17,20-tetraoxo-18-(sulfomethyl)-10,13-dioxa-3,6,16,19-tetraazadocosanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid. In some embodiments, (**L-1a'**) has a Chemical Abstracts Service (CAS) Registry Number of 2773415-17-9.

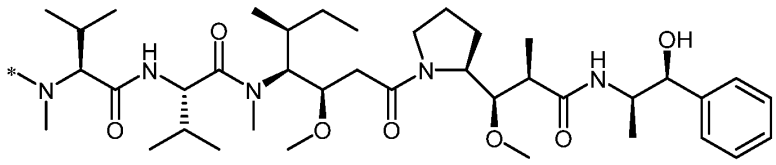
[00173] In one embodiment, one or more amino acids in one or both of the heavy chain constant regions and light chain constant regions is conjugated to a linker-drug comprising a linker of (**L-1**):



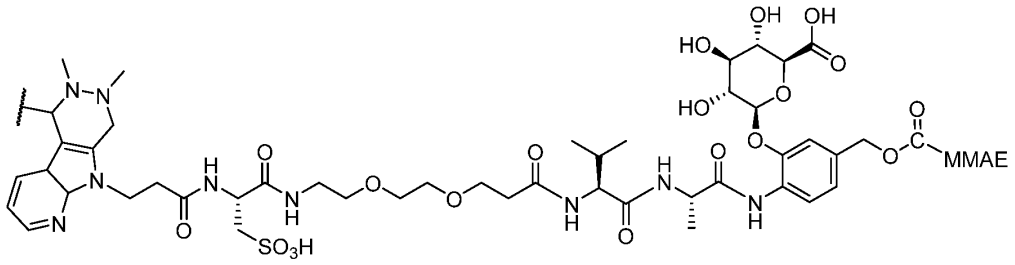
(**L-1**),

wherein ~~~ represents the point of conjugation to Ab and ## indicates the point of attachment to the MMAE; and

wherein the MMAE is represented by **Formula (III)**:

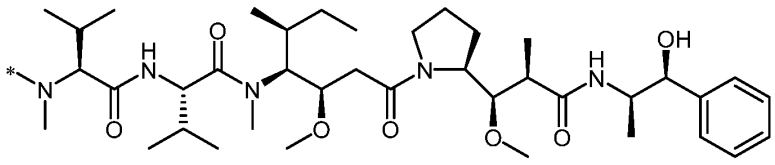


(**III**), wherein * indicates the point of attachment to (**L-1**). In one embodiment, the linker-drug comprising pre-conjugate linker **(L-1a')** or (**L-1a**) is a linker-drug of **Formula (II)**:



(**II**),

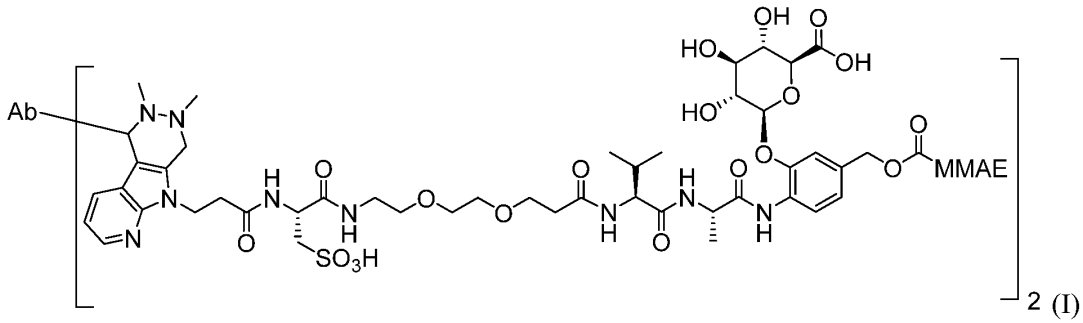
wherein the wavy line indicates the point of attachment to the Ab and MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment in **Formula (II)**.

[00174] In one embodiment, one or more amino acids in one or both of the heavy chain constant region and light chain constant region is conjugated to a linker-drug comprising a linker of (**L-1**). In one embodiment, one amino acid in both of the heavy chain constant regions is conjugated to a linker-drug comprising a linker of (**L-1**). A composition comprising such 5T4 ADCs may exhibit a drug-to-antibody ratio ("DAR") of about 1 to about 2. In other words, each 5T4 antibody is conjugated to one or two linker-drugs. Methods to determine DAR are well known to the skilled person and include methods using Reverse Phase Chromatography, or HPLC-MS, for example, those disclosed in WO2023220620, which is incorporated herein by reference in its entirety.

[00175] In one embodiment, provided herein is a 5T4 ADC as disclosed, *e.g.*, in International Patent Publication No. WO 2023/220620 A2 (International Patent Application No. PCT/US2023/066811), incorporated herein by reference in its entirety. In one embodiment, the 5T4 ADC is represented by **Formula (I)**, comprising an Ab conjugated to a monomethyl auristatin E (MMAE) by a linker-drug comprising linker (L-1):



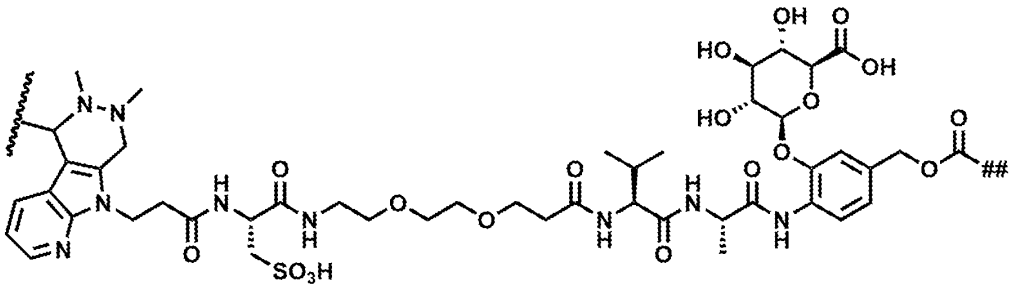
(I)

wherein Ab is a 5T4 antibody or an antigen binding fragment thereof. In one embodiment, the 5T4 ADC comprises a 5T4 antibody or an antigen binding fragment thereof comprising a VH region, VL region, heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and/or a light chain complementarity determining region 3 (LCDR3) as described in **Table 1**. In one embodiment, the 5T4 ADC comprises a 5T4 antibody or an antigen binding fragment thereof comprising a VH region comprising the amino acid sequence of SEQ ID NO: 11 and a VL region comprising the amino acid sequence of SEQ ID NO: 12. In one embodiment, the 5T4 antibody comprises a light chain having an amino acid sequence of:
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3).

[00176] In one embodiment, a linker-drug comprising linker (**L-1**) is conjugated to each of the heavy chain constant regions in the 5T4 antibody (*e.g.*, the 5T4 antibody is conjugated to two linker-drugs). In one embodiment, a linker-drug comprising linker (**L-1**) is conjugated to each of the heavy chains in the 5T4 antibody (*e.g.*, the 5T4 antibody is conjugated to two linker-drugs). In one embodiment, a linker-drug comprising linker (**L-1**) is conjugated to the CH3 region in each of the heavy chain constant regions in the 5T4 antibody. In one embodiment, a linker-drug comprising linker (**L-1**) is conjugated to the CH3 region in each of the heavy chains in the 5T4 antibody. In a further embodiment, a linker-drug comprising linker (**L-1**) is conjugated to the aldehyde in the formylglycine at C453 of each heavy chain of an anti-5T4 aldehyde-containing antibody (the formylglycine amino acid residue corresponding to the 453rd amino acid residue of C in SEQ ID NO: 40, also referred to herein as "C453"). In one embodiment, a linker-drug comprising linker (**L-1**) is conjugated to the aldehyde in the formylglycine corresponding to the 453rd amino acid residue of fGly in SEQ ID NO: 41). In one embodiment, a linker-drug comprising linker (**L-1**) is conjugated to the aldehyde in the formylglycine corresponding to the 453rd amino acid residue of fGly' in SEQ ID NO: 2). In such embodiments, each 5T4 ADC comprises two MMAE drugs. A composition comprising such ADCs will exhibit a DAR of 2. In some embodiments, a 5T4 ADC comprises a 5T4 antibody comprising a light chain comprising the amino acid sequence of

DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3) and a heavy chain having an amino acid sequence of:

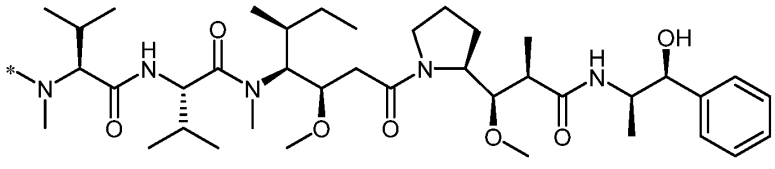
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL*n*TP
SRGS (SEQ ID NO: 42), wherein *n* is an amino acid having its side chain replaced by a linker-drug comprising linker (**L-1**):



(**L-1**),

wherein ~~~ represents the point of conjugation to Ab and ## indicates the point of attachment to the MMAE; and

wherein the MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment to (**L-1**). In some embodiments, the amino acid *n* in SEQ ID NO: 42 refers to *fGly'* as described herein.

[00177] In one embodiment, a 5T4 ADC is produced by conjugating a pre-conjugate linker (**L-1a'**) to the aldehyde in the formylglycine, such as at C453, of each heavy chain of an anti-5T4 aldehyde-containing antibody, wherein Ab is a 5T4 antibody or an antigen binding fragment thereof. In one embodiment, the 5T4 ADC comprises a 5T4 antibody or an antigen binding

fragment thereof comprising a VH region, VL region, heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and/or a light chain complementarity determining region 3 (LCDR3) as described in **Table 1**. In one embodiment, the 5T4 ADC comprises a 5T4 antibody or an antigen binding fragment thereof as disclosed herein. In one embodiment, the 5T4 ADC comprises a 5T4 antibody or an antigen binding fragment thereof comprising a VH region comprising the amino acid sequence of SEQ ID NO: 11 and a VL region comprising the amino acid sequence of SEQ ID NO: 12. In one embodiment, the 5T4 antibody comprises a light chain having an amino acid sequence of SEQ ID NO: 3. In some embodiments, a 5T4 ADC comprises a 5T4 antibody comprising a light chain comprising the amino acid sequence of SEQ ID NO: 3 and a heavy chain having an amino acid sequence of SEQ ID NO: 2.

[00178] In further embodiments, the antibody of the 5T4 ADC (*e.g.*, those of **Formula (I)** or **Formula (I')**, or the ADCs comprising **(L-1)**, or the ADCs produced by conjugating **(L-1a')**) comprises two heavy chains (HCs) and two light chains (LCs), and wherein each of the HCs comprises one motif of L(*fGly'*)TPSR (SEQ ID NO: 1). Accordingly, each 5T4 ADC comprises two MMAE drugs, and a composition comprising such ADCs exhibits a DAR of about 1 to about 2, such as 2.

[00179] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 13, (ii) SEQ ID NO: 19, (iii) SEQ ID NO: 24, (iv) SEQ ID NO: 25, and (v) SEQ ID NO: 30; (2) an HCDR2 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 14, (ii) SEQ ID NO: 20, (iii) SEQ ID NO: 26, (iv) SEQ ID NO: 31, and (v) SEQ ID NO: 36; and (3) an HCDR3 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 15, (ii) SEQ ID NO: 21, (iii) SEQ ID NO: 27, and (iv) SEQ ID NO: 32; and/or a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 16, (ii) SEQ ID NO: 22, (iii) SEQ ID NO: 28, and (iv) SEQ ID NO: 33; (2) an LCDR2 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 17, (ii) SEQ ID NO: 23, and (iii) SEQ ID NO: 34; and (3) an LCDR3 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 18, (ii) SEQ ID NO: 29, and (iii) SEQ ID NO: 35.

[00180] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 13, (ii) SEQ ID NO: 19, (iii) SEQ ID NO: 24, (iv) SEQ

ID NO: 25, and (v) SEQ ID NO: 30; (2) an HCDR2 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 14, (ii) SEQ ID NO: 20, (iii) SEQ ID NO: 26, (iv) SEQ ID NO: 31, and (v) SEQ ID NO: 36; and (3) an HCDR3 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 15, (ii) SEQ ID NO: 21, (iii) SEQ ID NO: 27, and (iv) SEQ ID NO: 32

[00181] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 16, (ii) SEQ ID NO: 22, (iii) SEQ ID NO: 28, and (iv) SEQ ID NO: 33; (2) an LCDR2 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 17, (ii) SEQ ID NO: 23, and (iii) SEQ ID NO: 34; and (3) an LCDR3 comprising an amino acid sequence of any one of: (i) SEQ ID NO: 18, (ii) SEQ ID NO: 29, and (iii) SEQ ID NO: 35.

[00182] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 13; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO: 14; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 15; and (b) a VL region comprising: (1) a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 16; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 17; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 18.

[00183] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 19; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO: 20; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 21; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of SEQ ID NO: 22; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 23; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 18.

[00184] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 24; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO: 14; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 15; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of SEQ ID NO: 16; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 17; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 18.

[00185] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 25; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO:

26; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 27; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of SEQ ID NO: 28; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 23; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 29.

[00186] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 30; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO: 31; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 32; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of SEQ ID NO: 33; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 34; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 35.

[00187] In some embodiments, a 5T4 ADC comprises an antibody that binds to 5T4, wherein the antibody comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 13; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO: 36; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 15; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of SEQ ID NO: 16; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 17; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 18.

[00188] In some embodiments, a 5T4 ADC can comprise a 5T4 antibody that comprises one or more (*e.g.*, one, two, three, four, or more) conservative sequence modifications. With respect to polypeptides that are 5T4 antibodies, such as human 5T4 antibodies, conservative sequence modifications include conservative amino acid substitutions in which an amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families are disclosed herein. Thus, in some embodiments, a predicted nonessential amino acid residue in a 5T4 antibody can be replaced with another amino acid residue from the same side chain family. Methods of identifying amino acid conservative substitutions which do not eliminate antigen binding and nucleotides encoding thereof are well known in the art (*see, e.g.*, Brummell et al., Biochem. 32:1180-1187 (1993); Kobayashi et al. Protein Eng. 12(10):879-884 (1999); and Burks et al. Proc. Natl. Acad. Sci. USA 94:412-417 (1997)). In some embodiments, the conservative sequence modifications described herein modify the amino acid sequences of a 5T4 antibody (*e.g.*, a human 5T4 antibody) by 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%. In some embodiments, the amino acid sequence modifications refer to at most one, two, three, four, five, or six amino acid substitutions to the CDRs, such as those described in **Table 1**.

Thus, for example, each such CDR can contain up to five conservative amino acid substitutions, for example up to (not more than) four conservative amino acid substitutions, for example up to (not more than) three conservative amino acid substitutions, for example up to (not more than) two conservative amino acid substitutions, or no more than one conservative amino acid substitution. In some embodiments, a 5T4 ADC comprises a 5T4 antibody that contains one or more, (*e.g.*, one, two, three, four, five, or six CDRs) having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the CDRs of **mAbA15** (*see, e.g.*, **Table 1**). In some embodiments, a 5T4 ADC comprises a 5T4 antibody that contains a VH and a VL comprising CDRs identical to those of **mAbA15** (*see, e.g.*, **Table 1**). In some embodiments, the amino acid sequence modifications do not include any modification within a specificity determining residue (SDR). In some embodiments, the amino acid sequence modifications do not include any modification within a CDR (such as CDR1, CDR2, CDR3, or any combination thereof). In further embodiments, the amino acid sequence modifications are in the framework or constant region.

[00189] In some embodiments, the antibody in a 5T4 ADC comprises a VH comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 11 and a VL comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 12, and the binding of the antibody to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). In further embodiments, the antibody in a 5T4 ADC comprises CDRs identical to those of **mAbA15** (*see, e.g.*, **Table 1**).

[00190] In some embodiments, the antibody in a 5T4 ADC comprises a heavy chain comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 2 and a light chain comprising an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 3, and the binding of the antibody to 5T4 (*e.g.,* human 5T4) is maintained (*e.g.*, substantially maintained, for example, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, or at least 95%). In further embodiments, the antibody in a 5T4 ADC comprises CDRs identical to those of **mAbA15** (*see, e.g.*, **Table 1**). In yet further embodiments, the motif of L(*fGly'*)TPSR (SEQ ID NO: 1) in the heavy chain is maintained.

[00191] In some embodiments, a 5T4 ADC can be represented by **Formula (I)** or **Formula (I')**, or comprise **(L-1)**, or be produced by conjugating **(L-1a')**, wherein Ab is an antibody that binds to 5T4 as disclosed herein. In some embodiments, the antibody comprises a VH comprising

one or more (such as one, two, or three) CDRs identical or substantially identical to the CDRs as described herein, such as in **Table 1**. Additionally or alternatively, in some embodiments, the antibody comprises a VL comprising one or more (such as one, two, or three) CDRs identical or substantially identical to the CDRs as described herein, such as in **Table 1**.

[00192] In some embodiments, a 5T4 ADC can be represented by **Formula (I)** or **Formula (I')**, or comprise **(L-1)**, or be produced by conjugating **(L-1a')**, wherein Ab is an antibody that binds to 5T4 as disclosed herein. In some embodiments, the antibody comprises a VH identical or substantially identical to a VH as described herein, such as in **Table 1**. Additionally or alternatively, in some embodiments, the antibody comprises a VL identical or substantially identical to a VL as described herein, such as in **Table 1**.

[00193] In some embodiments, a 5T4 ADC can be represented by **Formula (I)** or **Formula (I')**, or comprise **(L-1)**, or be produced by conjugating **(L-1a')**, wherein Ab is an antibody that binds to 5T4. In some embodiments, the antibody comprises a VH comprising the amino acid sequence of SEQ ID NO: 11 and a VL comprising the amino acid sequence of SEQ ID NO: 12.

[00194] In some embodiments, a 5T4 ADC can be represented by **Formula (I)** or **Formula (I')**, or comprise **(L-1)**, or be produced by conjugating **(L-1a')**, wherein Ab is an antibody that binds to 5T4 and comprises (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of any one SEQ ID NOs:13, 19, 24, 25, and 30; (2) an HCDR2 comprising an amino acid sequence of any one SEQ ID NOs:14, 20, 26, 31, and 36; and (3) an HCDR3 comprising an amino acid sequence of any one SEQ ID NOs:15, 21, 27, and 32; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of any one SEQ ID NOs:16, 22, 28, and 33; (2) an LCDR2 comprising an amino acid sequence of any one SEQ ID NOs:17, 23, and 34; and (3) an LCDR3 comprising an amino acid sequence of any one SEQ ID NOs:18, 29, and 35.

[00195] In some embodiments, a 5T4 ADC can be represented by **Formula (I)** or **Formula (I')**, or comprise **(L-1)**, or be produced by conjugating **(L-1a')**, wherein Ab is an antibody that binds to 5T4. In some embodiments, the Ab comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 13; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO: 14; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 15; and (b) a VL region comprising: (1) a VL CDR1 comprising an amino acid sequence of SEQ ID NO: 16; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 17; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 18. In some embodiments, the Ab comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 19; (2) an HCDR2 comprising an amino acid sequence of

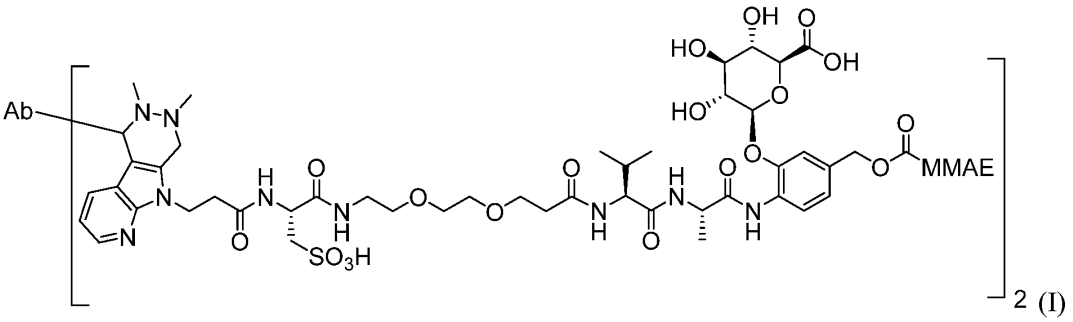
SEQ ID NO: 20; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 21; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of SEQ ID NO: 22; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 23; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 18. In some embodiments, the Ab comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 24; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO: 14; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 15; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of SEQ ID NO: 16; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 17; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 18. In some embodiments, the Ab comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 25; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO: 26; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 27; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of SEQ ID NO: 28; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 23; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 29. In some embodiments, the Ab comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 30; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO: 31; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 32; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of SEQ ID NO: 33; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 34; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 35. In some embodiments, the Ab comprises: (a) a VH region comprising: (1) an HCDR1 comprising an amino acid sequence of SEQ ID NO: 13; (2) an HCDR2 comprising an amino acid sequence of SEQ ID NO: 36; and (3) an HCDR3 comprising an amino acid sequence of SEQ ID NO: 15; and (b) a VL region comprising: (1) an LCDR1 comprising an amino acid sequence of SEQ ID NO: 16; (2) an LCDR2 comprising an amino acid sequence of SEQ ID NO: 17; and (3) an LCDR3 comprising an amino acid sequence of SEQ ID NO: 18.

[00196] In some embodiments, a 5T4 ADC can be represented by **Formula (I)** or **Formula (I')**, or comprise **(L-1)**, or be produced by conjugating **(L-1a')**, wherein Ab is an antibody that binds to 5T4. In some embodiments, the antibody further comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and/or a framework 4 (FR4) sequence as set forth in any one of SEQ ID NOs: 11 and 12.

[00197] In some embodiments, a 5T4 ADC can be represented by **Formula (I)** or **Formula (I')**, or comprise **(L-1)**, or be produced by conjugating **(L-1a')**, wherein Ab is an antibody that

binds to 5T4. In some embodiments, the Ab is **mAbA15**. In some embodiments, the Ab comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 2 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 3. In some embodiments, the Ab comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 42 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 3.

[00198] In one embodiment, the 5T4 ADC comprises an Ab conjugated to a monomethyl auristatin E (MMAE) by linker (**L-1**) and is represented by **Formula (I)** below:



(I)

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which a linker-drug comprising linker (**L-1**) is attached, and wherein **mAbA15** comprises:

a) a heavy chain comprising an amino acid sequence of:

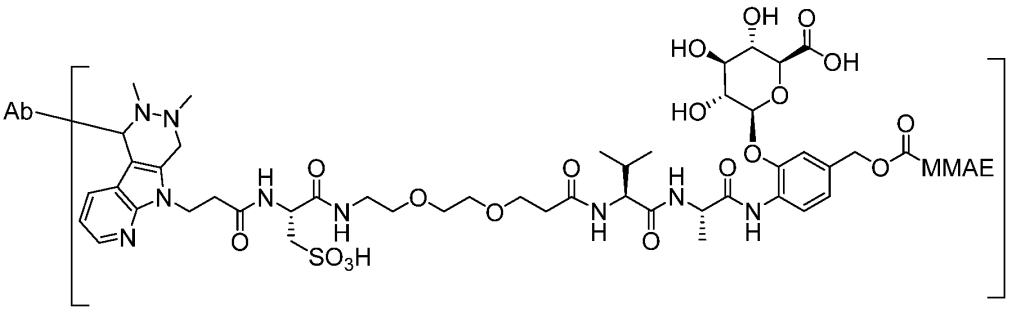
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL

(*fGly'*)TPSRGS (SEQ ID NO: 2); and

b) a light chain comprising an amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3).

[00199] In one embodiment, the 5T4 ADC is represented by **Formula (I)** below:



(I),

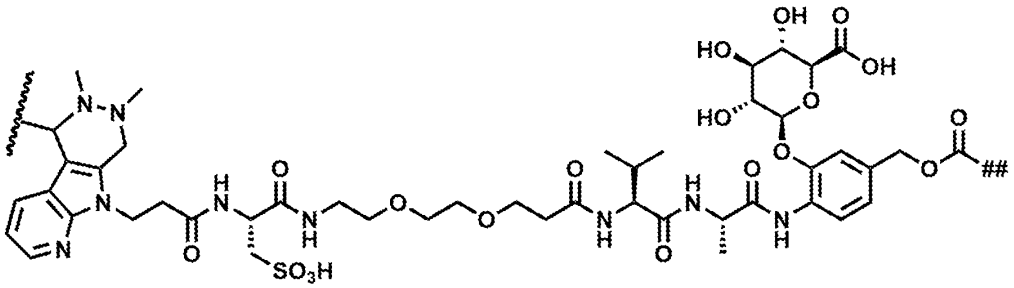
wherein Ab is a 5T4 antibody comprising:

a) a heavy chain comprising an amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL
(*fGly'*)TPSRGS (SEQ ID NO: 2); and

b) a light chain comprising an amino acid sequence of:

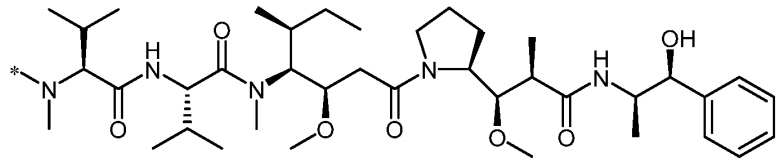
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3). In some embodiments, linker (**L-1**) is:



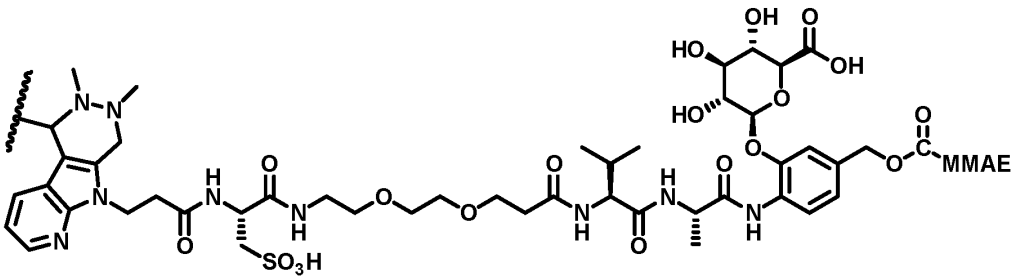
(**L-1**),

wherein the wavy line indicates the point of attachment to the Ab at (*fGly'*) and ## indicates the point of attachment to the MMAE; and

wherein the MMAE is represented by **Formula (III)**:

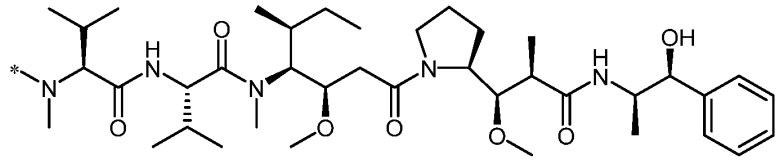


(**III**), wherein * indicates the point of attachment to (**L-1**). In one embodiment, a linker-payload of **Formula (II)** is conjugated to the Ab at (*fGly'*):



(**II**),

wherein the wavy line indicates the point of attachment to the Ab and MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment in **Formula (II).** This ADC is also referred to herein as "**5T4-ADC-1**."

[00200] In some embodiments, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**), carries two MMAE drug molecules per antibody component, resulting in a drug-to-antibody ratio (DAR) of 2:1.

[00201] The ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a'),** was evaluated in a 13-week, Good Laboratory Practice (GLP)-compliant, toxicology, toxicokinetic, and safety pharmacology study in a pharmacologically relevant species, cynomolgus monkeys. In some embodiments, the 5T4 ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**),carries two MMAE payload molecules per antibody component, resulting in a drug-to-antibody ratio (DAR) of 2:1. Without wishing to be bound by theory, it is believed that, mechanistically, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**), binds to 5T4 expressed on the surface of tumor cells and is subsequently internalized by the tumor cell. Subsequent enzymatic cleavage of the linker-payload from the antibody releases the MMAE cytotoxin. Free MMAE works inside the tumor cell to block polymerization of tubulin cell division but is also released from the initial tumor cell into the extracellular space to enter surrounding cells, including tumor cells, to inhibit cell division in those surrounding cells (commonly known as the bystander effect). This bystander effect efficiently kills both 5T4-expressing tumor cells and 5T4-negative tumor cells existing alongside 5T4-expressing cells.

[00202] Antibodies targeting the PD-1, a T-cell inhibitory checkpoint protein, have been demonstrated to enhance the immune response against tumor cells and are part of standard of care for a variety of cancers. Pembrolizumab is a humanized monoclonal immunoglobulin G4 (IgG4) kappa antibody specific for PD-1/PD-L1 cell surface membrane receptor that has demonstrated the ability to generate increased antitumor immune responses and improvements in survival of cancer patients. Pembrolizumab has been approved in multiple cancer types in the US and Europe (Keytruda US Prescribing Information [USPI] and European Medicines Agency [EMA] Summary of Product Characteristics [SmPC]). As illustrated in the Examples, combining a PD-L1 inhibitor or a PD-1 inhibitor, such as pembrolizumab, with the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**), enhances the anticancer effect of the ADC, which may be particularly useful in patients with advanced tumors or for those patients where other available therapies are ineffective and/or not well-tolerated. As such, in one aspect, the present disclosure also provides methods of using a combination of the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**), and a PD-L1/PD-1 inhibitor, such as pembrolizumab, in the treatment of cancer.

[00203] In some embodiments, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) ,is provided in the form of a pharmaceutical composition for administration to a subject. Such a pharmaceutical composition comprises a therapeutically effective amount of the ADC and one or more pharmaceutically acceptable excipients.

[00204] Accordingly, provided herein is a pharmaceutical composition comprising a 5T4 ADC as disclosed herein, such as **5T4-ADC-1**, and one or more pharmaceutically acceptable excipients.

[00205] Examples of suitable pharmaceutically acceptable excipients include solid or liquid fillers, diluents, other excipients, or encapsulating substances that are suitable for administration into a human or veterinary subject (*e.g.*, a physiologically acceptable and/or pharmacologically acceptable). The pharmaceutically acceptable excipient can be co-mingled with one or more of the active components, *e.g.*, a hybrid molecule, and with each other, when more than one pharmaceutically acceptable excipient is present in the pharmaceutical composition, in a manner so as not to substantially impair the desired pharmaceutical efficacy. Pharmaceutically acceptable materials typically are capable of administration to a subject without the production of significant undesirable physiological effects such as nausea, dizziness, rash, or gastric upset. It is, for example, desirable for a composition comprising a pharmaceutically acceptable excipient not to be immunogenic when administered to a human subject for therapeutic purposes.

[00206] In some embodiments, a pharmaceutical composition comprising the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**), additionally contains suitable buffering agents, including, for example, acetic acid in a salt, citric acid in a salt, boric acid in a salt, and phosphoric acid in a salt. In some embodiments, a pharmaceutical composition comprising the ADC also optionally contains suitable preservatives, such as benzalkonium chloride, chlorobutanol, parabens, and thimerosal. In some embodiments, a pharmaceutical composition comprising the ADC is provided in unit dosage form and can be prepared by any suitable method, many of which are well known in the art of pharmacy.

[00207] In some embodiments, a pharmaceutical composition comprising the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**), that is suitable for parenteral administration is in the form of a sterile aqueous preparation of the ADC, which preferably is isotonic with the blood of the recipient. In some embodiments, this aqueous preparation is formulated using known methods using suitable dispersing or wetting agents and suspending

agents. In some embodiments, a sterile injectable preparation is also a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Non-limiting examples of suitable vehicles and solvents include water, Ringer's solution, and isotonic sodium chloride solution. In some embodiments, sterile, fixed oils is employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed, such as synthetic mono-or di-glycerides. In addition, fatty acids, such as oleic acid, can be used in the preparation of an injectable formulation of the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**).

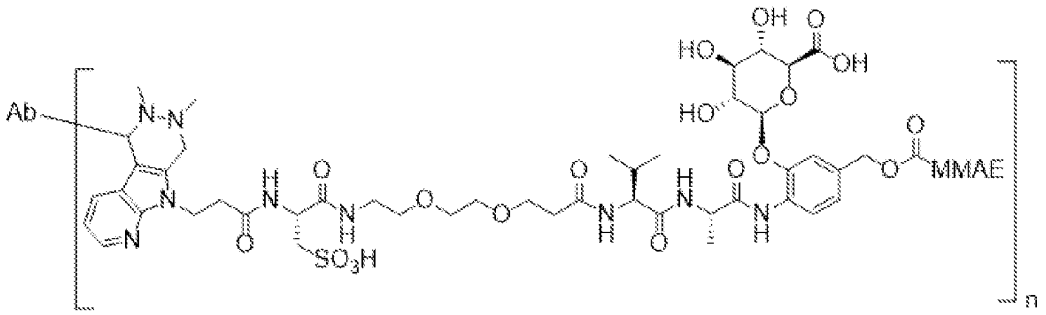
[00208] In some embodiments, a pharmaceutical composition of the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is suitably packaged, *e.g.,* in a vial, pouch, ampoule, and/or any like and appropriate container. In one embodiment, a pharmaceutical composition comprising the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is provided as a concentrate, which can be further diluted prior to use. In one embodiment, a pharmaceutical composition comprising the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is provided as a such as a lyophilizate, which can be reconstitute prior to use. In one embodiment, a pharmaceutical composition comprising the ADC of as disclosed herein, for example those **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is provided at the concentration intended for use. In some embodiments, a pharmaceutical composition comprising the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is provided in unit dosage form in sterilized containers, *e.g.*, as part of a kit, each container having a desired amount and concentration of the ADC.

7.4 METHODS, ADMINISTRATION, AND DOSING

[00209] In one aspect, provided herein is a method for treating cancer in a subject, the method comprising administering an ADC to the subject.

[00210] In some embodiments, the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer.

[00211] In some embodiments, the ADC is represented by **Formula (I')**:



(I'),

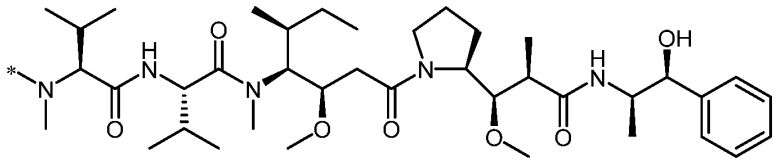
wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises:

(1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and

(2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12.

[00212] In some embodiments, the Ab further comprises one or more *fGly'*, wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I')** is attached.

[00213] In some embodiments, wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



(III),

wherein * indicates the point of attachment in **Formula (I')**.

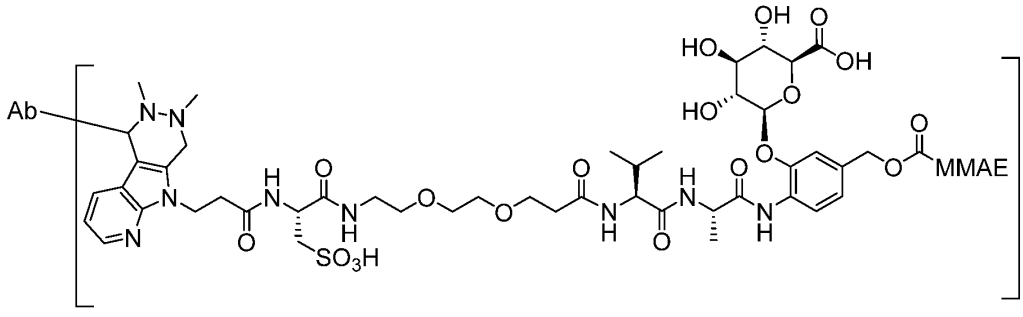
[00214] In some embodiments, n is an integer. In further embodiments, n is an integer selected from any one of 1 to 10. In yet further embodiments, n is 2. In other embodiments, n is 1.

[00215] In some embodiments, administered is a population of ADCs comprising a multiplicity of DAR species. In further embodiments, the population comprises a first ADC represented by **Formula (I')** wherein its n is 1 and a second ADC represented by **Formula (I')** wherein its n is 2.

[00216] In some embodiments, a pharmaceutical composition comprising a first ADC represented by **Formula (I')** and a pharmaceutically acceptable excipient is administered to the subject. In further embodiments, the pharmaceutical composition comprises a second ADC represented by **Formula (I')**, wherein a first n in the first ADC is different from the second n of the second ADC. In yet further embodiments, the first n is 2. Additionally or alternatively, the second n is 1. In some embodiments, such a pharmaceutical composition exhibits a drug-to-antibody ratio ("DAR") of about 1 to about 2. In further embodiments, the pharmaceutical composition exhibits a DAR of 1.8.

[00217] In some embodiments, the Ab in **Formula (I')** comprises at least one *fGly'*. In some embodiments, the Ab in **Formula (I')** further comprises one or more (such as one to ten) *fGly*. In further embodiments, the Ab in **Formula (I')** further comprises one or more (such as one to ten) motif of SEQ ID NO: 93.

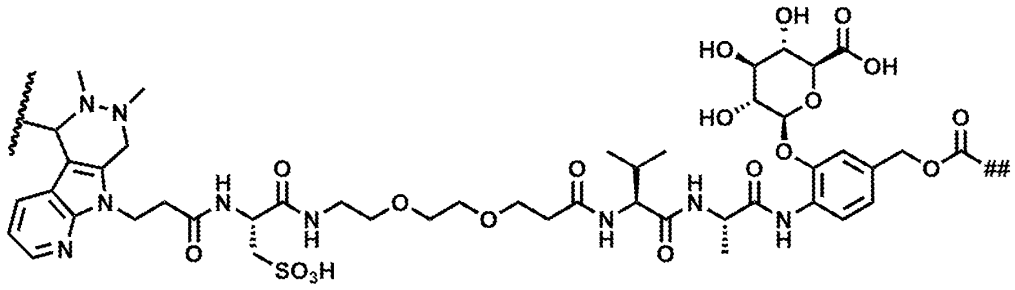
[00218] In another aspect, the ADC is represented by **Formula (I):**



(I),

wherein the * in **Formula (III)** indicates the point of attachment in **Formula (I)** and wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I)** is attached**.**

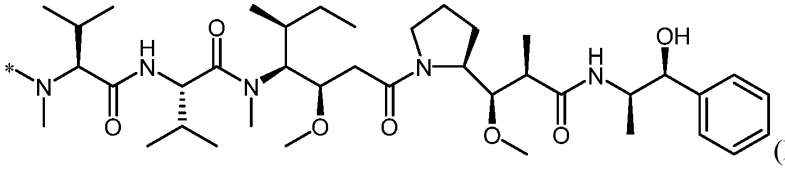
[00219] In some embodiments, the ADC comprises an antibody (Ab) conjugated to a monomethyl auristatin E (MMAE) by a linker (**L-1**):



(**L-1**),

wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE.

[00220] In some embodiments, the MMAE is represented by Formula (III):



(**III**),

wherein * indicates the point of attachment to (**L-1**).

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises:

(1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and

(2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; and

wherein the Ab further comprises one or more *fGly'* and wherein *fGly'* is a formylglycine amino acid residue to which linker (**L-1**) is attached.

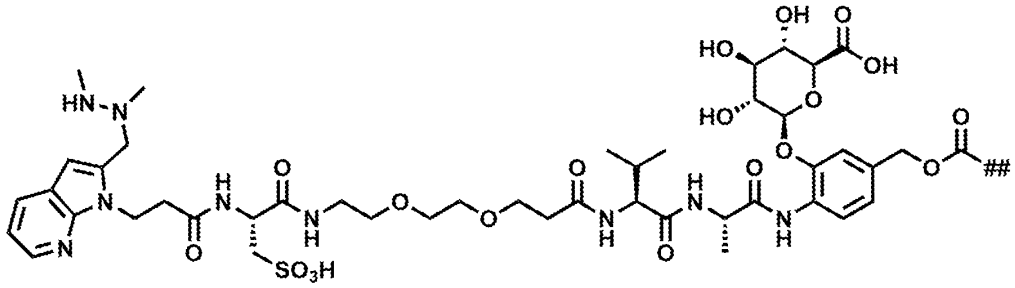
[00221] In some embodiments, one Ab is conjugated to one to ten MMAEs and wherein each MMAE is conjugated to Ab by one linker (**L-1**).

[00222] In some embodiments, one Ab is conjugated to two MMAEs and wherein each MMAE is conjugated to Ab by one linker (**L-1**).

[00223] In yet another aspect, provided is a method for treating cancer in a subject, the method comprising administering an ADC to the subject,

wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer,

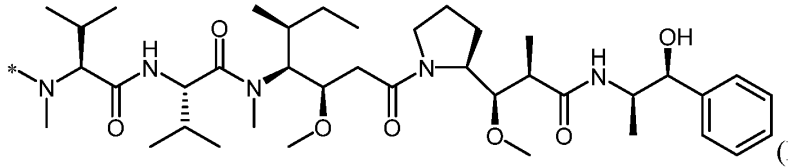
wherein the ADC is produced by conjugating an antibody (Ab) to a linker-drug comprising a pre-conjugate linker **(L-1a')** and a monomethyl auristatin E (MMAE):



(L-1a'),

wherein the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:



(III),

wherein * indicates the point of attachment to **(L-1a')**;

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises:

(1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and

(2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; and

wherein the Ab further comprises one or more *fGly'* and wherein *fGly'* is a formylglycine amino acid residue to which linker (**L-1a'**) is attached after conjugating the Ab and the linker-drug. In some embodiments, the Ab prior to conjugating to the linker-drug comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 41 and a light chain comprising an amino acid sequence of SEQ ID NO: 3.

[00224] In any of the embodiments and aspects as disclosed herein, one Ab is conjugated to one to ten linker-drugs.

[00225] In any of the embodiments and aspects as disclosed herein, one Ab is conjugated to two linker-drugs.

[00226] In any of the embodiments and aspects as disclosed herein, the Ab comprises one to ten *fGly'*.

[00227] In any of the embodiments and aspects as disclosed herein, the Ab comprises two *fGly'*.

[00228] In any of the embodiments and aspects as disclosed herein, the Ab comprises a motif of X^1(*fGly'*)$X^2Z^2X^3Z^3$ (SEQ ID NO: 67),

wherein:

Z^2 is either a proline (P) or alanine (A) residue;

Z^3 is a basic amino acid residue optionally selected from the group consisting of: arginine (R), lysine (K), and histidine (H), or an aliphatic amino acid residue optionally selected from the group consisting of: alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), and proline (P);

X^1 is present or absent and, when present, is any amino acid residue, with the proviso that when the sequence of SEQ ID NO: 67 is at the N-terminus of the antibody Ab, X^1 is present; and

X^2 and X^3 independently is any amino acid residue,

optionally wherein,

the motif of SEQ ID NO: 67 is selected from the group consisting of: L(*fGly'*)TPSR (SEQ ID NO: 1), M(*fGly'*)TPSR (SEQ ID NO: 45), V(*fGly'*)TPSR (SEQ ID NO: 46), L(*fGly'*)SPSR (SEQ ID NO: 47), L(*fGly'*)APSR (SEQ ID NO: 48), L(*fGly'*)VPSR (SEQ ID NO: 49), L(*fGly'*)GPSR (SEQ ID NO: 50), I(*fGly'*)TPAR (SEQ ID NO: 51), L(*fGly'*)TPSK (SEQ ID NO: 52), M(*fGly'*)TPSK (SEQ ID NO: 53), V(*fGly'*)TPSK (SEQ ID NO: 54), L(*fGly'*)SPSK (SEQ ID NO: 55), L(*fGly'*)APSK (SEQ ID NO: 56), L(*fGly'*)VPSK (SEQ ID NO: 57), L(*fGly'*)GPSK (SEQ ID NO: 58),

L(*fGly'*)TPSA (SEQ ID NO: 59), I(*fGly'*)TPAA (SEQ ID NO: 60), M(*fGly'*)TPSA (SEQ ID NO: 61), V(*fGly'*)TPSA (SEQ ID NO: 62), L(*fGly'*)SPSA (SEQ ID NO: 63), L(*fGly'*)APSA (SEQ ID NO: 64), L(*fGly'*)VPSA (SEQ ID NO: 65), and L(*fGly'*)GPSA (SEQ ID NO: 66).

[00229] In any of the embodiments and aspects as disclosed herein, the Ab comprises two heavy chains and two light chains, and wherein each of the heavy chains comprises a motif of SEQ ID NO: 67.

[00230] In any of the embodiments and aspects as disclosed herein, the Ab comprises an amino acid sequence of L(*fGly'*)TPSR (SEQ ID NO: 1).

[00231] In any of the embodiments and aspects as disclosed herein, the Ab comprises two heavy chains and two light chains, and wherein each of the heavy chains comprises a motif of L(*fGly'*)TPSR (SEQ ID NO: 1)

[00232] In any of the embodiments and aspects as disclosed herein, the *fGly'* of the motif is located in the heavy chain at a position corresponding to the 453rd amino acid residue of SEQ ID NO: 2 (also referred to herein as C453).

[00233] In any of the embodiments and aspects as disclosed herein,

(1) HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 19, 24, 25, and 30,

(2) HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 20, 26, 31, and 36,

(3) HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 21, 27, and 32,

(4) LCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22, 28, and 33,

(5) LCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 23, and 34, and

(6) LCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 29, and 35.

[00234] In any of the embodiments and aspects as disclosed herein,

(i) HCDR1 comprises the amino acid sequence of SEQ ID NO: 13, HCDR2 comprises the amino acid sequence of SEQ ID NO: 14, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO:

16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18; or

(ii) HCDR1 comprises the amino acid sequence of SEQ ID NO: 19, HCDR2 comprises the amino acid sequence of SEQ ID NO: 20, HCDR3 comprises the amino acid sequence of SEQ ID NO: 21, LCDR1 comprises the amino acid sequence of SEQ ID NO: 22, LCDR2 comprises the amino acid sequence of SEQ ID NO: 23, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18; or

(iii) HCDR1 comprises the amino acid sequence of SEQ ID NO: 24, HCDR2 comprises the amino acid sequence of SEQ ID NO: 14, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18; or

(iv) HCDR1 comprises the amino acid sequence of SEQ ID NO: 25, HCDR2 comprises the amino acid sequence of SEQ ID NO: 26, HCDR3 comprises the amino acid sequence of SEQ ID NO: 27, LCDR1 comprises the amino acid sequence of SEQ ID NO: 28, LCDR2 comprises the amino acid sequence of SEQ ID NO: 23, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 29; or

(v) HCDR1 comprises the amino acid sequence of SEQ ID NO: 30, HCDR2 comprises the amino acid sequence of SEQ ID NO: 31, HCDR3 comprises the amino acid sequence of SEQ ID NO: 32, LCDR1 comprises the amino acid sequence of SEQ ID NO: 33, LCDR2 comprises the amino acid sequence of SEQ ID NO: 34, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 35; or

(vi) HCDR1 comprises the amino acid sequence of SEQ ID NO: 13, HCDR2 comprises the amino acid sequence of SEQ ID NO: 36, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18.

[00235] In any of the embodiments and aspects as disclosed herein, the Ab comprises a VH comprising the amino acid sequence of SEQ ID NO: 11 and a VL comprising the amino acid sequence of SEQ ID NO: 12.

[00236] In any of the embodiments and aspects as disclosed herein, the Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 2 and a light chain comprising the amino acid sequence of SEQ ID NO: 3. Accordingly, the Ab prior to being conjugated to a linker

or linker-drug comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 41 and a light chain comprising the amino acid sequence of SEQ ID NO: 3.

[00237] In any of the embodiments and aspects as disclosed herein, the cancer is triple-negative breast cancer (TNBC) or hormone-receptor positive breast cancer (HR+ BC).

[00238] In any of the embodiments and aspects as disclosed herein, the cancer is pancreatic ductal adenocarcinoma (PDAC).

[00239] In any of the embodiments and aspects as disclosed herein, the cancer is non-small cell lung cancer (NSCLC).

[00240] In any of the embodiments and aspects as disclosed herein, the cancer is head and neck squamous cell carcinoma (HNSCC).

[00241] In any of the embodiments and aspects as disclosed herein, the method further comprises administrating to the subject means for inhibiting interaction between PD-1 and PD-L1. Exemplary means for inhibiting interaction between PD-1 and PD-L1 include a PD-L1/PD-1 inhibitor as disclosed herein, such as a pembrolizumab.

[00242] In any of the embodiments and aspects as disclosed herein, the ADC is administered concurrently with, after, or before the means for inhibiting interaction between PD-1 and PD-L1.

[00243] In any of the embodiments and aspects as disclosed herein, the method further comprises administering to the subject a PD-L1/PD-1 inhibitor.

[00244] In any of the embodiments and aspects as disclosed herein, the ADC is administered concurrently with, after, or before the PD-L1/PD-1 inhibitor.

[00245] In any of the embodiments and aspects as disclosed herein, the PD-L1/PD-1 inhibitor is an antibody or an antigen binding fragment thereof comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 of pembrolizumab.

[00246] In any of the embodiments and aspects as disclosed herein, the PD-L1/PD-1 inhibitor is pembrolizumab.

[00247] In any of the embodiments and aspects as disclosed herein, the PD-L1/PD-1 inhibitor is administered to the subject at a dose of about 200 mg.

[00248] In any of the embodiments and aspects as disclosed herein, the PD-L1/PD-1 inhibitor is administered intravenously.

[00249] In any of the embodiments and aspects as disclosed herein, the PD-L1/PD-1 inhibitor is administered once every 3 weeks (q3w).

[00250] In any of the embodiments and aspects as disclosed herein, the ADC is administered intravenously.

[00251] In any of the embodiments and aspects as disclosed herein, the ADC is administered once every 3 weeks (q3w).

[00252] In any of the embodiments and aspects as disclosed herein, the subject is human.

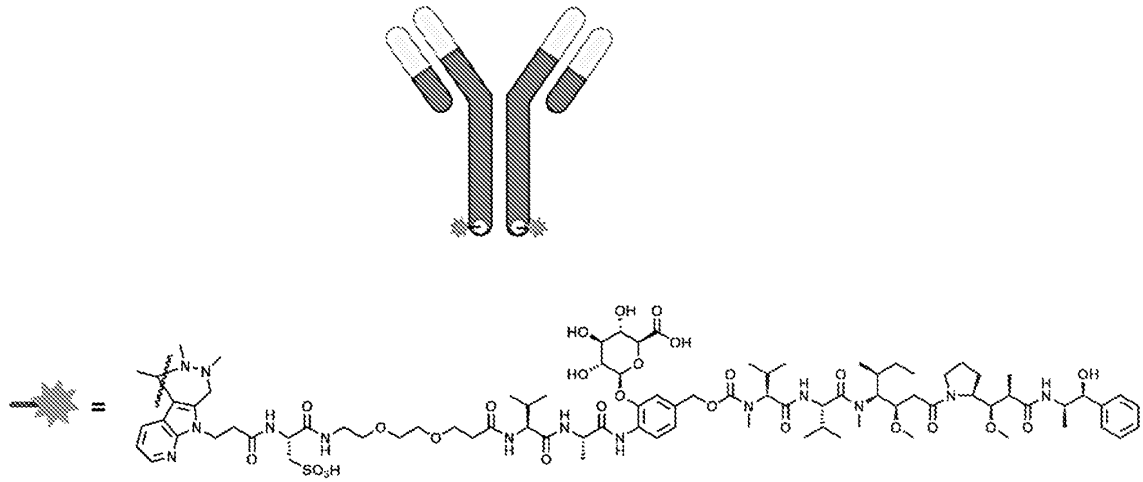
[00253] In any of the embodiments and aspects as disclosed herein, the ADC also refers to its stereoisomer, solvate, or salt.

[00254] In any of the embodiments and aspects as disclosed herein, a pharmaceutical composition comprising an ADC as described and a pharmaceutically acceptable excipient is administered to the subject. In further embodiments, the pharmaceutical composition comprises a multiplicity of DAR species of the ADC. In some embodiments, the average DAR of the pharmaceutical composition is about 1 to about 2. In further embodiments, the average DAR of the pharmaceutical composition is 1.8. In some embodiments, the pharmaceutical composition exhibits a DAR of about 1 to about 2. In further embodiments, the pharmaceutical composition exhibits a DAR of 1.8.

[00255] In any of the embodiments and aspects as disclosed herein, a pharmaceutical composition comprising a first ADC as described and a pharmaceutically acceptable excipient is administered to the subject. In some embodiments, the pharmaceutical composition comprises a second ADC as disclosed herein, wherein the two ADCs are different in the number of drugs, such as MMAEs, directly or indirectly conjugated to each Ab. In yet further embodiments, two drugs, such as MMAEs, are directly or indirectly conjugated to the Ab in the first ADC and one drug, such as MMAE, is directly or indirectly conjugated to the Ab in the second ADC. In some embodiments, such a pharmaceutical composition exhibits a drug-to-antibody ratio ("DAR") of about 1 to about 2. In further embodiments, the pharmaceutical composition exhibits a DAR of 1.8. In yet further embodiments, each 5T4 antibody is conjugated to one or two linker-drugs.

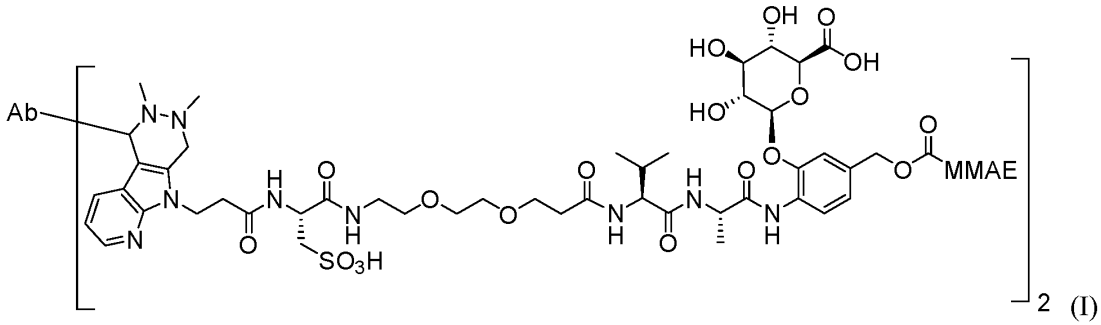
[00256] In any of the embodiments and aspects as disclosed herein, a population of the ADCs is administered to the subject. In further embodiments, such an ADC population exhibits a drug-to-antibody ratio ("DAR") of about 1 to about 2. In yet further embodiments, the ADC population exhibits a DAR of 1.8. In yet further embodiments, each 5T4 antibody is conjugated to one or two linker-drugs.

[00257] In some embodiments, the ADCs as disclosed herein (such as **5T4-ADC-1**) are represented by the illustration below. In further embodiments, the wavy line in the lower panel separates the Ab (illustrated as four bars forming a Y shape in the upper panel) and the linker-drug (illustrated as a solid star in both panels).



[00258] The present disclosure provides methods of treating cancer in a subject comprising administering a therapeutically effective dose of a 5T4 ADC as disclosed herein, such as an ADC of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (for example, **5T4-ADC-1)**.

[00259] In one embodiment, the 5T4 ADC is represented by **Formula (I)** below:

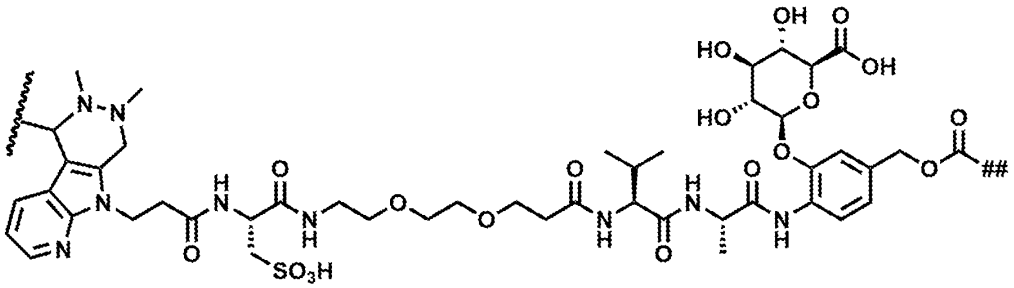


(I)

wherein Ab is a 5T4 antibody or an antigen binding fragment thereof. In one embodiment, the 5T4 ADC comprises a 5T4 antibody or an antigen binding fragment thereof, wherein the 5T4 antibody or antigen binding fragment thereof comprises a VH region, VL region, heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and/or a light chain complementarity determining region 3 (LCDR3) as described in **Table 1**. In one embodiment, the Ab is a 5T4 antibody as disclosed herein. In one embodiment, the 5T4 ADC comprises a 5T4 antibody comprising a VH region comprising the amino acid sequence of SEQ ID NO: 11 and a VL region comprising the amino acid sequence of

SEQ ID NO: 12. In one embodiment, the 5T4 antibody comprises a light chain having an amino acid sequence of:
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3).

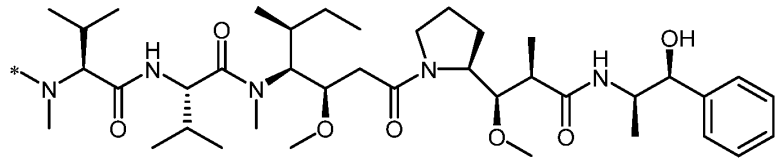
[00260] In one embodiment, a linker-drug comprising linker (**L-1**) is conjugated to each of the heavy chain constant regions in the 5T4 antibody (*e.g.*, the 5T4 antibody is conjugated to two linker-drugs). In one embodiment, a linker-drug comprising linker (**L-1**) is conjugated to each of the heavy chains in the 5T4 antibody (*e.g.*, the 5T4 antibody is conjugated to two linker-drugs). In one embodiment, a linker-drug comprising linker (**L-1**) is conjugated to the CH3 region in each of the heavy chain constant regions in the 5T4 antibody. In one embodiment, a linker-drug comprising linker (**L-1**) is conjugated to the CH3 region in each of the heavy chains in the 5T4 antibody. In a further embodiment, a linker-drug comprising linker (**L-1**) is conjugated to the aldehyde in the formylglycine at C453 of each heavy chain of an anti-5T4 aldehyde-containing antibody. In such embodiments, each 5T4 ADC comprises two MMAE drugs. A composition comprising such ADCs will exhibit a DAR of 2. In some embodiments, a 5T4 ADC comprises a 5T4 antibody comprising a light chain comprising the amino acid sequence of
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3) and a heavy chain having an amino acid sequence of:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL*n*TP
SRGS (SEQ ID NO: 42), wherein *n* is an amino acid conjugated to (*e.g.*, by having its side chain replaced by) a linker-drug comprising linker (**L-1**):



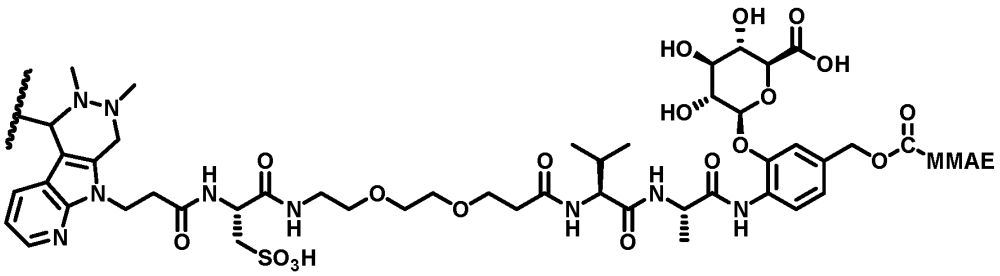
(**L-1**),

wherein ~~~ represents the point of conjugation to Ab and ## indicates the point of attachment to MMAE; and

wherein the MMAE is represented by **Formula (III)**:

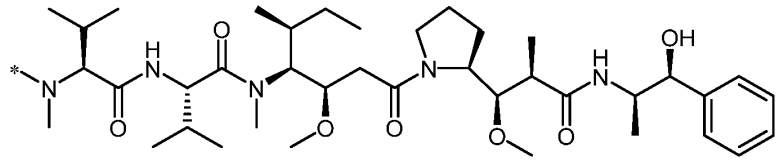


(**III**), wherein * indicates the point of attachment to (**L-1**). In some embodiments, the amino acid *n* in SEQ ID NO: 42 refers to *fGly'* as described herein. In some embodiments, *n* is an amino acid having its side chain replaced by **Formula (II)**:



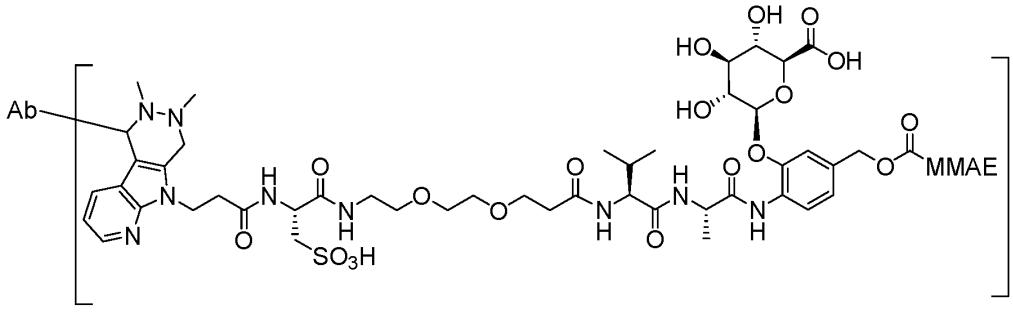
(**II**),

wherein the wavy line indicates the point of attachment to the Ab and MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment in **Formula (II)**.

[00261] In one embodiment, the ADC is represented by **Formula (I)**:

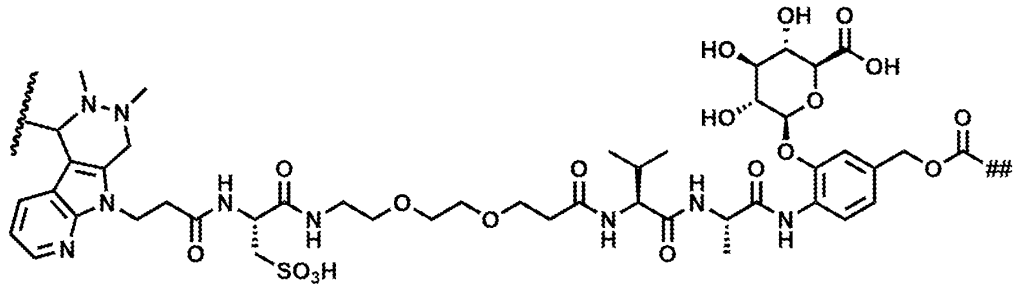


(I).

wherein Ab is a 5T4 antibody comprising:

a) a heavy chain having an amino acid sequence of:

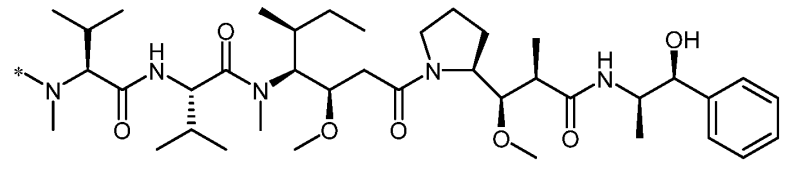
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL*n*TP
SRGS (SEQ ID NO: 42), wherein *n* is the amino acid to which a linker-drug comprising linker (**L-1**), as described herein, is attached:



(**L-1**)

wherein the wavy line indicates the point of attachment to the Ab at (*fGly'*) and ## indicates the point of attachment to the MMAE; and

wherein the MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment to linker (**L-1**); and

b) a light chain having an amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3). In some embodiments, the amino acid *n* in SEQ ID NO: 42 refers to *fGly'* as described herein.

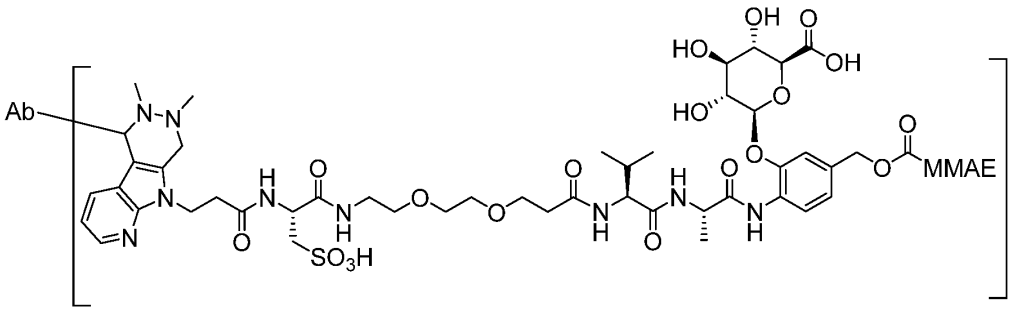
[00262] In some embodiments, a therapeutically effective dose of a PD-L1 inhibitor is administered to the subject in combination with the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**). In one embodiment, the PD-L1 inhibitor is pembrolizumab. In one embodiment, the therapeutically effective dose of the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered to the subject in a pharmaceutical composition, as described herein in Section 7.3.

[00263] In one embodiment, the cancer is a cancer selected from the group consisting of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, or bladder cancer. In one embodiment, the cancer is TNBC. In one embodiment, the cancer is HR+ BC. In one embodiment, the cancer is PDAC. In certain embodiments, the cancer is lung adenocarcinoma or squamous cell lung carcinoma. In one embodiment, the cancer is NSCLC. In some embodiments, the cancer is head and neck cancer. In one embodiment, the cancer is HNSCC. In one embodiment, the NSCLC is characterized by a mutation selected from the group consisting of an EGFR sensitizing mutation, an ALK rearrangement, a ROS1 rearrangement, a BRAF V600E mutation, a MET Exon 14 skipping mutation, and a RET rearrangement. In certain embodiments, the cancer is renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer. In some embodiments, the cancer is a genitourinary cancer. In some embodiments, the cancer is a gastrointestinal cancer. In some embodiments, the cancer is a gynecological cancer. Additionally or alternatively, the cancer is previously untreatable. Additionally or alternatively, the cancer is recurrent. Additionally or alternatively, the cancer is metastatic. Additionally or alternatively, the cancer is advanced, for example locally advanced. Additionally or alternatively, the cancer is inoperable.

[00264] A number of therapeutically effective administration and dosing regimens for treating cancer in a subject are contemplated and described herein below.

[00265] In one aspect, provided is a method for treating a cancer in a subject comprising administering an ADC as disclosed herein, *e.g.*, according to any embodiment in Section 7.3, to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg. In one embodiment, the ADC is

represented by **Formula (I)** and comprises an Ab conjugated to a monomethyl auristatin E (MMAE) by linker (**L-1**) as disclosed herein:



(I).

[00266] In further embodiments, the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**. In some embodiments, the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1). In further embodiments, *fGly'* is the amino acid residue to which a linker-drug comprising linker (**L-1**), as disclosed herein, is attached. In some embodiments, **mAbA15** comprises: a) a heavy chain comprising an amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL

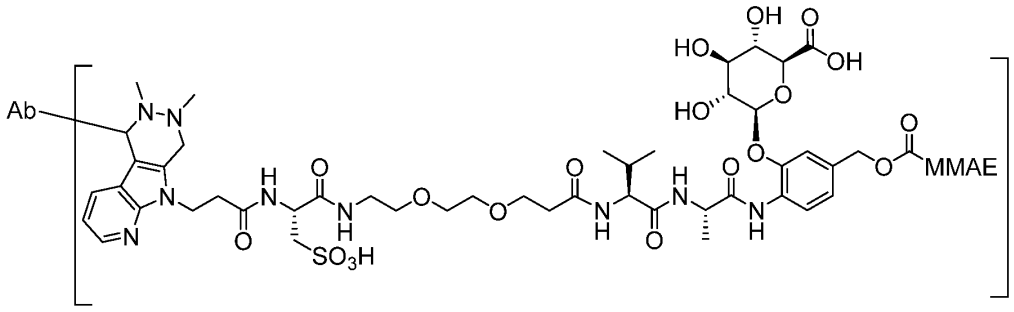
(*fGly'*)TPSRGS (SEQ ID NO: 2); and b) a light chain comprising an amino acid sequence of:

[00267] DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSG
VPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3). In some embodiments, the ADC is **5T4-ADC-1**. In some embodiments, the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, or bladder cancer. In certain embodiments, the cancer is pancreatic adenocarcinoma (PDAC, also

referred to herein as pancreatic ductal adenocarcinoma). In certain embodiments, the cancer is lung adenocarcinoma or squamous cell lung carcinoma. In certain embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the cancer is head and neck cancer. In certain embodiments, the cancer is head and neck squamous cell carcinoma (HNSCC). In certain embodiments, the cancer is renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer. In some embodiments, the cancer is a genitourinary cancer. In some embodiments, the cancer is a gastrointestinal cancer. In some embodiments, the cancer is a gynecological cancer. Additionally or alternatively, the cancer is previously untreatable. Additionally or alternatively, the cancer is recurrent. Additionally or alternatively, the cancer is metastatic. Additionally or alternatively, the cancer is advanced, for example locally advanced. Additionally or alternatively, the cancer is inoperable.

[00268] Also provided herein is a method for treating cancer in a subject comprising administering a 5T4 ADC as disclosed herein to the subject. In some embodiments, the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, or bladder cancer. In certain embodiments, the cancer is pancreatic adenocarcinoma (PDAC, also referred to herein as pancreatic ductal adenocarcinoma). In certain embodiments, the cancer is lung adenocarcinoma or squamous cell lung carcinoma. In certain embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the cancer is head and neck cancer. In certain embodiments, the cancer is head and neck squamous cell carcinoma (HNSCC). In certain embodiments, the cancer is renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer. In some embodiments, the cancer is a genitourinary cancer. In some embodiments, the cancer is a gastrointestinal cancer. In some embodiments, the cancer is a gynecological cancer. Additionally or alternatively, the cancer is previously untreatable. Additionally or alternatively, the cancer is recurrent. Additionally or alternatively, the cancer is metastatic. Additionally or alternatively, the cancer is advanced, for example locally advanced. Additionally or alternatively, the cancer is inoperable.

[00269] In some embodiments, the ADC is represented by **Formula (I)** and comprises an Ab conjugated to a monomethyl auristatin E (MMAE) by linker (**L-1**):



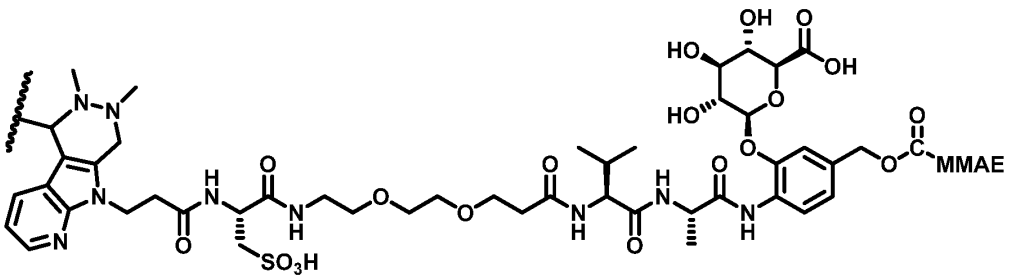
(I)

In further embodiments, the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**. In some embodiments, the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1). In further embodiments, *fGly'* is the amino acid residue to which a linker-drug comprising (**L-1**) is attached. In some embodiments, **mAbA15** comprises: a) a heavy chain comprising an amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL

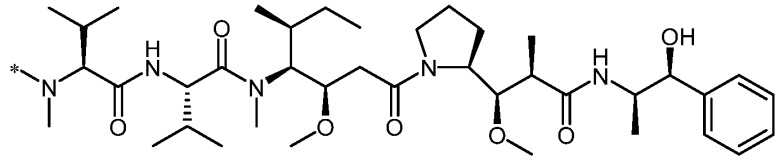
(*fGly'*)TPSRGS (SEQ ID NO: 2); and b) a light chain comprising an amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3). Additionally or alternatively, *fGly'* is attached to a linker-drug of **Formula (II)**:



(**II**),

wherein the wavy line indicates the point of attachment to the Ab and MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment in **Formula (II)**. In some embodiments, the ADC is **5T4-ADC-1**. In further embodiments, the ADC is administered to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg.

[00270] In some embodiments, the Ab comprises two heavy chains (HCs) and two light chains (LCs), and each of the HCs comprises one motif of L(*fGly'*)TPSR (SEQ ID NO: 1). In some embodiments, the Ab is **mAbA15**.

[00271] In some embodiments, the ADC is administered to the subject on the first day of a treatment cycle for one or more treatment cycles. In further embodiments, each treatment cycle is 3 weeks ± 3 days.

[00272] In some embodiments, the ADC is administered to the subject once every three weeks (q3w).

[00273] In some embodiments, the ADC is administered to the subject intravenously.

[00274] In some embodiments, a method as disclosed herein further comprises administering to the subject a PD-L1/PD-1 inhibitor. Additionally or alternatively, the subject is administered a PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered concurrently with the PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered after the PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered before the PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered on the same day with the PD-L1/PD-1 inhibitor. In some embodiments, the administration of the ADC and the PD-L1/PD-1 inhibitor is at least about 30 minutes apart or at least about 1 hour apart. In some embodiments, the PD-L1/PD-1 inhibitor is administered to the subject on the first day of a treatment cycle for one or more treatment cycles. In further embodiments, each treatment cycle is 3 weeks ± 3 days. In some embodiments,

the PD-L1/PD-1 inhibitor is administered to the subject once every three weeks (q3w). In some embodiments, the PD-L1/PD-1 inhibitor is administered to the subject intravenously. In some embodiments, the PD-L1/PD-1 inhibitor is an antibody or an antigen binding fragment thereof comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 of pembrolizumab. In further embodiments, the PD-L1/PD-1 inhibitor is pembrolizumab. In some embodiments, the PD-L1/PD-1 inhibitor is administered to the subject at a dose of about 200 mg.

[00275] In some embodiments, the ADC as disclosed herein is administered to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 0.1 mg/kg to about 9.5 mg/kg, about 0.1 mg/kg to about 9.0 mg/kg, about 0.1 mg/kg to about 8.5 mg/kg, about 0.1 mg/kg to about 8.0 mg/kg, about 0.1 mg/kg to about 7.5 mg/kg, about 0.1 mg/kg to about 7.0 mg/kg, about 0.1 mg/kg to about 6.5 mg/kg, about 0.1 mg/kg to about 6.0 mg/kg, about 0.1 mg/kg to about 5.5 mg/kg, about 0.1 mg/kg to about 5.0 mg/kg, about 0.1 mg/kg to about 4.5 mg/kg, about 0.1 mg/kg to about 4.0 mg/kg, about 0.1 mg/kg to about 3.5 mg/kg, about 0.1 mg/kg to about 3.0 mg/kg, about 0.1 mg/kg to about 2.5 mg/kg, about 0.1 mg/kg to about 2.0 mg/kg, about 0.1 mg/kg to about 1.5 mg/kg, about 0.1 mg/kg to about 1.0 mg/kg, about 0.1 mg/kg to about 0.5 mg/kg, or about 0.1 mg/kg to about 0.3 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 0.3 mg/kg to about 9.5 mg/kg, about 0.3 mg/kg to about 9.0 mg/kg, about 0.3 mg/kg to about 8.5 mg/kg, about 0.3 mg/kg to about 8.0 mg/kg, about 0.3 mg/kg to about 7.5 mg/kg, about 0.3 mg/kg to about 7.0 mg/kg, about 0.3 mg/kg to about 6.5 mg/kg, about 0.3 mg/kg to about 6.0 mg/kg, about 0.3 mg/kg to about 5.5 mg/kg, about 0.3 mg/kg to about 5.0 mg/kg, about 0.3 mg/kg to about 4.5 mg/kg, about 0.3 mg/kg to about 4.0 mg/kg, about 0.3 mg/kg to about 3.5 mg/kg, about 0.3 mg/kg to about 3.0 mg/kg, about 0.3 mg/kg to about 2.5 mg/kg, about 0.3 mg/kg to about 2.0 mg/kg, about 0.3 mg/kg to about 1.5 mg/kg, about 0.3 mg/kg to about 1.0 mg/kg, or about 0.3 mg/kg to about 0.5 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 0.5 mg/kg to about 9.5 mg/kg, about 0.5 mg/kg to about 9.0 mg/kg, about 0.5 mg/kg to about 8.5 mg/kg, about 0.5 mg/kg to about 8.0 mg/kg, about 0.5 mg/kg to about 7.5 mg/kg, about 0.5 mg/kg to about 7.0 mg/kg, about 0.5 mg/kg to about 6.5 mg/kg, about 0.5 mg/kg to about 6.0 mg/kg, about 0.5 mg/kg to about 5.5 mg/kg, about 0.5 mg/kg to about 5.0 mg/kg, about 0.5 mg/kg to about 4.5 mg/kg, about 0.5 mg/kg to about 4.0 mg/kg, about 0.5 mg/kg to about 3.5 mg/kg, about 0.5 mg/kg to about 3.0 mg/kg, about 0.5 mg/kg to about 2.5 mg/kg, about 0.5 mg/kg to about 2.0 mg/kg, about 0.5 mg/kg to about 1.5 mg/kg, or about 0.5 mg/kg to about 1.0 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 1.0 mg/kg to about 9.5 mg/kg, about 1.0 mg/kg to about 9.0

mg/kg, about 1.0 mg/kg to about 8.5 mg/kg, about 1.0 mg/kg to about 8.0 mg/kg, about 1.0 mg/kg to about 7.5 mg/kg, about 1.0 mg/kg to about 7.0 mg/kg, about 1.0 mg/kg to about 6.5 mg/kg, about 1.0 mg/kg to about 6.0 mg/kg, about 1.0 mg/kg to about 5.5 mg/kg, about 1.0 mg/kg to about 5.0 mg/kg, about 1.0 mg/kg to about 4.5 mg/kg, about 1.0 mg/kg to about 4.0 mg/kg, about 1.0 mg/kg to about 3.5 mg/kg, about 1.0 mg/kg to about 3.0 mg/kg, about 1.0 mg/kg to about 2.5 mg/kg, about 1.0 mg/kg to about 2.0 mg/kg, or about 1.0 mg/kg to about 1.5 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 1.5 mg/kg to about 9.5 mg/kg, about 1.5 mg/kg to about 9.0 mg/kg, about 1.5 mg/kg to about 8.5 mg/kg, about 1.5 mg/kg to about 8.0 mg/kg, about 1.5 mg/kg to about 7.5 mg/kg, about 1.5 mg/kg to about 7.0 mg/kg, about 1.5 mg/kg to about 6.5 mg/kg, about 1.5 mg/kg to about 6.0 mg/kg, about 1.5 mg/kg to about 5.5 mg/kg, about 1.5 mg/kg to about 5.0 mg/kg, about 1.5 mg/kg to about 4.5 mg/kg, about 1.5 mg/kg to about 4.0 mg/kg, about 1.5 mg/kg to about 3.5 mg/kg, about 1.5 mg/kg to about 3.0 mg/kg, about 1.5 mg/kg to about 2.5 mg/kg, or about 1.5 mg/kg to about 2.0 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 2.0 mg/kg to about 9.5 mg/kg, about 2.0 mg/kg to about 9.0 mg/kg, about 2.0 mg/kg to about 8.5 mg/kg, about 2.0 mg/kg to about 8.0 mg/kg, about 2.0 mg/kg to about 7.5 mg/kg, about 2.0 mg/kg to about 7.0 mg/kg, about 2.0 mg/kg to about 6.5 mg/kg, about 2.0 mg/kg to about 6.0 mg/kg, about 2.0 mg/kg to about 5.5 mg/kg, about 2.0 mg/kg to about 5.0 mg/kg, about 2.0 mg/kg to about 4.5 mg/kg, about 2.0 mg/kg to about 4.0 mg/kg, about 2.0 mg/kg to about 3.5 mg/kg, about 2.0 mg/kg to about 3.0 mg/kg, or about 2.0 mg/kg to about 2.5 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 2.5 mg/kg to about 9.5 mg/kg, about 2.5 mg/kg to about 9.0 mg/kg, about 2.5 mg/kg to about 8.5 mg/kg, about 2.5 mg/kg to about 8.0 mg/kg, about 2.5 mg/kg to about 7.5 mg/kg, about 2.5 mg/kg to about 7.0 mg/kg, about 2.5 mg/kg to about 6.5 mg/kg, about 2.5 mg/kg to about 6.0 mg/kg, about 2.5 mg/kg to about 5.5 mg/kg, about 2.5 mg/kg to about 5.0 mg/kg, about 2.5 mg/kg to about 4.5 mg/kg, about 2.5 mg/kg to about 4.0 mg/kg, about 2.5 mg/kg to about 3.5 mg/kg, or about 2.5 mg/kg to about 3.0 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 3.0 mg/kg to about 9.5 mg/kg, about 3.0 mg/kg to about 9.0 mg/kg, about 3.0 mg/kg to about 8.5 mg/kg, about 3.0 mg/kg to about 8.0 mg/kg, about 3.0 mg/kg to about 7.5 mg/kg, about 3.0 mg/kg to about 7.0 mg/kg, about 3.0 mg/kg to about 6.5 mg/kg, about 3.0 mg/kg to about 6.0 mg/kg, about 3.0 mg/kg to about 5.5 mg/kg, about 3.0 mg/kg to about 5.0 mg/kg, about 3.0 mg/kg to about 4.5 mg/kg, about 3.0 mg/kg to about 4.0 mg/kg, or about 3.0 mg/kg to about 3.5 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 3.5 mg/kg to about 9.5 mg/kg, about 3.5 mg/kg to about 9.0

mg/kg, about 3.5 mg/kg to about 8.5 mg/kg, about 3.5 mg/kg to about 8.0 mg/kg, about 3.5 mg/kg to about 7.5 mg/kg, about 3.5 mg/kg to about 7.0 mg/kg, about 3.5 mg/kg to about 6.5 mg/kg, about 3.5 mg/kg to about 6.0 mg/kg, about 3.5 mg/kg to about 5.5 mg/kg, about 3.5 mg/kg to about 5.0 mg/kg, about 3.5 mg/kg to about 4.5 mg/kg, or about 3.5 mg/kg to about 4.0 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 4.0 mg/kg to about 9.5 mg/kg, about 4.0 mg/kg to about 9.0 mg/kg, about 4.0 mg/kg to about 8.5 mg/kg, about 4.0 mg/kg to about 8.0 mg/kg, about 4.0 mg/kg to about 7.5 mg/kg, about 4.0 mg/kg to about 7.0 mg/kg, about 4.0 mg/kg to about 6.5 mg/kg, about 4.0 mg/kg to about 6.0 mg/kg, about 4.0 mg/kg to about 5.5 mg/kg, about 4.0 mg/kg to about 5.0 mg/kg, or about 4.0 mg/kg to about 4.5 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 4.5 mg/kg to about 9.5 mg/kg, about 4.5 mg/kg to about 9.0 mg/kg, about 4.5 mg/kg to about 8.5 mg/kg, about 4.5 mg/kg to about 8.0 mg/kg, about 4.5 mg/kg to about 7.5 mg/kg, about 4.5 mg/kg to about 7.0 mg/kg, about 4.5 mg/kg to about 6.5 mg/kg, about 4.5 mg/kg to about 6.0 mg/kg, about 4.5 mg/kg to about 5.5 mg/kg, or about 4.5 mg/kg to about 5.0 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 5.0 mg/kg to about 9.5 mg/kg, about 5.0 mg/kg to about 9.0 mg/kg, about 5.0 mg/kg to about 8.5 mg/kg, about 5.0 mg/kg to about 8.0 mg/kg, about 5.0 mg/kg to about 7.5 mg/kg, about 5.0 mg/kg to about 7.0 mg/kg, about 5.0 mg/kg to about 6.5 mg/kg, about 5.0 mg/kg to about 6.0 mg/kg, about 5.0 mg/kg to about 5.5 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 5.5 mg/kg to about 9.5 mg/kg, about 5.5 mg/kg to about 9.0 mg/kg, about 5.5 mg/kg to about 8.5 mg/kg, about 5.5 mg/kg to about 8.0 mg/kg, about 5.5 mg/kg to about 7.5 mg/kg, about 5.5 mg/kg to about 7.0 mg/kg, about 5.5 mg/kg to about 6.5 mg/kg, or about 5.5 mg/kg to about 6.0 mg/kg. In some embodiments, the ADC is administered to the subject at a dose of about 6.0 mg/kg to about 9.5 mg/kg, about 6.0 mg/kg to about 9.0 mg/kg, about 6.0 mg/kg to about 8.5 mg/kg, about 6.0 mg/kg to about 8.0 mg/kg, about 6.0 mg/kg to about 7.5 mg/kg, about 6.0 mg/kg to about 7.0 mg/kg, or about 6.0 mg/kg to about 6.5 mg/kg.

[00276] In some embodiments, the ADC as disclosed herein is administered to the subject at a dose of about 0.3 mg/kg to about 6.0 mg/kg.

[00277] In some embodiments, the ADC as disclosed herein is administered to the subject at a dose of about 0.3 mg/kg to about 2.0 mg/kg.

[00278] In some embodiments, the ADC as disclosed herein is administered to the subject at a dose of about 0.3 mg/kg, about 0.6 mg/kg, about 1.0 mg/kg, about 1.5 mg/kg, about 2.0 mg/kg, about 2.5 mg/kg, about 3.0 mg/kg, about 3.5 mg/kg, about 4.0 mg/kg, about 4.5 mg/kg, about 5.0

mg/kg, about 5.5 mg/kg, about 6.0 mg/kg, about 6.5 mg/kg, about 7.0 mg/kg, about 7.5 mg/kg, about 8.0 mg/kg, about 8.5 mg/kg, about 9.0 mg/kg, about 9.5 mg/kg, about 10.0 mg/kg.

[00279] As it would be understood by one of skill in the art, an equivalent dose, such as a dose as disclosed herein converted to ADC amount per body surface area, is also included herein. Methods of such a conversion can be found, for example, Nair AB, Jacob S. J Basic Clin Pharm. 2016 Mar;7(2):27-31, which is incorporated herein by reference in its entirety.

[00280] In some embodiments, the cancer is triple-negative breast cancer (TNBC) or hormone-receptor positive breast cancer (HR+ BC).

[00281] In some embodiments, the cancer is pancreatic adenocarcinoma (PDAC, also referred to herein as pancreatic ductal adenocarcinoma).

[00282] In some embodiments, the cancer is prostate cancer.

[00283] In some embodiments, the cancer is cervical cancer.

[00284] In some embodiments, the cancer is bladder cancer.

[00285] In some embodiments, the cancer is colorectal cancer.

[00286] In some embodiments, the cancer is non-small cell lung cancer (NSCLC). In some embodiments, the subject has one or more mutations selected from the group consisting of an EGFR sensitizing mutation, an ALK rearrangement, a ROS1 rearrangement, a BRAF V600E mutation, a MET Exon 14 skipping mutation, and a RET rearrangement. In some embodiments, the cancer of the subject has one or more mutations selected from the group consisting of an EGFR sensitizing mutation, an ALK rearrangement, a ROS1 rearrangement, a BRAF V600E mutation, a MET Exon 14 skipping mutation, and a RET rearrangement. In certain embodiments, the cancer is lung adenocarcinoma or squamous cell lung carcinoma.

[00287] In some embodiments, the cancer is head and neck squamous cell carcinoma (HNSCC).

[00288] In some embodiments, the ADC as disclosed herein is administered to the subject for about four to about twenty-four months. In some embodiments, the ADC is administered to the subject for about four to about eighteen months. In some embodiments, the ADC is administered to the subject for about four to about twelve months. In some embodiments, the ADC is administered to the subject for about four to about six months.

[00289] In some embodiments, the subject is human.

7.4.1 Monotherapy

[00290] In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**)

is administered at a therapeutically effective dose to a subject as a single agent (*e.g.*, not in combination with any other chemotherapeutic agents) to treat a cancer. In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered periodically to a subject (*e.g.*, once every treatment cycle) at a therapeutically effective dose, when the periodic administration is continued for a length of time ("length of treatment") and/or a certain number of treatment cycles.

[00291] In one embodiment, a method for treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 0.3 mg/kg to about 5.0 mg/kg for one or more treatment cycles. In one embodiment, each dose of the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered to the subject on the first day of each treatment cycle. Additionally or alternatively, each treatment cycle only contains one administration. In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered to the subject intravenously. In one embodiment, each treatment cycle is 3 weeks ± 3 days. Additionally or alternatively, the subject undergoes 1, or 2, or 3 or more treatment cycles and receives one administration on the first day of each treatment cycle. In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered to the subject at least 19 days apart. In one embodiment, a method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 0.3 mg/kg to about 3.5 mg/kg of the ADC. In one embodiment, a method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 1 mg/kg to about 3.5 mg/kg of the ADC. In one embodiment, a method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 0.3 mg/kg to about 2.0 mg/kg of the ADC. In one embodiment, a method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating

(L-1a') (such as **5T4-ADC-1**) to the subject at a dose of about 0.3 mg/kg of the ADC. In one embodiment, a method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 0.6 mg/kg of the ADC. In one embodiment, a method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 1 mg/kg of the ADC. In one embodiment, a method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 1.5 mg/kg of the ADC. In one embodiment, a method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 2 mg/kg of the ADC. As it would be understood by one of skill in the art, an equivalent dose, such as a dose as disclosed herein converted to ADC amount per body surface area, is also included herein. Methods of such a conversion can be found, for example, Nair AB, Jacob S. J Basic Clin Pharm. 2016 Mar;7(2):27-31, which is incorporated herein by reference in its entirety. In one embodiment, the length of treatment during which the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a'),** is administered to the subject is about four to about twenty-four months. In one embodiment, the length of treatment is about four to about eighteen months. In one embodiment, the length of treatment is about four to about twelve months. In one embodiment, the length of treatment is about four to about six months.

[00292] In one embodiment, the cancer treated by the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is a cancer selected from the group consisting of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, or bladder cancer. In one embodiment, the cancer is TNBC. In one embodiment, the cancer is HR+ BC. In one embodiment, the cancer is PDAC. In certain embodiments, the cancer is lung adenocarcinoma or squamous cell lung carcinoma. In one embodiment, the cancer is NSCLC. In some embodiments, the cancer is head and neck cancer. In one embodiment, the cancer is HNSCC. In one embodiment, the NSCLC is characterized by a mutation selected from the group consisting of an EGFR sensitizing mutation, an ALK rearrangement, a ROS1 rearrangement, a BRAF V600E mutation, a MET Exon 14 skipping mutation, and a RET

rearrangement. In certain embodiments, the cancer is renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer. In some embodiments, the cancer is a genitourinary cancer. In some embodiments, the cancer is a gastrointestinal cancer. In some embodiments, the cancer is a gynecological cancer. Additionally or alternatively, the cancer is previously untreatable. Additionally or alternatively, the cancer is recurrent. Additionally or alternatively, the cancer is metastatic. Additionally or alternatively, the cancer is advanced, for example locally advanced. Additionally or alternatively, the cancer is inoperable.

7.4.2 Combination Therapy

[00293] In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered at a therapeutically effective dose to a subject in combination with a therapeutically effective dose of a second chemotherapeutic agent to treat a cancer. In one embodiment, the second chemotherapeutic agent is a PD-1/PD-L1 inhibitor. In one embodiment, the PD-1/PD-L1 inhibitor is pembrolizumab. In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) and the second therapeutic agent, *e.g.*, a PD-1/PD-L1 inhibitor or pembrolizumab, is administered periodically to the subject (*e.g.*, once every treatment cycle) at a therapeutically effective dose, when the periodic administration is continued for a certain length of time ("length of treatment") and/or certain number of treatment cycles. In one embodiment, 200 mg of pembrolizumab is administered to the subject in combination with a therapeutically effective amount of the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**).

[00294] In one embodiment, a method for treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 0.3 mg/kg to about 5.0 mg/kg and a PD-1/PD-L1 inhibitor, such as 200 mg pembrolizumab, for one or more treatment cycles. In one embodiment, each dose of the ADC is administered to the subject on the first day of each treatment cycle. In one embodiment, the PD-1/PD-L1 inhibitor, *e.g.*, 200 mg pembrolizumab, is administered to the subject on the same day of each treatment cycle as the ADC. In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered to the subject intravenously. In one embodiment, pembrolizumab is

administered to the subject intravenously. In one embodiment, each treatment cycle is 3 weeks ± 3 days. In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered to the subject at least 19 days apart. In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered to the subject at a dose of about 0.3 mg/kg to about 3.5 mg/kg. In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered to the subject at a dose of about 1 mg/kg to about 3.5 mg/kg. In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered to the subject at a dose of about 0.3 mg/kg to about 2.0 mg/kg. In one embodiment, the method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 0.3 mg/kg of the ADC. In one embodiment, the method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 0.6 mg/kg of the ADC. In one embodiment, the method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 1 mg/kg of the ADC. In one embodiment, the method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 1.5 mg/kg of the ADC. In one embodiment, the method of treating cancer in a subject comprises administering the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) to the subject at a dose of about 2 mg/kg of the ADC. As it would be understood by one of skill in the art, an equivalent dose, such as a dose as disclosed herein converted to ADC amount per body surface area, is also included herein. Methods of such a conversion can be found, for example, Nair AB, Jacob S. J Basic Clin Pharm. 2016 Mar;7(2):27-31, which is incorporated herein by reference in its entirety. In one embodiment, the length of treatment during which the ADC (such as **5T4-ADC-1**) is administered to the subject is about four to about twenty-four months. In one embodiment, the length of treatment is about four to

about eighteen months. In one embodiment, the length of treatment is about four to about twelve months. In one embodiment, the length of treatment is about six months.

[00295] In one embodiment, the cancer treated by the combination of the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) and pembrolizumab is a cancer selected from the group consisting of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, or bladder cancer. In one embodiment, the cancer is TNBC. In one embodiment, the cancer is HR+ BC. In one embodiment, the cancer is PDAC. In certain embodiments, the cancer is lung adenocarcinoma or squamous cell lung carcinoma. In one embodiment, the cancer is NSCLC. In some embodiments, the cancer is head and neck cancer. In one embodiment, the cancer is HNSCC. In one embodiment, the NSCLC is characterized by a mutation selected from the group consisting of an EGFR sensitizing mutation, an ALK rearrangement, a ROS1 rearrangement, a BRAF V600E mutation, a MET Exon 14 skipping mutation, and a RET rearrangement. In certain embodiments, the cancer treated by the combination of the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) and pembrolizumab is a cancer selected from the group consisting of renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer. In some embodiments, the cancer is a genitourinary cancer. In some embodiments, the cancer is a gastrointestinal cancer. In some embodiments, the cancer is a gynecological cancer. Additionally or alternatively, the cancer is previously untreatable. Additionally or alternatively, the cancer is recurrent. Additionally or alternatively, the cancer is metastatic. Additionally or alternatively, the cancer is advanced, for example locally advanced. Additionally or alternatively, the cancer is inoperable.

7.4.3 Adverse Events

[00296] A subject that has been administered the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) and/or pembrolizumab according to any dosing regimen disclosed herein (*e.g.*, any as disclosed in any of sections 7.4.1 through 7.4.2) may, in any embodiment, undergo monitoring for adverse events (AEs). A subject may experience one or more treatment-related AEs due to the ADC and/or pembrolizumab. As such, it may be prudent to adjust the dose of the ADC and/or discontinue administration of the ADC and/or pembrolizumab until the subject recovers from the adverse event.

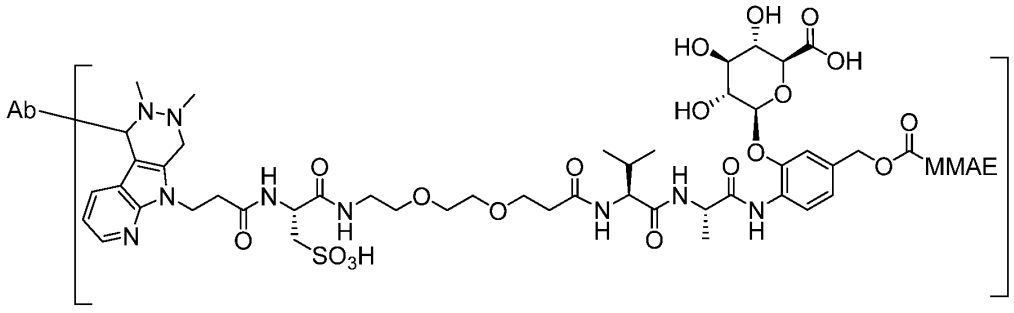
[00297] AEs are characterized as one of five grades—grade 1 is a mild AE; grade 2 is a moderate AE; grade 3 is a severe AE; grade 4 is a life-threatening or disabling AE; and grade 5 is death related to AE. The grading of AEs is based on the guidelines provided in the CTCAE version 6.0. Non-limiting examples of adverse events that may prompt dose adjustment and/or discontinuation of administration include Grade 3 thrombocytopenia with clinically significant bleeding, Grade 4 thrombocytopenia, Grade 3 or higher neutropenia with a measured fever of at least 38.3°C, Grade 3 or higher neutropenia with a sustained fever of at least 38°C for at least an hour, Grade 4 neutropenia for greater than 7 days, Grade 4 anemia, alanine transaminase or aspartate transaminase levels that are greater than 3 times the upper limit of normal and a total bilirubin level that is greater than 2 times the upper limit of normal in the absence of biliary obstruction or other explanation for liver abnormality (such as viral hepatitis, alcohol or autoimmune hepatitis, Gilbert Syndrome, or treatment with other hepatotoxic drug), and any Grade 5 adverse event.

[00298] In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered at a therapeutically effective dose optionally in combination with pembrolizumab for one or more treatment cycles to a subject to treat a cancer according to any dosing regimen disclosed herein (*e.g.*, any as disclosed in any of sections 7.4.1 through 7.4.2) and the subject experiences an AE. Administration of the ADC to the subject is then interrupted until such time as the subject has recovered from the AE, at which time the administration of the ADC is resumed. In some embodiments, when the subject is administered the ADC in combination with pembrolizumab, only administration of the ADC is interrupted until such time as the subject has recovered from the AE, at which time the administration of the ADC is resumed.

[00299] In one embodiment, the ADC as disclosed herein, for example those of **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')** (such as **5T4-ADC-1**) is administered at a therapeutically effective dose optionally in combination with pembrolizumab for one or more treatment cycles to a subject to treat a cancer according to any dosing regimen disclosed herein (*e.g.*, any as disclosed in any of sections 7.4.1 through 7.4.2) and the subject experiences an AE. The dose of the ADC is then reduced in one or more subsequent treatment cycles until such time as the subject has recovered from the AE, at which time the administration of the ADC at the dose that was administered prior to the AE is resumed. In some embodiments, when the subject is administered the ADC in combination with pembrolizumab, the dose of the ADC is reduced until such time as the subject has recovered from the AE, at which time the administration of the ADC at the dose that was administered prior to the AE is resumed.

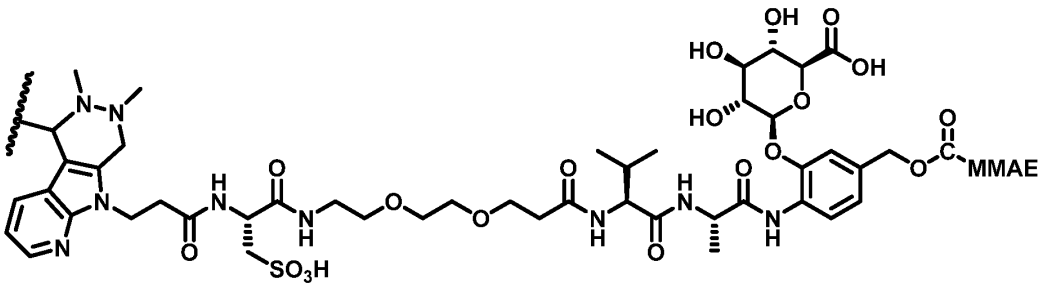
[00300] Recovery from the AE may be assessed by measurements, such as by measure of subject's body temperature, alanine transaminase or aspartate transaminase levels, total bilirubin level, and/or blood counts (*e.g.*, absolute neutrophil count (ANC) and/or platelet count). For example, a subject may be considered to have recovered from an AE when their ANC reaches a certain threshold, *e.g.*, ≥ 1,500 cells/μL, ≥ 1,000 cells/μL, or ≥ 500 cells/μL, and/or their platelet count reaches a certain threshold, *e.g.*, ≥ 100,000 cells/μL, ≥ 75,000 cells/μL, ≥ 50,000 cells/μL, or ≥ 25,000 cells/μL, for up to 14 days.

[00301] In one aspect, provided is a method of treating a cancer for two or more treatment cycles in a subject experiencing an adverse event when administered an ADC. The method comprises: (i) administering the ADC to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg in a first treatment cycle; (ii) delaying a further administration of the ADC to the subject upon the subject having an adverse event after the first treatment cycle and before the second treatment cycle; and (iii) resuming the administration of the ADC in a second treatment cycle. In some embodiments, the ADC is as disclosed herein, for example those represented by **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')**. Additionally or alternatively, the ADC comprises an Ab conjugated to a monomethyl auristatin E (MMAE) by linker (**L-1**).



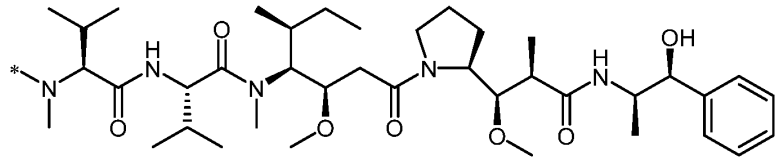
In some embodiments, the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**. In some embodiments, the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which a linker-drug comprising linker (**L-1**) is attached. In some embodiments, **mAbA15** comprises: a) a heavy chain comprising an amino acid sequence of: EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK

GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL
(*fGly'*)TPSRGS (SEQ ID NO: 2); and b) a light chain comprising an amino acid sequence of:
DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3). In some embodiments, the Ab is **mAbA15**. Additionally or alternatively, *fGly'* is the amino acid residue to which a linker-drug of **Formula (II)** is attached:



(**II**),

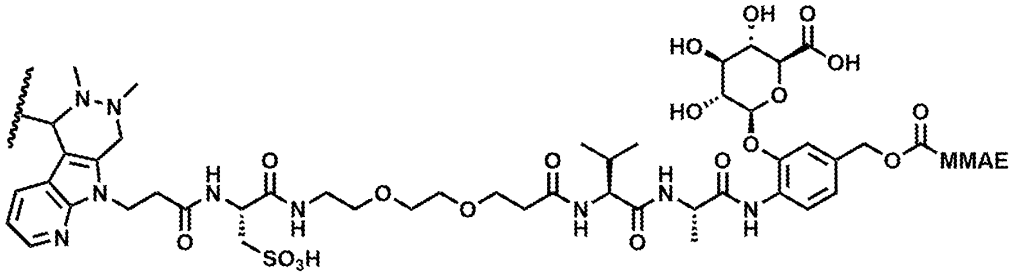
wherein the wavy line indicates the point of attachment to the Ab and MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment in **Formula (II)**. In some embodiments, the ADC is **5T4-ADC-1**. In some embodiments, the administration is resumed in step (iii) once the adverse event is alleviated or recovered. In some embodiments, the administration of the second treatment cycle is at the dose of the first treatment cycle. In some embodiments, the administration of the second treatment cycle is at a dose lower than that of the first treatment cycle.

[00302] In one aspect, provided is a method of treating a cancer for two or more treatment cycles in a subject experiencing an adverse event when administered an ADC. The method comprises: i) administering the ADC to the subject at a first dose of about 0.1 mg/kg to about 10.0 mg/kg in a first treatment cycle; ii) in a second treatment cycle, administering the ADC to

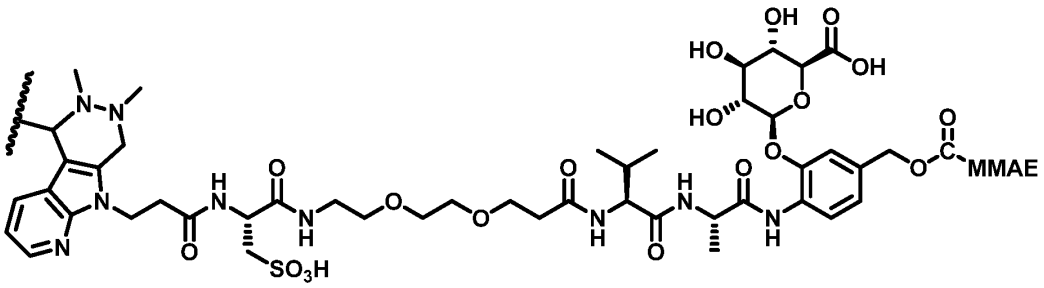
the subject at a second dose which is lower than the first dose upon the subject having an adverse event. In some embodiments, the ADC is as disclosed herein, for example those represented by **Formula (I)** or **Formula (I')**, or comprising **(L-1)**, or produced by conjugating **(L-1a')**. In some embodiments, the ADC comprises an Ab conjugated to a monomethyl auristatin E (MMAE) by linker (**L-1**) (also illustrated as **(L-1')**).



(L-I')

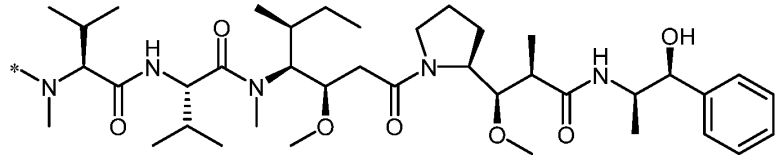
In some embodiments, the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**. In some embodiments, the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which a linker-drug comprising linker (**L-1**) is attached. In some embodiments, **mAbA15** comprises: a) a heavy chain comprising an amino acid sequence of: EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL (*fGly'*)TPSRGS (SEQ ID NO: 2); and b) a light chain comprising an amino acid sequence of: DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3). In some embodiments, the Ab is **mAbA15**.

Additionally or alternatively, *fGly'* is the amino acid residue to which a linker-drug of **Formula (II)** is attached:



(**II**),

wherein the wavy line indicates the point of attachment to the Ab and MMAE is represented by **Formula (III)**:



(**III**), wherein * indicates the point of attachment in **Formula (II)**. In some embodiments, the ADC is **5T4-ADC-1**. In some embodiments, the subject is administered at the second dose in step (ii) once the adverse event is alleviated or recovered. In some embodiments, the subject is administered with the ADC once every 3 weeks (q3w).

[00303] In some embodiments, the adverse event is selected from the group consisting of Grade 3 thrombocytopenia with clinically significant bleeding, Grade 4 thrombocytopenia, Grade 3 or higher neutropenia with a measured fever of at least 38.3°C, Grade 3 or higher neutropenia with a sustained fever of at least 38°C for at least an hour, Grade 4 neutropenia for greater than 7 days, Grade 4 anemia, alanine transaminase or aspartate transaminase levels that are greater than 3 times the upper limit of normal and a total bilirubin level that is greater than 2 times the upper limit of normal in the absence of biliary obstruction or other explanation for liver abnormality (such as viral hepatitis, alcohol or autoimmune hepatitis, Gilbert Syndrome, or treatment with other hepatotoxic drug), and a Grade 5 adverse event.

[00304] In some embodiments, the Ab comprises two heavy chains (HCs) and two light chains (LCs), and wherein each of the HCs comprises one motif of L(*fGly'*)TPSR (SEQ ID NO: 1). In some embodiments, the Ab is **mAbA15**.

[00305] In some embodiments, the ADC is administered to the subject on the first day of each treatment cycle. In further embodiments, each treatment cycle is 3 weeks ± 3 days.

[00306] In some embodiments, the ADC is administered to the subject intravenously.

[00307] In some embodiments, the method further comprises administering to the subject a PD-L1/PD-1 inhibitor. In some embodiments, the subject is administered with a PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered concurrently with the PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered after the PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered before the PD-L1/PD-1 inhibitor. In some embodiments, the ADC is administered on the same day with the PD-L1/PD-1 inhibitor. In some embodiments, the administrations of the ADC and the PD-L1/PD-1 inhibitor is at least about 30 minutes apart or at least about 1 hour apart. In some embodiments, the PD-L1/PD-1 inhibitor is administered to the subject on the first day of each treatment cycle. In some embodiments, the PD-L1/PD-1 inhibitor is administered to the subject once every three weeks (q3w). In some embodiments, the PD-L1/PD-1 inhibitor is administered to the subject intravenously. In some embodiments, the PD-L1/PD-1 inhibitor is an antibody or an antigen binding fragment thereof comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 of pembrolizumab. In some embodiments, the PD-L1/PD-1 inhibitor is pembrolizumab. In some embodiments, the PD-L1/PD-1 inhibitor is administered to the subject at a dose of about 200 mg.

[00308] In some embodiments, the ADC is administered to the subject in the first treatment cycle at a dose of about 0.3 mg/kg to about 6.0 mg/kg. In some embodiments, the ADC is administered to the subject in the first treatment cycle at a dose of about 0.3 mg/kg to about 2.0 mg/kg. In some embodiments, the ADC is administered to the subject in the first treatment cycle at a dose of about 0.3 mg/kg, about 0.6 mg/kg, about 1.0 mg/kg, about 1.5 mg/kg, or about 2.0 mg/kg.

[00309] In some embodiments, the cancer is breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, or bladder cancer. In some embodiments, the cancer is triple-negative breast cancer (TNBC) or hormone-receptor positive breast cancer (HR+ BC). In some embodiments, the cancer is pancreatic adenocarcinoma (PDAC, also referred to herein as pancreatic ductal adenocarcinoma). In certain embodiments, the cancer is lung adenocarcinoma or squamous cell lung carcinoma. In some embodiments, the cancer is non-small cell lung cancer (NSCLC). In further embodiments, the subject has one or more mutations selected from the group consisting of an EGFR sensitizing mutation, an ALK rearrangement, a ROS1 rearrangement, a BRAF V600E mutation, a MET Exon 14 skipping mutation, and a RET rearrangement. In some embodiments, the cancer of the subject has one or more mutations selected from the group consisting of an EGFR sensitizing mutation, an ALK rearrangement, a ROS1 rearrangement, a BRAF V600E mutation, a MET Exon 14 skipping mutation, and a RET rearrangement. In some embodiments, the cancer is head and neck cancer.

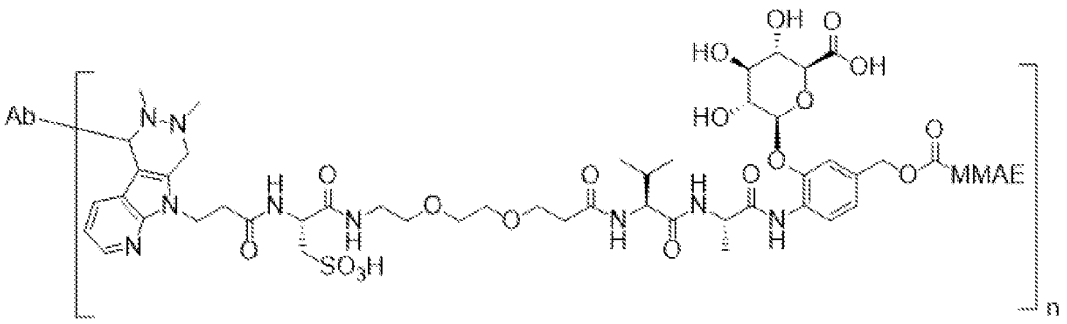
In some embodiments, the cancer is head and neck squamous cell carcinoma (HNSCC). In certain embodiments, the cancer is renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer. In some embodiments, the cancer is a genitourinary cancer. In some embodiments, the cancer is a gastrointestinal cancer. In some embodiments, the cancer is a gynecological cancer. Additionally or alternatively, the cancer is previously untreatable. Additionally or alternatively, the cancer is recurrent. Additionally or alternatively, the cancer is metastatic. Additionally or alternatively, the cancer is advanced, for example locally advanced. Additionally or alternatively, the cancer is inoperable.

[00310] In some embodiments, the ADC is administered to the subject for about four to about twenty-four months. In some embodiments, the ADC is administered to the subject for about four to about eighteen months. In some embodiments, the ADC is administered to the subject for about four to about twelve months. In some embodiments, the ADC is administered to the subject for about four to about six months.

[00311] In some embodiments, the subject is human.

8. EMBODIMENTS

[00312] 1. A method for treating cancer in a subject, the method comprising administering an ADC to the subject, wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer; wherein the ADC is represented by **Formula (I')**:



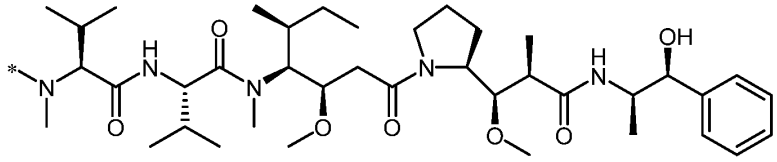
(I'),

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the

amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12;

wherein the Ab further comprises one or more *fGly'*, wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I')** is attached;

wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



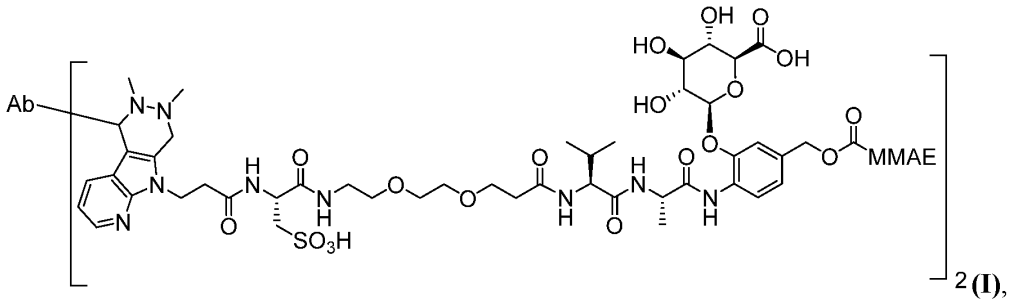
(III),

wherein * indicates the point of attachment in **Formula (I')**; and

wherein n is an integer.

[00313] 2. The method of embodiment 1, wherein n is an integer selected from any one of 1 to 10.

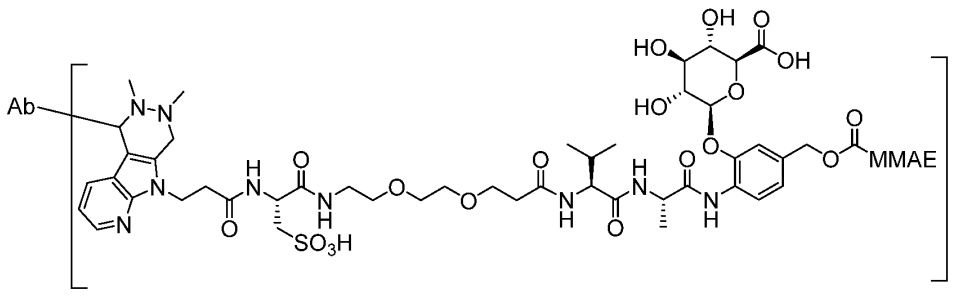
[00314] 3. The method of embodiment 1 or 2, wherein n is 2 and wherein the ADC is represented by **Formula (I):**



(I),

wherein the * in **Formula (III)** indicates the point of attachment in **Formula (I)** and wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I)** is attached.

[00315] 4. A method for treating cancer in a subject, the method comprising administering an ADC to the subject, wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer, wherein the ADC is represented by **Formula (I)**:



(I)

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which the non-Ab part of the ADC represented by **Formula (I)** is attached, and wherein **mAbA15** comprises:

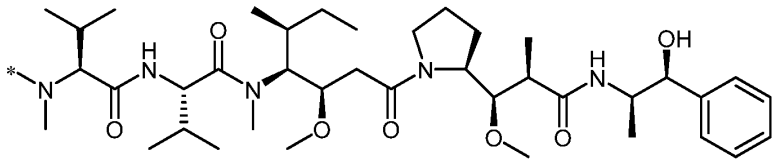
a) a heavy chain comprising an amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEYADSVK
GRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKC
KVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(*fGl*
y')TPSRGS (SEQ ID NO: 2); and

b) a light chain comprising an amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPSRFSGS
RSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE
VTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3); and

wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



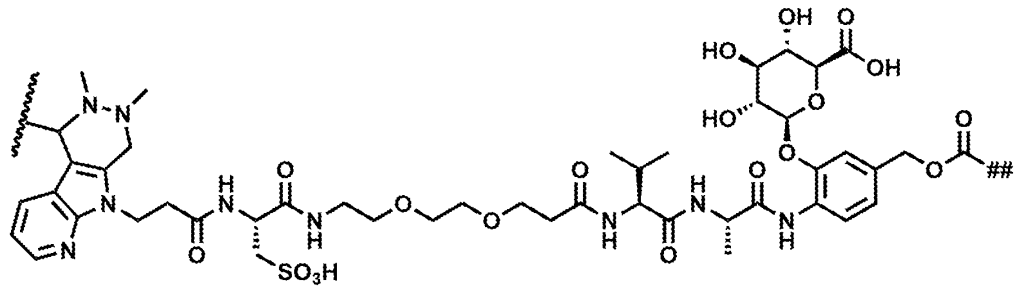
(**III**),

wherein * indicates the point of attachment in **Formula (I)**.

[00316] 5. A method for treating cancer in a subject, the method comprising administering an ADC to the subject,

wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer;

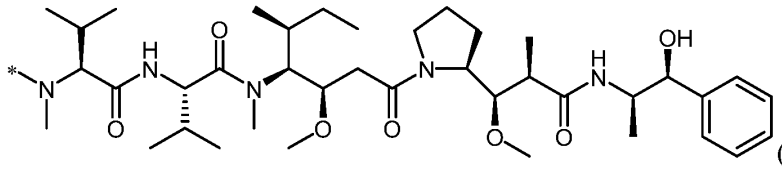
wherein the ADC comprises an antibody (Ab) conjugated to a monomethyl auristatin E (MMAE) by a linker (**L-1**):



(**L-1**),

wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:



(**III**),

wherein * indicates the point of attachment to (**L-1**);

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light

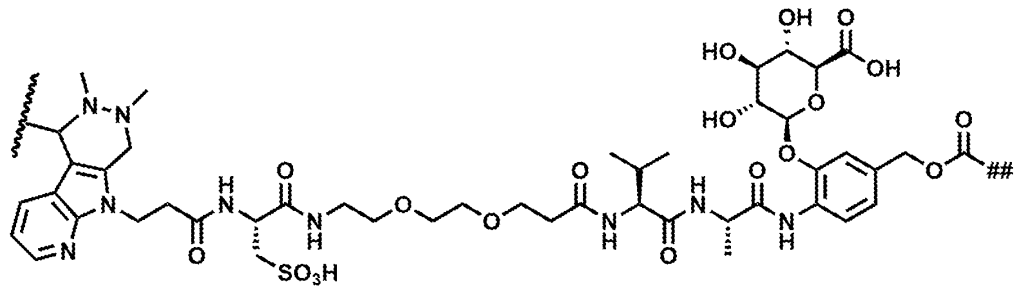
chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; and

wherein the Ab further comprises one or more *fGly'* and wherein *fGly'* is a formylglycine amino acid residue to which linker (**L-1**) is attached.

[00317] 6. The method of embodiment 5, wherein one Ab is conjugated to one to ten MMAEs and wherein each MMAE is conjugated to Ab by one linker (**L-1**).

[00318] 7. The method of embodiment 5 or 6, wherein one Ab is conjugated to two MMAEs and wherein each MMAE is conjugated to Ab by one linker (**L-1**).

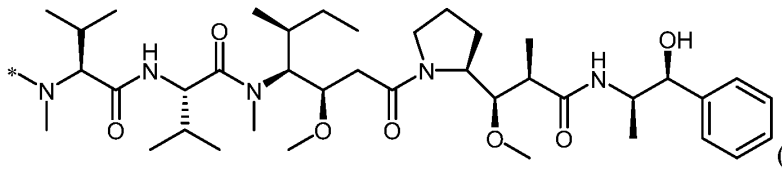
[00319] 8. A method for treating cancer in a subject, the method comprising administering an ADC to the subject, wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer, wherein the ADC comprises an antibody (Ab) conjugated to a monomethyl auristatin E (MMAE) by a linker (**L-1**):



(**L-1**),

wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:



(**III**),

wherein * indicates the point of attachment to (**L-1**); and

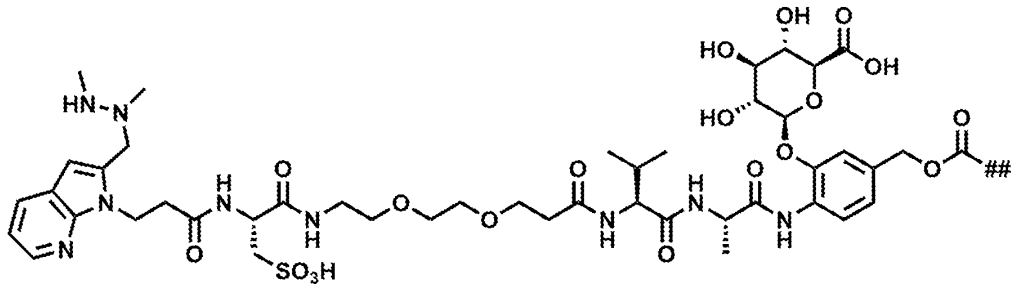
wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3

(LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which linker (**L-1**) is attached, and wherein **mAbA15** comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 2 and a light chain comprising an amino acid sequence of SEQ ID NO: 3.

[00320] 9. A method for treating cancer in a subject, the method comprising administering an ADC to the subject,

wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer,

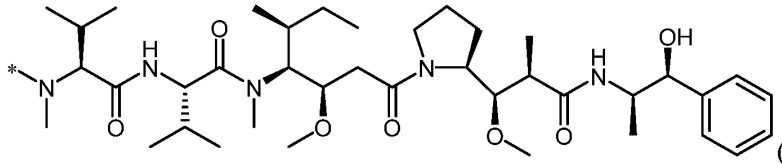
wherein the ADC is produced by conjugating an antibody (Ab) to a linker-drug comprising a pre-conjugate linker **(L-1a')** and a monomethyl auristatin E (MMAE):



(L-1a'),

wherein the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:



(III),

wherein * indicates the point of attachment to (**L-1a'**);

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light

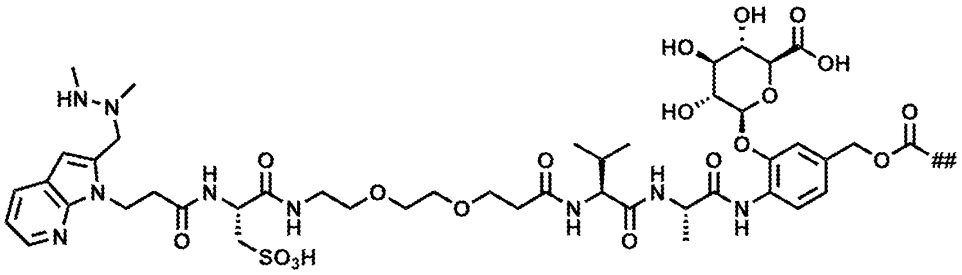
chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; and

wherein the Ab further comprises one or more *fGly'* and wherein *fGly'* is a formylglycine amino acid residue to which linker (**L-1a'**) is attached after conjugating the Ab and the linker-drug.

[00321] 10. The method of embodiment 9, wherein one Ab is conjugated to one to ten linker-drugs.

[00322] 11. The method of embodiment 9 or 10, wherein one Ab is conjugated to two linker-drugs.

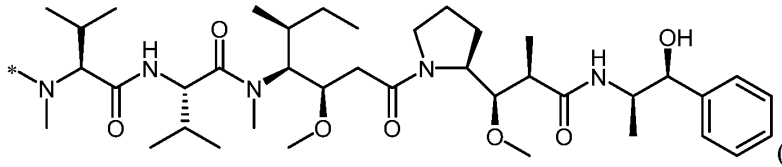
[00323] 12. A method for treating cancer in a subject, the method comprising administering an ADC to the subject, wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer, wherein the ADC is produced by conjugating an antibody (Ab) to a linker-drug comprising a pre-conjugate linker **(L-1a')** and a monomethyl auristatin E (MMAE):



(L-1a'),

wherein the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:



(III),

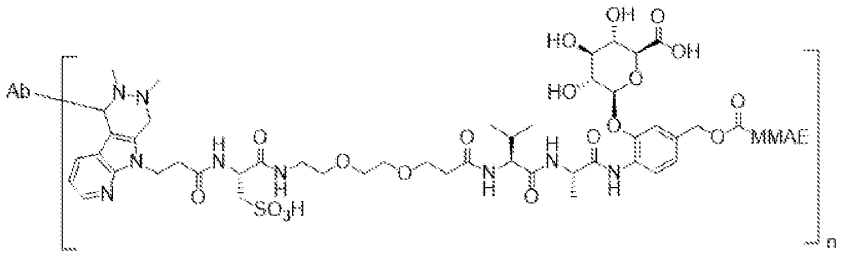
wherein * indicates the point of attachment to (**L-1a'**); and

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to

which (**L-1a'**) is attached after the conjugation, and wherein **mAbA15** comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 2 and a light chain comprising an amino acid sequence of SEQ ID NO: 3.

[00324] 13. The method of any one of embodiments 1-12, wherein the ADC is administered to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg.

[00325] 14. A method for treating cancer in a subject, the method comprising administering an ADC to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg, wherein the ADC is represented by **Formula (I')**:

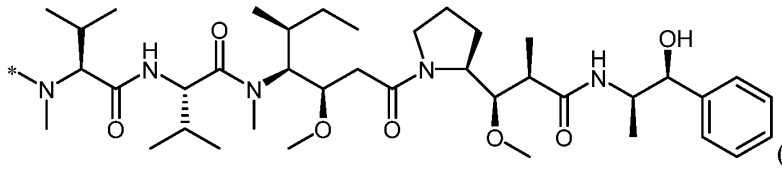


(I'),

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12;

wherein the Ab further comprises one or more *fGly'*, wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I')** is attached;

wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



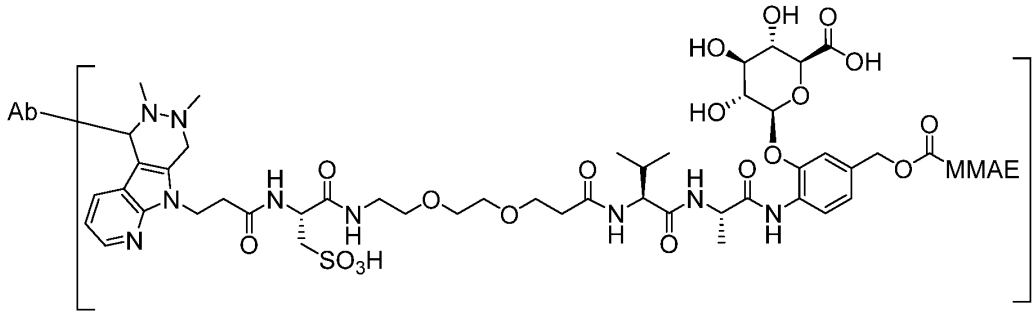
(III),

wherein * indicates the point of attachment in **Formula (I')**; and

wherein n is an integer.

[00326] 15. The method of embodiment 14, wherein n is an integer selected from any one of 1 to 10.

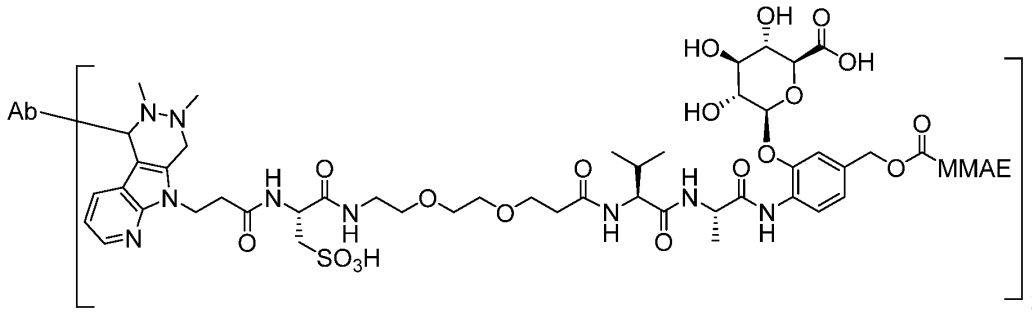
[00327] 16. The method of embodiment 14 or 15, wherein n is 2 and wherein the ADC is represented by **Formula (I):**



(I),

wherein the * in **Formula (III)** indicates the point of attachment in **Formula (I)** and wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I)** is attached.

[00328] 17. A method for treating cancer in a subject, the method comprising administering an ADC to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg, wherein the ADC is represented by **Formula (I)**:



(I),

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which the non-Ab part of the ADC represented by **Formula (I)** is attached, and wherein **mAbA15** comprises:

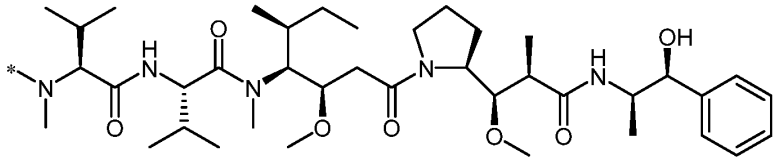
a) a heavy chain comprising an amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEY
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(*fGly*')TPSRGS (SEQ ID NO: 2); and

b) a light chain comprising an amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPS
RFSGSRSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3); and

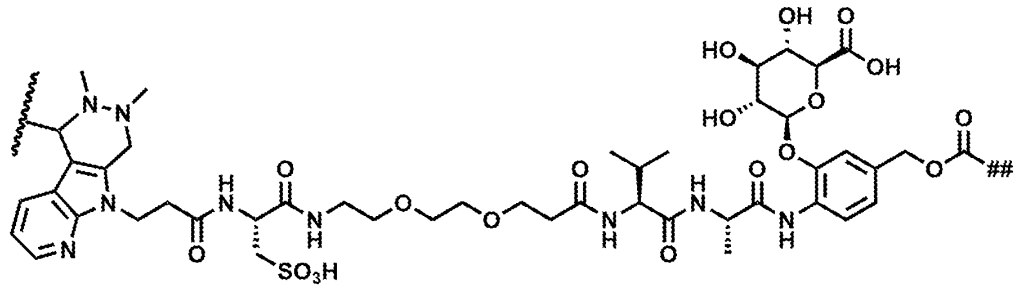
wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



(III),

wherein * indicates the point of attachment in **Formula (I)**.

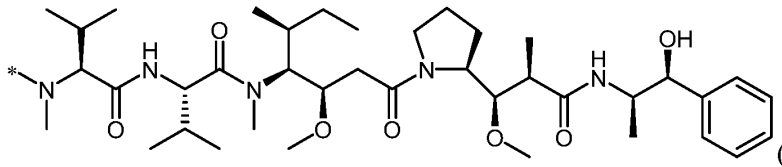
[00329] 18. A method for treating cancer in a subject, the method comprising administering an ADC to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg, wherein the ADC comprises an antibody (Ab) conjugated to a monomethyl auristatin E (MMAE) by a linker (**L-1**):



(L-1),

wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:



(**III**),

wherein * indicates the point of attachment to (**L-1**);

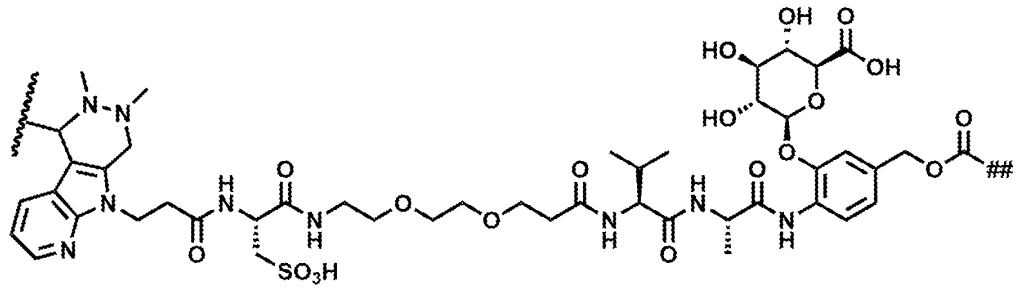
wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; and

wherein the Ab further comprises one or more *fGly'* and wherein *fGly'* is a formylglycine amino acid residue to which linker (**L-1**) is attached.

[00330] 19. The method of embodiment 18, wherein one Ab is conjugated to one to ten MMAEs and wherein each MMAE is conjugated to Ab by one linker (**L-1**).

[00331] 20. The method of embodiment 18 or 19, wherein one Ab is conjugated to two MMAEs and wherein each MMAE is conjugated to Ab by one linker (**L-1**).

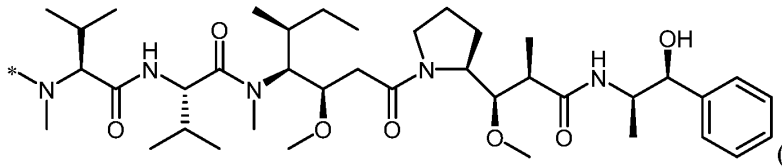
[00332] 21. A method for treating cancer in a subject, the method comprising administering an ADC to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg, wherein the ADC comprises an antibody (Ab) conjugated to a monomethyl auristatin E (MMAE) by a linker (**L-1**):



(**L-1**),

wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:

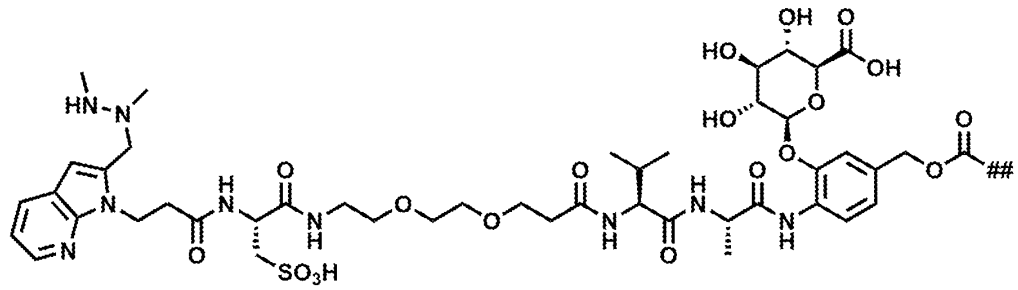


(**III**),

wherein * indicates the point of attachment to (**L-1**); and

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which linker (**L-1**) is attached, and wherein **mAbA15** comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 2 and a light chain comprising an amino acid sequence of SEQ ID NO: 3.

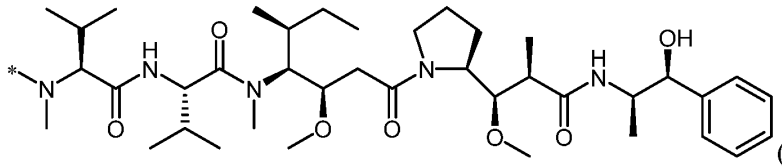
[00333] 22. A method for treating cancer in a subject, the method comprising administering an ADC to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg, wherein the ADC is produced by conjugating an antibody (Ab) to a linker-drug comprising a pre-conjugate linker **(L-1a')** and a monomethyl auristatin E (MMAE):



(**L-1a'**),

wherein the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:



(**III**),

wherein * indicates the point of attachment to (**L-1a'**);

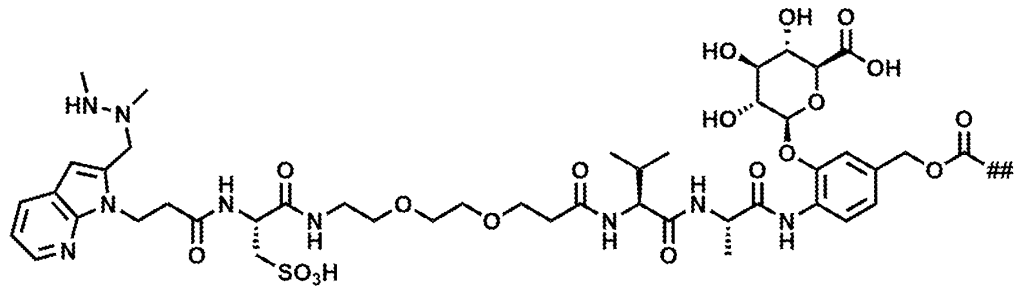
wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises: (1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and (2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; and

wherein the Ab further comprises one or more *fGly'* and wherein *fGly'* is a formylglycine amino acid residue to which linker (**L-1a'**) is attached after conjugating the Ab and the linker-drug.

[00334] 23. The method of embodiment 22, wherein one Ab is conjugated to one to ten linker-drugs.

[00335] 24. The method of embodiment 22 or 23, wherein one Ab is conjugated to two linker-drugs.

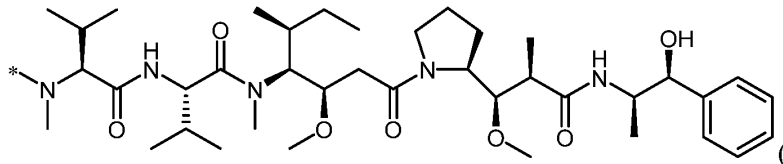
[00336] 25. A method for treating cancer in a subject, the method comprising administering an ADC to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg, wherein the ADC is produced by conjugating an antibody (Ab) to a linker-drug comprising a pre-conjugate linker **(L-1a')** and a monomethyl auristatin E (MMAE):



(L-1a'),

wherein the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:

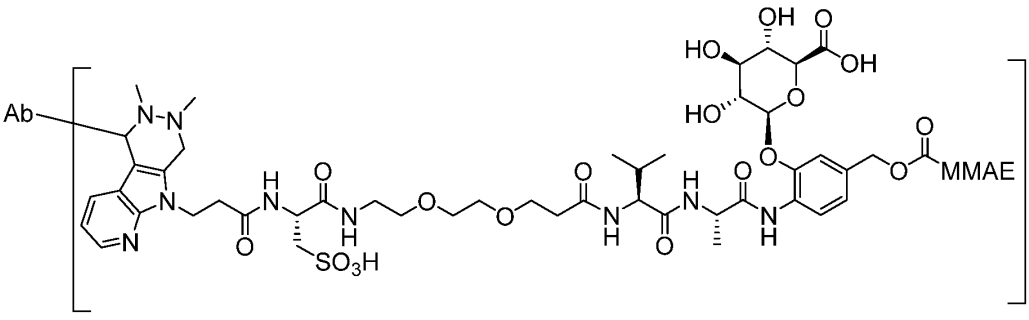


(III),

wherein * indicates the point of attachment to (**L-1a'**); and

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which (**L-1a'**) is attached after conjugating the Ab and the linker-drug, and wherein **mAbA15** comprises a heavy chain comprising an amino acid sequence of SEQ ID NO: 2 and a light chain comprising an amino acid sequence of SEQ ID NO: 3.

[00337] 26. A method of treating cancer for two or more treatment cycles in a subject experiencing an adverse event when or after being administered an ADC, the method comprising:
(i) administering the ADC to the subject at a dose of about 0.1 mg/kg to about 10.0 mg/kg in a first treatment cycle;
(ii) delaying a further administration of the ADC to the subject upon the subject having an adverse event after the first treatment cycle and before a second treatment cycle; and
(iii) resuming the administration of the ADC in the second treatment cycle,
wherein the ADC is represented by **Formula (I)**:



(I),

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which the non-Ab part of the ADC represented by **Formula (I)** is attached, and wherein **mAbA15** comprises:

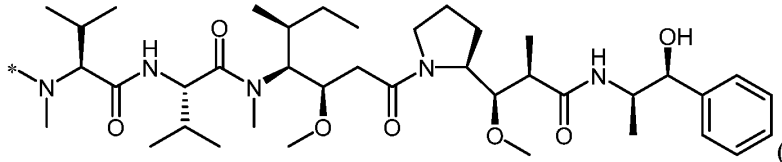
a) a heavy chain comprising an amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEY
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(*fGly'*)TPSRGS (SEQ ID NO: 2); and

b) a light chain comprising an amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPS
RFSGSRSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3); and

wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



(III),

wherein * indicates the point of attachment in **Formula (I)**.

[00338] 27. The method of embodiment 26, wherein the administration is resumed in step (iii) once the adverse event is alleviated or recovered.

[00339] 28. The method of embodiment 26 or 27, wherein the administration of the second treatment cycle is at the dose of the first treatment cycle.

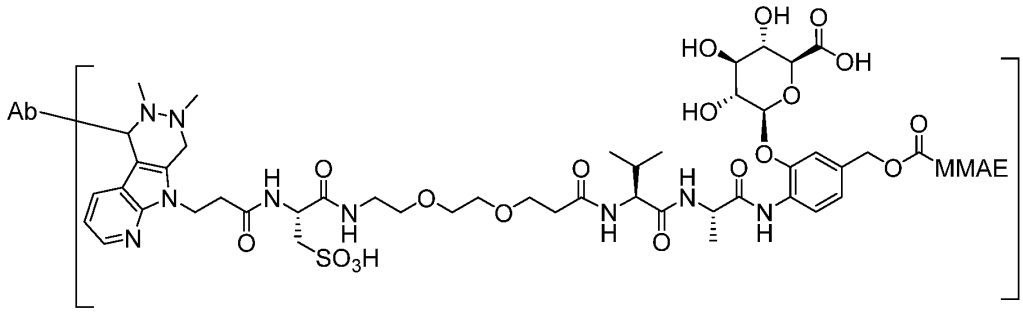
[00340] 29. The method of embodiment 26 or 27, wherein the administration of the second treatment cycle is at a dose lower than that of the first treatment cycle.

[00341] 30. A method of treating cancer for two or more treatment cycles in a subject experiencing an adverse event when or after being administered an ADC, the method comprising:

i) administering the ADC to the subject at a first dose of about 0.1 mg/kg to about 10.0 mg/kg in a first treatment cycle;

ii) in a second treatment cycle, administering the ADC to the subject at a second dose which is lower than the first dose upon the subject having an adverse event,

wherein the ADC is represented by **Formula (I)**:



(I),

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof and comprises a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), a heavy chain complementarity determining region 3 (HCDR3), a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) of an antibody designated **mAbA15**, wherein the Ab further comprises two motifs, each of which is L(*fGly'*)TPSR (SEQ ID NO: 1), wherein *fGly'* is the amino acid residue to which the non-Ab part of the ADC represented by **Formula (I)** is attached, and wherein **mAbA15** comprises:

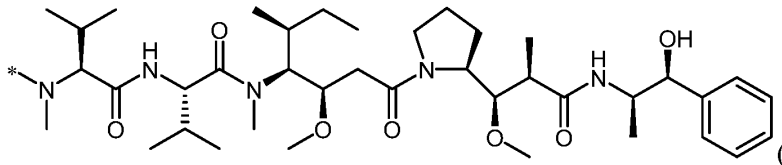
a) a heavy chain comprising an amino acid sequence of:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYIHWVRQAPGKGLEWVAYITSTGSYTEY
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYDRSTRYSGLDYWGQGTLVTVS
SASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(*fGly'*)TPSRGS (SEQ ID NO: 2); and

b) a light chain comprising an amino acid sequence of:

DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLYSGVPS
RFSGSRSGTDFTLTISSLQPEDFATYYCQQAYATPVTFGQGTKVEIKRTVAAPSVFIFPP
SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT
LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 3); and

wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



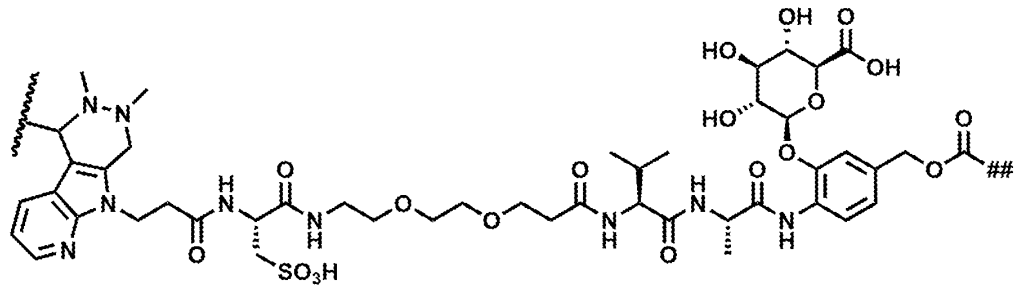
(III),

wherein * indicates the point of attachment in **Formula (I)**.

[00342] 31. The method of embodiment 30, wherein the subject is administered at the second dose in step (ii) once the adverse event is alleviated or recovered.

[00343] 32. The method of any one of embodiments 26-31, wherein the adverse event is selected from the group consisting of Grade 3 thrombocytopenia with clinically significant bleeding, Grade 4 thrombocytopenia, Grade 3 or higher neutropenia with a measured fever of at least 38.3°C, Grade 3 or higher neutropenia with a sustained fever of at least 38°C for at least an hour, Grade 4 neutropenia for greater than 7 days, Grade 4 anemia, alanine transaminase or aspartate transaminase levels that are greater than 3 times the upper limit of normal and a total bilirubin level that is greater than 2 times the upper limit of normal in the absence of biliary obstruction or other explanation for liver abnormality (such as viral hepatitis, alcohol or autoimmune hepatitis, Gilbert Syndrome, or treatment with other hepatotoxic drug), and a Grade 5 adverse event.

[00344] 33. The method of any one of embodiments 26-32, wherein the ADC comprises the Ab conjugated to the MMAE by a linker (**L-1**):



(**L-1**),

wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE.

[00345] 34. The method of any one of embodiments 14-33, wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, or bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer.

[00346] 35. The method of any one of embodiments 1, 2, 5, 6, 9, 10, 13-15, 18, 19, 22, 23, and 35, wherein the Ab comprises one to ten *fGly'*.

[00347] 36. The method of any one of embodiments 1-35, wherein the Ab comprises two *fGly'*.

[00348] 37. The method of any one of embodiments 1-36, wherein the Ab comprises a motif of X^1(*fGly'*)$X^2Z^2X^3Z^3$ (SEQ ID NO: 67),

wherein:

Z^2 is either a proline (P) or alanine (A) residue;

Z^3 is a basic amino acid residue optionally selected from the group consisting of: arginine (R), lysine (K), and histidine (H), or an aliphatic amino acid residue optionally selected from the group consisting of: alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), and proline (P);

X^1 is present or absent and, when present, is any amino acid residue, with the proviso that when the sequence of SEQ ID NO: 67 is at the N-terminus of the antibody Ab, X^1 is present; and

X^2 and X^3 independently is any amino acid residue,

optionally wherein,

the motif of SEQ ID NO: 67 is selected from the group consisting of: L(*fGly'*)TPSR (SEQ ID NO: 1), M(*fGly'*)TPSR (SEQ ID NO: 45), V(*fGly'*)TPSR (SEQ ID NO: 46), L(*fGly'*)SPSR (SEQ ID NO: 47), L(*fGly'*)APSR (SEQ ID NO: 48), L(*fGly'*)VPSR (SEQ ID NO: 49), L(*fGly'*)GPSR (SEQ ID NO: 50), I(*fGly'*)TPAR (SEQ ID NO: 51), L(*fGly'*)TPSK (SEQ ID NO: 52), M(*fGly'*)TPSK (SEQ ID NO: 53), V(*fGly'*)TPSK (SEQ ID NO: 54), L(*fGly'*)SPSK (SEQ ID NO: 55), L(*fGly'*)APSK (SEQ ID NO: 56), L(*fGly'*)VPSK (SEQ ID NO: 57), L(*fGly'*)GPSK (SEQ ID NO: 58), L(*fGly'*)TPSA (SEQ ID NO: 59), I(*fGly'*)TPAA (SEQ ID NO: 60), M(*fGly'*)TPSA (SEQ ID NO: 61), V(*fGly'*)TPSA (SEQ ID NO: 62), L(*fGly'*)SPSA (SEQ ID NO: 63), L(*fGly'*)APSA (SEQ ID NO: 64), L(*fGly'*)VPSA (SEQ ID NO: 65), and L(*fGly'*)GPSA (SEQ ID NO: 66).

[00349] 38. The method of embodiment 37, wherein the Ab comprises two heavy chains and two light chains, and wherein each of the heavy chains comprises a motif of SEQ ID NO: 67.

[00350] 39. The method of any one of embodiments 1-38, wherein the Ab comprises an amino acid sequence of L(*fGly'*)TPSR (SEQ ID NO: 1).

[00351] 40. The method of any one of embodiments 1-39, wherein the Ab comprises two heavy chains (HCs) and two light chains (LCs), and wherein each of the HCs comprises one motif of L(*fGly'*)TPSR (SEQ ID NO: 1).

[00352] 41. The method of embodiment 38 or 40, wherein the *fGly'* of the motif is located in the heavy chain at a position corresponding to the 453rd amino acid residue (*fGly'*) of SEQ ID NO: 2.

[00353] 42. The method of any one of embodiments 1-41, wherein

(1) HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 19, 24, 25, and 30,

(2) HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 20, 26, 31, and 36,

(3) HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 21, 27, and 32,

(4) LCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22, 28, and 33,

(5) LCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 23, and 34, and

(6) LCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 29, and 35.

[00354] 43. The method of any one of embodiments 1-42, wherein:

(i) HCDR1 comprises the amino acid sequence of SEQ ID NO: 13, HCDR2 comprises the amino acid sequence of SEQ ID NO: 14, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18; or

(ii) HCDR1 comprises the amino acid sequence of SEQ ID NO: 19, HCDR2 comprises the amino acid sequence of SEQ ID NO: 20, HCDR3 comprises the amino acid sequence of SEQ ID NO: 21, LCDR1 comprises the amino acid sequence of SEQ ID NO: 22, LCDR2 comprises the amino acid sequence of SEQ ID NO: 23, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18; or

(iii) HCDR1 comprises the amino acid sequence of SEQ ID NO: 24, HCDR2 comprises the amino acid sequence of SEQ ID NO: 14, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18; or

(iv) HCDR1 comprises the amino acid sequence of SEQ ID NO: 25, HCDR2 comprises the amino acid sequence of SEQ ID NO: 26, HCDR3 comprises the amino acid sequence of SEQ ID

NO: 27, LCDR1 comprises the amino acid sequence of SEQ ID NO: 28, LCDR2 comprises the amino acid sequence of SEQ ID NO: 23, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 29; or

(v) HCDR1 comprises the amino acid sequence of SEQ ID NO: 30, HCDR2 comprises the amino acid sequence of SEQ ID NO: 31, HCDR3 comprises the amino acid sequence of SEQ ID NO: 32, LCDR1 comprises the amino acid sequence of SEQ ID NO: 33, LCDR2 comprises the amino acid sequence of SEQ ID NO: 34, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 35; or

(vi) HCDR1 comprises the amino acid sequence of SEQ ID NO: 13, HCDR2 comprises the amino acid sequence of SEQ ID NO: 36, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18.

[00355] 44. The method of any one of embodiments 1-43, wherein the Ab comprises a VH comprising the amino acid sequence of SEQ ID NO: 11 and a VL comprising the amino acid sequence of SEQ ID NO: 12.

[00356] 45. The method of any one of embodiments 1-44, wherein the Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 2 and a light chain comprising the amino acid sequence of SEQ ID NO: 3.

[00357] 46. The method of any one of embodiments 1-45, wherein the Ab is **mAbA15**.

[00358] 47. The method of any one of embodiments 1-46, wherein the ADC is administered to the subject on the first day of a treatment cycle for one or more treatment cycles.

[00359] 48. The method of embodiment 47, wherein each treatment cycle is 3 weeks ± 3 days.

[00360] 49. The method of any one of embodiments 1-48, wherein the ADC is administered to the subject once every three weeks (q3w).

[00361] 50. The method of any one of embodiments 1-49, wherein the ADC is administered to the subject intravenously.

[00362] 51. The method of embodiments 1-50, further comprising administrating to the subject means for inhibiting interaction between PD-1 and PD-L1.

[00363] 52. The method of embodiments 1-51, wherein the subject is administrated with means for inhibiting interaction between PD-1 and PD-L1.

[00364] 53. The method of embodiment 51 or 52, wherein the ADC is administered concurrently with the means for inhibiting interaction between PD-1 and PD-L1.

[00365] 54. The method of embodiment 51 or 52, wherein the ADC is administered after the means for inhibiting interaction between PD-1 and PD-L1.

[00366] 55. The method of embodiment 51 or 52, wherein the ADC is administered before the means for inhibiting interaction between PD-1 and PD-L1.

[00367] 56. The method of embodiment 54 or 55, wherein the ADC is administered on the same day with the means for inhibiting interaction between PD-1 and PD-L1.

[00368] 57. The method of embodiment 56, wherein the administration of the ADC and the means for inhibiting interaction between PD-1 and PD-L1 is at least about 30 minutes apart or at least about 1 hour apart.

[00369] 58. The method of any one of embodiments 51-57, wherein the means for inhibiting interaction between PD-1 and PD-L1 are administered to the subject on the first day of a treatment cycle for one or more treatment cycles.

[00370] 59. The method of embodiment 58, wherein each treatment cycle is 3 weeks ± 3 days.

[00371] 60. The method of any one of embodiments 51-59, wherein the means for inhibiting interaction between PD-1 and PD-L1 are administered to the subject once every three weeks (q3w).

[00372] 61. The method of any one of embodiments 51-60, wherein the means for inhibiting interaction between PD-1 and PD-L1 are administered to the subject intravenously.

[00373] 62. The method of any one of embodiments 1-50, further comprising administering to the subject a PD-L1/PD-1 inhibitor.

[00374] 63. The method of any one of embodiments 1-50, wherein the subject is administered with a PD-L1/PD-1 inhibitor.

[00375] 64. The method of embodiment 62 or 63, wherein the ADC is administered concurrently with the PD-L1/PD-1 inhibitor.

[00376] 65. The method of embodiment 62 or 63, wherein the ADC is administered after the PD-L1/PD-1 inhibitor.

[00377] 66. The method of embodiment 62 or 63, wherein the ADC is administered before the PD-L1/PD-1 inhibitor.

[00378] 67. The method of embodiment 65 or 66, wherein the ADC is administered on the same day with the PD-L1/PD-1 inhibitor.

[00379] 68. The method of embodiment 67, wherein the administration of the ADC and the PD-L1/PD-1 inhibitor is at least about 30 minutes apart or at least about 1 hour apart.

[00380] 69. The method of any one of embodiments 62-68, wherein the PD-L1/PD-1 inhibitor is administered to the subject on the first day of a treatment cycle for one or more treatment cycles.

[00381] 70. The method of embodiment 69, wherein each treatment cycle is 3 weeks ± 3 days.

[00382] 71. The method of any one of embodiments 62-70, wherein the PD-L1/PD-1 inhibitor is administered to the subject once every three weeks (q3w).

[00383] 72. The method of any one of embodiments 62-71, wherein the PD-L1/PD-1 inhibitor is administered to the subject intravenously.

[00384] 73. The method of any one of embodiments 62-72, wherein the PD-L1/PD-1 inhibitor is an antibody or an antigen binding fragment thereof comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 of pembrolizumab.

[00385] 74. The method of embodiment 73, wherein the PD-L1/PD-1 inhibitor is pembrolizumab.

[00386] 75. The method of embodiment 73 or 74, wherein the PD-L1/PD-1 inhibitor is administered to the subject at a dose of about 200 mg.

[00387] 76. The method of any one of embodiments 1-75, wherein the ADC is administered to the subject at a dose of about 0.3 mg/kg to about 6.0 mg/kg.

[00388] 77. The method of any one of embodiments 1-76, wherein the ADC is administered to the subject at a dose of about 0.3 mg/kg to about 2.0 mg/kg.

[00389] 78. The method of any one of embodiments 1-77, wherein the ADC is administered to the subject at a dose of about 0.3 mg/kg, about 0.6 mg/kg, about 1.0 mg/kg, about 1.5 mg/kg, or about 2.0 mg/kg.

[00390] 79. The method of any one of embodiments 1-78, wherein the cancer is triple-negative breast cancer (TNBC) or hormone-receptor positive breast cancer (HR+ BC).

[00391] 80. The method of any one of embodiments 1-78, wherein the cancer is pancreatic ductal adenocarcinoma (PDAC).

[00392] 81. The method of any one of embodiments 1-78, wherein the cancer is non-small cell lung cancer (NSCLC).

[00393] 82. The method of embodiment 81, wherein the subject has one or more mutations selected from the group consisting of an EGFR sensitizing mutation, an ALK rearrangement, a ROS1 rearrangement, a BRAF V600E mutation, a MET Exon 14 skipping mutation, and a RET rearrangement.

[00394] 83. The method of any one of embodiments 1-78, wherein the cancer is head and neck squamous cell carcinoma (HNSCC).

[00395] 84. The method of any one of embodiments 1-83, wherein the ADC is administered to the subject for about four to about twenty-four months.

[00396] 85. The method of any one of embodiments 1-84, wherein the ADC is administered to the subject for about four to about eighteen months.

[00397] 86. The method of any one of embodiments 1-85, wherein the ADC is administered to the subject for about four to about twelve months.

[00398] 87. The method of any one of embodiments 1-86, wherein the ADC is administered to the subject for about four to about six months.

[00399] 88. The method of any one of embodiments 1-87, wherein the ADC also refers to its stereoisomer, solvate, or salt.

[00400] 89. The method of any one of embodiments 1-88, wherein a pharmaceutical composition comprising the ADC and a pharmaceutically acceptable excipient is administered to the subject.

[00401] 90. The method of embodiment 89, the pharmaceutical composition comprises a multiplicity of DAR species of the ADC.

[00402] 91. The method of embodiment 90, wherein the pharmaceutical composition exhibits a drug-to-antibody ratio (DAR) of about 2.

[00403] 92. The method of embodiment 90 or 91, wherein the pharmaceutical composition exhibits a DAR of 1.8.

[00404] 93. The method of any preceding embodiment, wherein the subject is human.

9. EXAMPLES

Example 1: Synthesis of the 5T4 ADC of Formula (I)

[00405] **1.1 Synthesis of Linker-Payload**

[00406] The synthesis of the linker-drug pre-conjugate of **Compound (IIa)** (*e.g.*, a form of **Formula (II)** ready to be conjugated to the 5T4 antibody) is shown in **FIG. 1**.

[00407] Compound **I-1** and 2,3,4,5,6-pentafluorophenol were obtained commercially from Shanghai Medicilon and used as received. Monomethyl auristatin E (MMAE) was purchased from BroadPharm (San Diego, CA, USA). All other reagents were obtained from commercial sources and used without purification.

[00408] Compound **I-1** (1.33 g, 1.67 mmol) was combined with 2,3,4,5,6-pentafluorophenol (1.23 g, 6.68 mmol) in 6.5 mL of anhydrous DMF. This mixture was treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide ("EDCI-HCl," 0.64 g, 3.34 mmol) in one portion at room temperature and stirred for 20 hours until Compound **I-1** was fully consumed, as evidenced by

HPLC analysis. The reaction mixture was loaded onto a C18 column (BIOTAGE®, 60 g with a samplet) and eluted with a 0-80% gradient (10 column volumes) of ACN/water + 0.05% TFA. Pure fractions were combined, concentrated on a rotary evaporator until slightly murky, and lyophilized to give intermediate **I-2** (1.40 g, 1.46 mmol, 87% yield) as a tan powder, which was stored under nitrogen at -20 °C until further use. LRMS (ESI) analysis of the tan powder revealed a peak at *m/z* 961.2 $[M+H]^+$, as expected for $C_{44}H_{45}F_5N_6O_{11}S$.

[00409] In a 20 mL glass vial, MMAE (720 mg, 1.0 mmol), 5 mL of anhydrous DMF, and 0.35 mL of DIPEA (2.0 mmol) were combined at room temperature. The resulting mixture was stirred and treated with compound **I-3** (1014 mg, 1.0 mmol) as a solid in a few small portions, followed by the addition of HOAt (136 mg, 1.0 mmol) in one portion at room temperature. The reaction mixture was stirred for 6 hours until the reaction was complete, as evidenced by HPLC analysis. The reaction mixture was poured into 30 mL of water. The resulting white precipitate was separated by spinning and collected, washed with 5 mL of water, and dried briefly under high vacuum to give 1.87 g of intermediate **I-4** as a yellow solid, which was further reacted according to the disclosure below without purification.

[00410] A solution of intermediate **I-4** (1.87 g) in 15 mL of THF was cooled down to 0 °C in an ice bath and treated slowly with 1 M aqueous lithium hydroxide solution (3 mL). The reaction mixture was stirred at 0 °C for 3 hours, then warmed up to ambient temperature, treated with 3 mL of 1 M aqueous lithium hydroxide and diluted with 3 mL of methanol. The resulting mixture was stirred at room temperature for 3 hours until hydrolysis was complete, as evidenced by HPLC analysis. The reaction was then quenched by adding 1 M aqueous HCl solution to bring the reaction mixture to a pH of 7. The reaction mixture was then concentrated under reduced pressure and washed with 10 mL of methyl tert-butyl ether ("MTBE"). The aqueous layer was purified by reversed-phase chromatography (C18 column, 0-40% acetonitrile-water + 0.05% TFA). Pure product fractions were combined, concentrated under reduced pressure, and lyophilized to give intermediate **I-5** as a white powder (735 mg, 0.60 mmol, 60% yield over two steps). LRMS (ESI) analysis of the white powder revealed a peak at *m/z* 1229.7 $[M+H]^+$, as expected for $C_{61}H_{96}N_8O_{18}$.

[00411] DIPEA (0.21 mL, 1.2 mmol) and a solution of intermediate **I-2** (575 mg, 0.60 mmol) in 2 mL of DMA were added to a stirred solution of intermediate **I-5** (735 mg, 0.60 mmol) in 3 mL of anhydrous DMA at room temperature. 1-Hydroxy-7-azabenzotriazole ("HOAt," 84 mg, 0.60 mmol) was then added. The resulting mixture was stirred for 30 minutes until coupling was complete, as evidenced by HPLC. The reaction mixture was then treated with 1.2 mL of piperidine at room temperature. After 15 minutes, the reaction mixture was purified by reversed-

phase chromatography (C18 column, 0-40% gradient of acetonitrile-water). Pure fractions were combined, concentrated under reduced pressure and room temperature, and lyophilized to give **Formula (IIa)** (808 mg, 0.45 mmol, 75% yield) as a white fluffy powder. LRMS (ESI) analysis of the white powder revealed a peak at 1783.9 $[M+H]^+$, as expected for $C_{84}H_{130}N_{14}O_{26}S$ *m/z*.

[00412] 1.2 Synthesis of the 5T4 Antibody

[00413] The gene encoding heavy chain and light chains of the 5T4 antibody in the ADC were cloned separately into mammalian expression vectors for expression as a full-length IgG in mammalian cells. The IgG antibody was purified from culture supernatant using Protein A resin. Tagged 5T4 antibody was prepared according to methods known in the art, such as those described in Rabuka D. et al., "Site-specific chemical protein conjugation using genetically encoded aldehyde tags," Nat Protoc., 2012; 7(6):1052-1067 and in U.S. Patent No. 7,985,783 B2, each of which is incorporated in its entirety herein by reference. Briefly, the 5T4 antibody, which contained a heterologous sulfatase motif was contacted with a formylglycine-generating enzyme to convert the cysteine in the motif to a formylglycine having an aldehyde moiety to generate an "aldehyde-tagged antibody."

[00414] 1.3 Conjugation

[00415] The 5T4 aldehyde-tagged antibody described in section 1.1 above (15 mg/mL) was conjugated to the synthesized linker-drug pre-conjugate **IIa** (8 mol. equivalents drug:antibody) by reacting the linker-drug and aldehyde-tagged antibody for 72 hours at 37 °C in 20 mM sodium citrate, pH 5.5, 50 mM sodium chloride containing 0.85% DMA. After conjugation, residual free drug was removed by diafiltration using a 115V Labscale Tangential Flow Filtration (TFF) system (Millipore, Cat. No. XX42LSS11) with a 30 kDa TFF cassette (Sigma-Aldrich, Cat. No. P3C030C00) and exchanging for 12 diavolumes (600 mL) into 20 mM sodium citrate, pH 5.5, 50 mM NaCl. Alternatively, residual free drug can also be removed using multiple rounds of dilution into 20 mM sodium citrate, pH 5.5, 50 mM sodium chloride and concentration using AMICON® 0.5 mL 30 kD molecular weight cut off (MWCO) centrifugal filters (Millipore Sigma, Cat. No. #UFC5030BK) or ZEBA® desalting columns (Fisher Scientific, Cat. No. PI87766).

Example 2: A Dose-Escalation and Expansion Study of the ADC of Example 1 as a Single Agent and in Combination Therapy in Subjects with Locally Advanced or Metastatic Solid Tumors

[00416] 2.1 Dose Escalation Stage

[00417] Subjects with advanced solid tumors are treated with the ADC of **Example 1** (**5T4-ADC-1**) in escalating doses either as a single agent (Single Agent Dose Escalation Cohort) or in combination with 200 mg pembrolizumab (Combination Dose Escalation Cohort). The starting dose of the ADC is 0.3 mg/kg body weight administered intravenously once every three weeks (q3w). For subjects with a body weight > 120 kg, the maximum total dose is calculated based on 120 kg body weight. In the "Combination Dose Escalation Cohort," pembrolizumab is also administered intravenously at the same time as the ADC.

[00418] Escalating dosing is determined using the Bayesian optimal interval (BOIN) design. The 3-week period following administration of the starting dose comprises a DLT-Evaluation Period. Escalated doses are initially based on a modified Fibonacci progression (increments of 100%, 67%, 50%, and then 33% over the prior dose), but smaller escalating doses may be instituted between cohorts based on evaluation of safety and PK results from the current and previous cohorts.

[00419] In this study, the target toxicity rate for the maximum tolerated dose (MTD) is ϕ= 0.25 and the maximum sample size is 45. The total number of subjects enrolled under the BOIN design, shown in **FIG. 2**, depends on the number of dose levels required to determine the MTD and/or recommended dose (RD). Enrollment cohorts of sizes up to 5 subjects are treated and observed. Subjects are deemed evaluable for purposes of making dose-escalation/de-escalation decisions if they experience a dose-limiting toxicity (DLT) or complete the DLT-Evaluation Period without experiencing a DLT.

[00420] Subjects who do not experience a DLT and do not complete the DLT-Evaluation Period for reasons other than safety are not considered evaluable. Given the DLT rate among the evaluable subjects at the dose level, the BOIN design uses the following rule, optimized to minimize the probability of incorrect dose assignment to guide dose-escalation/de-escalation.

[00421] According to **FIG. 2**:

- If the observed DLT rate at the current dose is $\leq$ 0.197, the dose is escalated to the next higher dose level;
- If the observed DLT rate at the current dose is > 0.298, the dose is de-escalated to the next lower dose level;

- Otherwise, the current dose is continued.

[00422] For the purpose of overdose control, doses j and higher levels are eliminated from further examination if Pr (p_j > 0.25 | data) > 0.95 and at least 3 evaluable subjects have been treated at dose level j, where p_j is the true DLT rate of dose level j. This posterior probability is evaluated based on the beta-binomial model $y_j|p_j$~binomial (p_j) with p_j~uniform (0, 1), where y_j is the number of subjects experiencing DLT at dose level j. When the lowest dose is eliminated, stop the trial for safety. The probability cutoff 0.95 is chosen to be consistent with the common practice that when the target DLT rate ≤ 1/6, a dose with 2 out of 3 subjects experiencing DLT is eliminated. The above dose-escalation/de-escalation and elimination rule can be equivalently presented in **Table 2**, which is used to conduct the trial.

Table 2: Dose Escalation and De-Escalation Rules for the BOIN Design

Number of evaluable subjects treated at current dose	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Escalate if # of DLT ≤	NA	NA	0	0	0	1	1	1	1	1	2	2
De-escalate if # of DLT ≥	1	1	1	2	2	2	3	3	3	3	4	4
Eliminate if # of DLT ≥	NA	NA	3	3	3	4	4	4	5	5	6	6
Number of evaluable subjects treated at current dose	**13**	**14**	**15**	-	-	-	-	-	-	-	-	-
Escalate if # of DLT ≤	2	2	2	-	-	-	-	-	-	-	-	-
De-escalate if # of DLT ≥	4	5	5	-	-	-	-	-	-	-	-	-
Eliminate if # of DLT ≥	6	7	7	-	-	-	-	-	-	-	-	-

[00423] Accrual to a Dose-Escalation Stage ends when either 12 evaluable subjects have already been observed in the dose level indicated as the next step in the enrollment algorithm or a total of 45 evaluable subjects have been treated and observed in the stage for a regimen, whichever comes first. Additional subjects may also be added at any dose level lower than the safe dose, simultaneously.

[00424] The following stopping rules are employed during the study, including in the dose escalation stages and expansion stages:

- Two or more Grade 5 treatment-related AEs that occur within one cohort (in nonrandomized cohorts) or within one treatment arm (in randomized cohorts);
- More than 30% of subjects within one cohort that discontinue study treatment due to treatment-related AE;
- More than 70% of subjects within one cohort that experience a ≥ Grade 3 treatment-related AE.

MTD Determination:

[00425] Determination of the MTD is based on isotonic regression as specified in Liu and Yuan, 2015, "Bayesian Optimal Interval Designs for Phase I Clinical Trials," Journal of the Royal Statistical Society, 64(3):507-523. This computation is implemented by the Shiny app "BOIN" (Zhou et al., 2020, "BOIN Suite: A Software Platform to Design and Implement Novel Early-Phase Clinical Trials," JCO Clin Cancer Inform., 5:91-10, available at http://www.trialdesign.org). Specifically, the dose selected as the MTD is the maximum isotonic estimate of the toxicity rate closest to the target toxicity rate. If there are ties, if the isotonic estimate is lower than the target toxicity rate, the higher dose level is selected. If the isotonic estimate is greater than or equal to the target toxicity rate, the lower dose level is selected.

[00426] The BOIN design (Liu and Yuan 2015; Yuan et al 2016) is used to identify the MTD of the ADC of **Example 1**. In this study, the target toxicity rate for the MTD is ϕ= 0.25. The maximum sample size is 45 for single-agent ADC based on up to 7 dose levels, and 30 in combination of pembrolizumab based on up to 4 dose levels. The total number of subjects enrolled under the BOIN design depends on the number of dose levels required to determine the MTD and/or RD.

RD Determination:

[00427] Independent of MTD determination, and upon review of all available safety, PK, and preliminary clinical activity data, the Cohort Review Committee chooses one RD level for the ADC at which the corresponding expansion cohort subjects are treated. The RD for the ADC is the MTD or a lower dose level at the Cohort Review Committee's discretion.

Magnitude of Dose-Escalation:

[00428] Dose-escalation to the next higher dose level proceeds only if at least 3 subjects enrolled under the BOIN design have been evaluated at the current dose level and a reason for discontinuing enrollment is not reached as discussed above. When selecting new higher dose levels for evaluation, dose-escalation is based on a modified Fibonacci progression, but the Cohort Review Committee may decide to institute a smaller escalation between cohorts (*e.g.*, dose for the next cohort may be less than that recommended by the Fibonacci sequence) based on evaluation of safety and PK results from the current and previous cohorts.

Intra-subject Dose-Escalation:

[00429] Prior to establishing the MTD, the Cohort Review Committee can allow an intra-subject dose-escalation of a subject to a safe ADC dose level (that is, DLT-evaluation has been completed for at least 3 subjects). Subjects who are allowed to dose-escalate do contribute to the DLT evaluation at that higher dose.

Intermediate Dose Level Cohorts:

[00430] Intermediate dose level cohorts can be inserted between previously opened dose levels and evaluated at the request of the Cohort Review Committee based on emerging safety and PK data. The total sample size can be expanded by up to 6 subjects per additional intermediate dose (a maximum of 2 intermediate doses may be explored). Data from intermediate dose levels is included in final regression models to determine the MTD.

Regimen Re-evaluation:

[00431] If the Cohort Review Committee is unable to select an acceptable RD for further evaluation of a regimen in the Cohort-Expansion Stage (*e.g.*, early termination for safety, insufficient toxicity data), alternative treatment schedules can be evaluated in a new Dose-Escalation Stage. Under these circumstances, data from the first dose-escalation evaluation is used to inform the dose level and/or treatment schedule choices, but enrollment and escalation/de-escalation decisions follows the BOIN design independent of the prior evaluation.

Treatment Extension Period:

[00432] After completing the 21-day DLT-Evaluation Period, subjects have the opportunity to continue to receive study treatment, including the combination therapy agent, if applicable, in the Treatment Extension Period for up to 24 months, provided none of the discontinuation criteria are met. Subjects can continue to receive the ADC for more than 24 months if the subject is deriving clear clinical benefit as determined by the Investigator and agreed by the Sponsor. Treatment can continue after first radiographic progression if the Investigator believes that the subject is still receiving clinical benefit from study treatment and that the potential benefit of continuing study treatment outweighs potential risk. Clinical judgment is used for allowing treatment beyond radiographic progression. Subjects with clinically significant symptomatic deterioration at the time of radiographic progression may not be suitable for further treatment. If there is evidence of subsequent progression, the subject must discontinue treatment.

[00433] After the first DLT-Evaluation period (days 22+), subjects may continue to receive treatment for up to 24 months or longer until radiographic PD as assessed by the Investigator or any other treatment discontinuation criteria are met.

[00434] Dose Limiting Toxicities (DLTs) are defined as any of the following treatment-emerging adverse events occurring during the DLT-Evaluation Period:

- Any treatment-emergent AE, that in the opinion of the Cohort Review Committee, is of potential clinical significance such that further dose-escalation would expose subjects to unacceptable risk, provided that the AE is not reasonably attributed to the subject's underlying disease, other medical condition or concomitant medications;

- Non-hematologic toxicities: any ≥ Grade 3 treatment-emergent AE (exceptions are described below);
- Hematologic toxicities: any of the following treatment-emergent AEs:
  - Grade 3 thrombocytopenia with clinically significant bleeding;
  - Grade 4 thrombocytopenia;
  - ≥ Grade 3 neutropenia of any duration with fever (single temperature of ≥ 38.3°C [101°F] or sustained temperature of ≥ 38.0°C [100°F] for ≥ 1 hour);
  - Grade 4 neutropenia of > 7 days duration; and
  - Grade 4 anemia;
- Alanine transaminase (ALT) or aspartate transaminase (AST) > 3 × upper limit of normal (ULN) AND total bilirubin > 2 × ULN (Hy's law) in the absence of evidence of biliary obstruction (*i.e.*, significant elevation of alkaline phosphatase [ALP]) or other explanation for liver abnormality (*e.g.*, viral hepatitis, alcohol or autoimmune hepatitis, Gilbert Syndrome, other hepatotoxic drugs);
- Any treatment-emergent AE that requires a dose reduction for the second infusion of the ADC or discontinuation of the ADC; and
- Grade 5 AE not clearly due to the underlying disease or extraneous causes.

[00435] Examples of AEs that are not considered DLTs:

- Grade 3 fatigue or anorexia lasting < 7 days; ≥ Grade 3 nausea, vomiting, diarrhea, or dehydration lasting ≤ 72 hours with maximal/optimal medical therapy; ≥ Grade 3 rash lasting ≤ 5 days;
- Single laboratory values that are out of normal range and do not have any clinical correlate. Normal values or baseline grade level must have been recorded on repeat testing within 1 week after the out-of-range value without direct intervention intended to correct the abnormality (*e.g.*, replacement of electrolytes for an electrolyte abnormality); and
- Tumor flare-related AEs (*i.e.*, localized pain, irritation at tumor sites).

[00436] For subjects who experience a DLT, administration of the ADC is held until the toxicity resolves to baseline or ≤ Grade 1 severity. At the Investigator's discretion and with the agreement of the Sponsor, subjects who recover within 21 days are allowed to resume the ADC at a maximum dose of one dose level below the dose that produced the DLT. If the reduced dose is tolerated without DLT or other unacceptable toxicity, the subject is eligible to proceed to the Treatment Extension Period at the reduced dose.

[00437] A scheme depicting the BOIN Design for escalating the doses is shown in **FIG. 3** for the Single Agent Dose Escalation Cohort (top) and Combination Dose Escalation Cohort (bottom).

[00438] **2.2 Cohort-Expansion Stage**

[00439] After the recommended dose of the ADC of **Example 1** has been determined in the Dose-Escalation Stage, safety and efficacy of the ADC as a single-agent (Single-Agent Cohort-Expansion Stage) or in combination with pembrolizumab (Combination Therapy Cohort-Expansion Stage) is evaluated in multiple tumor-specific expansion cohorts in the Single-Agent Cohort-Expansion Stage. Tumor specific cohorts include subjects with HR+ breast cancer (HR+ BC), non-small cell lung cancer (NSCLC), head and neck cancer (HNSCC), pancreatic cancer (PDAC), endometrial cancer (EC), or triple negative breast cancer (TNBC). In some embodiments, tumor specific cohorts further include subjects with prostate cancer, cervical cancer, bladder cancer. Additionally or alternatively, tumor specific cohorts further include subjects with renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer. A Simon's 2-Stage design is used for HNSCC, PDAC, TNBC, and EC cohorts.

Single-Agent Cohort-Expansion Stage

[00440] Two dose levels (the RD and a dose lower than the RD [RD-low]) of the ADC are administered q3w IV to subjects in select single-agent tumor cohorts to understand the exposure-safety and exposure-response relationships more completely. This facilitates determination of the optimal dose of the ADC in these indications and whether it can be generalized to other solid tumors. Enrollment to RD or RD-low cohorts is randomized.

Combination Therapy Cohort-Expansion Stage

[00441] The ADC of **Example 1** is administered at the recommended dose IV q3w and pembrolizumab is administered at a dose of 200 mg q3w to subjects in select single-agent tumor cohorts to understand the exposure-safety and exposure-response relationships more completely. This facilitates determination of the optimal dose of the ADC in these indications and whether it can be generalized to other solid tumors. Enrollment to RD or RD-low cohorts is randomized.

[00442] For NSCLC and HR+ BC expansion cohorts, two dose levels are evaluated to facilitate the determination of the optimal dose of ADC in these indications and whether it can be generalized to other solid tumors. Approximately 50 subjects with each tumor type are randomized using a 1:1 randomization ratio to allocate subjects to either the RD or RD-low levels.

[00443] For HNSCC, PDAC, EC, and TNBC, each of the expansion cohorts is evaluated at the RD using a Two-Stage design (see **Table 3**).

Table 3: Two-Stage Design for Expansion Cohorts Evaluated at RD

Cohort[a]	Tumor Type	Maximum Response Rate of "Ineffective" Drug (p0)	Target Response Rate (p1)	Total Number of Subjects	Number of Subjects in Stage I	Minimum Number of Responses Needed to Continue to Stage II	Minimum Total Responses Needed for Cohort "Success"	Probability of Stopping Study After Stage I if "Ineffective"	1-sided alpha	Power
Cohort D	HNSCC	15%	35%	40	12	2	10	44%	0.061	91%
Cohort E	PDAC	4%	15%	40	19	1	4	46%	0.071	85%
Cohort F	EC	12%	30%	38	10	1	8	28%	0.074	90%
Cohort G	TNBC	12%	30%	38	10	1	8	28%	0.074	90%
Abbreviations: EC, endometrial cancer; HNSCC, head and neck squamous cell carcinoma; PDAC, pancreatic adenocarcinoma; RD, recommended dose; TNBC, triple-negative breast cancer										

[00444] 2.3 Subjects

[00445] The number of subjects enrolled in this Phase 1 study evaluating the ADC as a single agent in subjects with advanced solid tumors is approximately 301 (approximately 45 subjects in dose-escalation cohorts and approximately 256 subjects in expansion cohorts (RD dose level)). Additionally, up to 30 subjects are enrolled in dose-escalation cohorts treated with the ADC in combination with pembrolizumab. The number of subjects in expansion cohorts treated with the ADC in combination with pembrolizumab may be different or comparable to that of the dose-escalation cohorts treated with the ADC as a single agent. The subject enrollment is shown in **Table 4**.

Table 4: Subject Enrollment

Dose-Escalation Stage			
Cohort[a]	**Tumor Type**	**ADC Dose**	**BOIN Design n =**
Cohort A1	Solid tumor	Dose mg/kg IV q3w[b]	3 to 12
Cohort A2	Solid tumor	TBD mg/kg IV q3w [c]	3 to 12 each
Total			**~45[d]**
Cohort-Expansion Stage: Randomized Cohorts			
Cohort	**Tumor Type**	**ADC Dose**	**Sample Size per Treatment Arm n =**
Cohort B	NSCLC	Arm B1: RD (TBD) Arm B2: RD-low (TBD)	~25 ~25
Cohort C	HR+ breast cancer	Arm C1: RD (TBD) Arm C2: RD-low (TBD)	~25 ~25
Cohort-Expansion Stage: Single-Arm Cohorts			
Cohort	**Tumor Type**	**ADC Dose**	**Two-Stage Design n =**

Cohort D	HNSCC	TBD mg/kg IV q3w[e]	40 (12 + 28)
Cohort E	PDAC	TBD mg/kg IV q3w[e]	40 (19 + 21)
Cohort F	EC	TBD mg/kg IV q3w[e]	38 (10 + 28)
Cohort G	TNBC	TBD mg/kg IV q3w[e]	38 (10 + 28)
Total			**~256**

Abbreviations: BOIN, Bayesian optimal interval; d, dose level of subjects enrolled; EC, endometrial cancer; HNSCC, head and neck squamous cell carcinoma; HR+ BC, hormone-receptor-positive breast cancer; IV, intravenous; NSCLC, non-small cell lung cancer; PDAC, pancreatic adenocarcinoma; RD, recommended dose; TNBC, triple-negative breast cancer; n, up to 7 cohorts for A; q3w, every 3 weeks; TBD, to be determined.

[a] All subjects treated at a single ADC dose level in the Dose-Escalation Stage is defined as a cohort (*e.g.*, Cohort A1 includes all subjects treated at the ADC starting dose).

[b] Starting dose level of the ADC.

[c] Maximum dose increment of 100% of previous dose for ADC escalation.

[d] In addition to these 45 subjects, if additional intermediate doses are explored the total sample size can be expanded. Additional subjects can also be added at any dose level being evaluated (up to a total of 12 at that dose level) if the Cohort Review Committee concludes that additional safety data should be obtained at this dose level.

[e] At the recommended dose for the ADC.

[00446] To be eligible for the study, the subject must meet all of the inclusion and none of the exclusion criteria as follow:

Inclusion Criteria

2) Subject-disease characteristics:

- Cytologically or histologically and radiologically confirmed solid tumor that is inoperable, locally advanced, metastatic, or recurrent:

Dose-Escalation Stage Cohort A (Solid Tumors):

- The subject has a histologically confirmed solid tumor that is unresectable or metastatic and for which standard curative measures do not exist or are no longer effective, and there are no known therapies to prolong survival.
- Note: Colorectal adenocarcinoma (CRC), clear cell renal cell carcinoma (ccRCC), hepatocellular carcinoma (HCC) tumor types, are excluded from Dose-Escalation Stage due to anticipated lack of benefit to the payload.

Cohort-Expansion Stage (Cohorts B-G):

- The subject has received standard life-prolonging therapies unless they do not exist, or available therapies are intolerable or no longer effective, as described below:

Cohort B (Non-Small Cell Lung Cancer):

- Subjects with Stage IV NSCLC require systemic therapy in the opinion of the principal investigator during or following their last systemic anticancer therapy.

- Subjects without an actionable mutation must have received platinum-based chemotherapy and a PD-(L)1 inhibitor, administered concurrently or sequentially for locally advanced or metastatic disease. Subjects who are not eligible to receive ICI therapy are eligible if they have received platinum-containing doublet chemotherapy.
- Subjects with an actionable mutation (*e.g.*, EGFR sensitizing mutation, ALK rearrangement, ROS1 rearrangement, BRAF V600E mutation, MET Exon 14 skipping mutation, RET rearrangement) must have received at least one targeted therapy for that specific genetic alteration unless such therapy is not available, or the subject is not qualified to receive such therapy.
- Subjects must have received no more than 3 lines of prior systemic anticancer therapy for locally advanced or metastatic disease (*e.g.*, chemotherapy, immunotherapy, molecular targeted therapy given either as single agents or in combination).

Cohort C (Hormone-Receptor-Positive Breast Cancer):

- Subjects with breast cancer that is hormone-receptor-positive (ER+ and/or PR+) and HER-2 negative or low and who require systemic therapy in the opinion of the Investigator during or following their last systemic anticancer therapy for inoperable locally advanced or metastatic disease.
- Note: Estrogen receptor and progesterone receptor positivity are defined as ≥ 1% of cells expressing hormonal receptors via IHC analysis.
- HER-2 negativity is defined as either of the following by local laboratory assessments: in situ hybridization (ISH) non-amplified (ratio of HER-2 to CEP17 < 2.0 or single probe average HER-2 gene copy number < 4 signals/cell), or IHC 0 or IHC 1+.
- Note: HER-2 low is defined as an IHC score of 1+ or 2+ with a negative ISH test. If more than one test result is available and they do not all meet the inclusion criterion definition, there is discussion with the Sponsor to establish eligibility of the subject.
- Subjects must have postmenopausal status due to either surgical or natural menopause or ovarian suppression with gonadotrophin-releasing hormone (GnRH) agonist (*e.g.*, goserelin)

- Subjects must have received at least one prior endocrine anticancer therapy and one prior CDK 4/6 inhibitor therapy either sequentially or concurrently for inoperable locally advanced or metastatic breast cancer.
- Subjects must not have received more than 3 lines of prior systemic anticancer therapies for locally advanced or metastatic disease (*e.g.*, CDK4/6 inhibitors, cytotoxic therapy, targeted therapy including ADCs).
- Note: There is no limit on number of prior lines of endocrine anticancer therapy. Examples include selective estrogen receptor degraders (SERD), selective estrogen receptor modulator (SERM), and steroidal and non-steroidal aromatase inhibitor (SAI, NSAI).

Cohort D (Head and Neck Cancer):

- Subjects with head and neck cancer (squamous cell histology) who require systemic therapy in the opinion of the principal investigator during or following their last systemic anticancer therapy. Allowed primary tumor locations are oral cavity, oropharynx, hypopharynx, and glottic larynx. Note: Excluded are subjects with primary tumor site of the nasopharynx.
- Subjects must have received prior platinum-containing chemotherapy (including chemoradiation) for inoperable locally advanced, recurrent, or metastatic disease. Subjects must have also received ICI therapy, if locally considered as standard of care and if eligible.
- Subjects must have received no more than 3 lines of prior systemic anticancer therapy for inoperable locally advanced recurrent, or metastatic disease setting (*e.g.*, chemotherapy, immunotherapy, targeted therapy given either as single-agents or in combination)

Cohort E (Pancreatic Cancer)

- Subjects with pancreatic cancer (adenocarcinoma histology) who require systemic therapy in the opinion of the principal investigator during or following their last systemic anticancer therapy.
- Subjects must have received prior treatment with gemcitabine in combination with albumin-bound paclitaxel (nab-paclitaxel) or FOLFIRINOX or have documented ineligibility for standard therapy.

- Subjects who have received at least 1 and no more than 2 prior lines of systemic anticancer therapy for inoperable locally advanced, recurrent, or metastatic disease.

Cohort F (Endometrial Cancer):

- i. Subjects with histologically confirmed, advanced, recurrent or metastatic endometrial cancer who require systemic therapy in the opinion of the principal investigator during or following their last systemic anticancer therapy.
- ii. Subjects must have received platinum-containing chemotherapy in the adjuvant, locally advanced, or metastatic disease setting.
- iii. Subjects must have received no more than 3 lines of prior systemic anticancer therapy for inoperable locally advanced recurrent, or metastatic disease setting (*e.g.*, chemotherapy, immunotherapy, targeted therapy given either as single-agents or in combination).

Cohort G (Triple-Negative Breast Cancer):

- Subjects with triple-negative (estrogen receptor negative [ER-]/progesterone receptor negative [PR-] and HER-2-) breast cancer who require systemic therapy in the opinion of the principal investigator during or following their last systemic anticancer therapy for inoperable locally advanced or metastatic disease.
- Estrogen receptor and progesterone receptor negativity are defined as < 1% of cells expressing hormonal receptors via IHC analysis.
- HER-2 negativity is defined as either of the following by local laboratory assessments: ISH non-amplified (ratio of HER-2 to CEP17 < 2.0 or single probe average HER-2 gene copy number < 4 signals/cell), or IHC 0 or IHC 1+.
- Note: HER-2 low, defined as an IHC score of 1+ or 2+ with a negative FISH test. If more than one test result is available and they do not all meet the inclusion criterion definition, there is discussion with the Sponsor to establish eligibility of the subject).
- Subjects must have received at least 1 line of prior systemic chemotherapy (anthracycline-, alkylator-, or taxane-based chemotherapy) but no more than 3 lines of prior systemic anticancer therapies for locally advanced or

metastatic disease (*e.g.*, cytotoxic therapy, targeted therapy including ADCs, immunotherapy).

3) Cohort-Expansion Stage Only: Measurable disease per RECIST 1.1 as determined by the Investigator.
4) Available archival tumor tissue collected no more than 2 years prior to consent. If archival tumor tissue is not available, a fresh tumor biopsy may be collected at least 7 days prior to first dose if it can be safely acquired. Specific requirements for tumor tissue samples are provided in the laboratory manual.
5) Recovery to baseline or ≤ Grade 1 severity (Common Terminology Criteria for Adverse Events version 5 [CTCAE v5]) from AEs, unless AEs are clinically nonsignificant (*e.g.*, alopecia) or stable.
6) Age 18 years or older on the day of consent.
7) Eastern Cooperative Oncology Group (ECOG) Performance Status of 0-1.
8) Adequate organ and marrow function, based upon meeting all the following laboratory criteria within 10 days before first dose of study treatment:
    a. Absolute neutrophil count (ANC) ≥ 1500/mm^3 (≥1.5 GI/L) without granulocyte colony-stimulating factor (G-CSF) support within 2 weeks prior to screening laboratory sample collection.
    b. Platelets ≥ 100,000/mm^3 (≥ 100 GI/L)] without transfusion within 2 weeks prior to screening laboratory sample collection.
    c. Hemoglobin ≥ 9 g/dL (≥ 9 g/L)] without transfusion within 2 weeks prior to screening laboratory sample collection.
    d. Prothrombin time (PT) or activated partial thromboplastin time (aPTT) ≤ 1.2 × ULN or International Normalized Ratio (INR) ≤ 1.5 without anticoagulation therapy (INR ≤ 3 if on stable oral coumarin-based anticoagulant).
    e. ALT and AST ≤ 3 × ULN or ≤ 5 with documented liver metastases.
    f. Total bilirubin ≤ 1.5 × ULN (for subjects with Gilbert's disease or unconjugated hyperbilirubinemia ≤ 3 × ULN).
    g. Serum creatinine ≤ 1.5 × ULN or calculated creatinine clearance ≥ 45 mL/min (≥ 0.75 mL/sec)] using the Cockcroft-Gault equation.
    h. Urine protein-to-creatinine ratio (UPCR) ≤ 1 mg/mg (≤ 113.2 mg/mmol)] creatinine.
9) Capable of understanding and complying with the protocol requirements and must have signed the informed consent document.

10) Sexually active fertile subjects and their partners must agree to highly effective methods of contraception that alone or in combination result in a failure rate of less than 1% per year when used consistently and correctly during the course of the study and for the following durations after the last dose of study treatment (whichever is later):
    - 9 months after the last dose of the ADC for females of childbearing potential or 4 months after the last dose of the ADC for men.
    - 186 days after the last dose of pembrolizumab for females of childbearing potential or 96 days after last dose for men
    - Note: An additional contraceptive method, such as a barrier method (*e.g.*, condom), is required.
    - Note: In addition, men must agree not to donate sperm and women must agree not to donate eggs (ova, oocyte) for the purpose of reproduction during these same periods.
    - Note: azoospermic males are exempt from contraceptive requirements. Females of childbearing potential who are continuously not heterosexually active are also exempt from contraceptive requirements, and still must undergo pregnancy testing.
11) Female subjects of childbearing potential must not be pregnant at screening. Female subjects are considered to be of childbearing potential unless one of the following criteria is met: permanent sterilization (hysterectomy, bilateral salpingectomy, or bilateral oophorectomy) or documented postmenopausal status (defined as 12 months of amenorrhea in a woman over 45 years-of-age in the absence of other biological or physiological causes. In addition, females under 55 years-of-age must have a serum follicle stimulating hormone (FSH) level > 40 mIU/mL to confirm menopause).

Exclusion Criteria

1) Single-Agent and Combination Dose-Escalation Stage: Tumor types that are unlikely to respond to antimitotic chemotherapies, including: colorectal adenocarcinoma, hepatocellular carcinoma, clear cell renal cell carcinoma.
2) Prior treatment with any 5T4-targeting antibody drug conjugate or auristatin derivate based antibody drug conjugate.
3) Receipt of any chemotherapy or anticancer antibody (*e.g.*, anti-VEGF mAb, antibody drug conjugate, or PD-1/PD-L1 mAb) within 21 days (nitrosoureas or mitomycin within 42 days) before first dose of study treatment

4) Receipt of any type of small molecule kinase inhibitor (including investigational kinase inhibitor) within 2 weeks before first dose of study treatment.
5) Receipt of any anticancer hormonal therapy within 2 weeks or within 5 half-lives of the last dose, whichever is shorter, before the first dose of study treatment. Note: Concomitant use of a luteinizing hormone-releasing hormone (LHRH) agonist or antagonist is permitted.
6) Radiation therapy within 2 weeks before first dose of study treatment. Subjects with clinically relevant ongoing complications from prior radiation therapy are not eligible.
7) Known brain metastases or cranial epidural disease unless adequately treated with radiotherapy and/or surgery (including radiosurgery) and stable for at least 4 weeks before first dose of study treatment. Eligible subjects must be neurologically asymptomatic and without corticosteroid treatment at the time of first dose of study treatment.
8) (For pembrolizumab combination only) Diagnosis of immunodeficiency or is receiving systemic steroid therapy or any other form of immunosuppressive therapy within 2 weeks prior to first dose of study treatment. Inhaled, topical, or otic corticosteroids and mineralocorticoids are allowed.
9) The subject has uncontrolled, significant intercurrent or recent illness including, but not limited to, the following conditions:
    a. Acute ocular infection, acute or chronic ulcerative/cicatricial condition of conjunctiva or cornea including but not limited to ocular disorders from autoimmune diseases (*e.g.*, mucous membrane pemphigoid, Sjogren's syndrome), severe conjunctiva/cornea scarring (*e.g.*, radiation keratopathy), severe dry eye disease (for Schirmer's test read $\leq$ 5 mm), history of corneal transplantation, monocularity;
    b. Peripheral neuropathy (sensory and/or motor) CTCAE Grade $\geq$ 2;
    c. Congestive heart failure New York Heart Association class 3 or 4, unstable angina pectoris, serious cardiac arrhythmias (*e.g.*, ventricular flutter, ventricular fibrillation, Torsade de pointes);
    d. Stroke (including transient ischemic attack [TIA]), myocardial infarction, or other ischemic event within 6 months before first dose;
    e. Thromboembolic events (*e.g.*, DVT or PE) within 3 months before first dose. Subjects must have received anticoagulation therapy and be asymptomatic at the time of first dose;

f. History of idiopathic pulmonary fibrosis, organizing pneumonia, drug-induced pneumonitis, or idiopathic pneumonitis;
g. Moderate to severe hepatic impairment (Child-Pugh B or C);
h. Active infection requiring systemic therapy;
i. Untreated known human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)-related illness. Subjects who are HIV positive but who have undetectable viral loads and who have been on anti-retroviral therapy for at least 30 days are eligible. Note: HIV testing is performed at screening if required by local regulation;
j. Acute or chronic hepatitis B or C infection; or
k. Requirement for peritoneal or hemodialysis.

10) Positive hepatitis B surface antigen (HBsAg) test.
11) Positive hepatitis C virus (HCV) antibody test.
- Note: Subjects with treated hepatitis C but with positive HCV antibody test followed by a negative HCV RNA test and no ongoing anti-HCV therapy are eligible.
- Note: The HCV RNA test is performed only for subjects who have a positive HCV antibody test.

12) Clinically significant hematuria, hematemesis, or hemoptysis or other history of significant bleeding (*e.g.*, pulmonary hemorrhage) within 4 weeks before first dose.
13) Active autoimmune disease or a history of autoimmune disease within the past 5 years requiring immunosuppressive therapy. Subjects with asthma that require intermittent use of bronchodilators are not excluded from study participation. Additional exceptions require Sponsor approval.
14) Uncompensated/symptomatic hypothyroidism.
15) History of solid organ or allogenic stem cell transplant.
16) Major surgery (*e.g.*, gastrointestinal [GI] surgery, removal or biopsy of brain metastasis) within 4 weeks before first dose of study treatment. Minor surgery (*e.g.*, simple excision, tooth extraction) within 7 days before first dose. Complete wound healing from major surgery must have occurred before first dose. Subjects with clinically relevant ongoing complications from prior surgery are not eligible.
17) Corrected QT interval calculated by the Fridericia formula (QTcF) > 480 ms per electrocardiogram (ECG) within 4 weeks before first dose of study treatment.

- Note: If a single ECG shows a QTcF with an absolute value > 480 ms, 2 additional ECGs at intervals of approximately 3 minutes must be performed within 30 minutes after the initial ECG, and the average of the 3 consecutive results for QTcF must be ≤ 480 ms for the subject to be eligible.

18) History of psychiatric illness likely to interfere with ability to comply with protocol requirements or give informed consent.

19) Pregnant or lactating females.

20) Previously identified allergy or hypersensitivity to components of the study treatment formulations.

21) Diagnosis of another malignancy within 2 years before first dose of study treatment, except for superficial skin cancers, or localized, low-grade tumors deemed cured and not treated with systemic therapy.

[00447] The ADC of **Example 1** is supplied in 10 mL vials filled with 3.4 mL of drug product formulated at 15 mg/mL (51 mg/vial). The extractable volume from each vial is ≥ 3.0 mL (≥ 45 mg). The drug product is provided as frozen liquid, and vials must be stored at ≤ -65 °C prior to use.

Objectives and Endpoints

[00448] **Table 5** summarizes the primary objectives of the study and the endpoints that are used to achieve and evaluate those objectives.

Table 5: Objectives and Endpoints

Objectives	Endpoints
Dose Escalation Stage (Single-Agent Cohorts)	
Primary	
• To determine the MTD and/or RD for further evaluation of IV administration of the ADC in subjects with advanced malignancies	• Incidence and severity of DLTs
Secondary	
• To evaluate safety and tolerability of the ADC	• Incidence of AEs, SAEs, AEs leading to discontinuation, and deaths
• To characterize PK of the ADC	• Evaluate the PK of the ADC (antibody conjugated to payload), total antibody (unconjugated and conjugated antibody), and free payload in plasma or serum at specified time points
• To assess the immunogenicity of the ADC	• The incidence of ADAs response to the ADC

Exploratory	
• To evaluate the antitumor activity of the ADC as measured by ORR, DOR, and PFS per RECIST 1.1 per INV	• ORR per RECIST 1.1 per INV • DOR per RECIST 1.1 per INV • PFS per RECIST 1.1 per INV
• To evaluate the relationship between PK and exploratory PD biomarkers, preliminary efficacy and safety outcomes	• Change from baseline in plasma protein biomarkers (such as, but not limited to, HMGB1, CDH2, SFRP1, PLAUR) in association with the ADC concentration. • Changes in the tumor (*e.g.*, IHC of 5T4, IHC of proliferation markers [*e.g.*, Ki67], IHC of mitotic marker[*e.g.*, phospho-histone H3], spatial transcriptomics analysis of tumor, stroma and interface) • Correlation of selected tumor (*e.g.*, IHC and/or genetics and genomics) and blood biomarkers (*e.g.*, soluble proteins, ctDNA) with clinical endpoints
Dose-Escalation Stage (Combination Therapy Cohorts)	
Primary	
• To determine the MTD and/or RD for further evaluation of IV administration of the ADC in combination with pembrolizumab in subjects with advanced malignancies	• Incidence and severity of DLTs
Secondary	
• To evaluate safety and tolerability of the ADC in combination with pembrolizumab	• Incidence of AEs, SAEs, imAEs, AEs leading to discontinuation, and deaths
• To characterize PK of the ADC in combination with pembrolizumab	• Evaluate the PK of the ADC (antibody conjugated to payload), total antibody (unconjugated and conjugated antibody), and free payload in plasma or serum at specified time points
• To characterize the immunogenicity of the ADC when administered in combination with pembrolizumab	• The incidence of ADAs response to the ADC
Exploratory	
• To evaluate the antitumor activity of the ADC in combination with pembrolizumab as measured by ORR, DOR, and PFS per RECIST 1.1 per INV	• ORR per RECIST 1.1 per INV • DOR per RECIST 1.1 per INV • PFS per RECIST 1.1 per INV
• To evaluate the relationship between PK and exploratory PD biomarkers, preliminary efficacy and safety outcomes	• Change from baseline in plasma protein biomarkers (such as, but not limited to, HMGB1, CDH2, SFRP1, PLAUR) in association with the ADC concentration.

	• Changes in the tumor (*e.g.*, IHC of 5T4, IHC of proliferation markers [*e.g.*, Ki67], IHC of mitotic marker[*e.g.*, phospho-Histone H3], spatial transcriptomics analysis of tumor, stroma and interface) • Correlation of selected tumor (*e.g.*, IHC and/or genetics and genomics) and blood biomarkers (*e.g.*, soluble proteins, ctDNA) with clinical endpoints
Cohort-Expansion Stage (Single-Agent and Combination Therapy Cohorts	
Primary	
• To evaluate preliminary efficacy of the ADC by estimating the ORR per RECIST 1.1 per INV	• ORR per RECIST per INV
Secondary	
• To evaluate safety and tolerability of the ADC	• Incidence of AEs, SAEs, AEs leading to discontinuation, deaths
• To evaluate the antitumor activity of the ADC as measured by ORR, DOR, OS, and PFS per RECIST 1.1 per INV for selected cohorts	• Evaluate the antitumor activity of the ADC as measured by DOR and PFS per RECIST 1.1 • ORR by INV • ORR for selected cohorts • OS
• Characterize PK of the ADC monotherapy and PK of the ADC when administered in combination with pembrolizumab	• Evaluate the PK of the ADC (antibody conjugated to payload), total antibody (unconjugated and conjugated antibody), and free payload in plasma or serum at specified time points.
• Characterize immunogenicity of the ADC	• The incidence of ADAs response to the ADC
Exploratory	
• To assess the effects of the ADC on tumor and blood biomarkers and correlation of tumor markers with clinical endpoints	• Change from baseline in plasma protein biomarkers (such as, but not limited to, HMGB1, CDH2, SFRP1, PLAUR) or in post-treatment tumor tissue (*e.g.*, IHC of 5T4, IHC of Ki67 and phospho-Histone H3, spatial transcriptomics analysis of different compartments in the tumor and stroma), in association with the ADC concentration. Correlation of selected tumor biomarkers (*e.g.*, IHC and/or genetics and genomics) and blood biomarkers (*e.g.*, soluble proteins, ctDNA, CTC) with clinical endpoints
Abbreviations Used: ADA, anti-drug antibody; AE, adverse event; ctDNA, circulating tumor DNA; DLT, dose-limiting toxicities; DOR, duration of response; IHC, immunohistochemistry; INV, investigator; IV, intravenous; MTD, maximum tolerated dose; ORR, objective response rate; PD, pharmacodynamic; PFS, progression-free survival; PK, pharmacokinetics; RD, recommended dose; SAE, serious adverse event	

Tumor Assessments

[00449] Tumor statuses are evaluated by contrast-enhanced computed tomography (CT) and/or magnetic resonance imaging (MRI) and treatment response is assessed according to RECIST 1.1 (Eisenhauer et al 2009). RECIST 1.1 assessments are not required for subjects with primary brain tumors, such as glioblastoma. The same imaging modalities used at screening are used for each subsequent tumor assessments after first dose of study treatment.

[00450] Radiographic assessments include the following:

[00451] Chest / Abdomen / Pelvis (CAP) Imaging: CT of CAP or CT chest and MRI abdomen/pelvis are performed in all subjects at screening and at every 6 weeks after initiation of study Upon completion of 42 weeks on study, these assessments are performed every 9 weeks.

[00452] Brain Imaging:

[00453] In subjects with brain metastases, MRI (or CT) of the brain is performed at screening in all subjects who have a history or clinical symptoms of brain metastasis in the Dose-Escalation Stage and in all subjects in the Cohort-Expansion Stage. After study treatment initiation, assessments are performed every 6 weeks in subjects with brain metastasis or if clinically indicated. Upon completion of 42 weeks on study, these assessments are performed every 9 weeks (± 7 days).

[00454] In subjects with primary brain tumors, such as glioblastoma, contrast-enhanced MRI of the brain is performed in all subjects with primary brain tumors at screening and every 6 weeks (± 5 days) after initiation of study treatment. Response assessment in neuro-oncology criteria (RANO) is used to assess treatment response (Huang and Wen, 2016, "Response Assessment in Neuro-Oncology Criteria and Clinical Endpoints," Magn Reason Imaging Clin N Am, 24(4):705-718). Upon completion of 42 weeks on study, these assessments are performed every 9 weeks (± 7 days).

[00455] Bone Imaging: Technetium bone scans or positron emission tomography (PET) imaging for bone metastasis (*e.g.*, 18F-NaF) are not part of study-mandated assessments but can be used per standard clinical practice to direct corroborative CT/MRI imaging per RECIST 1.1 for subjects with suspected or known bone metastases.

[00456] Radiographic tumor assessments per the Investigator may be used for treatment decisions. Study treatment(s) may be discontinued upon locally determined radiographic progression and/or if the subject is no longer felt to be clinically benefiting. Radiographic tumor assessments continue according to the protocol-defined schedule, regardless of whether study treatment is reduced, withheld, delayed, or discontinued until the later of radiographic progression or study treatment discontinuation.

Assessments

[00457] The schedule of required assessments for the Dose-Escalation Stage and Cohort-Expansion Stage for subjects receiving ADC treatment is presented in the following **Table 6** and **Table 7**.

[00458] The following are principles for procedures and assessments during the treatment period:

- Cycle 1, Day 1 is defined as the first IV study treatment date. ADC infusions are administered in "cycles," every 3 weeks ± 3 days (except when delayed due to AE) and always at least 19 days apart and no more than 42 days between doses. The start of a cycle is the day of IV study treatment infusion.
- During the treatment period, study visits to assess safety and laboratory studies are required prior (within 72 hours) to each treatment cycle and continue at least every 3 weeks or more frequently if clinically indicated, *i.e.*, during treatment delays.
- Visits are required during the first 2 cycles at Day 15 (± 1 day) after IV study drug administration to monitor AEs, vital signs, and certain safety labs.
- Radiographic tumor assessments occur with corresponding treatment cycles which are fixed from the date of randomization. Time points are not be modified as a result of delay, hold, or discontinuation of study treatment.
- Unscheduled visits for additional clinical and safety evaluations are allowed at any time.
- PK, immunogenicity, PD, and biomarker assessments are performed per **Tables 6** and **7**.

Table 6: Schedule of Assessments for the Dose Escalation Stage

Assessment	Pre-Treatment	21-Day DLT-Evaluation Period						Treatment Period[b]					Post-Treatment[c]			
		Cycle 1						Cycle 2			Cycle 3 and beyond		Safety FU (after the decision to discontinue study treatment)			Extended FU
	Screening[a]	Day 1	Day 2	Day 4[d]	Day 8 (± 1d)	Day 15 (± 1d)	Day 21 (± 1d)	Day 1	Day 2	Day 8 (± 1d)	Day 1	EOT	30-day follow- up (+14 d)	60-day follow- up (+14 d)	100-day[e] follow-up (+14 d)	(Q12W ± 14d)
Informed consent[e]	X															
Demographics, medical and cancer history	≤ 28 days															
Safety[f]																
Physical examination + weight[g]	≤ 28 days (+ height)	X	X		X	X	X	X			X	X	X	X	X	
ECOG PS	≤ 28 days	X						X			X	X	X	X	X	
Vital signs[h]	≤ 28 days	X			X	X		X		X	X	X	X	X	X	
Echocardiogram	≤ 10 days															
12-lead ECG[i]	≤ 10 days	X				X		X			X	X	X	X	X	
Concomitant medications	Document concomitant medication taken from 21 days before first dose of study treatment through 30 days after the date of the decision to discontinue study treatment.															
Adverse events	Document new or worsening AEs from informed consent through the 30-day follow-up visit. AE information i collected at study visits and may also be collected at any time over the phone or by spontaneous subject report. At the date of the first dose of study treatment, AEs are documented pre- and post-dose. Certain AEs and all SAEs that are ongoing after the 30-day follow-up visit are followed until resolution or determination by the Investigator that the event is stable or irreversible.															
Ophthalmologic Evaluation[k]	≤ 28 days										X					

Neurological and ocular symptom assessment	≤ 28 days										X					
Hematology	≤ 10 days	X[l]	X		X	X	X	X		X	X	X	X	X	X	
Serum chemistry	≤ 10 days	X[l]	X		X	X	X	X		X	X					
Urinalysis	≤ 10 days	X[l]	X		X	X		X		X	X	X	X	X	X	
Urine chemistry including UPCR	≤ 10 days	X[l]	X		X	X		X		X	X[a]	X	X	X	X	
Thyroid function test (TSH, fT3, fT4)	≤ 10 days	X[l]						X			X[k]	X	X	X	X	
HBV and HCV; HIV (if required per local regulation)	≤ 10 days															
INR and aPTT	≤ 10 days	X			X			X			X[n]	X	X	X	X	
Serum or urine pregnancy test[l]	≤ 7 days (serum)	X						X			X	X	X	X	X	
FSH[m]	≤ 10 days	X														
Study Treatment																
ADC IV infusion		Doses of ADC are administered every 3 weeks (+ 3 days) and at least 19 days apart. After ADC IV infusion, subjects are monitored in the clinic for at least 60 minutes (for the first infusion) and, if tolerated (with no IRRs), at least 30 minutes for subsequent infusions. Prophylaxis for ocular AEs is administered in relation to infusions as described herein.														
Pembrolizumab infusion		Doses of pembrolizumab are administered every 3 weeks (+ 3 days) and at least 19 days apart. After pembrolizumab IV infusion, subjects are monitored in the clinic for at least 60 minutes (for the first infusion) and, if tolerated (with no IRRs), at least 30 minutes for subsequent infusions. Prophylaxis for imAEs is administered in relation to infusions as described herein.														
Pharmacokinetic, Pharmacodynamic, and Biomarker Assessments																
All PK, PD, and biomarker analyses are collected at time points as indicated in the Schedule of Assessments in Table 8.																

Tumor Assessments			
CT/MRI[n] **Chest, Abdomen, Pelvis (CAP)**	≤ 28 days	Every 6 weeks (± 7 days) throughout the first 42 weeks on study. Upon completion of 42 weeks on study (Week 42 visit), these assessments are performed every 9 weeks (± 7 days).	
MRI/CT[o] **Brain**	≤ 28 days	Assessments after the first dose of study treatment are performed every 6 weeks (± 7 days) throughout the first 42 weeks on study. Upon completion of 42 weeks on study (Week 42 visit), these assessments are performed every 9 weeks (± 7 days).	
Technetium bone scan[p] **Bone**	≤ 28 days	Assessments are performed every 12 weeks (± 7 days) throughout the first 48 weeks and every 24 weeks (± 14 days) thereafter or as clinically indicated.	
Abbreviations: aPTT, activated partial thromboplastin; CAP, chest, abdomen, pelvis; CT, computed tomography; DLT, dose-limiting toxicity; ECG, electrocardiogram; ECOG, Eastern Cooperative Oncology Group; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; INR, international normalized ratio; IV, intravenous; MRI, magnetic resonance imaging; OS, overall survival; PD, pharmacodynamic; PK, pharmacokinetic; TSH, thyroid stimulating hormone; UPCR, urine protein-creatinine ratio **Footnotes** Note: Baseline is defined as the most recent assessment prior to receiving study drug(s). [a] Screening: Results of screening assessments are reviewed before first dose of study treatment to confirm that the subject meets the eligibility criteria. [b] Treatment Period: Subjects may continue administration of study treatment in the absence of dose-limiting toxicity (DLT) during the Treatment Extension Period (or if a DLT has resolved) if there is no clinical evidence of disease progression for up to 24 months with the agreement of the Sponsor. Subjects may continue to receive ADC for more than 24 months if the subject is deriving clear clinical benefit as determined by the Investigator and agreed by the Sponsor. [c] Post-treatment Period: Date of decision to discontinue study treatment is defined as the later of the date of the decision to permanently discontinue study treatment or the date of the last dose of study treatment. [d] Day 4 assessments are performed for PK samples. Further details are available in Table 8. [e] Informed consent: Informed consent must be provided before any study-specific procedures are performed; however, evaluations performed as part of routine care before informed consent can be utilized as screening evaluations if permitted by the site's IRB/EC policies. [f] Safety assessments: Safety assessments may be performed up to 72 hours prior to Day 1 of each cycle. g Physical examination: Symptom-directed physical examination is conducted on Day 1 before the first dose of study treatment and at subsequent safety assessments as clinically indicated. The actual bodyweight (consistently measured with or without shoes) is determined prior to each infusion in order to determine the dose of ADC. [h] Vital signs: Vital signs consist of 5-minute sitting or supine blood pressure, pulse rate, respiratory rate, and temperature. Vital signs are measured pre-dose (*e.g.*, within 60 minutes prior to administration of study treatment whenever possible). At study visits for which multiple assessments are planned at the same time point, ECGs are measured first, followed by vital signs, and then blood collection (initiation of PK sampling) whenever possible. [i] 12-lead ECG: On Day 1, 12-lead ECG is performed prior to administration of study treatment and 1 hour (± 30 min) post-dose. Post-dose ECG timepoints are relative to the end of study treatment infusion. ECG is not required pre-dose on the day of Dose 2 if done within 1 day prior. Subsequently, if performed on the day of study drug administration, 12-lead ECGs are performed prior to study treatment administration at indicated			

time points. Additional ECGs are performed if clinically indicated. In the event of re-initiation of ADC treatment due to QT prolongation, ECGs are repeated weekly for 2 weeks, then every 2 weeks for 1 month, then according to the protocol-defined time points.

[j] Day 1 assessment: This assessment intends to confirm suitability for treatment after screening and prior to first dose. If this assessment is performed during screening within 10 days (7 days for pregnancy test for women of childbearing potential) prior to first dose, this assessment does not need to be performed on Day 1, unless the subject's clinical status has changed (*e.g.*, onset of new symptoms indicative of clinical deterioration). If the assessment is performed pre-dose on Day 1, the results must be available to and reviewed by the Investigator prior to any treatment being administered.

[k] Ophthalmic examinations are performed by an ophthalmologist (or an optometrist if an ophthalmologist is not available): (1) during the screening period (baseline) within 21 days prior to first dose; (2) follow-up on treatment at least 1 week after the previous ADC dose and within 2 weeks prior to each next ADC dose; (3) promptly for worsening of ocular symptoms. Baseline ophthalmic examination includes ophthalmic history, documentation of current glasses prescription (if applicable), best corrected visual acuity, Schirmer's test, slit lamp, tonometry. Follow-up on treatment ophthalmic examinations include best corrected visual acuity and slit lamp. Additional on treatment ophthalmic examinations are performed as clinically indicated. Subjects with ongoing corneal adverse events at 60-Day follow-up visit (60-Day FU) are followed periodically (*e.g.*, monthly) until resolved or deemed clinically stable by an ophthalmologist (or an optometrist if an ophthalmologist is not available).

[l] Serum or urine pregnancy test: A serum pregnancy test (for females of childbearing potential) is repeated before dosing Day 1 unless a pregnancy evaluation was done during screening within 7 days prior to Day 1. Additional pregnancy tests (serum or urine) are performed for postbaseline pregnancy evaluation.

[m] FSH: For women under the age of 55 years to determine menopause status.

[n] The same imaging modality is used throughout the study. PR or CR per RECIST 1.1 at a given time point must be confirmed by repeat assessments ≥ 4 weeks after the criteria for response are first met. This may be done at the next scheduled assessment.

[o] Imaging of the brain is only required in subjects with documented, locally treated brain metastasis or if indicated by clinical evidence of new brain metastasis. In order to meet the eligibility requirements of the study, brain metastasis must have been treated and stable for at least 4 weeks before first dose.

[p] Technetium bone scans or positron emission tomography (PET) imaging for bone metastasis (*e.g.*, 18F-NaF) are not part of study-mandated assessments but can be used per standard clinical practice to direct corroborative CT/MRI imaging per RECIST 1.1 and may also be used to assess progression in prostate cancer patients (see Cohort I inclusion criteria). Bone lesions corroborated by CT/MRI must be reported as non-target or new lesions.

Table 7: Schedule of Assessments for the Cohort-Expansion Stage

Assessment	Pre-Treatment	Treatment Period – Cohort-Expansion Stage[b]											Post-Treatment[c]		
	Screening[a]	Cycle 1						Cycle 2			Cycle 3 and beyond	EOT	30-day follow-up (+14 d)	60-day follow- up (+14 d)	Extended FU (Q12W ± 14d)
		Day 1	Day 2	Day 4[d]	Day 8 (± 1d)	Day 15 (± 1d)	Day 21 (± 1d)	Day 1	Day 2	Day 8 (± 1d)	Day 1				
Informed consent[e]	X														
Demographics, medical and cancer history	≤ 28 days														
Safety[f]															
Physical examination + weight[g]	≤ 28 days (+ height)	X	X		X	X	X	X			X	X	X	X	
ECOG PS	≤ 28 days	X						X			X	X	X	X	
Vital signs[h]	≤ 28 days	X			X	X		X		X	X	X	X	X	
Echocardiogram	≤ 10 days														
12-lead ECG[i]	≤ 10 days	X				X		X			X	X	X	X	
Concomitant medications	Document concomitant medication taken from 21 days before first dose of study treatment through 30 days after the date of the decision to discontinue study treatment.														
Adverse events	Document new or worsening AEs from informed consent through the 30-day follow-up visit. AE information are collected at study visits and may also be collected at any time over the phone or by spontaneous subject report. At the date of the first dose of study treatment, AEs are documented pre- and post-dose. Certain AEs and all SAEs that are ongoing after the 30-day follow-up visit are to be followed until resolution or determination by the Investigator that the event is stable or irreversible.														
Ophthalmologic Evaluation[k]	≤ 28 days										X				

Neurological and ocular symptom assessment	≤ 28 days										X				
Hematology	≤ 10 days	X[j]	X		X	X	X	X		X	X	X	X	X	
Serum chemistry	≤ 10 days	X[j]	X		X	X	X	X		X	X				
Urinalysis	≤ 10 days	X[j]	X		X	X		X		X	X	X	X	X	
Urine chemistry including UPCR	≤ 10 days	X[j]	X		X	X		X		X	X[d]	X	X	X	
Thyroid function test (TSH, fT3, fT4)	≤ 10 days	X[j]						X			X[n]	X	X	X	
HBV and HCV; HIV (if required per local regulation)	≤ 10 days														
INR and aPTT	≤ 10 days	X			X			X			X[n]	X	X	X	
Serum or urine pregnancy test[l]	≤ 7 days (serum)	X						X			X	X	X	X	
FSH[m]	≤ 10 days	X													
Study Treatment															
ADC IV infusion		Doses of ADC are administered every 3 weeks (+ 3 days) and at least 19 days apart. After ADC IV infusion, subjects are monitored in the clinic for at least 60 minutes (for the first infusion) and, if tolerated (with no IRRs), at least 30 minutes for subsequent infusions. Prophylaxis for ocular AEs is administered in relation to infusions as described herein.													
Pharmacokinetic, Pharmacodynamic, and Biomarker Assessments															
All PK, PD, and biomarker analyses are collected at time points as indicated in the Schedule of Assessments in Table 9.															
Tumor Assessments															
CT/MRI[n] **Chest, Abdomen, Pelvis (CAP)**	≤ 28 days	Every 6 weeks (± 7 days) throughout the first 42 weeks on study. Upon completion of 42 weeks on study (Week 42 visit), these assessments are performed every 9 weeks (± 7 days).													

MRI/CT[o] **Brain**	≤ 28 days	Assessments after the first dose of study treatment are performed every 6 weeks (± 7 days) throughout the first 42 weeks on study. Upon completion of 42 weeks on study (Week 42 visit), these assessments are performed every 9 weeks (± 7 days).
Technetium bone scan[p] **Bone**	≤ 28 days	Assessments are performed every 12 weeks (± 7 days) throughout the first 48 weeks and every 24 weeks (± 14 days) thereafter or as clinically indicated.
Abbreviations and footnote are the same as in Table 6.		

[00459] Blood samples for PK assessments and tumor samples are obtained and analyzed from subjects in all treatment cohorts as shown in **Table 8** and **Table 9**:

Table 8: Timeline for Sample Collections for Dose Escalation Cohorts

		Pharmaco-kinetic	Immunogenicity	Biomarker			
		Blood		Blood (Plasma Biomarker)	Blood (CTC)	Tumor Tissue (Archival)	Tumor Tissue (fresh biopsy)
Pre-Treatment Period							
Screening (≤ 14 days)						X[a]	
Treatment Period The ADC is administered as an intravenous infusion every 3 weeks. The PK sample is taken ~30 min prior to dosing, within ± 5 min up to 2 h post-dose and within ±15 min of later post-dose timepoints.							
21-Day DLT-Evaluation Period							
Day 1 (C1D1)	Predose	X (All)	X (All)	X	X		
	Post-dose: 15 min	X (All)					
Day 2 (C1D2)	24 h post-C1D1 dose	X (All)		X			
Day 4 (C1D4)	72 h post-dose	X (All)	X	X			
Day 8 (C1D8)	168 h post-dose	X (All)	X	X			
Day 15 (C1D15)	336 h post-C2D1 dose	X (All)					
Day 22 (C2D1)	Predose	X (All)	X (All)	X			X[b]
	Post-dose: 15 min	X (All)					
Treatment Extension Period							
Day 23 (C2D2)	24 h post C2D1 dose	X (All)					
Day 25 (C2D4)	72 h post-C2D1 dose	X (All)					
Day 29 (C2D8)	168 h post-C2D1 dose	X (All)					
Day 36 (C2D15)	336 h post-C2D1 dose	X (All)					
C3D1 and beyond	Predose	X (All)	X (All)		X		
	Post-dose: 15 min	X (All)					
	Post-dose: 7 +/-2 days	X (All)					
At progression					X		
Post-Treatment Period							
30-day FU		X	X (All)				
60-day FU		X	X (All)				
100-day FU		X	X (All)				

All = for all subjects

[a] Available archival tumor tissue (obtained approximately 2 years prior to consent) are collected. If archival

tumor tissue is not available, fresh tumor biopsy may be performed at least 7 days (and up to 60 days) prior to the Day 1 visit, if consented and if it can be safely acquired.
[b] Optional tumor samples may be collected at the Day 22 visit (± 5 days).

Table 9: Timeline for Sample Collections for Cohort Expansion Stage

		Pharmaco-kinetic	Immunogenicity	Biomarker				
		Blood		Blood (Plasma Biomarker)	Blood (Germline DNA)	Blood (ctDNA)	Tumor Tissue (Archival)	Tumor Tissue (fresh biopsy)
Pre-Treatment Period								
Screening (≤ 14 days)							X[a]	
Treatment Period The ADC is administered as an intravenous infusion every 3 weeks. The PK sample is taken ~30 min prior to dosing, within ± 5 min up to 2 h post-dose and within ±15 min of later post-dose time points.								
21-Day DLT-Evaluation Period								
Day 1 (C1D1)	Predose	X (All)	X (All)		X	X	X	
	Post-dose: 15 min	X (All)						
Day 2 (C1D2)	24 h post-C1D1 dose	X (All)						
Day 4 (C1D4)	72 h post-dose	X (All)						
Day 8 (C1D8)	168 h post-dose	X (All)						
Day 15 (C1D15)	336 h post-C2D1 dose	X (All)						
Day 22 (C2D1)	Predose	X (All)	X (All)					X
	Post-dose: 15 min	X (All)						
Treatment Extension Period								
Day 23 (C2D2)	24 h post C2D1 dose	X (All)						
Day 25 (C2D4)	72 h post-C2D1 dose	X (All)						
Day 29 (C2D8)	168 h post-C2D1 dose	X (All)						
Day 36 (C2D15)	336 h post-C2D1 dose	X (All)						

C3D1 and beyond	Predose	X (All)	X (All)		X			
	Post-dose: 15 min	X (All)						
	Post-dose: 7 +/-2 days	X (All)						
At progression					X			
Post-Treatment Period								
30-day FU		X	X (All)					
FU 2		X	X (All)					
All = for all subjects [a] Available archival tumor tissue (obtained approximately 2 years prior to consent) are collected. If archival tumor tissue is not available, fresh tumor biopsy may be performed at least 7 days (and up to 60 days) prior to the Day 1 visit, if consented and if it can be safely acquired. [b] Optional tumor samples may be collected at the Day 22 visit (± 5 days).								

[00460] Exploratory biomarker analyses, including, but not limited to:

- Genomics (*e.g.*, mutation alterations, epigenetic modifications, copy number);
- Expression (*e.g.*, TPBG, Beta **III** Tubulins, drug efflux transporters);
- Pharmacodynamics analyses (*e.g.*, CDH2, SFRP1, PLAUR);
- Response biomarkers (*e.g.*, Soluble HMGB1, cytokines, cell cycle arrest and apoptosis markers in tumor tissue);
- ctDNA; and
- CTC.

Example 3: Preclinical Characterization of 5T4-ADC-1 for the Treatment of Solid Tumors that Targets Tumor-Associated Antigen 5T4

[00461] 3.1 Summary

[00462] *In vitro* and *in vivo* efficacy of **5T4-ADC-1** was assessed in cell-derived xenograft (CDX) breast cancer cell lines (MCF-7, MDA-MD-468) and in patient-derived xenograft (PDX) tumor models (squamous cell carcinoma of the head and neck [HNSCC], non-small cell lung cancer [NSCLC], and breast cancer). Additionally, the *in vivo* combinatorial efficacy of **5T4-ADC-1** + anti-PD-1 antibody was assessed in an MC38-h5T4 syngeneic colon cancer xenograft model.

[00463] The results showed that **5T4-ADC-1** demonstrated *in vitro* cytotoxic effects with subnanomolar potency in the MCF7 and MDA-MB-468 breast cancer cell lines and *in vivo* tumor growth inhibition (80-99%) compared with vehicle-treated animals in xenograft and

PDX models at doses ranging from 5-10 mg/kg **5T4-ADC-1**. In the syngeneic MC38-h5T4 expressing colon cancer xenograft model, **5T4-ADC-1** + anti-PD-1 showed improved efficacy compared to either agent administered alone.

[00464] Accordingly, **5T4-ADC-1** is a novel anti-5T4 ADC that exhibits potent anti-tumor activity, inhibiting cancer cell growth *in vitro* and tumor growth in various *in vivo* models. These findings support the evaluation of **5T4-ADC-1** in human clinical studies.

[00465] **3.2 Materials and Methods**

[00466] RP-HPLC analysis: All samples (**5T4-ADC-1** and MMAE payload [purchased from MedChemExpress, Catalog No. CAS: 474645-27-7]) were analyzed using an Agilent 1260 HPLC system equipped with an XBridge Shield RP 18, 4.6 x 150 mm, 3.5 µm (Waters). The operational conditions were set as follows: a flow rate of 1.0 mL/min at 50 °C, with mobile phase A (MPA) comprising 0.1% trifluoroacetic acid in water, and mobile phase B (MPB) containing 0.1% trifluoroacetic acid in acetonitrile. Absorbance was monitored at 280 nm using a reference wavelength of 450 nm. A volume of 20 µL of the sample was injected into the system for each analysis. The elution profile included a 30-minute linear gradient from 1% to 30% MPB, followed by a 5-minute wash with 99% MPB, then a final 2-minute re-equilibration at 1% MPB.

[00467] Cell lines: MCF-7 and MDA-MB-468 cells (human breast cancer lines which express 5T4) were from the American Type Culture Collection (ATCC) or the National Cancer Institute (NCI) and were authenticated using morphology, short tandem repeat profiling, or polymerase chain reaction-based methods. Cells were maintained at 5% CO_2 at 37 °C as recommended and did not exceed 18 passages in culture.

[00468] *In vitro* cytotoxicity: To assess the *in vitro* cytotoxicity of **5T4-ADC-1**, eight concentrations (3 pm to 50 nM) were applied to breast cancer cell lines expressing the 5T4 antigen (MDA-MB-468 and MCF7). Cell viability was measured after 120 h using the CellTiter-Glo® luminescent assay (Promega, Catalog No. G7573), where the percentage viability was calculated by comparing the luminescence of treated cells relative to untreated control cells (*i.e.*, viability% = $100\text{-}[lum_{avg\ untreated} - lum_{experimental}]/lum_{avg\ untreated} \times 100$).

[00469] *In vivo* efficacy (anti-tumor activity) of **5T4-ADC-1** - Cell-derived xenograft (CDX) models: *In vivo* studies were performed at AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) accredited facilities under protocols approved by the respective Institutional Animal Care and Use Committee. The *in vivo* efficacy of **5T4-ADC-1** was evaluated in two murine xenograft models of breast cancer: MCF7 (female athymic nude mice; n=10 per treatment group; Charles River Discovery Services) and MDA-

MB-468 (female severe combined immunodeficiency disease [SCID] beige mice; n=8 per treatment group; Charles River Discovery services). For both models, mice were inoculated with either MCF-7 or MDA-MB-468 cells via subcutaneous flank injection and randomized to treatment groups once the tumor had reached a size of 100–150 mm^3. After randomization, the mice received intravenous (IV) doses (either one dose on day 1 or two doses [one on day 1 and one on day 7] in the MCF-7 model; one dose on day 1 and day 4 for the MDA-MB-468 model]). Dose levels for the two MCF-7 studies were as follows: **5T4-ADC-1** (0.5, 2.5, or 5 mg/kg in the single-dose regimen or 0.25, 1.25, or 2.5 mg/kg for each of the two doses in the two-dose QW [once weekly] x2 regimen). For the MDA-MB-468 model, animals received 5 mg/kg doses on day 1 and day 4). In all studies, negative control (isotype-matched ADC matched to the highest **5T4-ADC-1** dose being evaluated), positive control (5T4-targeting mafodotin ADC matched to the highest **5T4-ADC-1** dose being evaluated), or vehicle (PBS or 20 mM sodium citrate/50 mM sodium chloride) were included. For the MDA-MB-468 study, the mAb from **5T4-ADC-1** was also included as a treatment group (5 mg/kg). Body weight and tumor size were measured bi-weekly throughout the studies, and animals were euthanized when the tumor size exceeded a specific threshold (700 mm^3 for MDA-MD-468; 1000 mm^3 for MCF-7) or there was a >20% body weight loss. The tumor growth inhibition percentage (TGI%) was assessed at day 84 for MDA-MD-468 and day 26/28 for MCF-7 (one or two-dose models, respectively).

[00470] *In vivo* efficacy (anti-tumor activity) of **5T4-ADC-1 - 5T4-ADC-1** in combination with an anti-PD-1 agent: To evaluate the efficacy of **5T4-ADC-1** in combination with an anti-programmed cell death protein 1 (anti-PD-1) checkpoint inhibitor, female C57BL/6 mice were inoculated with MC38-h5T4 cells (a syngeneic colon cancer model) via subcutaneous flank injection and randomized to treatment groups once the tumor had reached a size of 87 mm^2 (n=10 per group; Crown Bioscience). After randomization, the mice received either **5T4-ADC-1** vehicle + anti-PD-1 vehicle, **5T4-ADC-1** 5 mg/kg (single dose) + anti-PD-1 vehicle, **5T4-ADC-1** vehicle + anti-PD-1 0.5 mg/kg (twice weekly for 2 weeks), **5T4-ADC-1** vehicle + anti-PD-1 0.75 mg/kg (twice weekly for 2 weeks), **5T4-ADC-1** 5 mg/kg (single dose) + anti-PD-1 0.75 mg/kg (twice weekly for 2 weeks), or **5T4-ADC-1** 5 mg/kg (single dose) + anti-PD-1 0.5 mg/kg (twice weekly for 2 weeks). Tumor volumes were measured twice per week (in two dimensions) and animals were euthanized when the tumor size reached 2,000 mm^3, there was >20% body weight loss, or they reached day 55 (from randomization). TGI% was assessed at day 18.

[00471] *In vivo* efficacy (anti-tumor activity) of **5T4-ADC-1 -** Patient-derived xenograft (PDX) models: PDX models (Crown Bioscience; HNSCC, NSCLC, and breast cancer) were selected based on H scores for 5T4 expression (determined by immunohistochemistry methods) between 100 and 200. Single IV doses of 10 mg/kg **5T4-ADC-1** or vehicle were administered to female NOD [non-obese diabetic]/SCID or Balb/c mice (n=10/group/study). The tumor volume was monitored for at least 28 days post treatment. Animals were euthanized when the tumor size reached >3,000 mm^3, there was >20% body weight loss, or they reached the prespecified end of study. TGI% was assessed on the final respective study days for both treatment and vehicle groups.

[00472] Statistical analyses: For *in vitro* cytotoxicity assays, survival was calculated as the percentage of luminescence (lum) in experimental wells relative to the untreated control wells. The average luminescence of the untreated wells was considered 100% viability. Specifically, the formula for calculating the percentage viability of an individual experimental well was as follows: % viability = $100-(lum_{avg\ unntreated}-lum_{experimental})/ lum_{avg\ unntreated}$ x 100. Data were displayed graphically using GraphPad PRISM. To calculate absolute IC_{50}, the normalized data were analyzed using a dose-response curve fitted using a nonlinear regression model and a four-parameter dose response.

[00473] For the MCF-7 and MDA-MB-468 CDX *in vivo* xenograft efficacy studies, statistical analyses were performed using GraphPad PRISM software. Statistical analyses for the PDX and CDX combination efficacy studies were performed in the R language and environment for statistical computing and graphics. Depending on the normality of *in vivo* efficacy data, either an ANOVA or Kruskal Wallis test was conducted for comparisons, with appropriate *post hoc* tests performed in cases where there were three or more groups.

[00474] For the PDX studies, pairwise comparisons were performed using either the Mann Whitney t test or Welch's t test.

[00475] For the MCF-7, MDA-MB-468 and PDX studies, %TGI was defined as (1-Ti/Ci)×100, where Ti and Ci were the mean tumor volumes of the treatment and control groups, respectively (on a given day). For the combination CDX study in the MC38-h5T4 model, %TGI was defined as 1−(Vt/V0)/(Ct-C0)*100, where V was the treated tumor volume at time t or 0, and C was the control [vehicle] volume). The log-rank (Mantel-Cox) test was used for survival analysis. In all cases, p-values of <0.05 were considered statistically significant.

[00476] 3.3 Results

[00477] A composition comprising **5T4-ADC-1** used herein was tested using RP-HPLC as described above and exhibited a DAR of 1.8.

[00478] In vitro cytotoxicity: *In vitro* cytotoxicity studies in MDA-MD-468 and MCF-7 breast cancer lines demonstrated that **5T4-ADC-1** has potent cytotoxic activity, with IC_{50} values of 0.896 nM (95% CI: 0.53–1.5) and 0.185 nM (95% CI: 0.10–0.33), and maximum inhibition values of 90.2% and 77.8% for MDA-MB-468 and MCF-7 cell lines, respectively (**FIGs. 4A and 4B**). The 5T4-targeting mafodotin ADC, had 4–7-fold less potency than **5T4-ADC-1**, with IC_{50} values of 6.4 nM (95% CI: 4.7–8.8) and 0.78 nM (95% CI: undefined) in MDA-MD-468 and MCF-7 cells, respectively (**FIGs. 4A and 4B**). In contrast, an isotype-matched control ADC (*e.g.*, a non-5T4-binding IgG1 antibody conjugated to the same linker payload) had little to no effect on tumor cell viability, confirming that 5T4 binding is required for cytotoxicity.

[00479] *In vivo* efficacy: In an MCF-7 breast cancer xenograft model, IV administration of 0.5, 2.5 or 5 mg/kg **5T4-ADC-1** as a single dose or of 0.25, 1.25, or 2.5 mg/kg **5T4-ADC-1** in a multi-dose QWx2 schedule resulted in dose-related tumor regression within the first 20 days and inhibited tumor growth up to end of study (58 days; **FIGs. 5A and 5B**). In the single-dose study (**Table 10**, **FIG. 5C**), TGI% values versus vehicle increased with dose (11%, 50% [$P<0.05$] and 93% [$P<0.0001$] for 0.5, 2.5 and 5 mg/kg **5T4-ADC-1**, respectively), as did the number of complete responses (CRs, defined as a tumor volume <13.5 mm^3 for three consecutive days: 0/10, 2/10, and 7/10, respectively). The median survival time for vehicle-treated animals was 29.5 days, compared with 29, 47 and >58 days (last day of study) for animals treated with 0.5 mg/kg, 2.5 mg/kg, and 5 mg/kg **5T4-ADC-1**, respectively. The same trends were observed in the multi-dose study (**Table 11**, **FIG. 5D**), with TGI% values of 21%, 50% ($P<0.001$) and 91% ($P<0.001$) and CRs of 0/10, 1/10 and 3/10 for QWx2 doses of 0.25, 1.25 and 2.5 mg/kg **5T4-ADC-1**, respectively. The median survival time in the vehicle group was 28 days, compared with 29.5, 38.5 and >58 days (last day of study) for the 0.25 mg/kg, 1.25 mg/kg and 2.5 mg/kg **5T4-ADC-1** groups, respectively. In addition, both single- and multi-dose MCF-7 studies showed that the 5T4-targeting mafodotin ADC resulted in a lower TGI% and fewer CRs than the highest **5T4-ADC-1** dose tested (**Table 11**). The isotype-matched ADC control also showed no anti-tumor activity in either xenograft study (TGI% 4.9% and 18%, respectively; no CRs in either study).

Table 10. Tumor Growth Inhibition, Response Rates, and Survival Following IV Administration of 5T4-ADC-1 and Controls (Single Dose in MCF7 Xenograft Model)

Group	CR	TGI% vs vehicle (day 26)	*P* value vs vehicle[a]	Median survival (days)	Log-rank test (Mantel-Cox) *P* value vs vehicle
Vehicle	0	--	--	29.5	--
Isotype ADC, 5 mg/kg	0	4.9	NS	31	NS
5T4 targeting mafodotin-ADC, 5 mg/kg	1	80	<0.001	>58	<0.001
5T4-ADC-1, 0.5 mg/kg	0	11	NS	29	NS
5T4-ADC-1, 2.5 mg/kg	2	50	<0.05	47	<0.05
5T4-ADC-1, 5 mg/kg	7	93	<0.0001	>58	<0.0001

[a]Statistical differences for TGI% vs vehicle were determined using one-way ANOVA with Dunnett's *post hoc* test, as appropriate.

ANOVA, analysis of variance; CR, complete response (defined as a tumor volume <13.5 mm^3 for three consecutive measurements; IV, intravenous; NS, = $P>0.05$; TGI%, percent tumor growth inhibition (defined as [1-Ti/Ci] × 100, where Ti and Ci are the mean tumor volumes of the treatment and control groups, respectively, on a given day).

Table 11. Tumor Growth Inhibition, Response Rates, and Survival Following IV Administration of 5T4-ADC-1 and Controls (QWx2 Dosing in MCF7 Xenograft Model)

Group	CR	TGI% vs vehicle (day 28)	*P* value vs vehicle[a]	Median survival (days)	Log-rank test (Mantel-Cox) *P* value (vs vehicle)
Vehicle	0	--	--	28	--
Isotype ADC, 2.5 mg/kg	0	18	NS	29.5	NS
5T4 targeting mafodotin-ADC, 2.5 mg/kg	0	57	<0.001	42	<0.001
5T4-ADC-1, 0.25 mg/kg	0	21	NS	29.5	NS
5T4-ADC-1, 1.25 mg/kg	1	50	<0.001	38.5	<0.05

Group	CR	TGI% vs vehicle (day 28)	*P* value vs vehicle[a]	Median survival (days)	Log-rank test (Mantel-Cox) *P* value (vs vehicle)
5T4-ADC-1, 2.55 mg/kg	3	91	<0.001	>58	<0.0001

[a]Statistical differences for TGI% vs vehicle were determined using one-way ANOVA with Dunnett's *post hoc* test, as appropriate.

ANOVA, analysis of variance; CR, complete response (defined as a tumor volume <13.5 mm^3 for three consecutive measurements; IV, intravenous; NS, = $P>0.05$; TGI%, percent tumor growth inhibition (defined as [1-Ti/Ci] × 100, where Ti and Ci are the mean tumor volumes of the treatment and control groups, respectively, on a given day).

[00480] In the MDA-MD-468 breast cancer model, IV administration of 5 mg/kg **5T4-ADC-1** resulted in near complete tumor regression within 20 days and inhibited tumor growth for up to 100 days post-dose (**FIG. 5E**). In addition, the TGI% value versus vehicle was 93% ($P<0.0001$) and CRs were seen in 8/10 animals (**Table 12**). In contrast, the 5T4-targeting mafodotin ADC did not exhibit durable tumor control (*i.e.*, tumor regrowth was apparent approximately 14 days after the last dose), had a lower TGI% (46%; $P<0.01$) with 2/10 CRs (**Table 12, FIG. 5E**). Neither the isotype-matched ADC (5 mg/kg) nor the parent mAb (5 mg/kg) showed any anti-tumor activity (TGI% 5.8% and 4.9%, respectively; no CRs in either group; **Table 12**).

Table 12. Tumor Growth Inhibition and Response Rates Following IV Administration of 5T4-ADC-1 and Controls (MDA-MB-468 Xenograft Model)

Group	CR	TGI% vs vehicle (day 84)	*P* value vs vehicle
Vehicle	0	--	--
Isotype ADC, 5 mg/kg	0	5.8	NS
5T4 targeting mafodotin-ADC, 5 mg/kg	2	46	<0.01
5T4-ADC-1, 5 mg/kg	8	93	<0.0001
Parental Antibody of **5T4-ADC-1**, 5 mg/kg	0	4.9	NS

[a]Statistical differences for TGI% vs vehicle were determined using one-way ANOVA with Dunnett's *post hoc* test, as appropriate.

ANOVA, analysis of variance; CR, complete response (defined as a tumor volume <13.5 mm^3 for three consecutive measurements; IV, intravenous; NS, = P>0.05; TGI%, percent tumor growth inhibition (defined as [1-Ti/Ci] × 100, where Ti and Ci are the mean tumor volumes of the treatment and control groups, respectively, on a given day).

[00481] **5T4-ADC-1** in combination with an anti-PD-1 agent: In the murine h5T4 syngeneic colon cancer model, a single dose of 5 mg/kg **5T4-ADC-1** significantly inhibited tumor growth compared with control by day 18 (TGI: 71%; P<0.001; **FIGs. 6A and 6B; Table 13**). In addition, CRs (defined as a tumor volume of 0 mm^3 for 3 consecutive days) were seen in 4/10 animals. In the **5T4-ADC-1** + anti-PD-1 groups, the **5T4-ADC-1**-mediated inhibition of tumor growth was enhanced further, with TGI values of 97% and 96% for **5T4-ADC-1** + 0.75 mg/kg anti-PD-1 and **5T4-ADC-1** + 5 mg/kg anti-PD-1, respectively (both P<0.001 vs control) with CR rates of 9/10 and 5/10, respectively; **FIGs. 6A and 6B; Table 13**). This enhanced tumor inhibition was not observed with the anti-PD-1 agent alone (0.75 mg/kg or 0.5 mg/kg doses), with TGIs of 55% and 57% and CR rates of 0/10 and 0/10, respectively. The **5T4-ADC-1** + anti-PD-1 treatment groups also showed the longest median survival (>55 days for **5T4-ADC-1** + 0.75 mg/kg anti-PD-1 and **5T4-ADC-1** + 0.5 mg/kg anti-PD-1, respectively), with other active treatment groups (vehicle + 0.5 mg/kg anti-PD-1; vehicle + 0.75 mg/kg anti-PD-1). having a median survival of 31–39 days. The control group had a median survival of 22 days.

Table 13. Tumor Growth Inhibition, Response Rates, and Survival Following IV Administration of 5T4-ADC-1 and Controls (h5T4-MC38 Model)

Group	Dose level (mg/kg)	CR	TGI% vs vehicle (day 18)	*P* Value[a] vs vehicle	Median survival (days)	Log-rank test (Mantel-Cox) *P* value (vs vehicle)
Vehicle (PBS) Vehicle (**5T4-ADC-1**)	- -	0	--	--	--	--
Vehicle (PBS) **5T4-ADC-1**	- 5	4	71	<0.001	22	<0.001
Anti-PD-1 Vehicle (**5T4-ADC-1**)	0.75 -	0	55	NS	39	<0.001
Anti-PD-1	0.5 -	0	57	NS	30.5	<0.001

Group	Dose level (mg/kg)	CR	TGI% vs vehicle (day 18)	*P* Value[a] vs vehicle	Median survival (days)	Log-rank test (Mantel-Cox) *P* value (vs vehicle)
Vehicle (**5T4-ADC-1**)						
Anti-PD-1 **5T4-ADC-1**	0.75 5	9	97	<0.001	>55	<0.001
Anti-PD-1 **5T4-ADC-1**	0.5 5	5	96	<0.001	>55	<0.001

[a]Statistical differences for TGI% vs vehicle were determined using Kruskal Wallis ANOVA with Conover's *post hoc* test.

ANOVA, analysis of variance; CR, complete response (defined as a tumor volume of 0 mm^3 for three consecutive measurements; IV, intravenous; NS, = *P*>0.05; TGI%, percent tumor growth inhibition, defined as (1-(Vt/V0) / (Ct-C0)) × 100 on the day indicated, where V was the treated tumor volume at time t or 0, and C was the control (vehicle) volume).

[00482] PDX Models: A single IV dose of 10 mg/kg **5T4-ADC-1** led to significant tumor reduction in 5T4 expressing PDX models across multiple indications (**FIGs. 7A-7C**) with TGI% values of 100%, 97% and 81% for HNSCC, NSCLC and breast cancer, respectively. **5T4-ADC-1**-treated animals showed tumor suppression and regression starting at ~10 days post dose and lasting to at least 30 days post dose, with CRs in 8/10, 0/10 and 7/10 animals for the HNSCC, NSCLC and breast cancer PDX models, respectively.

[00483] **3.4 Discussion**

[00484] Preclinical characterization of the novel anti-5T4 ADC, **5T4-ADC-1**, and its parental mAb showed that both have high binding affinity to the membrane distal end of recombinant human and cynomolgus monkey 5T4, with **5T4-ADC-1** displaying potent, target-mediated activity as a tandem-cleavage, MMAE-containing ADC with a highly site-specific DAR.

[00485] The combination of **5T4-ADC-1** and an anti-PD-1 agent in a murine syngeneic colon cancer model enhanced tumor suppression compared with either compound administered alone, with mean tumor volumes reduced by approximately 30-fold compared with controls, as well as TGI% values >95% and CRs in up to 90% of animals tested. These promising findings highlight the potential of **5T4-ADC-1** as a combination partner in 5T4-expressing cancers.

[00486] In summary, **5T4-ADC-1** exhibited potent anti-tumor activity, inhibiting cancer cell growth *in vitro* and tumor growth in various *in vivo* murine xenograft models and PDX models. These efficacy data suggests that **5T4-ADC-1** has promising therapeutic potential in a range of solid tumor types and supports the evaluation of **5T4-ADC-1** in clinical studies, either as monotherapy or in combination with immunotherapies such as anti-PD-1 agents.

Example 4: 5T4 Expression Analysis by IHC on Patient Tissues

[00487] An immunohistochemistry (IHC) study was performed to assess 5T4 expression of patient tumor tissue samples in select cancer types and to identify target cancer for clinical efforts.

[00488] Briefly, the tested samples were obtained from US Biomax. The IHC was then performed by following the manufacturer's instructions and using the following kits and reagents: primary antibody: Abcam, Cat # Ab134162, Clone # EPR5529, concentration: 0.168 mg/mL, dilution 1:600; secondary antibody detection kit: Bond Polymer Refine, Leica, Cat # DS9800; Bond™ Epitope Retrieval Solution 1,1L(Bond ER1) (Leica, Cat # AR9961); Bond™ Epitope Retrieval Solution 2,1L(Bond ER2) (Leica, Cat # AR9640); Bond™ Dewax Solution,1L (Leica, Cat # AR9222); Bond™ Wash Solution 10x concentrate (Leica, Cat # AR9590); IHC grade dehydrated alcohol (Leica, Cat # 3803686); SignalStain Antibody Diluent (ready-to-use) (CST, Cat # 8112); Animal-Free Blocking Solution (5X) (CST, Cat # 15019); Bond RX automatic IHC&ISH system (Leica, Cat # Bond RX); and NanoZoomer Digital Slide System (Hamamatsu, Cat # NDP2.0-HT). Antigen retrieval was performed for 20 minutes (using epitope retrieval solution 1 (Citrate buffer pH 6.0) or epitope retrieval solution 2 (EDTA buffer, pH 9.0)), while the IHC procedure as detailed in **Table 14** were followed.

Table 14. IHC Procedures.

Step	Reagent	Time (minute)	Temperature (°C)	Step	Reagent	Time (minute)	Temperature (°C)
1	Dewax solution	0.5	72	23	Blocking Reagent	20	RT
2	Dewax solution	0	72	24	Primary Ab	60	RT
3	Dewax solution	0	RT	25	Bond Wash Buffer	0	RT
4	Alcohol	0	RT	26	Bond Wash Buffer	2	RT
5	Alcohol	0	RT	27	Bond Wash Buffer	2	RT

Step	Reagent	Time (minute)	Temperature (°C)	Step	Reagent	Time (minute)	Temperature (°C)
6	Alcohol	0	RT	28	Bond Wash Buffer	2	RT
7	Bond Wash Buffer	0	RT	29	Polymer	20	RT
8	Bond Wash Buffer	0	RT	30	Bond Wash Buffer	0	RT
9	Bond Wash Buffer	0	RT	31	Bond Wash Buffer	2	RT
10	Bond ER1/ER2	0	RT	32	Bond Wash Buffer	2	RT
11	Bond ER1/ER2	0	RT	33	Bond Wash Buffer	2	RT
12	Bond ER1/ER2	20	100	34	Deionized Water	0	RT
13	Bond ER1/ER2	0	RT	35	DAB Refine	0	RT
14	Bond Wash Buffer	0	RT	36	DAB Refine	5	RT
15	Bond Wash Buffer	0	RT	37	Deionized Water	0	RT
16	Bond Wash Buffer	0	RT	38	Deionized Water	0	RT
17	Bond Wash Buffer	0	RT	39	Deionized Water	0	RT
18	Bond Wash Buffer	3	RT	40	Hematoxylin	10	RT
19	Peroxide Block	10	RT	41	Deionized Water	0	RT
20	Bond Wash Buffer	0	RT	42	Bond Wash Buffer	0	RT
21	Bond Wash Buffer	0	RT	43	Deionized Water	0	RT
22	Bond Wash Buffer	0	RT				

RT: room temperature

Time = 0 means that the slide was rinsed with the solution quickly.

[00489] All stained sections were scanned using a NanoZoomer-HT 2.0 Image system using 40x magnification. High resolution images of the whole section images were generated and further analyzed by HALO™ image analysis platform.

[00490] The tumor area was scored and necrosis was excluded. The intensity of positive staining was scored at four levels, 0 (negative), 1+ (weak staining), 2+ (medium staining), 3+ (strong staining). The percentages of tumor area at different intensity levels were evaluated and H-Score was calculated as followed: H-Score = (% at 0) ×0 + (% at 1) ×1 + (% at 2)×2 + (% at 3)×3 (H-Score range was between 0 to 300).

[00491] The results show that 5T4 expression was seen in broad range of cancer types. While 5T4 staining can be observed in both tumor and stromal cells, more prominent staining was typically seen in tumor cells. See, for example, **FIGs. 8A and 8B** and **Table 15.**

[00492] The results also show that, among tested cancer types, breast, lung, mesothelial, cervical and head & neck cancers showed higher 5T4 expression. Prostate, kidney, ovarian, colon and gastric cancers showed relative lower 5T4 expression. See, for example, **FIGs. 8A and 8B**.

Table 15. IHC Results

Cancer Type	Total Tumor Cores Evaluated	5T4 Positive Tumor Core	5T4 Positivity Rate (%)	H Score Median	H Score Average
Breast	315	314	99.7	128.0	124.9
Lung	338	336	99.4	139.9	135.3
Pancreas	278	272	97.8	90.9	96.5
Bladder	190	186	97.9	110.1	105.9
Head and Neck	130	130	100.0	124.9	122.0
Prostate	103	86	83.5	7.5	31.5
Endometrium	97	97	100.0	134.2	121.0
Mesothelioma	86	86	100.0	116.6	132.0
Cervix	281	280	99.6	93.4	93.6
Kidney	391	376	96.2	46.0	42.2
Ovary	384	321	83.6	43.7	54.9
Colon	376	316	84.0	27.7	48.8
Stomach	178	163	91.6	57.5	64.1

* * * * *

[00493] Throughout this application, various publications, patents, patent applications and other documents have been referenced. The disclosures of these publications, patents, patent applications and other documents in their entireties are hereby incorporated by reference in this application for all purposes, including in order to more fully describe the state of the art

to which this the subject matter disclosed herein pertains. Although the disclosed subject matter has been described with reference to the examples provided above, it should be understood that various modifications could be made without departing from the spirit of the disclosed subject matter. Many variations will become apparent to those skilled in the art upon review of this specification.

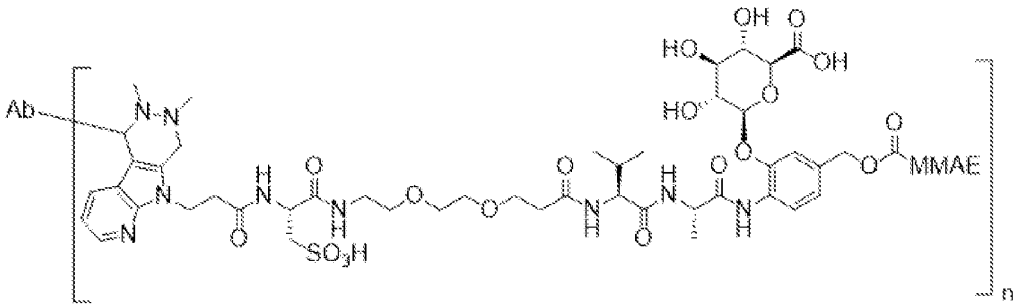
CLAIMS

What is claimed is:

1. A method for treating cancer in a subject, the method comprising administering an ADC to the subject,

wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer;

wherein the ADC is represented by **Formula (I')**:



(I'),

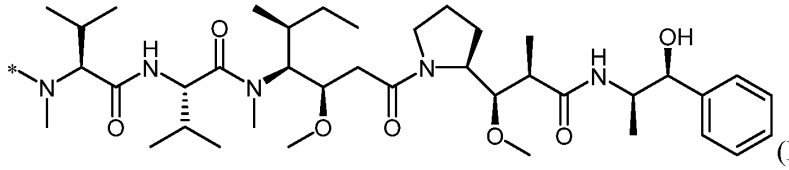
wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises:

(1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and

(2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12;

wherein the Ab further comprises one or more *fGly'*, wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I')** is attached;

wherein the MMAE is monomethyl auristatin E and represented by **Formula (III)**:



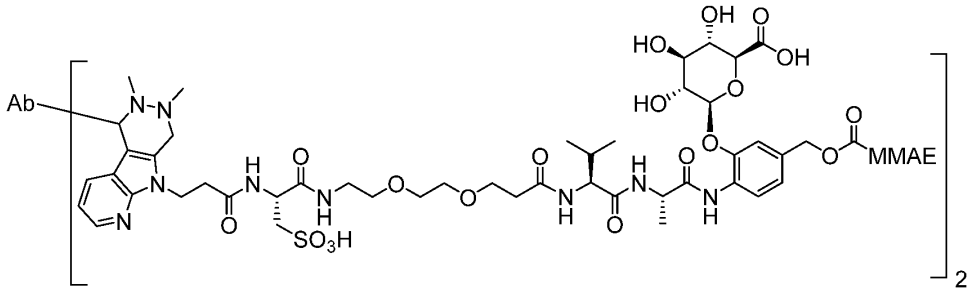
(III),

wherein * indicates the point of attachment in **Formula (I')**; and

wherein n is an integer.

2. The method of claim 1, wherein n is an integer selected from any one of 1 to 10.

3. The method of claim 1 or 2, wherein n is 2 and wherein the ADC is represented by **Formula (I):**



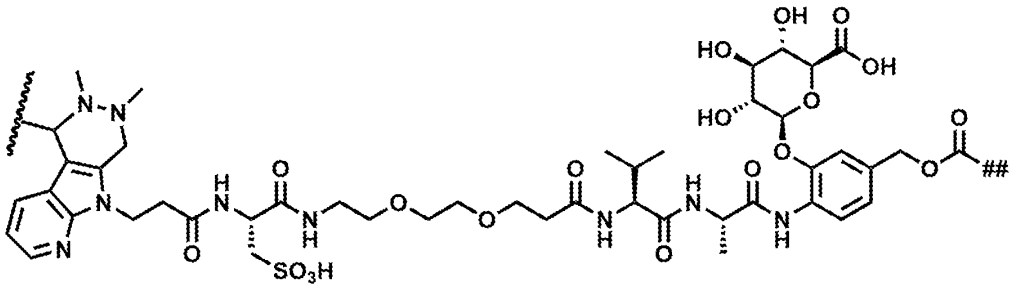
(I),

wherein the * in **Formula (III)** indicates the point of attachment in **Formula (I)** and wherein *fGly'* is a formylglycine amino acid residue to which the non-Ab part of **Formula (I)** is attached**.**

4. A method for treating cancer in a subject, the method comprising administering an ADC to the subject,

wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer;

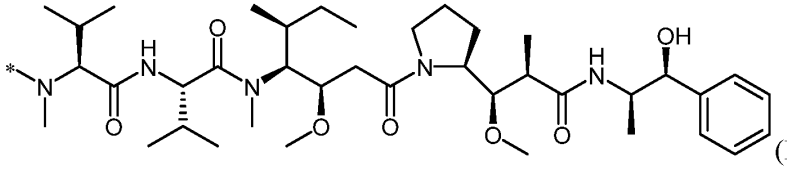
wherein the ADC comprises an antibody (Ab) conjugated to a monomethyl auristatin E (MMAE) by a linker (**L-1**):



(**L-1**),

wherein the wavy line indicates the point of attachment to the Ab and the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:



(**III**),

wherein * indicates the point of attachment to (**L-1**);

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises:

(1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and

(2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; and

wherein the Ab further comprises one or more *fGly'* and wherein *fGly'* is a formylglycine amino acid residue to which linker (**L-1**) is attached.

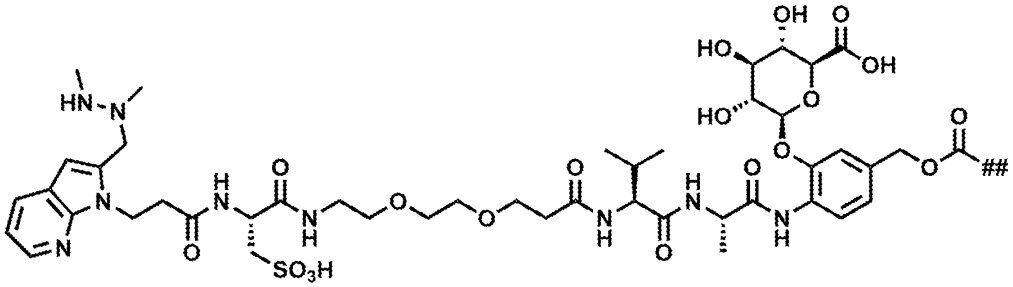
5. The method of claim 4, wherein one Ab is conjugated to one to ten MMAEs and wherein each MMAE is conjugated to Ab by one linker (**L-1**).

6. The method of claim 4 or 5, wherein one Ab is conjugated to two MMAEs and wherein each MMAE is conjugated to Ab by one linker (**L-1**).

7. A method for treating cancer in a subject, the method comprising administering an ADC to the subject,

wherein the cancer is any one or more of breast cancer, endometrial cancer, head and neck cancer, pancreatic cancer, lung cancer, prostate cancer, cervical cancer, bladder cancer, renal cell carcinoma (RCC), hepatocellular carcinoma (HCC), or colorectal cancer,

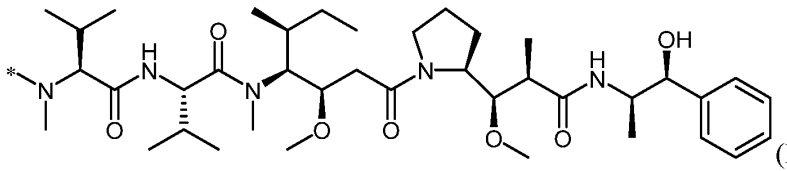
wherein the ADC is produced by conjugating an antibody (Ab) to a linker-drug comprising a pre-conjugate linker **(L-1a')** and a monomethyl auristatin E (MMAE):



(L-1a'),

wherein the ## indicates the point of attachment to the MMAE;

wherein the MMAE is represented by **Formula (III)**:



(III),

wherein * indicates the point of attachment to (**L-1a'**);

wherein the Ab is a 5T4 antibody or an antigen binding fragment thereof, and wherein the Ab comprises:

(1) a heavy chain complementarity determining region 1 (HCDR1), a heavy chain complementarity determining region 2 (HCDR2), and a heavy chain complementarity determining region 3 (HCDR3) as set forth in a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 11, and

(2) a light chain complementarity determining region 1 (LCDR1), a light chain complementarity determining region 2 (LCDR2), and a light chain

complementarity determining region 3 (LCDR3) as set forth in a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 12; and

wherein the Ab further comprises one or more *fGly'* and wherein *fGly'* is a formylglycine amino acid residue to which linker (**L-1a'**) is attached after conjugating the Ab and the linker-drug.

8. The method of claim 7, wherein one Ab is conjugated to one to ten linker-drugs.

9. The method of claim 7 or 8, wherein one Ab is conjugated to two linker-drugs.

10. The method of any one of claims 1, 2, 4, 5, 7, and 8, wherein the Ab comprises one to ten *fGly'*.

11. The method of any one of claims 1-10, wherein the Ab comprises two *fGly'*.

12. The method of any one of claims 1-11, wherein the Ab comprises a motif of X^1(*fGly'*)$X^2Z^2X^3Z^3$ (SEQ ID NO: 67),
wherein:

Z^2 is either a proline (P) or alanine (A) residue;

Z^3 is a basic amino acid residue optionally selected from the group consisting of: arginine (R), lysine (K), and histidine (H), or an aliphatic amino acid residue optionally selected from the group consisting of: alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), and proline (P);

X^1 is present or absent and, when present, is any amino acid residue, with the proviso that when the sequence of SEQ ID NO: 67 is at the N-terminus of the antibody Ab, X^1 is present; and

X^2 and X^3 independently is any amino acid residue,
optionally wherein,

the motif of SEQ ID NO: 67 is selected from the group consisting of: L(*fGly'*)TPSR (SEQ ID NO: 1), M(*fGly'*)TPSR (SEQ ID NO: 45), V(*fGly'*)TPSR (SEQ ID NO: 46), L(*fGly'*)SPSR (SEQ ID NO: 47), L(*fGly'*)APSR (SEQ ID NO: 48), L(*fGly'*)VPSR (SEQ ID NO: 49), L(*fGly'*)GPSR (SEQ ID NO: 50), I(*fGly'*)TPAR (SEQ ID NO: 51), L(*fGly'*)TPSK (SEQ ID NO: 52), M(*fGly'*)TPSK (SEQ ID NO: 53), V(*fGly'*)TPSK (SEQ ID NO: 54), L(*fGly'*)SPSK (SEQ ID NO: 55), L(*fGly'*)APSK (SEQ ID NO: 56), L(*fGly'*)VPSK (SEQ ID NO: 57), L(*fGly'*)GPSK (SEQ ID NO: 58), L(*fGly'*)TPSA (SEQ ID NO: 59), I(*fGly'*)TPAA (SEQ ID NO: 60), M(*fGly'*)TPSA (SEQ ID NO: 61), V(*fGly'*)TPSA (SEQ ID NO: 62), L(*fGly'*)SPSA

(SEQ ID NO: 63), L(*fGly'*)APSA (SEQ ID NO: 64), L(*fGly'*)VPSA (SEQ ID NO: 65), and L(*fGly'*)GPSA (SEQ ID NO: 66).

13. The method of claim 12, wherein the Ab comprises two heavy chains and two light chains, and wherein each of the heavy chains comprises a motif of SEQ ID NO: 67.

14. The method of any one of claims 1-13, wherein the Ab comprises an amino acid sequence of L(*fGly'*)TPSR (SEQ ID NO: 1).

15. The method of any one of claims 1-14, wherein the Ab comprises two heavy chains and two light chains, and wherein each of the heavy chains comprises a motif of L(*fGly'*)TPSR (SEQ ID NO: 1)

16. The method of claim 13 or 15, wherein the *fGly'* of the motif is located in the heavy chain at a position corresponding to the 453rd amino acid residue of SEQ ID NO: 2.

17. The method of any one of claims 1-16, wherein

(1) HCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 19, 24, 25, and 30,

(2) HCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 20, 26, 31, and 36,

(3) HCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 15, 21, 27, and 32,

(4) LCDR1 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 16, 22, 28, and 33,

(5) LCDR2 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 23, and 34, and

(6) LCDR3 comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 18, 29, and 35.

18. The method of any one of claims 1-17, wherein:

(i) HCDR1 comprises the amino acid sequence of SEQ ID NO: 13, HCDR2 comprises the amino acid sequence of SEQ ID NO: 14, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18; or

(ii) HCDR1 comprises the amino acid sequence of SEQ ID NO: 19, HCDR2 comprises the amino acid sequence of SEQ ID NO: 20, HCDR3 comprises the amino

acid sequence of SEQ ID NO: 21, LCDR1 comprises the amino acid sequence of SEQ ID NO: 22, LCDR2 comprises the amino acid sequence of SEQ ID NO: 23, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18; or

(iii) HCDR1 comprises the amino acid sequence of SEQ ID NO: 24, HCDR2 comprises the amino acid sequence of SEQ ID NO: 14, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18; or

(iv) HCDR1 comprises the amino acid sequence of SEQ ID NO: 25, HCDR2 comprises the amino acid sequence of SEQ ID NO: 26, HCDR3 comprises the amino acid sequence of SEQ ID NO: 27, LCDR1 comprises the amino acid sequence of SEQ ID NO: 28, LCDR2 comprises the amino acid sequence of SEQ ID NO: 23, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 29; or

(v) HCDR1 comprises the amino acid sequence of SEQ ID NO: 30, HCDR2 comprises the amino acid sequence of SEQ ID NO: 31, HCDR3 comprises the amino acid sequence of SEQ ID NO: 32, LCDR1 comprises the amino acid sequence of SEQ ID NO: 33, LCDR2 comprises the amino acid sequence of SEQ ID NO: 34, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 35; or

(vi) HCDR1 comprises the amino acid sequence of SEQ ID NO: 13, HCDR2 comprises the amino acid sequence of SEQ ID NO: 36, HCDR3 comprises the amino acid sequence of SEQ ID NO: 15, LCDR1 comprises the amino acid sequence of SEQ ID NO: 16, LCDR2 comprises the amino acid sequence of SEQ ID NO: 17, and LCDR3 comprises the amino acid sequence of SEQ ID NO: 18.

19. The method of any one of claims 1-18, wherein the Ab comprises a VH comprising the amino acid sequence of SEQ ID NO: 11 and a VL comprising the amino acid sequence of SEQ ID NO: 12.

20. The method of any one of claims 1-19, wherein the Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 2 and a light chain comprising the amino acid sequence of SEQ ID NO: 3.

21. The method of any one of claims 1-20, wherein the cancer is triple-negative breast cancer (TNBC) or hormone-receptor positive breast cancer (HR+ BC).

22. The method of any one of claims 1-20, wherein the cancer is pancreatic ductal adenocarcinoma (PDAC).

23. The method of any one of claims 1-20, wherein the cancer is non-small cell lung cancer (NSCLC).

24. The method of any one of claims 1-20, wherein the cancer is head and neck squamous cell carcinoma (HNSCC).

25. The method of any one of claims 1-24, further comprising administrating to the subject means for inhibiting interaction between PD-1 and PD-L1.

26. The method of claim 25, wherein the ADC is administered concurrently with, after, or before the means for inhibiting interaction between PD-1 and PD-L1.

27. The method of any one of claims 1-24, further comprising administering to the subject a PD-L1/PD-1 inhibitor.

28. The method of claim 27, wherein the ADC is administered concurrently with, after, or before the PD-L1/PD-1 inhibitor.

29. The method of claim 27 or 28, wherein the PD-L1/PD-1 inhibitor is an antibody or an antigen binding fragment thereof comprising HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 of pembrolizumab.

30. The method of any one of claims 27-29, wherein the PD-L1/PD-1 inhibitor is pembrolizumab.

31. The method of claim 29 or 30, wherein the PD-L1/PD-1 inhibitor is administered to the subject at a dose of about 200 mg.

32. The method of any one of claims 27-31, wherein the PD-L1/PD-1 inhibitor is administered intravenously.

33. The method of any one of claims 27-32, wherein the PD-L1/PD-1 inhibitor is administered once every 3 weeks (q3w).

34. The method of any one of claims 1-33, wherein the ADC is administered intravenously.

35. The method of any one of claims 1-34, wherein the ADC is administered once every 3 weeks (q3w).

36. The method of any one of claims 1-35, wherein the subject is human.