ACTIVATABLE TYROSINE-PROTEIN KINASE MEMBRANE RECEPTOR (ROR) ANTIBODY-DRUG CONJUGATES AND USES THEREOF

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[00001] This application claims the benefit of U.S. Provisional Patent Application No. 63/514,783, filed July 20, 2023, the disclosure of which is incorporated by reference herein in its entirety.

2. SEQUENCE LISTING

[00002] 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 "14529-147-228_SEQ_LISTING.xml", was created on July 17, 2024, and is 198,709 bytes in size.

3. FIELD

[00003] The present disclosure relates generally to activatable antibody-drug conjugates (ADCs) that bind to tyrosine-protein kinase membrane receptor (ROR, *e.g.*, human ROR) and methods of their use.

4. BACKGROUND

[00004] Various tumors can demonstrate cell-surface expression of tyrosine-protein kinase transmembrane receptor (ROR) antigens, as described in greater detail in Gentile, *et al.* (*Cancer Res*; 71(8) April 15, 2011), Rebagay, *et al.* (*Front. Oncol.*, 18 April 2012), Zhang, *et al.* (*American Journal of Pathology,* Vol. 181, No. 6, December 2012), Henry, *et al.* (*Oncotarget*, Vol. 6, No. 37 2015), Zhang, *et al.* (*PLoS ONE* 7(3): e31127.), and Bainbridge, *et al.* (*PLoS ONE* 9(7): e102695.), each herein incorporated by reference in their entirety. In addition, ROR expression may not be expressed, or only demonstrate limited expression, in normal, *e.g.*, non-cancerous, tissue as described in Balakrishnan *et al.* (*Clin Cancer Res.* 2017 Jun 15; 23(12): 3061–3071), herein incorporated in its entirety. Thus, ROR antigens can be used as a tumor-specific marker in certain tumors. Examples of tumors and cancers with demonstrated ROR expression include, but are not limited to, pancreatic cancer, ovarian cancer, breast cancer, lung cancer, gastric cancer, melanoma, Ewing sarcoma, chronic lymphocytic leukemia, mantle cell lymphoma, and B-ALL, as described in Gohil *et al.* (*Oncoimmunology*. 2017; 6(7): e1326437.), herein incorporated in its entirety. Other cancers include, but are not limited to, hematological cancer, prostate cancer, colon cancer, renal cancer, and uterine cancer.

[00005] Activatable antibodies and activatable antibody-drug conjugates (ADCs) are only capable of binding their targets in certain contexts (*e.g.*, in the protease-rich tumor microenvironment), and thus are useful for precision/context-dependent target-binding. However, the process of developing activatable antibodies and ADCs is slow, labor intensive, and costly.

[00006] There is, therefore, a need for activatable ROR antibodies and ADCs.

5. SUMMARY

[00007] The present disclosure provides ADCs comprising an antibody that binds tyrosine-protein kinase membrane receptor ("ROR-ADC"). Such ROR-ADCs, in some embodiments, bind to the same epitope of human ROR as an antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL) described herein.

[00008] Additionally, the present disclosure provides ADCs comprising an activatable antibody that binds tyrosine-protein kinase membrane receptor ("activatable ROR-ADC"). Such activatable ROR-ADCs, in some embodiments, bind to the same epitope of human ROR as an antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL) described herein.

[00009] The present disclosure also provides pharmaceutical compositions comprising an ROR-ADC (such as an activatable ROR-ADC) that comprises an activatable antibody or fragment thereof that binds to ROR (such as, "activatable ROR antibody") and a drug conjugated (directly or indirectly) thereto. Such pharmaceutical compositions, in some embodiments, include activatable ROR-ADCs comprising an antibody or fragment thereof that binds to essentially the same epitope of human ROR as an antibody comprising a VH and a VL described herein.

[00010] The present disclosure also provides methods of treating, preventing, or alleviating an ROR-mediated disease, disorder, or condition, such as alleviating one or more symptoms of the ROR-mediated disease, disorder, or condition with an ROR-ADC as disclosed herein, such as an activatable ROR-ADC.

[00011] More specifically, the present disclosure provides an ROR-ADC as disclosed herein, such as an activatable ROR-ADC, comprising (a) an ROR antibody, such as an activatable ROR antibody and (b) one or more pyridazine-pyrrolo coupling moieties comprising a drug conjugated to the pyridazine-pyrrolo coupling moiety through a linker, for example, using the Hydrazino-*iso*-Pictet-Spengler (HIPS) conjugation method.

[00012] Traditionally, the HIPS conjugation method has been used to produce conjugates carrying one payload per HIPS moiety per aldehyde tag, which produces antibody conjugates

with DAR values of up to 4. In some embodiments, an ROR-ADC as disclosed herein, such as an activatable ROR-ADC, as disclosed herein comprises branched HIPS linkers that carry two (or more) molecules of the same or different payload per one HIPS moiety and are therefore capable of conjugating two (or more) small molecule payloads per one aldehyde group in a protein in a single conjugation step. Consequently, the usage of such branched linkers allows the generation of higher DAR site-specific conjugates (*e.g.*, DAR up to 8) with controlled payload placement, which in the context of therapeutic ADCs would result in larger quantities of pharmaceutical agent delivered to the targeted tissue.

[00013] The present disclosure provides an ROR-ADC as disclosed herein, such as activatable ROR-ADC structures, which comprises (a) an ROR antibody, such as an activatable antibody that binds to tyrosine-protein kinase membrane receptor (ROR), (b) a branched HIPS linker, and (c) a drug. The disclosure also encompasses compounds and methods for production of such conjugates, as well as methods of using the conjugates.

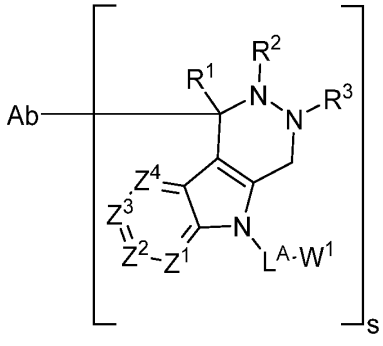
[00014] Aspects of the present disclosure include an activatable ROR-ADC comprising (a) an activatable ROR antibody; and (b) one or more pyridazine-pyrrolo coupling moieties comprising one or more drugs conjugated to the pyridazine-pyrrolo coupling moiety via one or more linkers.

[00015] Also provided are an activated activatable ROR-ADC. In some embodiments, the ROR-ADC comprises (a) an activated activatable ROR antibody and (b) one or more pyridazine-pyrrolo coupling moieties comprising a drug conjugated to the pyridazine-pyrrolo coupling moiety through a linker, for example, using the Hydrazino-*iso*-Pictet-Spengler (HIPS) conjugation method. In some embodiments, the ROR-ADC comprises (a) an activated activatable antibody that binds to tyrosine-protein kinase membrane receptor (ROR), (b) a branched HIPS linker, and (c) a drug. In some embodiments, the ROR-ADC comprises (a) an activated activatable ROR antibody; and (b) one or more pyridazine-pyrrolo coupling moieties comprising one or more drugs conjugated to the pyridazine-pyrrolo coupling moiety via one or more linkers.

[00016] In some embodiments, an ROR-ADC (*e.g.*, an activatable ROR-ADC) is represented by Formula (I), the ROR-ADC comprising:

a. an antibody that binds to tyrosine-protein kinase membrane receptor (ROR) (for example, an activatable antibody that binds to ROR); and

b. two or more drugs conjugated to a pyridazine-pyrrolo coupling moiety, each via a linker



(I)

wherein:

Ab represents the antibody that binds to ROR (*e.g.*, the activatable antibody that binds to ROR);

Z^1, Z^2, and Z^4 are each independently CR^4;

Z^3 is C-L^B-W^2;

R^1, R^2, R^3, and R^4 are each selected from hydrogen and alkyl;

L^A is a first linker comprising:

$$-(T^1\text{-}V^1)_a\text{-}(T^2\text{-}V^2)_b\text{-}(T^3\text{-}V^3)_c\text{-}(T^4\text{-}V^4)_d\text{-}(T^5\text{-}V^5)_e\text{-}(T^6\text{-}V^6)_f\text{-},$$

wherein:

a, b, c, d, e, and f are each independently 0 or 1, provided at least one of a, b, c, d, e, and f are 1;

T^1, T^2, T^3, T^4, T^5 and T^6 are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x$-, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12;

V^1, V^2, V^3, V^4,V^5, and V^6 are each independently selected from the group consisting of a covalent bond, -CO-, $-NR^{15}$-, $-NR^{15}(CH_2)_q$-, $-NR^{15}(C_6H_4)$-, $-CONR^{15}$-, $-NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, $-SO_2$-, $-SO_2NR^{15}$-, $-NR^{15}SO_2$- and -P(O)OH-, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

L^B is a second linker comprising:

$$-(T^7\text{-}V^7)_g\text{-}(T^8\text{-}V^8)_h\text{-}(T^9\text{-}V^9)_i\text{-}(T^{10}\text{-}V^{10})_j\text{-}(T^{11}\text{-}V^{11})_k\text{-}(T^{12}\text{-}V^{12})_l\text{-}(T^{13}\text{-}V^{13})_m\text{-},$$

wherein:

g, h, i, j, k, l, and m are each independently 0 or 1, provided that at least one of g, h, i, j, k, l, and m is 1;

T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} are each independently selected from a covalent bond, $(C_1\text{-}C_{12})$alkyl, substituted $(C_1\text{-}C_{12})$alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x\text{-}$, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12;

V^7, V^8, V^9, V^{10},V^{11}, V^{12}, and V^{13} are each independently selected from the group consisting of a covalent bond, -CO-, $-NR^{15}\text{-}$, $-NR^{15}(CH_2)_q\text{-}$, $-NR^{15}(C_6H_4)\text{-}$, $-CONR^{15}\text{-}$, $-NR^{15}CO\text{-}$, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, $-SO_2\text{-}$, $-SO_2NR^{15}\text{-}$, $-NR^{15}SO_2\text{-}$ and -P(O)OH-, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

s is an integer from 1 to 10;

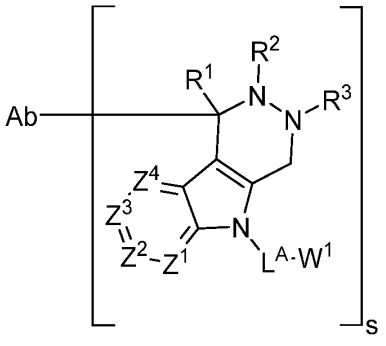
W^1 is a first drug; and

W^2 is a second drug.

[00017] In some embodiments, an activatable ROR-ADC is represented by Formula (I), the activatable ROR-ADC comprising:

a. an activatable antibody that binds to tyrosine-protein kinase membrane receptor (ROR); and

b. two or more drugs conjugated to a pyridazine-pyrrolo coupling moiety, each via a linker



(I)

wherein:

Ab represents the activatable antibody that binds to ROR;

Z^1, Z^2, and Z^4 are each independently CR^4;

Z^3 is C-L^B-W^2;

R^1, R^2, R^3, and R^4 are each selected from hydrogen and alkyl;

L^A is a first linker comprising:

$$-(T^1\text{-}V^1)_a\text{-}(T^2\text{-}V^2)_b\text{-}(T^3\text{-}V^3)_c\text{-}(T^4\text{-}V^4)_d\text{-}(T^5\text{-}V^5)_e\text{-}(T^6\text{-}V^6)_f\text{-},$$

wherein:

a, b, c, d, e, and f are each independently 0 or 1, provided at least one of a, b, c, d, e, and f is 1;

T^1, T^2, T^3, T^4, T^5 and T^6 are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x-$, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene

glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12;

V^1, V^2, V^3, V^4,V^5, and V^6 are each independently selected from the group consisting of a covalent bond, -CO-, -NR^{15}-, -$NR^{15}(CH_2)_q$-, -$NR^{15}(C_6H_4)$-, -$CONR^{15}$-, -$NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$- and -P(O)OH-, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

L^B is a second linker comprising:

$$-(T^7\text{-}V^7)_g\text{-}(T^8\text{-}V^8)_h\text{-}(T^9\text{-}V^9)_i\text{-}(T^{10}\text{-}V^{10})_j\text{-}(T^{11}\text{-}V^{11})_k\text{-}(T^{12}\text{-}V^{12})_l\text{-}(T^{13}\text{-}V^{13})_m\text{-},$$

wherein:

g, h, i, j, k, l, and m are each independently 0 or 1, provided that at least one of g, h, i, j, k, l, and m is 1;

T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, -$(CR^{13}OH)_x$-, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12;

V^7, V^8, V^9, V^{10},V^{11}, V^{12}, and V^{13} are each independently selected from the group consisting of a covalent bond, -CO-, -NR^{15}-, -$NR^{15}(CH_2)_q$-, -$NR^{15}(C_6H_4)$-, -$CONR^{15}$-, -$NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$- and -P(O)OH-, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

s is an integer from 1 to 10;

W^1 is a first drug; and

W^2 is a second drug.

[00018] In some embodiments, Z^1 is CR^4.

[00019] In some embodiments, Z^3 is $C\text{-}L^B\text{-}W^2$.

[00020] In some embodiments, one or both of W^1 and W^2 are camptothecin analogues, for example, belotecan.

[00021] In some embodiments, L^A comprises:

$$-(T^1\text{-}V^1)_a\text{-}(T^2\text{-}V^2)_b\text{-}(T^3\text{-}V^3)_c\text{-}(T^4\text{-}V^4)_d\text{-}(T^5\text{-}V^5)_e\text{-}(T^6\text{-}V^6)_f\text{-},$$

wherein:

a, b, c, d, e, and f are each independently 0 or 1, provided at least one of a, b, c, d, e, and f is 1;

T^1, T^2, T^3, T^4, T^5 and T^6 are each independently selected from a covalent bond, $(C_1\text{-}C_{12})$alkyl, substituted $(C_1\text{-}C_{12})$alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x-$, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12;

V^1, V^2, V^3, V^4,V^5, and V^6 are each independently selected from the group consisting of a covalent bond, -CO-, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$ and -P(O)OH-, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

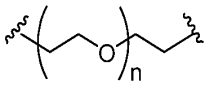
each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[00022] In some embodiments of L^A:

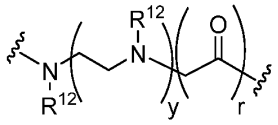
T^1 is selected from a $(C_1\text{-}C_{12})$alkyl and a substituted $(C_1\text{-}C_{12})$alkyl;

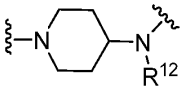
T^2, T^3, T^4, T^5 and T^6 are each independently selected from a covalent bond, $(C_1\text{-}C_{12})$alkyl, substituted $(C_1\text{-}C_{12})$alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x-$, 4-amino-piperidine (4AP), MABO, MABC, PABO, PABC, PAB, PABA, PAP, PHP, an acetal group, a hydrazine, and an ester; and

V^1, V^2, V^3, V^4,V^5, and V^6 are each independently selected from the group consisting of a covalent bond, -CO-, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$, and -P(O)OH-; wherein:

$(PEG)_n$ is , where n is an integer from 1 to 30;

EDA is an ethylene diamine moiety having the following structure:

, where y is an integer from 1 to 6 and r is 0 or 1;

4-amino-piperidine (4AP) is ; and

each R^{12} is independently selected from hydrogen, an alkyl, a substituted alkyl, a polyethylene glycol moiety, an aryl, and a substituted aryl, wherein any two adjacent R^{12} groups may be cyclically linked to form a piperazinyl ring;

In further embodiments, a, b, c, and d are each 1; and

e and f are 0.

[00023] In some embodiments, T^1, T^2, T^3, T^4, T^5 and T^6 are each optionally substituted with a glycoside.

[00024] In some embodiments, MABO, MABC, PABO, PABC, PAB, PABA, PAP and PHP are each optionally substituted with a glycoside.

[00025] In some embodiments, the glycoside is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00026] In some embodiments, L^A is a linker wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is substituted (C_1-C_{12})alkyl and V^2 is -CO-;

T^3 is $(AA)_p$ and V^3 is absent (*e.g.*, a covalent bond);

T^4 is PABC and V^4 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10;

a, b, c, and d are each 1; and

e and f are each 0.

[00027] In further embodiments, the PABC is substituted with a glycoside, for example, a hydrogen of PABC is replaced with a glycoside, such as a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00028] In some embodiments, L^B comprises:

$$-(T^7\text{-}V^7)_g\text{-}(T^8\text{-}V^8)_h\text{-}(T^9\text{-}V^9)_i\text{-}(T^{10}\text{-}V^{10})_j\text{-}(T^{11}\text{-}V^{11})_k\text{-}(T^{12}\text{-}V^{12})_l\text{-}(T^{13}\text{-}V^{13})_m\text{-},$$

wherein:

g, h, i, j, k, l, and m are each independently 0 or 1, provided at least one of g, h, i, j, k, l, and m is 1;

T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x$-, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12;

V^7, V^8, V^9, V^{10},V^{11}, V^{12}, and V^{13} are each independently selected from the group consisting of a covalent bond, -CO-, $-NR^{15}$-, $-NR^{15}(CH_2)_q$-, $-NR^{15}(C_6H_4)$-, $-CONR^{15}$-,

-$NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$- and -P(O)OH-, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[00029] In some embodiments, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} are each optionally substituted with a glycoside.

[00030] In some embodiments, MABO, MABC, PABO, PABC, PAB, PABA, PAP and PHP are each optionally substituted with a glycoside.

[00031] In some embodiments, the glycoside is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

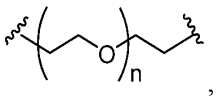
[00032] In some embodiments of L^B:

T^7 is a covalent bond;

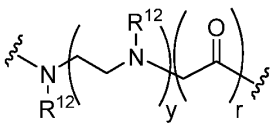
T^8, T^9, T^{10}, T^{11} and T^{12} are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, -$(CR^{13}OH)_x$-, 4-amino-piperidine (4AP), MABO, MABC, PABO, PABC, PAB, PABA, PAP, PHP, an acetal group, a hydrazine, and an ester; and

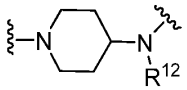
V^7, V^8, V^9, V^{10},V^{11} and V^{12} are each independently selected from the group consisting of a covalent bond, -CO-, -NR^{15}-, -$NR^{15}(CH_2)_q$-, -$NR^{15}(C_6H_4)$-, -$CONR^{15}$-, -$NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$-, and -P(O)OH-;

wherein:

$(PEG)_n$ is , where n is an integer from 1 to 30;

EDA is an ethylene diamine moiety having the following structure:

, where y is an integer from 1 to 6 and r is 0 or 1;

4-amino-piperidine (4AP) is  ; and

each R^{12} is independently selected from hydrogen, an alkyl, a substituted alkyl, a polyethylene glycol moiety, an aryl, and a substituted aryl, wherein any two adjacent R^{12} groups may be cyclically linked to form a piperazinyl ring;

g, h, i, j, and k are each 1; and

l and m are each 0.

[00033] In some embodiments, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, and T^{12} are each optionally substituted with a glycoside.

[00034] In some embodiments, MABO, MABC, PABO, PABC, PAB, PABA, PAP and PHP are each optionally substituted with a glycoside.

[00035] In some embodiments, the glycoside is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00036] In some embodiments, L^B is a linker wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is $(C_1\text{-}C_{12})$alkyl and V^8 is -CONH-;

T^9 is substituted $(C_1\text{-}C_{12})$alkyl and V^9 is -CO-;

T^{10} is $(AA)_p$ and V^{10} is absent (*e.g.*, a covalent bond);

T^{11} is PABC and V^{11} is absent (*e.g.*, a covalent bond);

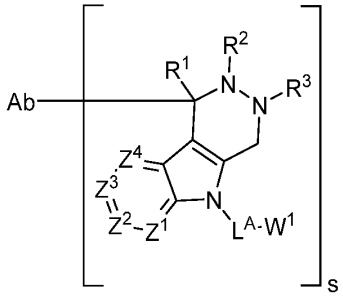
p is an integer from 1 to 10;

g, h, i, j, and k are each 1; and

l and m are each 0.

[00037] In further embodiments, the PABC is substituted with a glycoside, for example, a hydrogen of PABC is replaced with a glycoside, such as a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00038] In some embodiments, an ROR-ADC (such as an activatable ROR-ADC) is represented by Formula (I):



(I)

wherein:

Ab represents an antibody that binds to ROR (such as an activatable antibody that binds to ROR);

Z^1, Z^2, and Z^4 are each independently CR^4;

Z^3 is C-L^B-W^2;

R^1, R^2, R^3 and R^4 are each selected from hydrogen and (C_1-C_{12})alkyl;

L^A is a first linker wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is substituted (C_1-C_{12})alkyl and V^2 is -CO-;

T^3 is $(AA)_p$ where p is an integer from 1-20 and V^3 is a covalent bond;

T^4 is PABC and V^4 is a covalent bond;

a, b, c, and d are each 1;

e and f are each 0; and

L^B is a second linker wherein:

T^7 is a covalent bond and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is -CONH-;

T^9 is substituted (C_1-C_{12})alkyl and V^9 is -CO-;

T^{10} is $(AA)_p$ where p is an integer from 1-20 and V^{10} is a covalent bond;

T^{11} is PABC and V^{11} is a covalent bond; and

g, h, i, j, and k are each 1; and

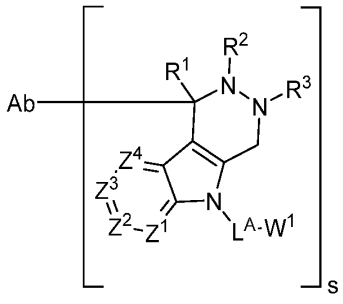
l and m are each 0;

s is an integer from 1 to 10;

W^1 is a first drug; and

W^2 is a second drug.

[00039] In some embodiments, an activatable ROR-ADC is represented by Formula (I):



(I)

wherein:

Ab represents an activatable antibody that binds to ROR;

Z^1, Z^2, and Z^4 are each independently CR^4;

Z^3 is C-L^B-W^2;

R^1, R^2, R^3 and R^4 are each selected from hydrogen and (C_1-C_{12})alkyl;

L^A is a first linker wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is substituted (C_1-C_{12})alkyl and V^2 is -CO-;

T^3 is $(AA)_p$ where p is an integer from 1-20 and V^3 is a covalent bond;

T^4 is PABC and V^4 is a covalent bond;

a, b, c, and d are each 1;

e and f are each 0; and

L^B is a second linker wherein:

T^7 is a covalent bond and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is -CONH-;

T^9 is substituted (C_1-C_{12})alkyl and V^9 is -CO-;

T^{10} is $(AA)_p$ where p is an integer from 1-20 and V^{10} is a covalent bond;

T^{11} is PABC and V^{11} is a covalent bond; and

g, h, i, j, and k are each 1; and

l and m are each 0;

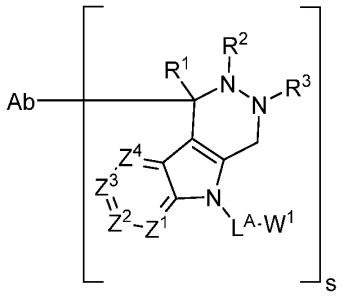
s is an integer from 1 to 10;

W^1 is a first drug; and

W^2 is a second drug.

[00040] In some embodiments, one or both of W^1 and W^2 are camptothecin analogues, for example, belotecan.

[00041] In some embodiments, an ROR-ADC (such as an activatable ROR-ADC) is represented by Formula (I):



(I)

wherein:

Ab represents an antibody that binds to ROR (such as an activatable antibody that binds to ROR);

Z^1, Z^2, and Z^4 are each independently CR^4;

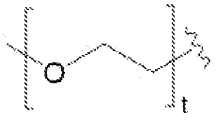
Z^3 is C-L^B-W^2;

R^1, R^2, R^3 and R^4 are each selected from hydrogen and (C_1-C_{12})alkyl;

L^A is a linker wherein:

T^1 is (C_1-C_6)alkyl and V^1 is -CONH-;

T^2 is (C_1-C_6)alkylene substituted with -NHCO$(PEG)_t$, wherein $(PEG)_t$ is



and t is an integer from 2 to 10, optionally 8, and V^2 is -CO-;

T^3 is $(AA)_2$ and V^3 is a covalent bond;

T^4 is PABC substituted with a glycoside and V^4 is a covalent bond;

a, b, c, and d are each 1; and

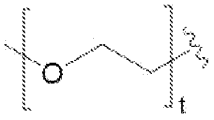
e and f are each 0; and

L^B is a linker wherein:

T^7 is a covalent bond and V^7 is -NHCO-;

T^8 is (C_1-C_6)alkyl and V^8 is -CONH-;

T^9 is (C_1-C_6)alkylene substituted with -NHCO$(PEG)_t$, wherein $(PEG)_t$ is



and t is an integer from 2 to 10, optionally 8, and V^9 is -CO-;

T^{10} is $(AA)_2$ and V^{10} is a covalent bond;

T^{11} is PABC substituted with a glycoside and V^{11} is a covalent bond;

g, h, i, j, and k are each 1; and

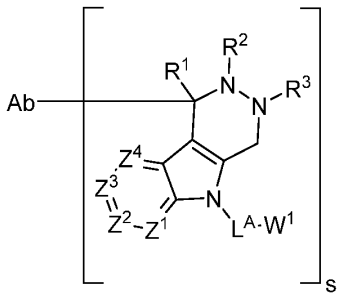
l and m are each 0;

s is an integer from 1 to 10;

W^1 is a first drug; and

W^2 is a second drug.

[00042] In some embodiments, an activatable ROR-ADC is represented by Formula (I):



(I)

wherein:

Ab represents an activatable antibody that binds to ROR;

Z^1, Z^2, and Z^4 are each independently CR^4;

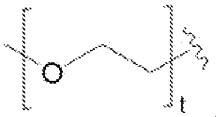
Z^3 is C-L^B-W^2;

R^1, R^2, R^3 and R^4 are each selected from hydrogen and (C_1-C_{12})alkyl;

L^A is a linker wherein:

T^1 is (C_1-C_6)alkyl and V^1 is -CONH-;

T^2 is (C_1-C_6)alkylene substituted with -NHCO$(PEG)_t$, wherein $(PEG)_t$ is



and t is an integer from 2 to 10, optionally 8, and V^2 is -CO-;

T^3 is $(AA)_2$ and V^3 is a covalent bond;

T^4 is PABC substituted with a glycoside and V^4 is a covalent bond;

a, b, c, and d are each 1; and

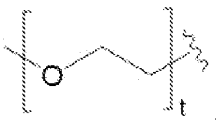
e and f are each 0; and

L^B is a linker wherein:

T^7 is a covalent bond and V^7 is -NHCO-;

T^8 is (C_1-C_6)alkyl and V^8 is -CONH-;

T^9 is (C_1-C_6)alkylene substituted with -NHCO$(PEG)_t$, wherein $(PEG)_t$ is



and t is an integer from 2 to 10, optionally 8, and V^9 is -CO-;

T^{10} is $(AA)_2$ and V^{10} is a covalent bond;

T^{11} is PABC substituted with a glycoside and V^{11} is a covalent bond;

g, h, i, j, and k are each 1; and

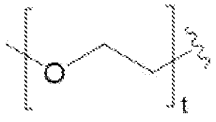
l and m are each 0;

s is an integer from 1 to 10;

W^1 is a first drug; and

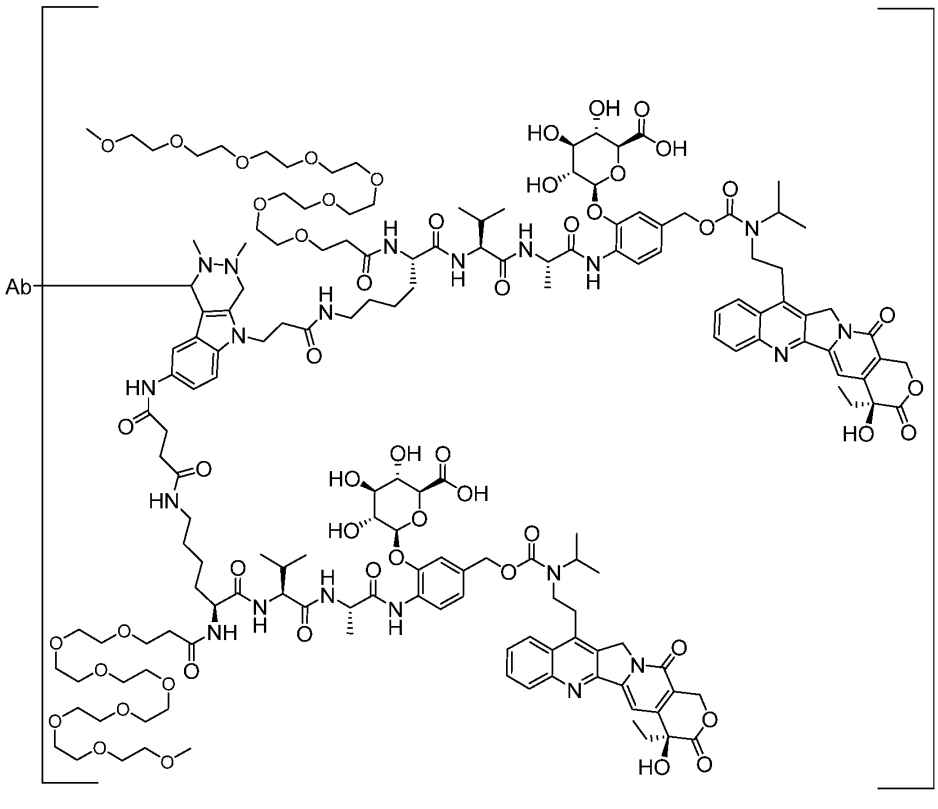
W^2 is a second drug.

[00043] In some embodiments, the PABC of one or both of T^4 and T^{11} is substituted with a glucuronide. In some embodiments, one or both of T^1 and T^8 is ethyl. In some embodiments, one or both of T^2 and T^9 is C_5 alkylene substituted with -$NHCO(PEG)_t$, wherein $(PEG)_t$ is



and t is an integer from 5-10, optionally 8. In some embodiments, one or both of W^1 and W^2 are camptothecin analogues, for example, belotecan.

[00044] In some embodiments, an ROR-ADC (such as an activatable ROR-ADC) is represented by Formula (II):



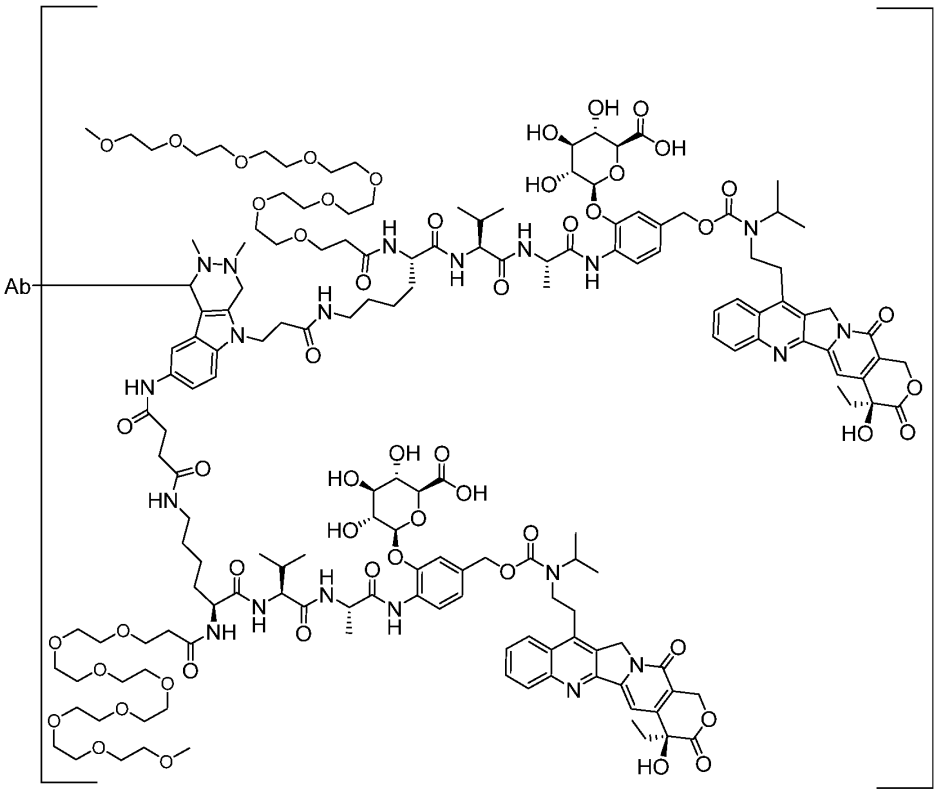
(II),

wherein:

Ab represents an antibody that binds to ROR (also referred to herein as an ROR antibody, such as an activatable antibody that binds to ROR); and

s is an integer from 1 to 10.

[00045] In some embodiments, an activatable ROR-ADC is represented by Formula (II):



(II),

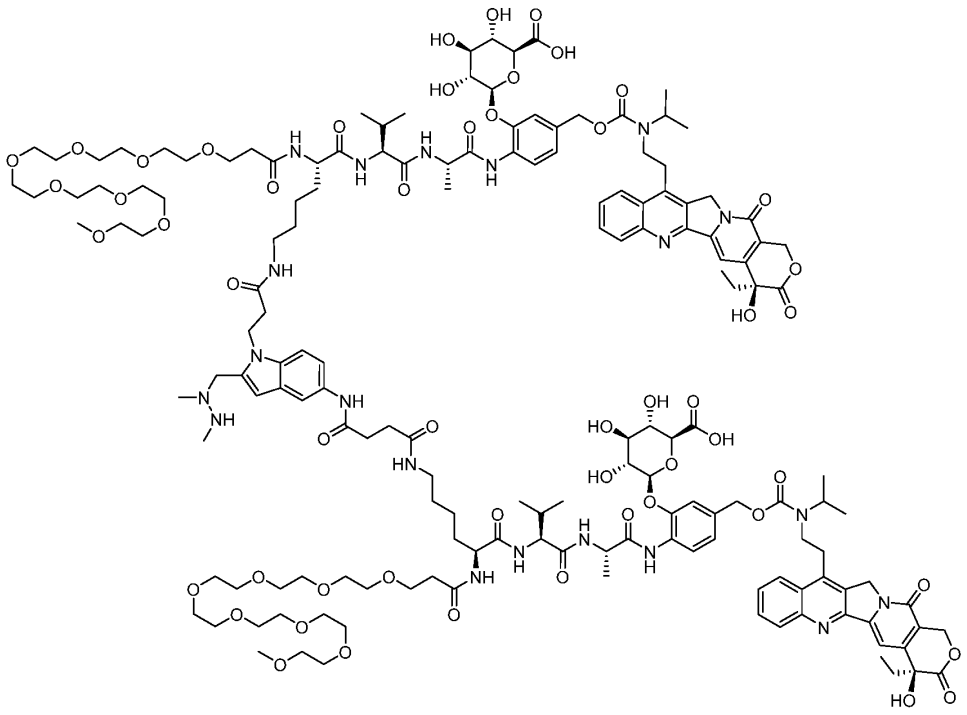
wherein:

Ab represents an activatable antibody that binds to ROR; and

s is an integer from 1 to 10.

[00046] In some embodiments, s is an integer from 1 to 8. In some embodiments, s is 4.

[00047] Formula (II) may be prepared by conjugating one or more linker-payloads of Formula (IIa), shown below, with an ROR antibody (such as an activatable ROR antibody):



(IIa)

[00048] In some embodiments, an ROR-ADC (such as an activatable ROR-ADC) is represented by Formula (I) or (II), wherein the ROR antibody (Ab) (such as an activatable ROR antibody) comprises: an antibody light chain variable (VL) region, and an antibody heavy chain variable (VH) region. In some embodiments, the VH region comprise a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:25, and the VL region comprises a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a VL comprising the amino acid sequence of SEQ ID NO:26.

[00049] In further embodiments, Ab further comprises a masking peptide. In yet further embodiments, the masking peptide can be cleaved, for example in a tumor microenvironment, thus activating the ROR-ADC. Accordingly, Ab is an activatable ROR antibody. In some embodiments, the masking peptide comprises an amino acid sequence of any one of SEQ ID NO:29, 30 or 31. In some embodiments, the activatable antibody comprises a polypeptide comprising, from N-terminus to C-terminus, the masking peptide and the antibody VL region.

[00050] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II), wherein the activatable antibody (Ab) comprises: a masking peptide, an antibody light chain variable (VL) region, and an antibody heavy chain variable (VH) region.

[00051] In some embodiments, the masking peptide can be cleaved, for example in a tumor microenvironment, thus activating the ROR-ADC.

[00052] In further embodiments, the masking peptide comprises an amino acid sequence of any one of SEQ ID NO:29, 30 or 31. In further embodiments, the VH region comprise a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:25, and the VL region comprises a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a VL comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the activatable antibody comprises a polypeptide comprising, from N-terminus to C-terminus, the masking peptide and the antibody VL region.

[00053] In some embodiments, an ROR-ADC (such as an activatable ROR-ADC) is represented by Formula (I) or (II), wherein Ab, such as the activatable ROR antibody,) comprises: (i) a VH region comprising a VH CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 7, 12, 13, 18, and 27, a VH CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 8, 14, 19, and 24; and a VH CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 9, 15, 20, and 28; and (ii) a VL region comprising a VL CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 10, 16, and 21, a VL CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 11, and 22, and a VL CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 17, and 23.

[00054] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II), wherein the activatable antibody (Ab) comprises: (i) a VH region comprising a VH CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 7, 12, 13, 18, and 27, a VH CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 8, 14, 19, and 24; and a VH CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 9, 15, 20, and 28; and (ii) a VL region comprising a VL CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 10, 16, and 21, a VL CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 11, and 22, and a VL CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 17, and 23.

[00055] In some embodiments, an ROR-ADC is represented by Formula (I) or (II), wherein the antibody (Ab) competes with any one of the ROR antibodies or activatable ROR antibodies as disclosed herein in binding to ROR.

[00056] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II), wherein the activatable antibody (Ab) competes with any one of the ROR antibodies or activatable ROR antibodies as disclosed herein in binding to ROR.

[00057] In some embodiments, an ROR-ADC (such as an activatable ROR-ADC) is represented by Formula (I) or (II) wherein Ab comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and/or a framework 4 (FR4) sequence, for example, as set forth in any one of SEQ ID NOs: 25 and 26.

[00058] In some embodiments, an ROR-ADC (such as an activatable ROR-ADC) is represented by Formula (I) or (II) wherein Ab comprises human framework sequences.

[00059] In some embodiments, an ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises (i) a VH comprising the amino acid sequence of SEQ ID NO:25 and a VL comprising the amino acid sequence of SEQ ID NO:26.

[00060] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises (i) a VH comprising the amino acid sequence of SEQ ID NO:25 and a VL comprising the amino acid sequence of SEQ ID NO:26.

[00061] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises the masking peptide, and wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29.

[00062] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises the polypeptide, and the wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:34 or 35.

[00063] In further embodiments, Ab further comprises a heavy chain comprising the amino acid sequences of SEQ ID NO:48 or 171.

[00064] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises the masking peptide, and wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:30.

[00065] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises the polypeptide, and wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:36 or 37.

[00066] In further embodiments, Ab further comprises a heavy chain comprising the amino acid sequences of SEQ ID NO:48 or 171.

[00067] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises the masking peptide, and wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:31.

[00068] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises the polypeptide, and wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:38 or 39.

[00069] In further embodiments, Ab further comprises a heavy chain comprising the amino acid sequences of SEQ ID NO:48 or 171.

[00070] In some embodiments, the antibody Ab comprises a sequence of Formula (VIII)

$$X^1(fGly')X^2Z^{20}X^3Z^{30} \qquad (VIII)$$

wherein:

fGly' is the amino acid residue coupled to the drug through a linker;

Z^{20} is either a proline (P) or alanine (A) residue;

Z^{30} 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, can be any amino acid residue, with the proviso that when the sequence of Formula (VIII) is at the N-terminus of the antibody Ab, X^1 is present; and

X^2 and X^3 independently can be any amino acid residue.

In further embodiments, the sequence of Formula (VIII) is selected from the group consisting of: L(fGly')TPSR (SEQ ID NO:146), M(fGly')TPSR (SEQ ID NO:147), V(fGly')TPSR (SEQ ID NO:148), L(fGly')SPSR (SEQ ID NO:149), L(fGly')APSR (SEQ ID NO:150), L(fGly')VPSR (SEQ ID NO:151), L(fGly')GPSR (SEQ ID NO:152), I(fGly')TPAR (SEQ ID NO:153), L(fGly')TPSK (SEQ ID NO:154), M(fGly')TPSK (SEQ ID NO:155), V(fGly')TPSK (SEQ ID NO:156), L(fGly')SPSK (SEQ ID NO:157), L(fGly')APSK (SEQ ID NO:158), L(fGly')VPSK (SEQ ID NO:159), L(fGly')GPSK (SEQ ID NO:160), L(fGly')TPSA (SEQ ID NO:161), I(fGly')TPAA (SEQ ID NO:162), M(fGly')TPSA (SEQ ID NO:163), V(fGly')TPSA (SEQ ID NO:164), L(fGly')SPSA (SEQ ID NO:165), L(fGly')APSA (SEQ ID NO:166), L(fGly')VPSA (SEQ ID NO:167), and L(fGly')GPSA (SEQ ID NO:168). In yet further embodiments, the sequence of Formula (VIII) comprises L(fGly')TPSR (SEQ ID NO:146).

[00071] In some embodiments, Ab is an IgG1 antibody, optionally an IgG1 kappa antibody.

[00072] In some embodiments, an ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:32, or a variant thereof engineered to conjugate to the linker payload, or SEQ ID NO:42 or a variant

thereof conjugated to the linker payload, or SEQ ID NO:48. In further embodiments, Ab comprises a peptide of any one of SEQ ID NOs: 33, 34, 36, or 38.

[00073] In some embodiments, an ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:169, or a variant thereof engineered to conjugate to the linker payload, or SEQ ID NO:170 or a variant thereof conjugated to the linker payload, or SEQ ID NO:171. In further embodiments, Ab comprises a peptide of any one of SEQ ID NOs: 33, 34, 36, or 38.

[00074] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:32, or a variant thereof engineered to conjugate to the linker payload, or SEQ ID NO:42 or a variant thereof conjugated to the linker payload, or SEQ ID NO:48.

[00075] In further embodiments, Ab comprises a peptide of any one of SEQ ID NOs: 34, 36, or 38.

[00076] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:169, or a variant thereof engineered to conjugate to the linker payload, or SEQ ID NO:170 or a variant thereof conjugated to the linker payload, or SEQ ID NO:171. In further embodiments, Ab comprises a peptide of any one of SEQ ID NOs: 34, 36, or 38.

[00077] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:42 or a variant thereof.

[00078] In further embodiments, Ab comprises a peptide of any one of SEQ ID NOs: 34, 36, or 38.

[00079] In some embodiments, an ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:48. In further embodiments, Ab comprises a peptide of any one of SEQ ID NOs: 33, 34, 36, or 38.

[00080] In some embodiments, an ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:171. In further embodiments, Ab comprises a peptide of any one of SEQ ID NOs: 33, 34, 36, or 38.

[00081] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:48.

[00082] In further embodiments, Ab comprises a peptide of any one of SEQ ID NOs: 34, 36, or 38.

[00083] In some embodiments, an activatable ROR-ADC is represented by Formula (I) or (II) wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:171. In further embodiments, Ab comprises a peptide of any one of SEQ ID NOs: 34, 36, or 38.

[00084] In some embodiments, an activatable ROR-ADC is represented by Formula (II), wherein s is 4 and wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:48 and a polypeptide comprising the amino acid sequence of SEQ ID NO:34. This ROR-ADC is also referred to herein as **ADC-11**.

[00085] In some embodiments, an activatable ROR-ADC is represented by Formula (II), wherein s is 4 and wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:48 and a polypeptide comprising the amino acid sequence of SEQ ID NO:36. This ROR-ADC is also referred to herein as **ADC-13**.

[00086] In some embodiments, an activatable ROR-ADC is represented by Formula (II), wherein s is 4 and wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:48 and a polypeptide comprising the amino acid sequence of SEQ ID NO:38. This ROR-ADC is also referred to herein as **ADC-M3**.

[00087] In some embodiments, an activatable ROR-ADC is represented by Formula (II), wherein s is 4 and wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:171 and a polypeptide comprising the amino acid sequence of SEQ ID NO:34. This ROR-ADC is also referred to herein as **ADC-11-sFc**.

[00088] In some embodiments, an activatable ROR-ADC is represented by Formula (II), wherein s is 4 and wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:171 and a polypeptide comprising the amino acid sequence of SEQ ID NO:36. This ROR-ADC is also referred to herein as **ADC-13-sFc**.

[00089] In some embodiments, an activatable ROR-ADC is represented by Formula (II), wherein s is 4 and wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:171 and a polypeptide comprising the amino acid sequence of SEQ ID NO:38. This ROR-ADC is also referred to herein as **ADC-M3-sFc**.

[00090] In some embodiments, an ROR-ADC is represented by Formula (I) or Formula (II) as disclosed herein, wherein the ROR-ADC comprises an activatable ROR antibody, and wherein the activatable antibody comprises (a) a masking peptide comprising a masking moiety (MM) and a cleavable moiety (CM); and (b) a target binding moiety (TBM). In further embodiments, TBM comprises an antibody light chain variable (VL) region, and an antibody heavy chain variable (VH) region. In yet further embodiments, the VH region

comprises a VH complementarity determining region 1 (CDR1), a VH complementarity determining region 2 (CDR2), and a VH complementarity determining region 3 (CDR3) as set forth in a VH comprising the amino acid sequence of SEQ ID NO:25, and the VL region comprises a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a VL comprising the amino acid sequence of SEQ ID NO:26. Additionally or alternatively, the activatable antibody is capable of binding to ROR when the CM is cleaved.

[00091] In some embodiments, an ROR-ADC is represented by Formula (I) or Formula (II) as disclosed herein, wherein Ab binds to both ROR1 and ROR2. In further embodiments, Ab binds to both human ROR1 and human ROR2. Additionally or alternatively, Ab comprises a silent Fc (sFc). In further embodiments, Ab comprises an sFc comprising an amino acid sequence as set forth in SEQ ID NO:44.

[00092] The present disclosure also provides a pharmaceutical composition comprising an activatable ROR-ADC, wherein the activatable ROR-ADC is represented by Formula (I) or Formula (II), and a pharmaceutically acceptable excipient, wherein the activatable ROR antibody (activatable ROR Ab or activatable Ab) is as described in any embodiment described herein. In some embodiments, such a pharmaceutical composition exhibits a drug-to-antibody ratio (DAR) of the activatable ROR-ADC of about 1 to about 20, for example, a DAR of about 2 to about 8, about 1 to about 4, about 2 to about 4, about 3 to about 4, about 4, about 1 to about 8, about 2 to about 8, about 3 to about 8, about 4 to about 8, about 5 to about 8, about 6 to about 8, about 6.5 to about 8, about 6 to about 7, about 6.5 to about 7.5, about 7 to about 8, about 6.5, about 7, about 7.5, or about 8.

[00093] The present disclosure also provides an ROR-ADC, which is produced by activating an activatable ROR-ADC as disclosed herein. Additionally or alternatively, the present disclosure also provides an ROR-ADC, which is an activatable ROR-ADC as disclosed herein having its activatable antibody Ab activated. In some embodiments, the ADC or Ab was activated by treating the ADC comprising the activatable antibody Ab with one or more proteases, wherein the Ab comprises a masking peptide, and wherein the one or more proteases cleave within the masking peptide. In some embodiments, the one or more proteases comprises MMP-9. In some embodiments, Ab comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:48 or 171 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:33. In further embodiments, Ab comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:33.

[00094] In other embodiments, Ab comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:171 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:33.

[00095] The present disclosure also provides a method for treating a cancer or a tumor in a subject comprising administering to the subject an ROR-ADC as disclosed herein, such as an activatable ROR-ADC as disclosed herein, or a pharmaceutical composition as -disclosed herein. In some embodiments, the ROR-ADC is represented by Formula (I) or (II). In further embodiments, the activatable ROR-ADC is represented by Formula (I) or (II) or the pharmaceutical composition comprising an activatable ROR-ADC of Formula (I) or (II) and a pharmaceutically acceptable excipient, wherein the activatable ROR antibody is as described in any embodiment herein.

[00096] Provided herein is a kit comprising the antibody-drug conjugate as disclosed herein or the pharmaceutical composition as disclosed herein, and instructions for use.

6. BRIEF DESCRIPTION OF THE DRAWINGS

[00097] **FIG. 1** shows a schematic of an exemplary selection process for self-blocking peptides, as further illustrated in **Example 1**.

[00098] **FIGs. 2A-2F** provide exemplary results of the tested antibodies' binding to antigens, as further illustrated in **Example 1**. **FIG. 2A** shows the binding of tested antibodies (**P0** and **M1**) to ROR1-Fc measured by ELISA. **FIG. 2B** shows the binding of tested antibodies (**P0, M2,** and **M3**) to ROR1-Fc measured by ELISA. **FIG. 2C** shows the binding of tested antibodies (**P0, M1, M2,** and **M3**) to ROR2-Fc measured by ELISA. **FIG. 2D** shows the binding of tested antibodies (**P0, M1, M2,** and **M3**) to HT-29 measured by FACS. **FIG. 2E** shows the binding of tested antibodies (**P0, M1, M2,** and **M3**) to H226 measured by FACS. **FIG. 2F** shows the binding of tested antibodies (**P0, M1, M2,** and **M3**) to 293F measured by FACS.

[00099] **FIGs. 3A-3B** provide exemplary differential scanning fluorimetry (DSF) results of the tested antibodies (**M1, M2,** and **M3**), as further illustrated in **Example 2**. **FIG. 3A** plots fluorescence, while **FIG. 3B** plots d(fluorescence)/dT.

[00100] **FIGs. 4A-4D** provide exemplary capillary electrophoresis sodium dodecyl sulfate (CE-SDS) results of the tested antibodies (**P0**: **FIG. 4A**, **M1**: **FIG. 4B**, **M2**: **FIG. 4C**, and **M3**: **FIG. 4D**), as further illustrated in **Example 2**.

[00101] **FIGs. 5A-5D** provide exemplary capillary isoelectric focusing (cIEF) results of the tested antibodies (**P0**: **FIG. 5A**, **M1**: **FIG. 5B**, **M2**: **FIG. 5C**, and **M3**: **FIG. 5D**), as further illustrated in **Example 2**.

[00102] **FIG. 6** provides exemplary hydrophobic interaction chromatography (HIC) results of the tested antibodies (**P0**, **M1**, **M2**, and **M3**), as further illustrated in **Example 2**.

[00103] **FIGs. 7A-7D** provide exemplary accelerated stability assessment results under 40 °C incubation of the tested antibodies (**P0**: **FIG. 7A**, **M1**: **FIG. 7B**, **M2**: **FIG. 7C**, and **M3**: **FIG. 7D**), as further illustrated in **Example 2**.

[00104] **FIGs. 8A-8D** provide exemplary accelerated stability assessment results after freezing and thawing (3 rounds: FT3; and 6 rounds: FT6) of the tested antibodies (**P0**: **FIG. 8A**, **M1**: **FIG. 8B**, **M2**: **FIG. 8C**, and **M3**: **FIG. 8D**), as further illustrated in **Example 2**.

[00105] **FIGs. 9A-9D** provide exemplary accelerated stability assessment results at low pH of the tested antibodies (**P0**: **FIG. 9A**, **M1**: **FIG. 9B**, **M2**: **FIG. 9C**, and **M3**: **FIG. 9D**), as further illustrated in **Example 2**.

[00106] **FIGs. 10A-10D** provide exemplary accelerated stability assessment results under oxidation of the tested antibodies (**P0**: **FIG. 10A**, **M1**: **FIG. 10B**, **M2**: **FIG. 10C**, and **M3**: **FIG. 10D**), as further illustrated in **Example 2**.

[00107] **FIGs. 11A-11C** provide exemplary plasma or serum stability results of the tested antibodies, as further illustrated in **Example 2**. **FIG. 11A** plots the plasma stability shown by the total antibody concentrations. **FIG. 11B** plots the serum stability shown by the total antibody concentrations. **FIG. 11C** plots the stability shown by the activated antibody concentrations.

[00108] **FIG. 12** provides an exemplary western blotting result showing human IgG from mice injected with 3 mg/kg or 10 mg/kg of **P0** or **M2**, as further illustrated in **Example 4**.

[00109] **FIGs. 13A-13C** provide exemplary western blotting results showing human IgG from mice injected with 10 mg/kg of **M1** (**FIG. 13A**), **M2** (**FIG. 13B**), or **M3** (**FIG. 13C**), as further illustrated in **Example 4**.

[00110] **FIG. 14** plots an exemplary intratumoral receptor occupancy (RO) result of mice 14 hours or 96 hours after dosing with 10 mg/kg **P0**, **M1**, **M2**, or **M3**, as further illustrated in **Example 5**.

[00111] **FIGs. 15A-15B** provide exemplary RO flow cytometry plots of mice 14 hours after dosing with 10 mg/kg an isotype control, **P0**, **M1**, **M2**, or **M3**, as further illustrated in **Example 5**. **FIG. 15A** provides plots relating to an isotype control, **P0**, and **M1**, while **FIG. 15B** provides plots relating to **M2** and **M3.**

[00112] **FIGs. 16A-16B** provide exemplary RO flow cytometry plots of mice 96 hours after dosing with 10 mg/kg an isotype control, **P0**, **M1**, **M2**, or **M3**, as further illustrated in

Example 5. **FIG. 16A** provides plots relating to an isotype control, **P0**, and **M1**, while **FIG. 16B** provides plots relating to **M2** and **M3.**

[00113] FIG. 17 provides exemplary IHZ and immunofluorescence (IF) staining result, as further illustrated in **Example 6**. White arrow heads show ROR1 staining outside of islets, while white arrows show ROR1 staining inside islets.

[00114] FIGs. 18A-18D provide exemplary IHZ staining results, as further illustrated in **Example 6**. **FIG. 18A** shows IHZ staining of ROR on HT-29 and MCF7 cells. **FIG. 18B** shows IHZ staining of ROR and insulin in normal human pancreas samples #3 and #5. **FIG. 18C** shows IHZ staining of ROR and insulin in normal human pancreas samples #6 and #7. **FIG. 18D** shows IHZ staining of ROR and insulin in normal human pancreas samples #8 and #9. In the P0+insulin panels, white arrow heads show ROR1 staining in pancreas, while white arrows show ROR1 staining inside islets. In the other insulin panels, white arrows show pancreatic islets.

[00115] FIGs. 19A-19C provide exemplary *in vitro* cytotoxicity results of masked and non-masked ROR-ADCs +/- MMP-9 in cell lines as detailed in **Example 10**. **FIG. 19A** plots results of ADC-00, ADC-11, and ADC-13 with or without MMP-9 activation in hROR1 expressing HEK cells, while **FIG. 19B** and **FIG. 19C** plot results of ADC-13 and ADC-13-sFc with or without MMP-9 activation in hROR1 expressing HEK cells (**FIG. 19B**) and hROR2 expressing HEK cells (**FIG. 19C**), respectively.

[00116] FIGs. 20A-20D provide exemplary binding results as measured by ELISA of ADCs -/+ MMP-9 activation to ROR1 and ROR2 as detailed in **Example 11**. **FIG. 20A** plots binding of the tested ADCs (*e.g.*, ADC-00, ADC-11, and ADC-13) without MMP-9 activation to ROR1. **FIG. 20B** plots binding of the tested ADCs (*e.g.*, ADC-00, ADC-11, and ADC-13) with MMP-9 activation to ROR1. **FIG. 20C** plots binding of the tested ADCs (*e.g.*, ADC-00, ADC-11, and ADC-13) without MMP-9 activation to ROR2. **FIG. 20D** plots binding of the tested ADCs (*e.g.*, ADC-00, ADC-11, and ADC-13) with MMP-9 activation to ROR2.

[00117] FIGs. 21A-21B provide exemplary *in vivo* efficacy data in MDA-MB-231 triple-negative breast cancer (TNBC) xenograft model, as detailed in **Example 12**. **FIG. 21A** plots tumor volumes, while **FIG. 21B** plots tumor growth inhibition percentages (%TGI) over the isotype control on Day 29.

[00118] FIGs. 22A-22B provide exemplary *in vivo* efficacy data in JEKO-1 mantle cell lymphoma (MCL) xenograft model, as detailed in **Example 13**. **FIG. 22A** plots tumor

volumes, while **FIG. 22B** plots tumor growth inhibition percentages (%TGI) over the isotype control on Day 25.

[00119] FIG. 23 provides exemplary pharmacokinetics (PK) results in rats as detailed in **Example 15.**

[00120] FIG. 24 provides exemplary toxicokinetics (TK) results in rats as detailed in **Example 16.**

[00121] FIG. 25 summarizes binding, xenograft efficacy and rat tox profiles of ADC-11 and ADC-13.

[00122] FIG. 26 provides exemplary *in vivo* efficacy data in a non-small cell lung cancer (NSCLC) patient-derived xenograft (PDX) model, as detailed in **Example 14**.

7. DETAILED DESCRIPTION

[00123] The present disclosure provides antibody-drug conjugates (ADCs) (such as activatable ADCs) that bind to ROR and a drug conjugated (directly or indirectly) thereto. Such ROR ADCs (such as activatable ROR-ADCs) are useful in compositions and in methods of treating, preventing, or alleviating an ROR-mediated disease, disorder, or condition, including one or more symptoms of the disease, disorder, or condition. ROR-mediated diseases, disorders, and conditions include a variety of cancers, including, but not limited to, any cancer wherein the tumor cells express or overexpress an ROR antigen. In addition, activatable ROR-ADCs are useful for the killing and/or removal of tumor cells. Activatable ROR-ADCs described herein are useful in compositions and in methods for treating cancer.

7.1 DEFINITIONS

[00124] Techniques and procedures described or referenced herein include those that are generally well understood and/or commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual (3d ed. 2001); Current Protocols in Molecular Biology (Ausubel et al. eds., 2003); Therapeutic Monoclonal Antibodies: From Bench to Clinic (An ed. 2009); Monoclonal Antibodies: Methods and Protocols (Albitar ed. 2010); and Antibody Engineering Vols 1 and 2 (Kontermann and Dübel eds., 2d ed. 2010). Unless otherwise defined herein, technical and scientific terms used in the present description have the meanings that are commonly understood by those of ordinary skill in the art. For purposes of interpreting this specification, the following description of terms will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa.

In the event that any description of a term set forth conflicts with any document incorporated herein by reference, the description of the term set forth below shall control.

[00125] The following terms have the following meanings unless otherwise indicated. Any undefined terms have their art-recognized meanings.

[00126] "Alkyl" refers to monovalent saturated aliphatic hydrocarbyl groups having from 1 to 10 carbon atoms and such as 1 to 6 carbon atoms, or 1 to 5, or 1 to 4, or 1 to 3 carbon atoms. This term includes, by way of example, linear and branched hydrocarbyl groups such as methyl (CH_3-), ethyl (CH_3CH_2-), n-propyl ($CH_3CH_2CH_2$-), isopropyl (($CH_3)_2CH$-), n-butyl ($CH_3CH_2CH_2CH_2$-), isobutyl (($CH_3)_2CHCH_2$-), sec-butyl (($CH_3)(CH_3CH_2)CH$-), t-butyl (($CH_3)_3C$-), n-pentyl ($CH_3CH_2CH_2CH_2CH_2$-), and neopentyl (($CH_3)_3CCH_2$-).

[00127] The term "substituted alkyl" refers to an alkyl group as defined herein wherein one or more carbon atoms in the alkyl chain (except the C_1 carbon atom) have been optionally replaced with a heteroatom such as -O-, -N-, -S-, -$S(O)_n$- (where n is 0 to 2), -NR- (where R is hydrogen or alkyl) and having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-aryl, -SO_2-heteroaryl, and -NR^aR^b, wherein R' and R" may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

[00128] "Alkylene" refers to divalent aliphatic hydrocarbyl groups preferably having from 1 to 6 and more preferably 1 to 3 carbon atoms that are either straight-chained or branched, and which are optionally interrupted with one or more groups selected from -O-, -NR^{10}-, -$NR^{10}C(O)$-, -$C(O)NR^{10}$- and the like. This term includes, by way of example, methylene (-CH_2-), ethylene (-CH_2CH_2-), n-propylene (-$CH_2CH_2CH_2$-), iso-propylene (-$CH_2CH(CH_3)$-), (-$C(CH_3)_2CH_2CH_2$-), (-$C(CH_3)_2CH_2C(O)$-), (-$C(CH_3)_2CH_2C(O)NH$-), (-$CH(CH_3)CH_2$-), and the like.

[00129] "Substituted alkylene" refers to an alkylene group having from 1 to 3 hydrogens replaced with substituents as described for carbons in the definition of "substituted" below.

[00130] The term "alkane" refers to alkyl group and alkylene group, as defined herein.

[00131] The term "alkylaminoalkyl," "alkylaminoalkenyl" and "alkylaminoalkynyl" refers to the groups R'NHR"- where R' is alkyl group as defined herein and R" is alkylene, alkenylene or alkynylene group as defined herein.

[00132] The term "alkaryl" or "aralkyl" refers to the groups -alkylene-aryl and -substituted alkylene-aryl where alkylene, substituted alkylene and aryl are defined herein.

[00133] "Alkoxy" refers to the group –O-alkyl, wherein alkyl is as defined herein. Alkoxy includes, by way of example, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, t-butoxy, sec-butoxy, n-pentoxy, and the like. The term "alkoxy" also refers to alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein.

[00134] The term "substituted alkoxy" refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O- where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

[00135] The term "alkoxyamino" refers to the group –NH-alkoxy, wherein alkoxy is defined herein.

[00136] The term "haloalkoxy" refers to alkyl-O- wherein one or more hydrogen atoms on the alkyl group have been substituted with a halo group and include, by way of examples, groups such as trifluoromethoxy, and the like.

[00137] The term "haloalkyl" refers to a substituted alkyl group as described above, wherein one or more hydrogen atoms on the alkyl group have been substituted with a halo group. Examples of such groups include, without limitation, fluoroalkyl groups, such as trifluoromethyl, difluoromethyl, trifluoroethyl and the like.

[00138] The term "alkylalkoxy" refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl, and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein.

[00139] The term "alkylthioalkoxy" refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein.

[00140] "Alkenyl" refers to straight chain or branched hydrocarbyl groups having from 2 to 6 carbon atoms and preferably 2 to 4 carbon atoms and having at least 1 and preferably from 1 to 2 sites of double bond unsaturation. This term includes, by way of example, bi-vinyl, allyl, and but-3-en-1-yl. Included within this term are the cis and trans isomers or mixtures of these isomers.

[00141] The term "substituted alkenyl" refers to an alkenyl group as defined herein having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl.

[00142] "Alkynyl" refers to straight or branched monovalent hydrocarbyl groups having from 2 to 6 carbon atoms and preferably 2 to 3 carbon atoms and having at least 1 and preferably from 1 to 2 sites of triple bond unsaturation. Examples of such alkynyl groups include acetylenyl (-C≡CH), and propargyl (-CH_2C≡CH).

[00143] The term "substituted alkynyl" refers to an alkynyl group as defined herein having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, and -SO_2-heteroaryl.

[00144] "Alkynyloxy" refers to the group –O-alkynyl, wherein alkynyl is as defined herein. Alkynyloxy includes, by way of example, ethynyloxy, propynyloxy, and the like.

[00145] "Acyl" refers to the groups H-C(O)-, alkyl-C(O)-, substituted alkyl-C(O)-, alkenyl-C(O)-, substituted alkenyl-C(O)-, alkynyl-C(O)-, substituted alkynyl-C(O)-, cycloalkyl-C(O)-, substituted cycloalkyl-C(O)-, cycloalkenyl-C(O)-, substituted cycloalkenyl-C(O)-, aryl-C(O)-, substituted aryl-C(O)-, heteroaryl-C(O)-, substituted heteroaryl-C(O)-, heterocyclyl-C(O)-, and substituted heterocyclyl-C(O)-, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein. For example, acyl includes the "acetyl" group CH_3C(O)-

[00146] "Acylamino" refers to the groups $–NR^{20}C(O)$alkyl, $-NR^{20}C(O)$substituted alkyl, N $R^{20}C(O)$cycloalkyl, $-NR^{20}C(O)$substituted cycloalkyl, -$NR^{20}C(O)$cycloalkenyl, $-NR^{20}C(O)$substituted cycloalkenyl, $-NR^{20}C(O)$alkenyl, $-NR^{20}C(O)$substituted alkenyl, $-NR^{20}C(O)$alkynyl, $-NR^{20}C(O)$substituted alkynyl, $-NR^{20}C(O)$aryl, $-NR^{20}C(O)$substituted aryl, $-NR^{20}C(O)$heteroaryl, $-NR^{20}C(O)$substituted heteroaryl, $-NR^{20}C(O)$heterocyclic, and $-NR^{20}C(O)$substituted heterocyclic, wherein R^{20} is hydrogen or alkyl and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00147] "Aminocarbonyl" or the term "aminoacyl" refers to the group $-C(O)NR^{21}R^{22}$, wherein R^{21} and R^{22} independently are selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic and where R^{21} and R^{22} are optionally joined together with the nitrogen bound thereto to form a heterocyclic or substituted heterocyclic group, and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00148] "Aminocarbonylamino" refers to the group $–NR^{21}C(O)NR^{22}R^{23}$ where R^{21}, R^{22}, and R^{23} are independently selected from hydrogen, alkyl, aryl or cycloalkyl, or where two R groups are joined to form a heterocyclyl group.

[00149] The term "alkoxycarbonylamino" refers to the group -NRC(O)OR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclyl wherein alkyl, substituted alkyl, aryl, heteroaryl, and heterocyclyl are as defined herein.

[00150] The term "acyloxy" refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclyl-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclyl are as defined herein.

[00151] "Aminosulfonyl" refers to the group $–SO_2NR^{21}R^{22}$, wherein R^{21} and R^{22} independently are selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted

heteroaryl, heterocyclic, substituted heterocyclic and where R^{21} and R^{22} are optionally joined together with the nitrogen bound thereto to form a heterocyclic or substituted heterocyclic group and alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[00152] "Sulfonylamino" refers to the group $-NR^{21}SO_2R^{22}$, wherein R^{21} and R^{22} independently are selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic and where R^{21} and R^{22} are optionally joined together with the atoms bound thereto to form a heterocyclic or substituted heterocyclic group, and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00153] "Aryl" or "Ar" refers to a monovalent aromatic carbocyclic group of from 6 to 18 carbon atoms having a single ring (such as is present in a phenyl group) or a ring system that has multiple condensed rings (examples of such aromatic ring systems include naphthyl, anthryl and indanyl) which condensed rings may or may not be aromatic, provided that the point of attachment is through an atom of an aromatic ring. This term includes, by way of example, phenyl and naphthyl. Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents, or from 1 to 3 substituents, selected from acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halogen, nitro, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl, $-SO_2$-heteroaryl and trihalomethyl.

[00154] "Aryloxy" refers to the group –O-aryl, wherein aryl is as defined herein, including, by way of example, phenoxy, naphthoxy, and the like, including optionally substituted aryl groups as also defined herein.

[00155] "Amino" refers to the group -NH_2.

[00156] The term "substituted amino" refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl, and heterocyclyl provided that at least one R is not hydrogen.

[00157] The term "azido" refers to the group -N_3.

[00158] "Carboxyl," "carboxy" or "carboxylate" refers to –CO_2H or salts thereof.

[00159] "Carboxyl ester" or "carboxy ester" or the terms "carboxyalkyl" or "carboxylalkyl" refers to the groups -C(O)O-alkyl, -C(O)O-substituted alkyl, -C(O)O-alkenyl, -C(O)O-substituted alkenyl, -C(O)O-alkynyl, -C(O)O-substituted alkynyl, -C(O)O-aryl, -C(O)O-substituted aryl, -C(O)O-cycloalkyl, -C(O)O-substituted cycloalkyl, -C(O)O-cycloalkenyl, -C(O)O-substituted cycloalkenyl, -C(O)O-heteroaryl, -C(O)O-substituted heteroaryl, -C(O)O-heterocyclic, and -C(O)O-substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00160] "(Carboxyl ester)oxy" or "carbonate" refers to the groups –O-C(O)O-alkyl, -O-C(O)O-substituted alkyl, -O-C(O)O-alkenyl, -O-C(O)O-substituted alkenyl, -O-C(O)O-alkynyl, -O-C(O)O-substituted alkynyl, -O-C(O)O-aryl, -O-C(O)O-substituted aryl, -O-C(O)O-cycloalkyl, -O-C(O)O-substituted cycloalkyl, -O-C(O)O-cycloalkenyl, -O-C(O)O-substituted cycloalkenyl, -O-C(O)O-heteroaryl, -O-C(O)O-substituted heteroaryl, -O-C(O)O-heterocyclic, and -O-C(O)O-substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00161] "Cyano" or "nitrile" refers to the group -CN.

[00162] "Cycloalkyl" refers to cyclic alkyl groups of from 3 to 10 carbon atoms having single or multiple cyclic rings including fused, bridged, and spiro ring systems. Examples of suitable cycloalkyl groups include, for instance, adamantyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl and the like. Such cycloalkyl groups include, by way of example, single ring structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

[00163] The term "substituted cycloalkyl" refers to cycloalkyl groups having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkyl, substituted alkyl, alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

[00164] "Cycloalkenyl" refers to non-aromatic cyclic alkyl groups of from 3 to 10 carbon atoms having single or multiple rings and having at least one double bond and preferably from 1 to 2 double bonds.

[00165] The term "substituted cycloalkenyl" refers to cycloalkenyl groups having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, $-SO_2$-alkyl, $-SO_2$-substituted alkyl, $-SO_2$-aryl and $-SO_2$-heteroaryl.

[00166] "Cycloalkynyl" refers to non-aromatic cycloalkyl groups of from 5 to 10 carbon atoms having single or multiple rings and having at least one triple bond.

[00167] "Cycloalkoxy" refers to –O-cycloalkyl.

[00168] "Cycloalkenyloxy" refers to –O-cycloalkenyl.

[00169] "Halo" or "halogen" refers to fluoro, chloro, bromo, and iodo.

[00170] "Hydroxy" or "hydroxyl" refers to the group –OH.

[00171] "Heteroaryl" refers to an aromatic group of from 1 to 15 carbon atoms, such as from 1 to 10 carbon atoms and 1 to 10 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur within the ring. Such heteroaryl groups can have a single ring (such as, pyridinyl, imidazolyl or furyl) or multiple condensed rings in a ring system (for example as in groups such as, indolizinyl, quinolinyl, benzofuran, benzimidazolyl or benzothienyl), wherein at least one ring within the ring system is aromatic. To satisfy valence requirements, any heteroatoms in such heteroaryl rings may or may not be bonded to H or a substituent group,

e.g., an alkyl group or other substituent as described herein. In certain embodiments, the nitrogen and/or sulfur ring atom(s) of the heteroaryl group are optionally oxidized to provide for the N-oxide (N→O), sulfinyl, or sulfonyl moieties. This term includes, by way of example, pyridinyl, pyrrolyl, indolyl, thiophenyl, and furanyl. Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents, or from 1 to 3 substituents, selected from acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halogen, nitro, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl and -SO_2-heteroaryl, and trihalomethyl.

[00172] The term "heteroaralkyl" refers to the groups -alkylene-heteroaryl where alkylene and heteroaryl are defined herein. This term includes, by way of example, pyridylmethyl, pyridylethyl, indolylmethyl, and the like.

[00173] "Heteroaryloxy" refers to –O-heteroaryl.

[00174] "Heterocycle," "heterocyclic," "heterocycloalkyl," and "heterocyclyl" refer to a saturated or unsaturated group having a single ring or multiple condensed rings, including fused bridged and spiro ring systems, and having from 3 to 20 ring atoms, including 1 to 10 hetero atoms. These ring atoms are selected from nitrogen, sulfur, or oxygen, where, in fused ring systems, one or more of the rings can be cycloalkyl, aryl, or heteroaryl, provided that the point of attachment is through the non-aromatic ring. In certain embodiments, the nitrogen and/or sulfur atom(s) of the heterocyclic group are optionally oxidized to provide for the N-oxide, -S(O)-, or -SO_2- moieties. To satisfy valence requirements, any heteroatoms in such heterocyclic rings may or may not be bonded to one or more H or one or more substituent group(s), *e.g.*, an alkyl group or other substituent as described herein.

[00175] Examples of heterocycles and heteroaryls include, but are not limited to, azetidine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, dihydroindole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, phthalimide,

1,2,3,4-tetrahydroisoquinoline, 4,5,6,7-tetrahydrobenzo[b]thiophene, thiazole, thiazolidine, thiophene, benzo[b]thiophene, morpholinyl, thiomorpholinyl (also referred to as thiamorpholinyl), 1,1-dioxothiomorpholinyl, piperidinyl, pyrrolidine, tetrahydrofuranyl, and the like.

[00176] Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-aryl, -SO_2-heteroaryl, and fused heterocycle.

[00177] "Heterocyclyloxy" refers to the group –O-heterocyclyl.

[00178] The term "heterocyclylthio" refers to the group heterocyclic-S-.

[00179] The term "heterocyclene" refers to the diradical group formed from a heterocycle, as defined herein.

[00180] The term "hydroxyamino" refers to the group -NHOH.

[00181] "Nitro" refers to the group –NO_2.

[00182] "Oxo" refers to the atom (=O).

[00183] "Sulfonyl" refers to the group -SO_2-alkyl, -SO_2-substituted alkyl, -SO_2-alkenyl, -SO_2-substituted alkenyl, -SO_2-cycloalkyl, -SO_2-substituted cylcoalkyl, -SO_2-cycloalkenyl, -SO_2-substituted cylcoalkenyl, -SO_2-aryl, -SO_2-substituted aryl, -SO_2-heteroaryl, -SO_2-substituted heteroaryl, -SO_2-heterocyclic, and -SO_2-substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein. Sulfonyl includes, by way of example, methyl-SO_2-, phenyl-SO_2-, and 4-methylphenyl-SO_2-.

[00184] "Sulfonyloxy" refers to the group -OSO_2-alkyl, -OSO_2-substituted alkyl, -OSO_2-alkenyl, -OSO_2-substituted alkenyl, -OSO_2-cycloalkyl, -OSO_2-substituted cylcoalkyl, -OSO_2-cycloalkenyl, -OSO_2-substituted cylcoalkenyl, -OSO_2-aryl, -OSO_2-substituted aryl, -OSO_2-heteroaryl, -OSO_2-substituted heteroaryl, -OSO_2-heterocyclic, and -OSO_2-substituted

heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00185] "Sulfate" or "sulfate ester" refers the group -O-SO_2-OH, -O-SO_2-O-alkyl, -O-SO_2-O-substituted alkyl, -O-SO_2-O-alkenyl, -O-SO_2-O-substituted alkenyl, -O-SO_2-O-cycloalkyl, -O-SO_2-O-substituted cylcoalkyl, -O-SO_2-O-cycloalkenyl, -O-SO_2-O-substituted cylcoalkenyl, -O-SO_2-O-aryl, -O-SO_2-O-substituted aryl, -O-SO_2-O-heteroaryl, -O-SO_2-O-substituted heteroaryl, -O-SO_2-O-heterocyclic, and -O-SO_2-O-substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00186] The term "aminocarbonyloxy" refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

[00187] "Thiol" refers to the group -SH.

[00188] "Thioxo" or the term "thioketo" refers to the atom (=S).

[00189] "Alkylthio" or the term "thioalkoxy" refers to the group -S-alkyl, wherein alkyl is as defined herein. In certain embodiments, sulfur may be oxidized to -S(O)-. The sulfoxide may exist as one or more stereoisomers.

[00190] The term "substituted thioalkoxy" refers to the group -S-substituted alkyl.

[00191] The term "thioaryloxy" refers to the group aryl-S- wherein the aryl group is as defined herein including optionally substituted aryl groups also defined herein.

[00192] The term "thioheteroaryloxy" refers to the group heteroaryl-S- wherein the heteroaryl group is as defined herein including optionally substituted aryl groups as also defined herein.

[00193] The term "thioheterocyclooxy" refers to the group heterocyclyl-S- wherein the heterocyclyl group is as defined herein including optionally substituted heterocyclyl groups as also defined herein.

[00194] In the disclosure herein, the term "substituted," when used to modify a specified group or radical, can also mean that one or more hydrogen atoms of the specified group or radical are each, independently of one another, replaced with the same or different substituent groups as defined below.

[00195] In addition to the groups disclosed with respect to the individual terms herein, substituent groups for substituting for one or more hydrogens (any two hydrogens on a single carbon can be replaced with =O, =NR^{70}, =N-OR^{70}, =N_2 or =S) on saturated carbon atoms in the specified group or radical are, unless otherwise specified, -R^{60}, halo, =O, -OR^{70}, -SR^{70}, -$NR^{80}R^{80}$, trihalomethyl, -CN, -OCN, -SCN, -NO, -NO_2, =N_2, -N_3, -SO_2R^{70}, -$SO_2O^-M^+$, -SO_2OR^{70}, -OSO_2R^{70}, -$OSO_2O^-M^+$, -OSO_2OR^{70}, -$P(O)(O^-)_2(M^+)_2$, -$P(O)(OR^{70})O^-M^+$, -$P(O)(OR^{70})_2$, -$C(O)R^{70}$, -$C(S)R^{70}$, -$C(NR^{70})R^{70}$, -$C(O)O^-M^+$, -$C(O)OR^{70}$, -$C(S)OR^{70}$, -$C(O)NR^{80}R^{80}$, -$C(NR^{70})NR^{80}R^{80}$, -$OC(O)R^{70}$, -$OC(S)R^{70}$, -$OC(O)O^-M^+$, -$OC(O)OR^{70}$, -$OC(S)OR^{70}$, -$NR^{70}C(O)R^{70}$, -$NR^{70}C(S)R^{70}$, -$NR^{70}CO_2^-M^+$, -$NR^{70}CO_2R^{70}$, -$NR^{70}C(S)OR^{70}$, -$NR^{70}C(O)NR^{80}R^{80}$, -$NR^{70}C(NR^{70})R^{70}$ and -$NR^{70}C(NR^{70})NR^{80}R^{80}$, where R^{60} is selected from the group consisting of optionally substituted alkyl, cycloalkyl, heteroalkyl, heterocycloalkylalkyl, cycloalkylalkyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl, each R^{70} is independently hydrogen or R^{60}; each R^{80} is independently R^{70} or alternatively, two R^{80}'s, taken together with the nitrogen atom to which they are bonded, form a 5-, 6- or 7-membered heterocycloalkyl which may optionally include from 1 to 4 of the same or different additional heteroatoms selected from the group consisting of O, N and S, of which N may have -H or C_1-C_3 alkyl substitution; and each M^+ is a counter ion with a net single positive charge. Each M^+ may independently be, for example, an alkali ion, such as K^+, Na^+, Li^+; an ammonium ion, such as $^+N(R^{60})_4$; or an alkaline earth ion, such as $[Ca^{2+}]_{0.5}$, $[Mg^{2+}]_{0.5}$, or $[Ba^{2+}]_{0.5}$ ("subscript 0.5 means that one of the counter ions for such divalent alkali earth ions can be an ionized form of a compound of the invention and the other a typical counter ion such as chloride, or two ionized compounds disclosed herein can serve as counter ions for such divalent alkali earth ions, or a doubly ionized compound of the invention can serve as the counter ion for such divalent alkali earth ions). As specific examples, -$NR^{80}R^{80}$ is meant to include -NH_2, -NH-alkyl, *N*-pyrrolidinyl, *N*-piperazinyl, 4*N*-methyl-piperazin-1-yl and *N*-morpholinyl.

[00196] In addition to the disclosure herein, substituent groups for hydrogens on unsaturated carbon atoms in "substituted" alkene, alkyne, aryl and heteroaryl groups are, unless otherwise specified, -R^{60}, halo, -O^-M^+, -OR^{70}, -SR^{70}, -S^-M^+, -$NR^{80}R^{80}$, trihalomethyl, -CF_3, -CN, -OCN, -SCN, -NO, -NO_2, -N_3, -SO_2R^{70}, -SO_3M^+, -SO_3R^{70}, -OSO_2R^{70}, -$OSO_3^-M^+$, -OSO_3R^{70}, -$PO_3^{-2}(M^+)_2$, -$P(O)(OR^{70})O^-M^+$, -$P(O)(OR^{70})_2$, -$C(O)R^{70}$, -$C(S)R^{70}$, -$C(NR^{70})R^{70}$, -$CO_2^-M^+$, -CO_2R^{70}, -$C(S)OR^{70}$, -$C(O)NR^{80}R^{80}$, -$C(NR^{70})NR^{80}R^{80}$, -$OC(O)R^{70}$, -$OC(S)R^{70}$, -$OCO_2^-M^+$, -OCO_2R^{70}, -$OC(S)OR^{70}$, -$NR^{70}C(O)R^{70}$, -$NR^{70}C(S)R^{70}$, -$NR^{70}CO_2^-M^+$, -$NR^{70}CO_2R^{70}$, -$NR^{70}C(S)OR^{70}$,

$-NR^{70}C(O)NR^{80}R^{80}$, $-NR^{70}C(NR^{70})R^{70}$ and $-NR^{70}C(NR^{70})NR^{80}R^{80}$, where R^{60}, R^{70}, R^{80} and M^+ are as previously defined, provided that in case of substituted alkene or alkyne, the substituents are not $-O^-M^+$, $-OR^{70}$, $-SR^{70}$, or $-S^-M^+$.

[00197] In addition to the groups disclosed with respect to the individual terms herein, substituent groups for hydrogens on nitrogen atoms in "substituted" heteroalkyl and cycloheteroalkyl groups are, unless otherwise specified, $-R^{60}$, $-O^-M^+$, $-OR^{70}$, $-SR^{70}$, $-S^-M^+$, $-NR^{80}R^{80}$, trihalomethyl, $-CF_3$, $-CN$, $-NO$, $-NO_2$, $-S(O)_2R^{70}$, $-S(O)_2O^-M^+$, $-S(O)_2OR^{70}$, $-OS(O)_2R^{70}$, $-OS(O)_2O^-M^+$, $-OS(O)_2OR^{70}$, $-P(O)(O^-)_2(M^+)_2$, $-P(O)(OR^{70})O^-M^+$, $-P(O)(OR^{70})(OR^{70})$, $-C(O)R^{70}$, $-C(S)R^{70}$, $-C(NR^{70})R^{70}$, $-C(O)OR^{70}$, $-C(S)OR^{70}$, $-C(O)NR^{80}R^{80}$, $-C(NR^{70})NR^{80}R^{80}$, $-OC(O)R^{70}$, $-OC(S)R^{70}$, $-OC(O)OR^{70}$, $-OC(S)OR^{70}$, $-NR^{70}C(O)R^{70}$, $-NR^{70}C(S)R^{70}$, $-NR^{70}C(O)OR^{70}$, $-NR^{70}C(S)OR^{70}$, $-NR^{70}C(O)NR^{80}R^{80}$, $-NR^{70}C(NR^{70})R^{70}$ and $-NR^{70}C(NR^{70})NR^{80}R^{80}$, where R^{60}, R^{70}, R^{80} and M^+ are as previously defined.

[00198] In addition to the disclosure herein, in a certain embodiment, a group that is substituted has 1, 2, 3, or 4 substituents, 1, 2, or 3 substituents, 1 or 2 substituents, or 1 substituent.

[00199] It is understood that in all substituted groups defined above, polymers arrived at by defining substituents with further substituents to themselves (*e.g.*, substituted aryl having a substituted aryl group as a substituent which is itself substituted with a substituted aryl group, which is further substituted by a substituted aryl group, etc.) are not intended for inclusion herein. In such cases, the maximum number of such substitutions is three. For example, serial substitutions of substituted aryl groups specifically contemplated herein are limited to substituted aryl-(substituted aryl)-substituted aryl.

[00200] Unless indicated otherwise, the nomenclature of substituents that are not explicitly defined herein are arrived at by naming the terminal portion of the functionality followed by the adjacent functionality toward the point of attachment. For example, the substituent "arylalkyloxycarbonyl" refers to the group (aryl)-(alkyl)-O-C(O)-.

[00201] As to any of the groups disclosed herein which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the subject compounds include all stereochemical isomers arising from the substitution of these compounds.

[00202] The term "pharmaceutically acceptable salt" means a salt which is acceptable for administration to a patient, such as a mammal (salts with counterions having acceptable

mammalian safety for a given dosage regime). Such salts can be derived from pharmaceutically acceptable inorganic or organic bases and from pharmaceutically acceptable inorganic or organic acids. "Pharmaceutically acceptable salt" refers to pharmaceutically acceptable salts of a compound, which salts are derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, formate, tartrate, besylate, mesylate, acetate, maleate, oxalate, and the like.

[00203] The term "salt thereof" means a compound formed when a proton of an acid is replaced by a cation, such as a metal cation or an organic cation and the like. Where applicable, the salt is a pharmaceutically acceptable salt, although this is not required for salts of intermediate compounds that are not intended for administration to a patient. By way of example, salts of the present compounds include those wherein the compound is protonated by an inorganic or organic acid to form a cation, with the conjugate base of the inorganic or organic acid as the anionic component of the salt.

[00204] "Solvate" refers to a complex formed by combination of solvent molecules with molecules or ions of the solute. The solvent can be an organic compound, an inorganic compound, or a mixture of both. Some examples of solvents include, but are not limited to, methanol, *N*,*N*-dimethylformamide, tetrahydrofuran, dimethylsulfoxide, and water. When the solvent is water, the solvate formed is a hydrate.

[00205] "Stereoisomer" and "stereoisomers" refer to compounds that have same atomic connectivity but different atomic arrangement in space. Stereoisomers include cis-trans isomers, *E* and *Z* isomers, enantiomers, and diastereomers.

[00206] "Tautomer" refers to alternate forms of a molecule that differ only in electronic bonding of atoms and/or in the position of a proton, such as enol-keto and imine-enamine tautomers, or the tautomeric forms of heteroaryl groups containing a -N=C(H)-NH- ring atom arrangement, such as pyrazoles, imidazoles, benzimidazoles, triazoles, and tetrazoles. A person of ordinary skill in the art would recognize that other tautomeric ring atom arrangements are possible.

[00207] It will be appreciated that the term "or a salt or solvate or stereoisomer thereof" is intended to include all permutations of salts, solvates and stereoisomers, such as a solvate of a pharmaceutically acceptable salt of a stereoisomer of subject compound.

[00208] In some embodiments, an "activatable" antibody refers to an antibody that exhibits a first level of binding to a target when in an inhibited, masked, and/or uncleaved state, and exhibits a second level of binding to the target in an uninhibited, unmasked, and/or cleaved state, where the second level of target binding is greater than the first level of target binding. In some embodiments, access to the target by the activatable antibody is greater after cleavage within the cleavable moiety (*e.g.,* by one or more proteases).

[00209] "Pharmaceutically effective amount" and "therapeutically effective amount" refer to an amount of a compound sufficient to treat a specified disorder or disease or one or more of its symptoms and/or to prevent the occurrence of the disease or disorder. In reference to tumorigenic proliferative disorders, a pharmaceutically or therapeutically effective amount comprises an amount sufficient to, among other things, cause the tumor to shrink or decrease the growth rate of the tumor.

[00210] A "prophylactically effective amount" is an amount of a pharmaceutical composition that, when administered to a subject, will have the intended prophylactic effect, *e.g.,* preventing or delaying the onset (or reoccurrence) of a disease, disorder, or condition, or reducing the likelihood of the onset (or reoccurrence) of a disease, disorder, or condition or associated symptom(s).

[00211] The full therapeutic or prophylactic effect does not necessarily occur by administration of one dose and may occur only after administration of a series of doses. Thus, a therapeutically or prophylactically effective amount may be administered in one or more administrations.

[00212] "Patient" refers to human and non-human subjects, especially mammalian subjects.

[00213] The term "treating" or "treatment" as used herein means the treating or treatment of a disease or medical condition in a patient, 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 patient; (c) suppressing the disease or medical condition, for example by, slowing or arresting the development of the disease or medical condition in a patient; or (d) alleviating a symptom of the disease or medical condition in a patient.

[00214] In some embodiments, the term "treating," or "treatment" excludes a prophylactic treatment.

[00215] By "reactive partner" is meant a molecule or molecular moiety that specifically reacts with another reactive partner to produce a reaction product. Exemplary reactive

partners include a cysteine or serine of a sulfatase motif and Formylglycine Generating Enzyme (FGE), which react to form a reaction product of a converted aldehyde tag containing a formylglycine (fGly) in lieu of cysteine or serine in the motif. Other exemplary reactive partners include an aldehyde of an fGly residue of a converted aldehyde tag (*e.g.*, a reactive aldehyde group) and an "aldehyde-reactive reactive partner," which comprises an aldehyde-reactive group and a moiety of interest, and which reacts to form a reaction product of a polypeptide having the moiety of interest conjugated to the polypeptide through the fGly residue.

[00216] "N-terminus" refers to the terminal amino acid residue of a polypeptide having a free amine group, which amine group in non-N-terminus amino acid residues normally forms part of the covalent backbone of the polypeptide.

[00217] "C-terminus" refers to the terminal amino acid residue of a polypeptide having a free carboxyl group, which carboxyl group in non-C-terminus amino acid residues normally forms part of the covalent backbone of the polypeptide.

[00218] By "internal site" as used in referenced to a polypeptide or an amino acid sequence of a polypeptide means a region of the polypeptide that is not at the N-terminus or at the C-terminus.

[00219] The term "subject" refers to human and non-human subjects, especially mammalian subjects.

[00220] The terms "native amino acid sequence" as used herein refers to the amino acid sequence of a polypeptide prior to modification to include a modified amino acid residue.

[00221] The terms "amino acid analog," "unnatural amino acid," and the like is used interchangeably, and include amino acid-like compounds that are similar in structure and/or overall shape to one or more amino acids commonly found in naturally occurring proteins (*e.g.*, Ala or A, Cys or C, Asp or D, Glu or E, Phe or F, Gly or G, His or H, Ile or I, Lys or K, Leu or L, Met or M, Asn or N, Pro or P, Gln or Q, Arg or R, Ser or S, Thr or T, Val or V, Trp or W, Tyr or Y). Amino acid analogs also include natural amino acids with modified side chains or backbones. Amino acid analogs also include amino acid analogs with the same stereochemistry as in the naturally occurring D-form, as well as the L-form of amino acid analogs. In some embodiments, the amino acid analogs share backbone structures, and/or the side chain structures of one or more natural amino acids, with difference(s) being one or more modified groups in the molecule. Such modification can include, but is not limited to, substitution of an atom (such as N) for a related atom (such as S), addition of a group (such as methyl, or hydroxyl, and the like) or an atom (such as Cl or Br, and the like), deletion of a

group, substitution of a covalent bond (single bond for double bond, and the like), or combinations thereof. For example, amino acid analogs can include α-hydroxy acids, and α-amino acids, and the like.

[00222] The term "amino acid side chain" is used to refer to the substituent attached to the α-carbon of an amino acid residue, including natural amino acids, unnatural amino acids, and amino acid analogs. An amino acid side chain can also include an amino acid side chain as described in the context of the modified amino acids and/or conjugates described herein.

[00223] The term "carbohydrate" is used to refer to monomer units and/or polymers of monosaccharides, disaccharides, oligosaccharides, and polysaccharides. The term sugar is be used to refer to the smaller carbohydrates, such as monosaccharides, disaccharides. The term "carbohydrate derivative" includes compounds where one or more functional groups of a carbohydrate of interest are substituted (replaced by any convenient substituent), modified (converted to another group using any convenient chemistry) or absent (*e.g.*, eliminated or replaced by H). A variety of carbohydrates and carbohydrate derivatives are available and can be adapted for use in the subject compounds and conjugates.

[00224] The term "glycoside" or "glycosyl" refers to a sugar molecule or group bound to a moiety via a glycosidic bond. For example, the moiety that the glycoside is bound to can be a cleavable linker as described herein. A glycosidic bond can link the glycoside to the other moiety through various types of bonds, such as, but not limited to, an O-glycosidic bond (an O-glycoside), an N-glycosidic bond (a glycosylamine), an S-glycosidic bond (a thioglycoside), or C-glycosidic bond (a C-glycoside or C-glycosyl). In some cases, glycosides can be cleaved from the moiety they are attached to, such as by chemically mediated hydrolysis or enzymatically mediated hydrolysis.

[00225] As used herein, "ROR antigens" refer to members of the tyrosine-protein kinase transmembrane receptor (ROR) family, including members ROR1 and ROR2. In certain embodiments, the activatable ROR antibody has antigen binding sites that specifically bind to ROR1 only. In other embodiments, the activatable ROR antibody has antigen binding sites that specifically bind to ROR2 only. In other embodiments, the activatable ROR antibody has antigen binding sites that are cross-reactive and specifically bind to both ROR1 and ROR2.

[00226] Thus, in one embodiment, the term "ROR" or "ROR antigen" as used herein refers to ROR1 (*e.g.*, human ROR1). In another embodiment, the term "ROR" or "ROR antigen" as used herein refers to ROR2 (*e.g.*, human ROR2). In another embodiment, the term "ROR" or "ROR antigen" as used herein refers to both ROR1 and ROR2 (*e.g.*, both human ROR1 and human ROR2).

[00227] 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 ROR binding characteristics. 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 ROR. 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.

[00228] The term "humanized antibody" or "humanized immunoglobulin" refers to a non-human (*e.g.*, mouse or rabbit) antibody containing one or more amino acids (in a framework region, a constant region or a CDR, for example) that have been substituted with a correspondingly positioned amino acid from a human antibody. In general, humanized antibodies produce a reduced immune response in a human host, as compared to a non-humanized version of the same antibody. Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332). In certain embodiments, framework substitutions are identified by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions (see, *e.g.*, U.S. Pat. No. 5,585,089; Riechmann et al., Nature 332:323 (1988)). Additional methods for humanizing antibodies contemplated for use in the present invention are described in U.S. Pat. Nos. 5,750,078; 5,502,167; 5,705,154; 5,770,403; 5,698,417; 5,693,493; 5,558,864; 4,935,496; and 4,816,567, and PCT publications WO 98/45331 and WO 98/45332. In particular embodiments, a subject rabbit antibody may be humanized according to the methods set forth in US20040086979 and US20050033031. Accordingly, the antibodies described above may be humanized using methods that are well known in the art.

[00229] The term "chimeric antibodies" refer to antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from antibody variable and constant region genes belonging to different species. For example, the variable segments of

the genes from a mouse monoclonal antibody may be joined to human constant segments, such as gamma 1 and gamma 3. An example of a therapeutic chimeric antibody is a hybrid protein composed of the variable or antigen-binding domain from a mouse antibody and the constant or effector domain from a human antibody, although domains from other mammalian species may be used.

[00230] The term "monospecific," as used herein denotes an antibody that has one or more binding sites each of which binds to the same epitope of the same antigen.

[00231] The term "multispecific" when used in reference to an antibody means that the antibody is able to specifically bind to at least two distinct epitopes, for example two binding sites each formed by a pair of an antibody heavy chain variable domain (VH) and an antibody light chain variable domain (VL) or each formed by a pair of VHH domains binding to different antigens or to different epitopes on the same antigen. Such a bispecific antibody may have a 1+1 format (comprising one binding site for a first antigen or epitope and one binding site for a second antigen or epitope). Other bispecific antibody formats may be 2+1 or 1+2 formats (comprising two binding sites for a first antigen or epitope and one binding site for a second antigen or epitope) or 2+2 format (comprising two binding sites for a first antigen or epitope and two binding sites for a second antigen or epitope). When a bispecific antibody comprises two antigen-binding sites, each may bind to a different epitope. Such a bispecific antibody may bind to two different epitopes on the same antigen (*e.g.*, epitopes on ROR).

[00232] 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.

[00233] 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.

[00234] 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.

[00235] 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 an ROR antigen (*e.g.*, a human ROR antigen), or a fragment thereof.

[00236] 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 ROR. 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.

[00237] 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.

[00238] As used herein, the terms "specifically binds," "specifically recognizes," "immunospecifically binds," "selectively binds," "immunospecifically recognizes" and "immunospecific" are analogous terms in the context of antibodies and refer to molecules that bind to an antigen (*e.g.*, epitope) as understood by one skilled in the art.

[00239] In some embodiments, "specifically binds" means, for instance, that a polypeptide or molecule interacts more frequently, more rapidly, with greater duration, with greater

affinity, or with some combination of the above to the epitope, protein, or target molecule than with alternative substances, including related and unrelated proteins. For example, a molecule that specifically binds to an antigen can bind to other peptides or polypeptides, generally with lower affinity as determined by, *e.g.*, immunoassays, BIACORE™, KinExA 3000 instrument (Sapidyne Instruments, Boise, ID), the OctetQK384 system (ForteBio, Menlo Park, CA), or other assays known in the art. In some embodiments, an antibody or antigen binding region binds to or specifically binds to an antigen when it binds to the antigen with higher affinity than to any cross-reactive antigen as determined using experimental techniques, such as radioimmunoassays (RIAs) and enzyme linked immunosorbent assays (ELISAs). Typically, a specific or selective reaction will be at least twice background signal or noise and can be more than ten times background. See, *e.g.*, Fundamental Immunology 332-36 (Paul ed., 2d ed. 1989) for a discussion regarding binding specificity. In some embodiments, the extent of binding of an antibody or antigen-binding region to a "non-target" protein is less than about 10% of the binding of the antibody or antigen-binding region to its target antigen, for example, as determined by fluorescence activated cell sorting (FACS) analysis or RIAs. In some embodiments, molecules that specifically bind to an antigen bind to the antigen with a K_A that is at least 2 logs, 2.5 logs, 3 logs, 4 logs or greater than the K_A when the molecules bind to another antigen. In some embodiments, molecules that specifically bind to an antigen do not cross react with other proteins. In another specific embodiment, molecules that specifically bind to an antigen do not cross react with other non-ROR antigens. In some embodiments, "specifically binds" means, for instance, that a polypeptide or molecule binds a protein or target with a K_D of about 0.1 mM or less, but more usually less than about 1 μM. In some embodiments, "specifically binds" means that a polypeptide or molecule binds a target with a K_D of at least about 0.1 μM or less, at least about 0.01 μM or less, or at least about 1 nM or less. Because of the sequence identity between homologous proteins in different species, specific binding can include a polypeptide or molecule that recognizes a protein or target in more than one species. Likewise, because of homology within certain regions of polypeptide sequences of different proteins, specific binding can include a polypeptide or molecule that recognizes more than one protein or target. It is understood that, in some embodiments, a polypeptide or molecule that specifically binds a first target may or may not specifically bind a second target. As such, "specific binding" does not necessarily require (although it can include) exclusive binding, *e.g.*, binding to a single target. Thus, in some embodiments, a polypeptide or molecule can specifically bind more than one target. In some embodiments, multiple targets can be bound

by the same antigen-binding site on the polypeptide or molecule. For example, in some embodiments, an antibody can comprise two identical antigen-binding sites, each of which specifically binds the same epitope on two or more proteins. In alternative embodiments, an antibody can be bispecific and comprise at least two antigen-binding sites with differing specificities. Generally, but not necessarily, reference to "binding" means "specific binding."

[00240] The term "binding affinity" generally refers to the strength of the sum of noncovalent interactions between a single binding site of a molecule (*e.g.,* antibody) and its binding partner (*e.g.,* an antigen such as ROR). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair (*e.g.,* antibody and antigen). The affinity of a binding molecule X for its binding partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity is known in the art, any of which can be used for purposes of the present disclosure. In one embodiment, the "K_D" or "K_D value" can be measured by biolayer interferometry (BLI) using, for example, the OctetQK384 system (ForteBio, Menlo Park, CA). Alternatively, the K_D may also be measured in a radiolabeled antigen binding assay (RIA), for example, performed with the Fab version of an antibody of interest and its antigen (Chen et al., (1999) J. Mol Biol 293:865-881) or using surface plasmon resonance (SPR) assays by BIACORE™, using, for example, a BIACORE™-2000 or a BIACORE™-3000 (BIAcore, Inc., Piscataway, NJ). An "on-rate" or "rate of association" or "association rate" or "k_{on}," as well as an "off-rate" or "rate of dissociation" or "dissociation rate" or "k_{off}," can also be determined with the same SPR or BLI techniques described herein using, for example, the OctetQK384 system (ForteBio, Menlo Park, CA) or a BIACORE™-2000 or a BIACORE™-3000 (BIACORE™, Inc., Piscataway, NJ), respectively.

[00241] The term "compete," when used in the context of an activatable ROR antibody, describes an antibody that, in the presence of another antibody, is at least partially inhibited from binding to an epitope or binding site due to binding of the other antibody. Competition can be determined by an assay in which the antibody under study prevents or inhibits the specific binding of a reference molecule (*e.g.*, a reference ligand, or reference antigen binding protein, such as a reference antibody) to a common antigen (*e.g.*, ROR). Numerous types of competitive binding assays can be used to determine if a test antibody competes with a

reference molecule for binding to ROR (*e.g.*, human ROR). Examples of assays that can be employed include solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (*see, e.g.*, Stahli et al., (1983) Methods in Enzymology 9:242-253); solid phase direct biotin-avidin EIA (*see, e.g.*, Kirkland et al., (1986) J. Immunol. 137:3614-3619 or Cheung et al., (1990) Virology 176:546-552); solid phase direct labeled assay, solid phase direct labeled sandwich assay (*see, e.g.*, Harlow and Lane, (1988) Antibodies, A Laboratory Manual, Cold Spring Harbor Press); solid phase direct label RIA using I-125 label (*see, e.g.*, Morel et al., (1988) Molec. Immunol. 25:7-15); and direct labeled RIA (Moldenhauer et al., (1990) Scand. J. Immunol. 32:77-82). Typically, such an assay involves the use of a purified antigen (*e.g.*, ROR, such as human ROR) bound to a solid surface or cells bearing either of an unlabeled test antigen binding protein (*e.g.*, test ROR antibody or ADC) or a labeled reference antigen binding protein (*e.g.*, reference ROR antibody or ADC). Competitive inhibition can be measured by determining the amount of label bound to the solid surface or cells in the presence of the test antigen binding protein. Usually, the test antigen binding protein is present in excess. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and/or antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference for antibodies steric hindrance to occur (*e.g.*, similar epitope or overlapping epitope). Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a common antigen by at least 20%, for example, at least 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, or 75%. In some embodiments, binding is inhibited by at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or more.

[00242] 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.

[00243] Antibody “effector functions” refer to those biological activities attributable to the Fc region (*e.g.,* a native sequence Fc region or amino acid sequence variant Fc region) of an antibody and which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody-

dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.,* B cell receptor); and B cell activation.

[00244] 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 = underline text): CPPCP**APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAK**GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG K (SEQ ID NO:43).

[00245] 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.

[00246] 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.

[00247] 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 (also referred to herein as "sFc")).

[00248] In some embodiments, a variant Fc region comprises an alanine (Ala, A) residue at position Leu234 (L234) according to the EU numbering system, an alanine (Ala, A) residue at position Leu235 (L235) according to the EU numbering system, and a lysine (Lys, K) residue at position Pro329 (P329) according to the EU numbering system (also referred to herein as "LALAPK" or "L234A/L235A/P329K").

[00249] An exemplary variant Fc region ("silent Fc") sequence is provided below (CH2 domain = bold text with amino acid changes underlined; CH3 domain = underline text): CPPCP**APEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALKAPIE KTISKAK**GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG K (SEQ ID NO:44).

[00250] Additionally or alternatively, a variant Fc region has a reduced potential immunogenicity. In further embodiments, a variant Fc region comprises a glutamic acid (Glu, E) residue at position Asp356 (D356) according to the EU numbering system, a glutamic acid (Glu, E) residue at position Glu357 (E357) according to the EU numbering system, and a methionine (Met, M) residue position Leu358 (L358) according to the EU numbering system (also referred to herein as "EEM" or "D356E/E357E/L358M").

[00251] 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.

[00252] 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. In one embodiment, a "chain" (*e.g.*, a heavy chain or a light chain) is itself a molecule (*e.g.*, a polypeptide). In another embodiment, a "chain" (*e.g.*, a heavy chain or a light chain) is part of a molecule (*e.g.*, a polypeptide), for example, is directly or indirectly conjugated to the remaining part of the molecule (such as polypeptide).

[00253] 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.

[00254] 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.

[00255] 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 ROR.

[00256] An 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, an activatable ROR 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.

[00257] In some embodiments, an antibody is a 4-chain antibody unit comprising two heavy (H) chain / light (L) chain pairs. In further embodiments, the amino acid sequences of the H chains are identical and the amino acid sequences of the L chains are identical. In other embodiments, the amino acid sequences of the H chains are different from each other.

Additionally or alternatively, the amino acid sequences of the L chains are different from each other. For example, an antibody comprises a first H / L chain pair and a second H / L chain pair, wherein the first H / L chain pair binds to an ROR antigen and the second H/ L chain pair binds to another ROR antigen or a non-ROR antigen. In some embodiments, an antibody is a 2-chain antibody unit comprising a VHH-VHH pair. In further embodiments, the amino acid sequences of the VHH are identical. In other embodiments, the amino acid sequences of the VHH are different from each other. For example, an antibody comprises a first VHH and a second VHH, wherein the first VHH binds to an ROR antigen and the second VHH binds to another ROR antigen or a non-ROR antigen. 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 such antibodies is a kappa or lambda light chain constant region, for example, a human kappa or lambda light chain constant region. In some embodiments, the H chain constant region of such antibodies comprises a gamma heavy chain constant region, for example, a human gamma heavy chain constant region. In some embodiments, such antibodies comprise IgG constant regions, for example, human IgG constant regions (*e.g.,* IgG1, IgG2, IgG3, and/or IgG4 constant regions).

[00258] As used herein, "ROR antibody" and "antibody that binds to ROR" are used interchangeably and refer to an antibody that preferentially binds to ROR. An antibody or fragment thereof can preferentially bind to ROR, such as human ROR, which means that the antibody or fragment thereof binds to ROR, such as human ROR, with greater affinity than it binds to an unrelated control protein. For example, the antibody or fragment thereof can specifically recognize and bind to ROR or a portion thereof. "Specific binding" means that the ROR antibody or fragment thereof binds to ROR 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 ROR antibody or fragment thereof can bind ROR substantially exclusively (*e.g.*, is able to distinguish ROR from other known polypeptides, for example, by virtue of measurable differences in binding affinity). In some embodiments, an ROR antibody can react with ROR sequences other than human ROR sequences (*e.g.,* cynomolgus ROR sequences).

[00259] 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.

[00260] The term "hypervariable region," "HVR," "HV," "complementarity determining region, "and "CDR" when used herein refers 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, H2 or VH CDR2, and H3 or VH CDR3), and three in the VL (L1 or VL CDR1, L2 or VL CDR2, and L3 or VL CDR3). 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.

[00261] 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 and 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

[00262] 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.

[00263] As used herein the term "isolated" is meant to describe a compound of interest that is in an environment different from that in which the compound naturally occurs. "Isolated" is meant to include compounds that are within samples that are substantially enriched for the compound of interest and/or in which the compound of interest is partially or substantially purified.

[00264] The terms "ROR-mediated disease," "ROR-mediated disorder," and "ROR-mediated condition" are used interchangeably and refer to any disease, disorder or condition associated with or characterized by ROR-expressing cells, such as ROR-expressing tumor cells. An ROR-mediated disease includes a cancer including, but not limited to, cancers that express or overexpress ROR.

[00265] 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.

[00266] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth.

[00267] The term "ADC" or "activatable-ADC" (the terms are used interchangeably) refers to an antibody-drug conjugate, which in the context of the present invention refers to an activatable ROR antibody, which is coupled to another moiety which includes a drug, as described herein.

[00268] As used herein, "drug" refers to a compound that has biological activity, such as a cytotoxic compound (*e.g.*, a cytotoxic small molecule, a cytotoxic synthetic peptide, and the like).

[00269] Examples of drugs include small molecule drugs, such as a cancer chemotherapeutic agent. For example, where the polypeptide is an antibody (or fragment thereof) that has specificity for a tumor cell, the antibody can be modified as described herein to include a modified amino acid, which can be subsequently conjugated to a cancer chemotherapeutic agent. Cancer chemotherapeutic agents include non-peptidic (*e.g.*, non-proteinaceous) compounds that reduce proliferation of cancer cells and encompass cytotoxic agents and cytostatic agents. Non-limiting examples of chemotherapeutic agents include alkylating

agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones. Peptidic compounds can also be used.

[00270] Suitable cancer chemotherapeutic agents include dolastatin and active analogs and derivatives thereof; and auristatin and active analogs and derivatives thereof (*e.g.*, Monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), and the like). See, *e.g.*, WO 96/33212, WO 96/14856, and U.S. 6,323,315. For example, dolastatin 10 or auristatin PE can be included in an activatable ROR-ADC of the present disclosure. Suitable cancer chemotherapeutic agents also include maytansinoids and active analogs and derivatives thereof (see, *e.g.*, EP 1391213; and Liu et al (1996) *Proc. Natl. Acad. Sci. USA* 93:8618-8623); duocarmycins and active analogs and derivatives thereof (*e.g.*, including the synthetic analogues, KW-2189 and CB 1-TM1); and benzodiazepines and active analogs and derivatives thereof (*e.g.*, pyrrolobenzodiazepine (PBD)).

[00271] Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (CYNOTAN™), melphalan (L-sarcolysin), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, uracil mustard, chlormethine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.

[00272] Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U®), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, 10-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatin, and gemcitabine.

[00273] Suitable natural products and their derivatives, (*e.g.*, vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (TAXOL®), docetaxel (TAXOTERE®), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, *e.g.* vincristine, vinblastine, vinorelbine, vindesine, and the like; podophyllotoxins, *e.g.* etoposide, teniposide, and the like; antibiotics, *e.g.* anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, and the like; phenoxizone biscyclopeptides, *e.g.* dactinomycin; basic glycopeptides, *e.g.* bleomycin; anthraquinone

glycosides, *e.g.* plicamycin (mithramycin); anthracenediones, *e.g.* mitoxantrone; azirinopyrrolo indolediones, *e.g.* mitomycin; macrocyclic immunosuppressants, *e.g.* cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, and the like; and the like.

[00274] Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrazole, letrazole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

[00275] Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (*e.g.*, NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (TAXOL®), TAXOL® derivatives, docetaxel (TAXOTERE®), thiocolchicine (NSC 361792), trityl cysterin, vinblastine sulfate, vincristine sulfate, natural and synthetic epothilones including but not limited to, eopthilone A, epothilone B, discodermolide; estramustine, nocodazole, and the like.

[00276] Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, *e.g.* prednisone, dexamethasone, and the like; estrogens and pregestins, *e.g.* hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; and the like; adrenocortical suppressants, *e.g.* aminoglutethimide; 17α-ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, flutamide (DROGENIL®), toremifene (FARESTON®), and goserelin (ZOLADEX®), and the like. Estrogens stimulate proliferation and differentiation; therefore, compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids can inhibit T cell proliferation.

[00277] Other suitable chemotherapeutic agents include metal complexes, *e.g.* cisplatin (cis-DDP), carboplatin, and the like; ureas, *e.g.* hydroxyurea; hydrazines, *e.g.* N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur; and the like Other anti-proliferative agents of interest include immunosuppressants, *e.g.* mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); gefitinib (IRESSA®, ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)quinazoline); and the like.

[00278] Taxanes are suitable for use. “Taxanes” include paclitaxel, as well as any active taxane derivative or pro-drug. “Paclitaxel” (which should be understood herein to include

analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL®, TAXOTERE® (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3'N-desbenzoyl-3'N-t-butoxycarbonyl analogs of paclitaxel) can be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076; U.S. Pat. Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; and EP 590,267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from *Taxus brevifolia*; or T-1912 from *Taxus yannanensis*). Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (*e.g.*, TAXOTERE® docetaxel, as noted herein) and paclitaxel conjugates (*e.g.*, paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylose).

[00279] Also included within the term "taxane" are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but are not limited to, galactose and mannose derivatives described in International Patent Application No. WO 99/18113; piperazino and other derivatives described in WO 99/14209; taxane derivatives described in WO 99/09021, WO 98/22451, and U.S. Patent No. 5,869,680; 6-thio derivatives described in WO 98/28288; sulfenamide derivatives described in U.S. Patent No. 5,821,263; and taxol derivative described in U.S. Patent No. 5,415,869. It further includes prodrugs of paclitaxel including, but not limited to, those described in WO 98/58927; WO 98/13059; and U.S. Patent No. 5,824,701.

[00280] Biological response modifiers suitable for use include, but are not limited to, (1) inhibitors of tyrosine kinase (RTK) activity; (2) inhibitors of serine/threonine kinase activity; (3) tumor-associated antigen antagonists, such as antibodies that bind specifically to a tumor antigen; (4) apoptosis receptor agonists; (5) interleukin-2; (6) IFN-α; (7) IFN-γ; (8) colony-stimulating factors; and (9) inhibitors of angiogenesis.

[00281] An "effective amount" is generally an amount sufficient to reduce the severity and/or frequency of symptoms, eliminate the symptoms and/or underlying cause, prevent the occurrence of symptoms and/or their underlying cause, and/or improve or remediate the damage that results from or is associated with a disease, disorder, or condition. In some embodiments, the effective amount is a therapeutically effective amount.

[00282] 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.,* an activatable ROR antibody or activatable 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.

[00283] In some embodiments, the drug is a microtubule affecting agent that has anti-proliferative activity, such as a maytansinoid. In some embodiments, the drug is an antimitotic agent, such as an auristatin or an active auristatin analog or derivative thereof. In some embodiments, the drug is a DNA alkylating agent.

[00284] 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.

[00285] "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 an activatable ROR-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.

[00286] 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.

[00287] As used herein, comparative terms as used herein, such as reduce, decrease, increase, or any grammatical variation thereof, can refer to certain variation from the reference. In some embodiments, such variation can refer to about 10%, or about 20%, or about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 1 fold, or about 2 fold, or about 3 fold, or about 4 fold, or about 5 fold, or about 10 fold, or about 20 fold, or about 30 fold, or about 40 fold, or about 100 fold or higher than the reference. In some embodiments, such variation can refer to about 1%, or about 2%,

or about 3%, or about 4%, or about 5%, or about 6%, or about 7%, or about 8%, or about 9%, or about 10%, or about 20%, or about 30%, or about 40%, or about 50%, or about 60%, or about 70%, or about 80%, or about 90%, or about 95%, or about 96%, or about 97%, or about 98%, or about 99% of the reference.

[00288] As used in the present disclosure and claims, the singular forms "a", "an" and "the" include plural forms unless the context clearly dictates otherwise.

[00289] 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.

[00290] 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.

[00291] 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.

[00292] 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).

[00293] 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.

[00294] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[00295] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller

ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[00296] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed, to the extent that such combinations embrace subject matter that are, for example, compounds that are stable compounds (*e.g.*, compounds that can be made, isolated, characterized, and tested for biological activity). In addition, all sub-combinations of the various embodiments and elements thereof (*e.g.*, elements of the chemical groups listed in the embodiments describing such variables) are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[00297] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[00298] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination.

[00299] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior

invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

7.2 ROR-ADCs

[00300] An antibody that binds to tyrosine-protein kinase membrane receptor (ROR) (also referred to herein as "ROR antibody," "anti-ROR antibody," "ROR Ab," "Ab," or "antibody"), such as an activatable ROR antibody (also referred to herein as "activatable ROR antibody," "activatable anti-ROR antibody," "activatable ROR Ab," "activatable Ab," or "activatable antibody"), and a drug can be linked directly or indirectly to each other via a pyridazine-pyrrolo coupling moiety to form an ROR-ADC, such as an activatable ROR-ADC as described herein. In certain embodiments, the ROR antibody, such as an activatable ROR antibody, and the two or more drugs or active agents are bound to each other through one or more functional groups and covalent bonds. For example, the one or more functional groups and covalent bonds can include a branched linker as described herein.

[00301] Moieties of interest (*e.g.*, drugs or active agents) can be conjugated to the ROR antibody, such as the activatable ROR antibody, at any desired site of the antibody. Thus, the present disclosure provides, for example, an ROR antibody, such as an activatable ROR antibody, that has moieties conjugated at two or more sites on the antibody, such as a site at or near the C-terminus of the antibody, a position at or near the N-terminus of the antibody, and a position between the C-terminus and the N-terminus of the antibody (*e.g.*, at an internal site of the antibody). Combinations of the above conjugation sites are also possible.

[00302] In certain embodiments, a conjugate of the present disclosure includes two (or more) drugs or active agents conjugated to an amino acid residue of an ROR antibody, such as an activatable ROR antibody, at the α-carbon of an amino acid residue. Stated another way, a conjugate includes an ROR antibody, such as an activatable ROR antibody, where the side chain of an amino acid residue in the antibody has been modified and attached to two (or more) drugs or active agents (*e.g.*, attached to two drugs or active agents through a branched linker as described herein). For example, a conjugate includes an ROR antibody, such as an activatable ROR antibody, where the α-carbon of an amino acid residue in the antibody has been modified and attached to two drugs or active agents (*e.g.*, attached to two drugs or active agents through a branched linker as described herein).

[00303] Embodiments of the present disclosure include conjugates where an ROR antibody, such as an activatable ROR antibody, is conjugated to two or more moieties, such as 3 moieties, 4 moieties, 5 moieties, 6 moieties, 7 moieties, 8 moieties, 9 moieties, 10 moieties, 11 moieties, 12 moieties, 13 moieties, 14 moieties, 15 moieties, 16 moieties, 17 moieties, 18

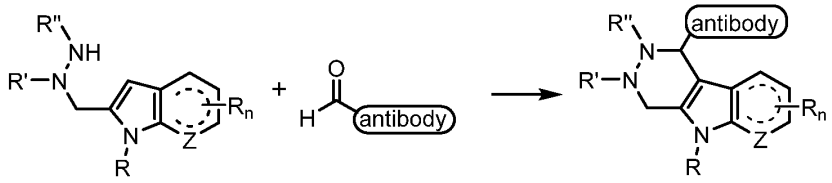
moieties, 19 moieties, or 20 or more moieties. The moieties may be conjugated to the ROR antibody, such as the activatable ROR antibody, at multiple sites in the antibody. In some embodiments, two moieties may be conjugated to a single amino acid residue of the activatable ROR antibody. For instance, two moieties may be conjugated to the same amino acid residue of the activatable ROR antibody. In other embodiments, two moieties are conjugated to a first amino acid residue of the activatable ROR antibody and two other moieties are conjugated to a second amino acid residue of the activatable ROR antibody. For example, an activatable ROR antibody can be conjugated to first and second moieties at a first amino acid residue and conjugated to third and fourth moieties at a second amino acid residue, etc. In some cases, two or more amino acid residues in the activatable ROR antibody are each conjugated to a pair of moieties (*e.g.*, two moieties), where each pair of moieties is conjugated to the activatable ROR antibody through a branched linker as described herein. In some cases, 1 amino acid residue in the activatable ROR antibody is conjugated to a pair of moieties through a branched linker as described herein. In other instances, 2 or more amino acid residues, such as 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid residues in the activatable ROR antibody are each conjugated to a pair of moieties through a branched linker as described herein.

[00304] The one or more amino acid residues of the ROR antibody, such as the activatable ROR antibody, that are conjugated to the moieties of interest may be naturally occurring amino acids, unnatural amino acids, or combinations thereof. For instance, the conjugate may include moieties of interest (*e.g.*, drugs or active agents) conjugated to a naturally occurring amino acid residue of the activatable ROR antibody. In other instances, the conjugate may include moieties of interest conjugated to an unnatural amino acid residue of the activatable ROR antibody. The moieties of interest may be conjugated to the activatable ROR antibody at a single natural or unnatural amino acid residue as described above. One or more natural or unnatural amino acid residues in the activatable ROR antibody may be conjugated to the moieties of interest as described herein. For example, two (or more) amino acid residues (*e.g.*, natural or unnatural amino acid residues) in the activatable ROR antibody may each be conjugated to two moieties through a branched linker, such that multiple sites in the activatable ROR antibody are conjugated to the moieties of interest.

[00305] As described herein, an ROR antibody, such as an activatable ROR antibody, may be conjugated to two or more moieties of interest. In certain embodiments, the moiety of interest is a payload, for instance, a chemical entity, such as a drug, an active agent, or a detectable label. For example, drugs (or active agents, such as cytokines) may be conjugated

to the activatable ROR antibody, or in other embodiments, detectable labels may be conjugated to the activatable ROR antibody. In other embodiments, combinations of different payloads may be conjugated to the activatable ROR antibody. Thus, for instance, embodiments of the present disclosure include, but are not limited to, the following: a conjugate of an activatable ROR antibody and two or more drugs; a conjugate of an activatable ROR antibody and two or more active agents, such as cytokines; a conjugate of an activatable ROR antibody and two or more detectable labels; and combinations thereof.

[00306] In certain embodiments, the ROR antibody, such as the activatable ROR antibody, and the moieties of interest (*e.g.*, drugs or active agents) are conjugated through a conjugation moiety. For example, the ROR antibody, such as the activatable ROR antibody, and the moieties of interest may each be bound (*e.g.*, covalently bonded) to the conjugation moiety, thus indirectly binding the antibody and the moieties of interest together through the conjugation moiety. In some cases, the conjugation moiety includes a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl compound, or a derivative of a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl compound. For instance, a general scheme for coupling moieties of interest to an ROR antibody, such as an activatable ROR antibody, through a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety is shown in the general reaction scheme below. Hydrazinyl-indolyl and hydrazinyl-pyrrolo-pyridinyl conjugation moieties are also referred to herein as a hydrazino-*iso*-Pictet-Spengler (HIPS) conjugation moiety and an aza-hydrazino-*iso*-Pictet-Spengler (azaHIPS) conjugation moiety, respectively.



[00307] In the reaction scheme above, each R independently includes a moiety of interest (*e.g.*, drug or active agent) that is conjugated to the ROR antibody, such as the activatable ROR antibody, (*e.g.*, conjugated to the activatable ROR antibody through a linker as described herein), where n is an integer from 1 to 4. As shown in the reaction scheme above, a conjugation moiety (*e.g.*, a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety) is attached to two or more drugs or active agents, R. An ROR antibody, such as an activatable ROR antibody, that includes a 2-formylglycine residue (fGly) is reacted with the conjugation moiety to produce an activatable ROR antibody conjugate, thus

attaching the two or more drugs or active agents to the antibody through the conjugation moiety.

[00308] As described herein, the moieties can be any of a variety of moieties such as, but not limited to, chemical entities, such as detectable labels, or drugs or active agents. R' and R" may 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, and substituted heterocyclyl. Z may be CR^{21}, NR^{22}, N, O or S, where R^{21} and R^{22} are each independently selected from any of the substituents described for R' and R" above.

[00309] Other hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moieties are also possible, as shown in the conjugates and compounds described herein. For example, the hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moieties may be attached (*e.g.*, covalently attached) to two or more linkers. As such, embodiments of the present disclosure include a hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety attached to two or more drugs or active agents each through a corresponding linker. Thus, conjugates of the present disclosure may include two or more linkers, where each linker attaches a corresponding drug or active agent to the hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety. Accordingly, the hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety and two or more linkers may be viewed overall as a "branched linker," where the hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety is attached to two of more "branches," where each branch includes a linker attached to a drug or active agent.

[00310] Combinations of the same or different payloads may be conjugated to the ROR antibody, such as the activatable ROR antibody, through the branched linker. In certain embodiments, the two payloads (*e.g.*, drugs, active agents, or detectable labels) attached to the branched linker are the same payload (*e.g.*, drug, active agent, or detectable label). For example, a first branch of a branched linker may be attached to a payload (*e.g.*, drug, active agent, or detectable label) and a second branch of the branched linker may be attached to the same payload (*e.g.*, drug, active agent, or detectable label) as the first branch.

[00311] In other embodiments, the two payloads (*e.g.*, drugs, active agents, or detectable labels) attached to the branched linker are different payloads (*e.g.*, drugs, active agents, or

detectable labels). For example, a first branch of a branched linker may be attached to a first payload (*e.g.*, a first drug, active agent, or detectable label) and a second branch of the branched linker may be attached to a second payload (*e.g.*, a second drug, active agent, or detectable label) different from the first payload (*e.g.*, the first drug, active agent, or detectable label) attached to the first branch.

[00312] In some embodiments, where two different drugs or active agents are attached to the branched linker, the drugs or active agents may be selected from drugs and active agents that have a synergistic therapeutic effect. By "synergistic," "synergism" or "synergy" is meant a therapeutic effect that is greater than the sum of the effects of the drugs or active agents taken separately. For example, in some instances, the use of two different drugs or active agents attached to the branched linker may provide a lower therapeutically effective concentration at which both payloads act, thereby increasing overall potency of the ADC.

[00313] In some embodiments, where two different drugs or active agents are attached to the branched linker, the drugs or active agents may be selected from drugs and active agents that provide an enhanced therapeutic benefit as compared to the use of the drugs or active agents separately, For example, the drugs or active agents may provide an increased effect on drug delivery of the ADC (*e.g.*, some payloads, such as the iRGD peptide, can increase extravasation into tissues and augment tumor penetration).

[00314] In some embodiments, where two different drugs or active agents are attached to the branched linker, the drugs or active agents may be selected from drugs and active agents that use different mechanisms of action. In some cases, this may provide a decrease in tumor drug resistance by targeting multiple pathways. Examples of payload combinations can include, but are not limited to, cytotoxic drugs, immunomodulatory molecules to activate or inhibit immune cell populations, cytokines, hormones, chelating agents loaded with radioisotopes, and the like.

[00315] In some embodiments, where two different payloads are attached to the branched linker, the payloads may be selected from combinations of drugs or active agents and detectable labels. For example, a first payload may be a detectable label that is used as an imaging agent or tracer to detect the location of the ADC *in vivo*, while a second payload may be a drug or active agent that provides a therapeutic activity.

[00316] Various embodiments of the linkers that may couple the hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety to the drugs or active agents are described in detail herein. For example, in some instances, the linker is a cleavable linker, such as a cleavable linker as described herein.

[00317] In certain embodiments, the ROR antibody, such as the activatable ROR antibody, may be conjugated to two or more moieties of interest, where one or more amino acids of the antibody are modified before conjugation to the moieties of interest. Modification of one or more amino acids of the antibody may produce an antibody that contains one or more reactive groups suitable for conjugation to the moieties of interest. In some cases, the ROR antibody, such as the activatable ROR antibody, may include one or more modified amino acid residues to provide one or more reactive groups suitable for conjugation to the moieties of interest (*e.g.*, where two or more moieties are attached to a conjugation moiety, such as a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety as described above). For example, an amino acid of the antibody may be modified to include a reactive aldehyde group (*e.g.*, a reactive aldehyde). A reactive aldehyde may be included in an "aldehyde tag" or "ald-tag", which, as used herein, refers to an amino acid sequence derived from a sulfatase motif (*e.g.*, L(C/S)TPSR, SEQ ID NO:99) 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 may also be referred to as a "formylglycine." Stated differently, the term "aldehyde tag" is used herein to refer to an amino acid sequence that includes a "converted" sulfatase motif (*e.g.*, a sulfatase motif in which a cysteine or serine residue has been converted to fGly by action of an FGE, *e.g.*, L(fGly)TPSR, SEQ ID NO:123). A converted sulfatase motif may be produced from an amino acid sequence that includes an "unconverted" sulfatase motif (*e.g.*, a sulfatase motif in which the cysteine or serine residue has not been converted to fGly by an FGE, but is capable of being converted, *e.g.*, an unconverted sulfatase motif with the sequence: LCTPSR, SEQ ID NO:100). By "conversion" as 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). Additional aspects of aldehyde tags and uses thereof in site-specific protein modification are described in U.S. Patent No. 7,985,783 and U.S. Patent No. 8,729,232, the disclosures of each of which are incorporated herein by reference.

[00318] In some cases, to produce the conjugate, the ROR antibody, such as the activatable ROR antibody containing the fGly residue may be conjugated to the moieties of interest by reaction of the fGly with a compound (*e.g.*, a compound containing a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety, as described above). For example, an fGly-containing ROR antibody may be contacted with a reactive partner under conditions suitable to provide for conjugation of two or more drugs to the ROR antibody. In some instances, the

reactive partner may include a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety as described above. For example, two or more drugs or active agents may be attached to a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety. In some cases, the drugs or active agents are attached to a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety, such as covalently attached to a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl, where each drug or active agent is attached through a corresponding linker to the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety. Accordingly, the fGly residue conjugated to the moieties of interest after the reaction is referred to herein as fGly'.

[00319] In certain embodiments, a conjugate of the present disclosure includes an ROR antibody, such as an activatable ROR antibody, having at least one amino acid residue that has been attached to two or more moieties of interest (*e.g.*, drugs or active agents). In order to make the conjugate, an amino acid residue of the ROR antibody, such as the activatable ROR antibody, may be modified and then coupled to two or more drugs or active agents attached to a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety as described above. In certain embodiments, an amino acid residue of the ROR antibody, such as the activatable ROR antibody, is a cysteine or serine residue that is modified to an fGly residue, as described above. In certain embodiments, the modified amino acid residue (*e.g.*, fGly residue) is conjugated to two or more drugs or active agents containing a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety as described above to provide a conjugate of the present disclosure where the two or more drugs or active agents are conjugated to the antibody through the hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety. As used herein, the term fGly' refers to the amino acid residue of the antibody that is coupled to the moieties of interest (*e.g.*, drugs or active agents).

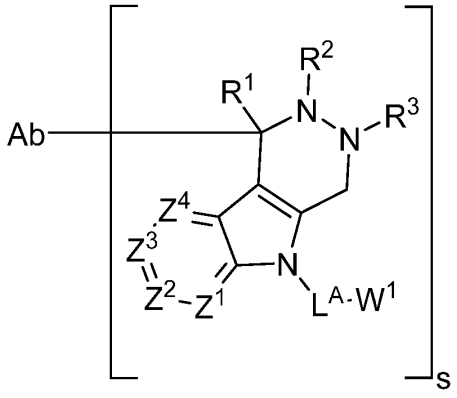
[00320] In certain embodiments, the conjugate includes an ROR antibody, such as an activatable ROR antibody, having at least one amino acid residue attached to a branched linker as described herein, which in turn is attached to two or more drugs or active agents. For instance, the conjugate may include an ROR antibody, such as an activatable ROR antibody, having at least one amino acid residue (fGly') that is conjugated to the moieties of interest (*e.g.*, drugs or active agents) as described above.

[00321] In some embodiments, an ROR-ADC (*e.g.*, an activatable ROR-ADC) comprises:

a. an antibody that binds to tyrosine-protein kinase membrane receptor (ROR) (for example, an activatable antibody that binds to ROR); and

b. two or more drugs conjugated to a pyridazine-pyrrolo coupling moiety, each via a linker.

[00322] In some embodiments, an ROR-ADC is represented by a conjugate of Formula (I):



(I)

wherein:

Ab represents an antibody that binds to ROR, such as an activatable antibody that binds to ROR;

Z^1, Z^2, Z^3 and Z^4 are each independently selected from CR^4, N and C-L^B-W^2, wherein at least one Z^1, Z^2, Z^3 and Z^4 is C-L^B-W^2;

R^1 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

R^2 and R^3 are each independently selected from 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, and substituted heterocyclyl, or R^2 and R^3 are optionally cyclically linked to form a 5 or 6-membered heterocyclyl;

each R^4 is independently selected from hydrogen, halogen, 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, and substituted heterocyclyl;

L^A is a first linker;

L^B is a second linker;

s is an integer from 1 to 10;

W^1 is a first drug; and

W^2 is a second drug.

[00323] The substituents related to conjugates of Formula (I) are described in more detail below.

[00324] In certain embodiments, Z^1, Z^2, Z^3 and Z^4 are each independently selected from CR^4, N and C-L^B-W^2, wherein at least one Z^1, Z^2, Z^3 and Z^4 is C-L^B-W^2. In certain embodiments, Z^1 is CR^4. In certain embodiments, Z^1 is N. In certain embodiments, Z^1 is C-L^B-W^2. In certain embodiments, Z^2 is CR^4. In certain embodiments, Z^2 is N. In certain embodiments, Z^2 is C-L^B-W^2. In certain embodiments, Z^3 is CR^4. In certain embodiments, Z^3 is N. In certain embodiments, Z^3 is C-L^B-W^2. In certain embodiments, Z^4 is CR^4. In certain embodiments, Z^4 is N. In certain embodiments, Z^4 is C-L^B-W^2. In some embodiments, each of Z^1, Z^3, and Z^4 is CR^4. In some embodiments, Z^3 is C-L^B-W^2.

[00325] Combinations of various Z^1, Z^2, Z^3 and Z^4 are possible. For example, in some instances, Z^1 is C-L^B-W^2, Z^2 is CR^4, Z^3 is CR^4, and Z^4 is CR^4. In some instances, Z^1 is CR^4, Z^2 is C-L^B-W^2, Z^3 is CR^4, and Z^4 is CR^4. In some instances, Z^1 is CR^4, Z^2 is CR^4, Z^3 is C-L^B-W^2, and Z^4 is CR^4. In some instances, Z^1 is CR^4, Z^2 is CR^4, Z^3 is CR^4, and Z^4 is C-L^B-W^2.

[00326] In certain embodiments, R^1 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, substituted heterocyclyl. In certain embodiments, R^1 is hydrogen. In certain embodiments, R^1 is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^1 is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^1 is alkynyl or substituted alkynyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^1 is aryl or substituted aryl, such as C_{5-8} aryl or C_{5-8} substituted aryl, such as a C_5 aryl or C_5 substituted aryl, or a C_6 aryl or C_6 substituted aryl. In certain embodiments, R^1 is heteroaryl or substituted heteroaryl, such as C_{5-8} heteroaryl or C_{5-8} substituted heteroaryl, such as a C_5 heteroaryl or C_5 substituted heteroaryl, or a C_6 heteroaryl or C_6 substituted heteroaryl. In certain embodiments, R^1 is cycloalkyl or substituted cycloalkyl, such as C_{3-8} cycloalkyl or C_{3-8} substituted cycloalkyl, such as a C_{3-6} cycloalkyl or C_{3-6} substituted cycloalkyl, or a C_{3-5}

cycloalkyl or C_{3-5} substituted cycloalkyl. In certain embodiments, R^1 is heterocyclyl or substituted heterocyclyl, such as C_{3-8} heterocyclyl or C_{3-8} substituted heterocyclyl, such as a C_{3-6} heterocyclyl or C_{3-6} substituted heterocyclyl, or a C_{3-5} heterocyclyl or C_{3-5} substituted heterocyclyl.

[00327] In certain embodiments, R^2 and R^3 are each independently selected from 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, and substituted heterocyclyl, or R^2 and R^3 are optionally cyclically linked to form a 5 or 6-membered heterocyclyl.

[00328] In certain embodiments, R^2 is selected from 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, and substituted heterocyclyl. In certain embodiments, R^2 is hydrogen. In certain embodiments, R^2 is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^2 is methyl. In certain embodiments, R^2 is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^2 is alkynyl or substituted alkynyl. In certain embodiments, R^2 is alkoxy or substituted alkoxy. In certain embodiments, R^2 is amino or substituted amino. In certain embodiments, R^2 is carboxyl or carboxyl ester. In certain embodiments, R^2 is acyl or acyloxy. In certain embodiments, R^2 is acyl amino or amino acyl. In certain embodiments, R^2 is alkylamide or substituted alkylamide. In certain embodiments, R^2 is sulfonyl. In certain embodiments, R^2 is thioalkoxy or substituted thioalkoxy. In certain embodiments, R^2 is aryl or substituted aryl, such as C_{5-8} aryl or C_{5-8} substituted aryl, such as a C_5 aryl or C_5 substituted aryl, or a C_6 aryl or C_6 substituted aryl. In certain embodiments, R^2 is heteroaryl or substituted heteroaryl, such as C_{5-8} heteroaryl or C_{5-8} substituted heteroaryl, such as a C_5 heteroaryl or C_5 substituted heteroaryl, or a C_6 heteroaryl or C_6 substituted heteroaryl. In certain embodiments, R^2 is cycloalkyl or substituted cycloalkyl, such as C_{3-8} cycloalkyl or C_{3-8} substituted cycloalkyl, such as a C_{3-6} cycloalkyl or C_{3-6} substituted cycloalkyl, or a C_{3-5} cycloalkyl or C_{3-5} substituted cycloalkyl. In certain

embodiments, R^2 is heterocyclyl or substituted heterocyclyl, such as a C_{3-6} heterocyclyl or C_{3-6} substituted heterocyclyl, or a C_{3-5} heterocyclyl or C_{3-5} substituted heterocyclyl.

[00329] In certain embodiments, R^3 is selected from 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, and substituted heterocyclyl. In certain embodiments, R^3 is hydrogen. In certain embodiments, R^3 is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^3 is methyl. In certain embodiments, R^3 is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^3 is alkynyl or substituted alkynyl. In certain embodiments, R^3 is alkoxy or substituted alkoxy. In certain embodiments, R^3 is amino or substituted amino. In certain embodiments, R^3 is carboxyl or carboxyl ester. In certain embodiments, R^3 is acyl or acyloxy. In certain embodiments, R^3 is acyl amino or amino acyl. In certain embodiments, R^3 is alkylamide or substituted alkylamide. In certain embodiments, R^3 is sulfonyl. In certain embodiments, R^3 is thioalkoxy or substituted thioalkoxy. In certain embodiments, R^3 is aryl or substituted aryl, such as C_{5-8} aryl or C_{5-8} substituted aryl, such as a C_5 aryl or C_5 substituted aryl, or a C_6 aryl or C_6 substituted aryl. In certain embodiments, R^3 is heteroaryl or substituted heteroaryl, such as C_{5-8} heteroaryl or C_{5-8} substituted heteroaryl, such as a C_5 heteroaryl or C_5 substituted heteroaryl, or a C_6 heteroaryl or C_6 substituted heteroaryl. In certain embodiments, R^3 is cycloalkyl or substituted cycloalkyl, such as C_{3-8} cycloalkyl or C_{3-8} substituted cycloalkyl, such as a C_{3-6} cycloalkyl or C_{3-6} substituted cycloalkyl, or a C_{3-5} cycloalkyl or C_{3-5} substituted cycloalkyl. In certain embodiments, R^3 is heterocyclyl or substituted heterocyclyl, such as C_{3-8} heterocyclyl or C_{3-8} substituted heterocyclyl, such as a C_{3-6} heterocyclyl or C_{3-6} substituted heterocyclyl, or a C_{3-5} heterocyclyl or C_{3-5} substituted heterocyclyl.

[00330] In certain embodiment, both R^2 and R^3 are methyl.

[00331] In certain embodiments, R^2 and R^3 are optionally cyclically linked to form a 5 or 6-membered heterocyclyl. In certain embodiments, R^2 and R^3 are cyclically linked to form a 5 or 6-membered heterocyclyl. In certain embodiments, R^2 and R^3 are cyclically linked to form a 5-membered heterocyclyl. In certain embodiments, R^2 and R^3 are cyclically linked to form a 6-membered heterocyclyl.

[00332] In certain embodiments, each R^4 is independently selected from hydrogen, halogen, 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, and substituted heterocyclyl.

[00333] The various possibilities for each R^4 are described in more detail as follows. In certain embodiments, R^4 is hydrogen. In certain embodiments, each R^4 is hydrogen. In certain embodiments, R^4 is halogen, such as F, Cl, Br, or I. In certain embodiments, R^4 is F. In certain embodiments, R^4 is Cl. In certain embodiments, R^4 is Br. In certain embodiments, R^4 is I. In certain embodiments, R^4 is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^4 is methyl. In certain embodiments, R^4 is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^4 is alkynyl or substituted alkynyl. In certain embodiments, R^4 is alkoxy or substituted alkoxy. In certain embodiments, R^4 is amino or substituted amino. In certain embodiments, R^4 is carboxyl or carboxyl ester. In certain embodiments, R^4 is acyl or acyloxy. In certain embodiments, R^4 is acyl amino or amino acyl. In certain embodiments, R^4 is alkylamide or substituted alkylamide. In certain embodiments, R^4 is sulfonyl. In certain embodiments, R^4 is thioalkoxy or substituted thioalkoxy. In certain embodiments, R^4 is aryl or substituted aryl, such as C_{5-8} aryl or C_{5-8} substituted aryl, such as a C_5 aryl or C_5 substituted aryl, or a C_6 aryl or C_6 substituted aryl (*e.g.*, phenyl or substituted phenyl). In certain embodiments, R^4 is heteroaryl or substituted heteroaryl, such as C_{5-8} heteroaryl or C_{5-8} substituted heteroaryl, such as a C_5 heteroaryl or C_5 substituted heteroaryl, or a C_6 heteroaryl or C_6 substituted heteroaryl. In certain embodiments, R^4 is cycloalkyl or substituted cycloalkyl, such as C_{3-8} cycloalkyl or C_{3-8} substituted cycloalkyl, such as a C_{3-6} cycloalkyl or C_{3-6} substituted cycloalkyl, or a C_{3-5} cycloalkyl or C_{3-5} substituted cycloalkyl. In certain embodiments, R^4 is heterocyclyl or substituted heterocyclyl, such as C_{3-8} heterocyclyl or C_{3-8} substituted heterocyclyl, such as a C_{3-6} heterocyclyl or C_{3-6} substituted heterocyclyl, or a C_{3-5} heterocyclyl or C_{3-5} substituted heterocyclyl.

[00334] In certain embodiments, L^A is a first linker. Examples of linkers that can be used in the conjugates of the present disclosure are described in more detail below.

[00335] In certain embodiments, L^B is a second linker. Examples of linkers that can be used in the conjugates of the present disclosure are described in more detail below.

[00336] In certain embodiments, W^1 is a first drug (or a first active agent). Examples of drugs and active agents that can be used in the conjugates of the present disclosure are described in more detail below.

[00337] In certain embodiments, W^2 is a second drug (or a second active agent). Examples of drugs and active agents that can be used in the conjugates of the present disclosure are described in more detail below.

[00338] In certain embodiments, Ab represents an antibody that binds to ROR ("ROR antibody"). In certain embodiments, Ab represents an activatable antibody that binds to ROR ("activatable ROR antibody"). In certain embodiments, Ab comprises one or more fGly' residues as described herein. In certain embodiments, the ROR antibody is attached to the rest of the conjugate through an fGly' residue as described herein. In certain embodiments, the activatable ROR antibody is attached to the rest of the conjugate through an fGly' residue as described herein. Examples of ROR antibodies that can be used in the conjugates of the present disclosure are described in more detail below.

[00339] In certain embodiments, the conjugate of Formula (I) includes a first linker, L^A. The first linker, L^A, may be utilized to bind a first moiety of interest (*e.g.*, a first drug or active agent) to an ROR antibody, such as an activatable ROR antibody, through a conjugation moiety. The first linker, L^A, may be bound (*e.g.*, covalently bonded) to the conjugation moiety (*e.g.*, as described herein). For example, the first linker, L^A, may attach a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety to a first drug. The hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety may be used to conjugate the first linker, L^A, (and thus the first drug) to an ROR antibody, such as an activatable ROR antibody.

[00340] For example, as shown in Formula (I) above, L^A is attached to Ab through a conjugation moiety, and thus Ab is indirectly bonded to the linker L^A through the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety. As described above, Ab is an ROR antibody, such as an activatable ROR antibody, and thus L^A is attached through the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety to the ROR antibody, such as the activatable ROR antibody, *e.g.*, the linker L^A is indirectly bonded to the ROR antibody, such as the activatable ROR antibody, through the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety.

[00341] Any convenient linker may be utilized for the first linker L^A in the subject conjugates and compounds. In certain embodiments, the first linker L^A may include a group selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl amino, alkylamide, substituted alkylamide, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, the first linker L^A may include an alkyl or substituted alkyl group. In certain embodiments, the first linker L^A may include an alkenyl or substituted alkenyl group. In certain embodiments, the first linker L^A may include an alkynyl or substituted alkynyl group. In certain embodiments, the first linker L^A may include an alkoxy or substituted alkoxy group. In certain embodiments, the first linker L^A may include an amino or substituted amino group. In certain embodiments, the first linker L^A may include a carboxyl or carboxyl ester group. In certain embodiments, the first linker L^A may include an acyl amino group. In certain embodiments, the first linker L^A may include an alkylamide or substituted alkylamide group. In certain embodiments, the first linker L^A may include an aryl or substituted aryl group. In certain embodiments, the first linker L^A may include a heteroaryl or substituted heteroaryl group. In certain embodiments, the first linker L^A may include a cycloalkyl or substituted cycloalkyl group. In certain embodiments, the first linker L^A may include a heterocyclyl or substituted heterocyclyl group.

[00342] In certain embodiments, the first linker L^A may include a polymer. For example, the polymer may include a polyalkylene glycol and derivatives thereof, including polyethylene glycol, methoxypolyethylene glycol, polyethylene glycol homopolymers, polypropylene glycol homopolymers, copolymers of ethylene glycol with propylene glycol (*e.g.*, where the homopolymers and copolymers are unsubstituted or substituted at one end with an alkyl group), polyvinyl alcohol, polyvinyl ethyl ethers, polyvinylpyrrolidone, combinations thereof, and the like. In certain embodiments, the polymer is a polyalkylene glycol. In certain embodiments, the polymer is a polyethylene glycol. Other linkers are also possible, as shown in the conjugates and compounds described in more detail below.

[00343] In some embodiments, L^A is a first linker described by the formula:

$$-(L^1)_a-(L^2)_b-(L^3)_c-(L^4)_d-(L^5)_e-(L^6)_f-,$$

wherein L^1, L^2, L^3, L^4, L^5 and L^6 are each independently a linker subunit, and a, b, c, d, e, and f are each independently 0 or 1, provided that at least one of a, b, c, d, e, and f is 1.

[00344] In certain embodiments, the sum of a, b, c, d, e, and f is 1 to 6. In certain embodiments, the sum of a, b, c, d, e, and f is 1. In certain embodiments, the sum of a, b, c, d,

e, and f is 2. In certain embodiments, the sum of a, b, c, d, e, and f is 3. In certain embodiments, the sum of a, b, c, d, e, and f is 4. In certain embodiments, the sum of a, b, c, d, e, and f is 5. In certain embodiments, the sum of a, b, c, d, e, and f is 6. In certain embodiments, a, b, c, d, e, and f are each 1. In certain embodiments, a, b, c, d, and e are each 1 and f is 0. In certain embodiments, a, b, c, and d are each 1 and e and f are each 0. In certain embodiments, a, b, and c are each 1 and d, e and f are each 0. In certain embodiments, a and b are each 1 and c, d, e, and f are each 0. In certain embodiments, a is 1 and b, c, d, e, and f are each 0.

[00345] In certain embodiments, the linker subunit L^1 is attached to the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety (*e.g.*, as shown in Formula (I) above). In certain embodiments, the linker subunit L^2, if present, is attached to the first drug or active agent W^1. In certain embodiments, the linker subunit L^3, if present, is attached to the first drug or active agent W^1. In certain embodiments, the linker subunit L^4, if present, is attached to the first drug or active agent W^1. In certain embodiments, the linker subunit L^5, if present, is attached to the first drug or active agent W^1. In certain embodiments, the linker subunit L^6, if present, is attached to the first drug or active agent W^1.

[00346] Any convenient linker subunits may be utilized in the first linker L^A. Linker subunits of interest include, but are not limited to, units of polymers such as polyethylene glycols, polyethylenes and polyacrylates, amino acid residue(s), carbohydrate-based polymers or carbohydrate residues and derivatives thereof, polynucleotides, alkyl groups, aryl groups, heterocyclic groups, combinations thereof, and substituted versions thereof. In some embodiments, each of L^1, L^2, L^3, L^4, L^5 and L^6 (if present) comprise one or more groups independently selected from a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, and a diamine (*e.g.*, a linking group that includes an alkylene diamine).

[00347] In some embodiments, L^1 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^1 comprises a polyethylene glycol. In some embodiments, L^1 comprises a modified polyethylene glycol. In some embodiments, L^1 comprises an amino acid residue. In some embodiments, L^1 comprises an alkyl group or a substituted alkyl. In some embodiments, L^1 comprises an aryl group or a substituted aryl group. In some embodiments, L^1 comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00348] In some embodiments, L^2 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^2 comprises a polyethylene glycol. In some embodiments, L^2 comprises a modified polyethylene glycol. In some embodiments, L^2 comprises an amino acid residue. In some embodiments, L^2 comprises an alkyl group or a substituted alkyl. In some embodiments, L^2 comprises an aryl group or a substituted aryl group. In some embodiments, L^2 comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00349] In some embodiments, L^3 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^3 comprises a polyethylene glycol. In some embodiments, L^3 comprises a modified polyethylene glycol. In some embodiments, L^3 comprises an amino acid residue. In some embodiments, L^3 comprises an alkyl group or a substituted alkyl. In some embodiments, L^3 comprises an aryl group or a substituted aryl group. In some embodiments, L^3 comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00350] In some embodiments, L^4 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^4 comprises a polyethylene glycol. In some embodiments, L^4 comprises a modified polyethylene glycol. In some embodiments, L^4 comprises an amino acid residue. In some embodiments, L^4 comprises an alkyl group or a substituted alkyl. In some embodiments, L^4 comprises an aryl group or a substituted aryl group. In some embodiments, L^4 comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00351] In some embodiments, L^5 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^5 comprises a polyethylene glycol. In some embodiments, L^5 comprises a modified polyethylene glycol. In some embodiments, L^5 comprises an amino acid residue. In some embodiments, L^5 comprises an alkyl group or a substituted alkyl. In some embodiments, L^5 comprises an aryl group or a substituted aryl group. In some embodiments, L^5 comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00352] In some embodiments, L^6 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group,

a substituted aryl group, or a diamine. In some embodiments, L^6 comprises a polyethylene glycol. In some embodiments, L^6 comprises a modified polyethylene glycol. In some embodiments, L^6 comprises an amino acid residue. In some embodiments, L^6 comprises an alkyl group or a substituted alkyl. In some embodiments, L^6 comprises an aryl group or a substituted aryl group. In some embodiments, L^6 comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00353] In some embodiments, L^A is a first linker comprising:

$$-(L^1)_a-(L^2)_b-(L^3)_c-(L^4)_d-(L^5)_e-(L^6)_f-,$$

wherein:

$-(L^1)_a-$ is $-(T^1-V^1)_a-$;

$-(L^2)_b-$ is $-(T^2-V^2)_b-$;

$-(L^3)_c-$ is $-(T^3-V^3)_c-$;

$-(L^4)_d-$ is $-(T^4-V^4)_d-$;

$-(L^5)_e-$ is $-(T^5-V^5)_e-$; and

$-(L^6)_f-$ is $-(T^6-V^6)_f-$,

wherein:

T^1, T^2, T^3, T^4, T^5 and T^6, if present, are tether groups;

V^1, V^2, V^3, V^4, V^5, and V^6, if present, are covalent bonds or linking functional groups; and

a, b, c, d, e, and f are each independently 0 or 1, provided that at least one of a, b, c, d, e, and f is 1.

[00354] In certain embodiments, the sum of a, b, c, d, e, and f is 1 to 6. In certain embodiments, the sum of a, b, c, d, e, and f is 1. In certain embodiments, the sum of a, b, c, d, e, and f is 2. In certain embodiments, the sum of a, b, c, d, e, and f is 3. In certain embodiments, the sum of a, b, c, d, e, and f is 4. In certain embodiments, the sum of a, b, c, d, e, and f is 5. In certain embodiments, the sum of a, b, c, d, e, and f is 6. In certain embodiments, a, b, c, d, e, and f are each 1. In certain embodiments, a, b, c, d, and e are each 1 and f is 0. In certain embodiments, a, b, c, and d are each 1 and e and f are each 0. In certain embodiments, a, b, and c are each 1 and d, e and f are each 0. In certain embodiments, a and b are each 1 and c, d, e, and f are each 0. In certain embodiments, a is 1 and b, c, d, e, and f are each 0.

[00355] As described above, in certain embodiments, L^1 is attached to the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety (*e.g.*, as shown in Formula (I) above). As such, in certain embodiments, T^1 is attached to the hydrazinyl-indolyl or a hydrazinyl-

pyrrolo-pyridinyl conjugation moiety (*e.g.*, as shown in Formula (I) above). In certain embodiments, V^1 is attached to the first drug or active agent. In certain embodiments, L^2, if present, is attached to the first drug or active agent. As such, in certain embodiments, T^2, if present, is attached to the first drug or active agent, or V^2, if present, is attached to the first drug or active agent. In certain embodiments, L^3, if present, is attached to the first drug or active agent. As such, in certain embodiments, T^3, if present, is attached to the first drug or active agent, or V^3, if present, is attached to the first drug or active agent. In certain embodiments, L^4, if present, is attached to the first drug or active agent. As such, in certain embodiments, T^4, if present, is attached to the first drug or active agent, or V^4, if present, is attached to the first drug or active agent. In certain embodiments, L^5, if present, is attached to the first drug or active agent. As such, in certain embodiments, T^5, if present, is attached to the first drug or active agent, or V^5, if present, is attached to the first drug or active agent. In certain embodiments, L^6, if present, is attached to the first drug or active agent. As such, in certain embodiments, T^6, if present, is attached to the first drug or active agent, or V^6, if present, is attached to the first drug or active agent.

[00356] In certain embodiments, the conjugate of Formula (I) includes a second linker, L^B. The second linker, L^B, may be utilized to bind a second moiety of interest (*e.g.*, a second drug or active agent) to an ROR antibody, such as an activatable ROR antibody, through a conjugation moiety. The second linker, L^B, may be bound (*e.g.*, covalently bonded) to the conjugation moiety (*e.g.*, as described herein). For example, the second linker, L^B, may attach a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety to a second drug. The hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety may be used to conjugate the second linker, L^B, (and thus the second drug) to an ROR antibody, such as an activatable ROR antibody.

[00357] For example, as shown in Formula (I) above, L^B is attached to Ab through a conjugation moiety, and thus Ab is indirectly bonded to the second linker L^B through the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety. As described above, Ab is an ROR antibody, such as an activatable ROR antibody, and thus L^B is attached through the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety to the ROR antibody, such as the activatable ROR antibody, *e.g.*, the linker L^B is indirectly bonded to the ROR antibody, such as the activatable ROR antibody, through the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety.

[00358] Any convenient linker may be utilized for the second linker L^B in the subject conjugates and compounds. In certain embodiments, the second linker L^B may include a

group selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl amino, alkylamide, substituted alkylamide, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, the second linker L^B may include an alkyl or substituted alkyl group. In certain embodiments, the second linker L^B may include an alkenyl or substituted alkenyl group. In certain embodiments, the second linker L^B may include an alkynyl or substituted alkynyl group. In certain embodiments, the second linker L^B may include an alkoxy or substituted alkoxy group. In certain embodiments, the second linker L^B may include an amino or substituted amino group. In certain embodiments, the second linker L^B may include a carboxyl or carboxyl ester group. In certain embodiments, the second linker L^B may include an acyl amino group. In certain embodiments, the second linker L^B may include an alkylamide or substituted alkylamide group. In certain embodiments, the second linker L^B may include an aryl or substituted aryl group. In certain embodiments, the second linker L^B may include a heteroaryl or substituted heteroaryl group. In certain embodiments, the second linker L^B may include a cycloalkyl or substituted cycloalkyl group. In certain embodiments, the second linker L^B may include a heterocyclyl or substituted heterocyclyl group.

[00359] In certain embodiments, the second linker L^B may include a polymer. For example, the polymer may include a polyalkylene glycol and derivatives thereof, including polyethylene glycol, methoxypolyethylene glycol, polyethylene glycol homopolymers, polypropylene glycol homopolymers, copolymers of ethylene glycol with propylene glycol (*e.g.*, where the homopolymers and copolymers are unsubstituted or substituted at one end with an alkyl group), polyvinyl alcohol, polyvinyl ethyl ethers, polyvinylpyrrolidone, combinations thereof, and the like. In certain embodiments, the polymer is a polyalkylene glycol. In certain embodiments, the polymer is a polyethylene glycol. Other linkers are also possible, as shown in the conjugates and compounds described in more detail below.

[00360] In some embodiments, L^B is a second linker described by the formula:

$$-(L^7)_g-(L^8)_h-(L^9)_i-(L^{10})_j-(L^{11})_k-(L^{12})_l-(L^{13})_m,$$

wherein L^7, L^8, L^9, L^{10}, L^{11}, L^{12} and L^{13} are each independently a linker subunit, and g, h, i, j, k, l, and m are each independently 0 or 1, provided at least one of g, h, i, j, k, l, and m is 1.

[00361] In certain embodiments, the sum of g, h, i, j, k, l, and m is 1 to 7. In certain embodiments, the sum of g, h, i, j, k, l, and m is 1. In certain embodiments, the sum of g, h, i, j, k, l, and m is 2. In certain embodiments, the sum of g, h, i, j, k, l, and m is 3. In certain embodiments, the sum of g, h, i, j, k, l, and m is 4. In certain embodiments, the sum of g, h, i,

j, k, l, and m is 5. In certain embodiments, the sum of g, h, i, j, k, l, and m is 6. In certain embodiments, the sum of g, h, i, j, k, l, and m is 7. In certain embodiments, g, h, i, j, k, l, and m are each 1. In certain embodiments, g, h, i, j, k and l are each 1 and m is 0. In certain embodiments, g, h, i, j and k are each 1 and l and m are each 0. In certain embodiments, g, h, i and j are each 1 and k, l, and m are each 0. In certain embodiments, g, h, and i are each 1 and j, k, l, and m are each 0. In certain embodiments, g and h are each 1 and i, j, k, l, and m are each 0. In certain embodiments, g is 1 and h, i, j, k, l, and m are each 0. In certain embodiments, g, h, i, j, k, l, and m are each 0.

[00362] In certain embodiments, the linker subunit L^7 is attached to the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety (*e.g.*, as shown in Formula (I) above). In certain embodiments, the linker subunit L^8, if present, is attached to the second drug or active agent W^2. In certain embodiments, the linker subunit L^9, if present, is attached to the second drug or active agent W^2. In certain embodiments, the linker subunit L^{10}, if present, is attached to the second drug or active agent W^2. In certain embodiments, the linker subunit L^{11}, if present, is attached to the second drug or active agent W^2. In certain embodiments, the linker subunit L^{12}, if present, is attached to the second drug or active agent W^2. In certain embodiments, the linker subunit L^{13}, if present, is attached to the second drug or active agent W^2.

[00363] Any convenient linker subunits may be utilized in the second linker L^B. Linker subunits of interest include, but are not limited to, units of polymers such as polyethylene glycols, polyethylenes and polyacrylates, amino acid residue(s), carbohydrate-based polymers or carbohydrate residues and derivatives thereof, polynucleotides, alkyl groups, aryl groups, heterocyclic groups, combinations thereof, and substituted versions thereof. In some embodiments, each of L^7, L^8, L^9, L^{10}, L^{11}, L^{12} and L^{13} (if present) comprise one or more groups independently selected from a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, and a diamine (*e.g.*, a linking group that includes an alkylene diamine).

[00364] In some embodiments, L^7 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^7 comprises a polyethylene glycol. In some embodiments, L^7 comprises a modified polyethylene glycol. In some embodiments, L^7 comprises an amino acid residue. In some embodiments, L^7 comprises an alkyl group or a substituted alkyl. In some embodiments, L^7 comprises an aryl group or a

substituted aryl group. In some embodiments, L^7 comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00365] In some embodiments, L^8 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^8 comprises a polyethylene glycol. In some embodiments, L^8 comprises a modified polyethylene glycol. In some embodiments, L^8 comprises an amino acid residue. In some embodiments, L^8 comprises an alkyl group or a substituted alkyl. In some embodiments, L^8 comprises an aryl group or a substituted aryl group. In some embodiments, L^8 comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00366] In some embodiments, L^9 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^9 comprises a polyethylene glycol. In some embodiments, L^9 comprises a modified polyethylene glycol. In some embodiments, L^9 comprises an amino acid residue. In some embodiments, L^9 comprises an alkyl group or a substituted alkyl. In some embodiments, L^9 comprises an aryl group or a substituted aryl group. In some embodiments, L^9 comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00367] In some embodiments, L^{10} (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^{10} comprises a polyethylene glycol. In some embodiments, L^{10} comprises a modified polyethylene glycol. In some embodiments, L^{10} comprises an amino acid residue. In some embodiments, L^{10} comprises an alkyl group or a substituted alkyl. In some embodiments, L^{10} comprises an aryl group or a substituted aryl group. In some embodiments, L^{10} comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00368] In some embodiments, L^{11} (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^{11} comprises a polyethylene glycol. In some embodiments, L^{11} comprises a modified polyethylene glycol. In some embodiments, L^{11} comprises an amino acid residue. In some embodiments, L^{11} comprises an alkyl group or a substituted alkyl. In some embodiments, L^{11} comprises an aryl group or a substituted aryl group. In some embodiments, L^{11} comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00369] In some embodiments, L^{12} (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^{12} comprises a polyethylene glycol. In some embodiments, L^{12} comprises a modified polyethylene glycol. In some embodiments, L^{12} comprises an amino acid residue. In some embodiments, L^{12} comprises an alkyl group or a substituted alkyl. In some embodiments, L^{12} comprises an aryl group or a substituted aryl group. In some embodiments, L^{12} comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00370] In some embodiments, L^{13} (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^{13} comprises a polyethylene glycol. In some embodiments, L^{13} comprises a modified polyethylene glycol. In some embodiments, L^{13} comprises an amino acid residue. In some embodiments, L^{13} comprises an alkyl group or a substituted alkyl. In some embodiments, L^{13} comprises an aryl group or a substituted aryl group. In some embodiments, L^{13} comprises a diamine (*e.g.*, a linking group comprising an alkylene diamine).

[00371] In some embodiments, L^B is a second linker comprising:

$$-(L^7)_g-(L^8)_h-(L^9)_i-(L^{10})_j-(L^{11})_k-(L^{12})_l-(L^{13})_m-,$$

wherein:

$-(L^7)_g-$ is $-(T^7-V^7)_g-$;

$-(L^8)_h-$ is $-(T^8-V^8)_h-$;

$-(L^9)_i-$ is $-(T^9-V^9)_i-$;

$-(L^{10})_j-$ is $-(T^{10}-V^{10})_j-$;

$-(L^{11})_k-$ is $-(T^{11}-V^{11})_k-$;

$-(L^{12})_l-$ is $-(T^{12}-V^{12})_l-$; and

$-(L^{13})_m-$ is $-(T^{13}-V^{13})_m-$,

wherein:

T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} if present, are tether groups;

V^7, V^8, V^9, V^{10}, V^{11}, V^{12}, and V^{13}, if present, are covalent bonds or linking functional groups; and

g, h, i, j, k, l, and m are each independently 0 or 1, provided at least one of g, h, i, j, k, l, and m is 1.

[00372] In certain embodiments, the sum of g, h, i, j, k, l, and m is 1 to 7. In certain embodiments, the sum of g, h, i, j, k, l, and m is 1. In certain embodiments, the sum of g, h, i,

j, k, l, and m is 2. In certain embodiments, the sum of g, h, i, j, k, l, and m is 3. In certain embodiments, the sum of g, h, i, j, k, l, and m is 4. In certain embodiments, the sum of g, h, i, j, k, l, and m is 5. In certain embodiments, the sum of g, h, i, j, k, l, and m is 6. In certain embodiments, the sum of g, h, i, j, k, l, and m is 7. In certain embodiments, g, h, i, j, k, l, and m are each 1. In certain embodiments, g, h, i, j, k, and l are each 1 and m is 0. In certain embodiments, g, h, i, j and k are each 1 and l, and m are each 0. In certain embodiments, g, h, i and j are each 1 and k, l, and m are each 0. In certain embodiments, g, h, and i are each 1 and j, k, l, and m are each 0. In certain embodiments, g and h are each 1 and i, j, k, l, and m are each 0. In certain embodiments, g is 1 and h, i, j, k, l, and m are each 0. In certain embodiments, g, h, i, j, k, l, and m are each 0.

[00373] As described above, in certain embodiments, L^7 is attached to the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety (*e.g.*, as shown in Formula (I) above). As such, in certain embodiments, T^7 is attached to the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety (*e.g.*, as shown in Formula (I) above). In certain embodiments, V^7 is attached to the second drug or active agent. In certain embodiments, L^8, if present, is attached to the second drug or active agent. As such, in certain embodiments, T^8, if present, is attached to the second drug or active agent, or V^8, if present, is attached to the second drug or active agent. In certain embodiments, L^9, if present, is attached to the second drug or active agent. As such, in certain embodiments, T^9, if present, is attached to the second drug or active agent, or V^9, if present, is attached to the second drug or active agent. In certain embodiments, L^{10}, if present, is attached to the second drug or active agent. As such, in certain embodiments, T^{10}, if present, is attached to the second drug or active agent, or $V10^4$, if present, is attached to the second drug or active agent. In certain embodiments, L^{11}, if present, is attached to the second drug or active agent. As such, in certain embodiments, T^{11}, if present, is attached to the second drug or active agent, or V^{11}, if present, is attached to the second drug or active agent. In certain embodiments, L^{12}, if present, is attached to the second drug or active agent. As such, in certain embodiments, T^{12}, if present, is attached to the second drug or active agent, or V^{12}, if present, is attached to the second drug or active agent. In certain embodiments, L^{13}, if present, is attached to the second drug or active agent. As such, in certain embodiments, T^{13}, if present, is attached to the second drug or active agent, or V^{13}, if present, is attached to the second drug or active agent.

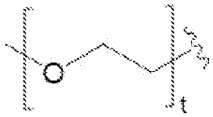
[00374] Regarding the tether groups, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13}, any convenient tether groups may be utilized in the subject linkers. In some embodiments, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} each comprise one or more groups

independently selected from a covalent bond, a (C_1-C_{12})alkyl, a substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x-$, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, where each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12.

[00375] In certain embodiments, the tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes a (C_1-C_{12})alkyl or a substituted (C_1-C_{12})alkyl. In certain embodiments, (C_1-C_{12})alkyl is a straight chain or branched alkyl group that includes from 1 to 12 carbon atoms, such as 1 to 10 carbon atoms, or 1 to 8 carbon atoms, or 1 to 6 carbon atoms, or 1 to 5 carbon atoms, or 1 to 4 carbon atoms, or 1 to 3 carbon atoms. In some instances, (C_1-C_{12})alkyl may be an alkyl or substituted alkyl, such as C_1-C_{12} alkyl, or C_1-C_{10} alkyl, or C_1-C_6 alkyl, or C_1-C_3 alkyl. In some instances, (C_1-C_{12})alkyl is a C_2-alkyl. For example, (C_1-C_{12})alkyl may be an alkylene or substituted alkylene, such as C_1-C_{12} alkylene, or C_1-C_{10} alkylene, or C_1-C_6 alkylene, or C_1-C_3 alkylene. In some instances, (C_1-C_{12})alkyl is a C_1-alkylene (*e.g.*, CH_2). In some instances, (C_1-C_{12})alkyl is a C_2-alkylene (*e.g.*, CH_2CH_2). In some instances, (C_1-C_{12})alkyl is a C_3-alkylene (*e.g.*, $CH_2CH_2CH_2$).

[00376] In certain embodiments, substituted (C_1-C_{12})alkyl is a straight chain or branched substituted alkyl group that includes from 1 to 12 carbon atoms, such as 1 to 10 carbon atoms, or 1 to 8 carbon atoms, or 1 to 6 carbon atoms, or 1 to 5 carbon atoms, or 1 to 4 carbon atoms, or 1 to 3 carbon atoms. In some instances, substituted (C_1-C_{12})alkyl may be a substituted alkyl, such as substituted C_1-C_{12} alkyl, or substituted C_1-C_{10} alkyl, or substituted C_1-C_6 alkyl, or substituted C_1-C_3 alkyl. In some instances, substituted (C_1-C_{12})alkyl is a substituted C_2-alkyl. For example, substituted (C_1-C_{12})alkyl may be a substituted alkylene, such as substituted C_1-C_{12} alkylene, or substituted C_1-C_{10} alkylene, or substituted C_1-C_6 alkylene, or substituted C_1-C_3 alkylene. In some instances, substituted (C_1-C_{12})alkyl is a substituted C_1-alkylene (*e.g.*, C_1-alkylene substituted with $-SO_3H$). In some instances, substituted (C_1-C_{12})alkyl is a substituted C_2-alkylene. In some instances, substituted (C_1-C_{12})alkyl is a substituted C_3-alkylene. For example, substituted (C_1-C_{12})alkyl may include C_1-C_{12} alkylene (*e.g.*, C_3-alkylene or C_5-alkylene) substituted with a $(PEG)_k$ group as described herein (*e.g.*,$-CONH(PEG)_k$, such as $-CONH(PEG)_3$ or $-CONH(PEG)_5$; or $-NHCO(PEG)_k$,

such as -NHCO(PEG)$_7$), or may include C_1-C_{12} alkylene (*e.g.*, C_3-alkylene) substituted with a -CONHCH$_2$CH$_2$SO$_3$H group, or may include C_1-C_{12} alkylene (*e.g.*, C_5-alkylene) substituted with a -NHCOCH$_2$SO$_3$H group.

[00377] In some embodiments, substituted (C_1-C_{12})alkyl may include C_1-C_{12} alkylene (*e.g.*, C_3-alkylene or C_5-alkylene) substituted with a (PEG)$_t$ group as described herein (*e.g.*, -NHCO(PEG)$_t$, wherein (PEG)$_t$ is 

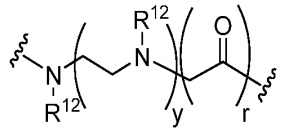
, ~~~ indicates the point of attachment to carbonyl group of -NHCO-, and t is an integer), such as -NHCO(CH$_2$CH$_2$O)$_3$CH$_3$ or -NHCO(CH$_2$CH$_2$O)$_5$CH$_3$ or -NHCO(CH$_2$CH$_2$O)$_8$CH$_3$.

[00378] In certain embodiments, the tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes an aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl. In some instances, the tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes an aryl or substituted aryl. For example, the aryl can be phenyl. In some cases, the substituted aryl is a substituted phenyl. The substituted phenyl can be substituted with one or more substituents selected from (C_1-C_{12})alkyl, a substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In some instances, the substituted aryl is a substituted phenyl, where the substituent includes a cleavable moiety as described herein (*e.g.*, an enzymatically cleavable moiety, such as a glycoside or glycoside derivative).

[00379] In some instances, the tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes a heteroaryl or substituted heteroaryl, such triazolyl (*e.g.*, 1,2,3-triazolyl). In some instances, the tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes a cycloalkyl or substituted cycloalkyl. In some instances, the tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes a heterocyclyl or substituted heterocyclyl. In some instances, the substituent on the substituted heteroaryl, substituted cycloalkyl or substituted heterocyclyl includes a cleavable moiety as described herein (*e.g.*, an enzymatically cleavable moiety, such as a glycoside or glycoside derivative).

[00380] In certain embodiments, the tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes an ethylene diamine (EDA) moiety, *e.g.*, an EDA containing tether group. In certain embodiments, (EDA)$_w$ includes one or more EDA moieties, such as where w is an integer from 1 to 50, such as from 1 to 40, from 1 to 30, from 1 to 20, from 1 to 12 or from 1 to 6, such as 1, 2, 3, 4, 5 or 6). The linked ethylene diamine (EDA) moieties

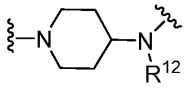
may optionally be substituted at one or more convenient positions with any convenient substituents, *e.g.*, with an alkyl, a substituted alkyl, an acyl, a substituted acyl, an aryl, or a substituted aryl. In certain embodiments, the EDA moiety is described by the structure:



,

where y is an integer from 1 to 6, r is 0 or 1, and each R^{12} is independently selected from 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, and substituted heterocyclyl. In certain embodiments, y is 1, 2, 3, 4, 5 or 6. In certain embodiments, y is 1 and r is 0. In certain embodiments, y is 1 and r is 1. In certain embodiments, y is 2 and r is 0. In certain embodiments, y is 2 and r is 1. In certain embodiments, each R^{12} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl. In certain embodiments, any two adjacent R^{12} groups of the EDA may be cyclically linked, *e.g.*, to form a piperazinyl ring. In certain embodiments, y is 1 and the two adjacent R^{12} groups are an alkyl group, cyclically linked to form a piperazinyl ring. In certain embodiments, y is 1 and the adjacent R^{12} groups are selected from hydrogen, an alkyl (*e.g.*, methyl) and a substituted alkyl (*e.g.*, lower alkyl-OH, such as ethyl-OH or propyl-OH).

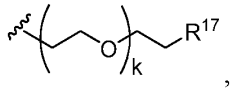
[00381] In certain embodiments, the tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes a 4-amino-piperidine (4AP) moiety (also referred to herein as piperidin-4-amino, P4A). The 4AP moiety may optionally be substituted at one or more convenient positions with any convenient substituents, *e.g.*, with an alkyl, a substituted alkyl, a polyethylene glycol moiety, an acyl, a substituted acyl, an aryl, or a substituted aryl. In certain embodiments, the 4AP moiety is described by the structure:



where R^{12} is selected from hydrogen, alkyl, substituted alkyl, a polyethylene glycol moiety (*e.g.*, a polyethylene glycol or a modified polyethylene glycol), 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, and substituted heterocyclyl. In certain embodiments, R^{12} is a polyethylene glycol moiety. In certain embodiments, R^{12} is a carboxy modified polyethylene glycol.

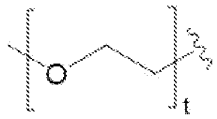
[00382] In certain embodiments, R^{12} includes a polyethylene glycol moiety described by the formula: $(PEG)_k$, which may be represented by the structure:



,

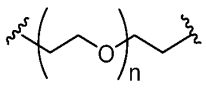
where k is an integer from 1 to 20, such as from 1 to 18, or from 1 to 16, or from 1 to 14, or from 1 to 12, or from 1 to 10, or from 1 to 8, or from 1 to 6, or from 1 to 4, or 1 or 2, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some instances, k is 2. In certain embodiments, R^{17} is selected from OH, COOH, OR, or COOR, where R is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R^{17} is COOH. In certain embodiments, R^{17} is OH. In certain embodiments, R^{17} is OCH_3.

[00383] In certain embodiments, $(PEG)_k$ is $(PEG)_t$ having the following structure:



wherein t is an integer from 2 to 10. In certain embodiments, t is 8.

[00384] In certain embodiments, a tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes $(PEG)_n$, wherein $(PEG)_n$ is a polyethylene glycol or a modified polyethylene glycol linking unit. In certain embodiments, $(PEG)_n$ is described by the structure:



,

where n is an integer from 1 to 50, such as from 1 to 40, from 1 to 30, from 1 to 20, from 1 to 12 or from 1 to 6, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some instances, n is 2. In some instances, n is 3. In some instances, n is 6. In some instances, n is 12.

[00385] In certain embodiments, a tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes $(AA)_p$, where AA is an amino acid residue. Any convenient

amino acids may be utilized. Amino acids of interest include but are not limited to, L- and D-amino acids, naturally occurring amino acids such as any of the 20 primary alpha-amino acids and beta-alanine, non-naturally occurring amino acids (*e.g.*, amino acid analogs), such as a non-naturally occurring alpha-amino acid or a non-naturally occurring beta-amino acid, etc. In certain embodiments, p is an integer from 1 to 50, such as from 1 to 40, from 1 to 30, from 1 to 20, from 1 to 12 or from 1 to 6, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In certain embodiments, p is 1. In certain embodiments, p is 2.

[00386] In further embodiments, $(AA)_p$ comprises a dipeptide of valine-alanine.

[00387] In certain embodiments, a tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes an amino acid analog. Amino acid analogs include compounds that are similar in structure and/or overall shape to one or more amino acids commonly found in naturally occurring proteins (*e.g.*, Ala or A, Cys or C, Asp or D, Glu or E, Phe or F, Gly or G, His or H, Ile or I, Lys or K, Leu or L, Met or M, Asn or N, Pro or P, Gln or Q, Arg or R, Ser or S, Thr or T, Val or V, Trp or W, Tyr or Y). Amino acid analogs also include natural amino acids with modified side chains or backbones. Amino acid analogs also include amino acid analogs with the same stereochemistry as in the naturally occurring D-form, as well as the L-form of amino acid analogs. In some instances, the amino acid analogs share backbone structures, and/or the side chain structures of one or more natural amino acids, with difference(s) being one or more modified groups in the molecule. Such modification may include, but is not limited to, substitution of an atom (such as N) for a related atom (such as S), addition of a group (such as methyl, or hydroxyl, etc.) or an atom (such as Cl or Br, etc.), deletion of a group, substitution of a covalent bond (single bond for double bond, etc.), or combinations thereof. For example, amino acid analogs may include α-hydroxy acids, and α-amino acids, and the like. Examples of amino acid analogs include, but are not limited to, sulfoalanine, and the like.

[00388] In certain embodiments, a tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes a moiety described by the formula $-(CR^{13}OH)_x-$, where x is 0 or x is an integer from 1 to 50, such as from 1 to 40, from 1 to 30, from 1 to 20, from 1 to 12 or from 1 to 6, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12. In certain embodiments, x is 1. In certain embodiments, x is 2. In certain embodiments, R^{13} is selected from 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

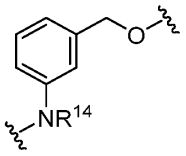
cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R^{13} is hydrogen. In certain embodiments, R^{13} is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^{13} is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^{13} is alkynyl or substituted alkynyl. In certain embodiments, R^{13} is alkoxy or substituted alkoxy. In certain embodiments, R^{13} is amino or substituted amino. In certain embodiments, R^{13} is carboxyl or carboxyl ester. In certain embodiments, R^{13} is acyl or acyloxy. In certain embodiments, R^{13} is acyl amino or amino acyl. In certain embodiments, R^{13} is alkylamide or substituted alkylamide. In certain embodiments, R^{13} is sulfonyl. In certain embodiments, R^{13} is thioalkoxy or substituted thioalkoxy. In certain embodiments, R^{13} is aryl or substituted aryl, such as C_{5-8} aryl or C_{5-8} substituted aryl, such as a C_5 aryl or C_5 substituted aryl, or a C_6 aryl or C_6 substituted aryl. In certain embodiments, R^{13} is heteroaryl or substituted heteroaryl, such as C_{5-8} heteroaryl or C_{5-8} substituted heteroaryl, such as a C_5 heteroaryl or C_5 substituted heteroaryl, or a C_6 heteroaryl or C_6 substituted heteroaryl. In certain embodiments, R^{13} is cycloalkyl or substituted cycloalkyl, such as C_{3-8} cycloalkyl or C_{3-8} substituted cycloalkyl, such as a C_{3-6} cycloalkyl or C_{3-6} substituted cycloalkyl, or a C_{3-5} cycloalkyl or C_{3-5} substituted cycloalkyl. In certain embodiments, R^{13} is heterocyclyl or substituted heterocyclyl, such as C_{3-8} heterocyclyl or C_{3-8} substituted heterocyclyl, such as a C_{3-6} heterocyclyl or C_{3-6} substituted heterocyclyl, or a C_{3-5} heterocyclyl or C_{3-5} substituted heterocyclyl.

[00389] In certain embodiments, R^{13} is selected from hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl. In these embodiments, alkyl, substituted alkyl, aryl, and substituted aryl are as described above for R^{13}.

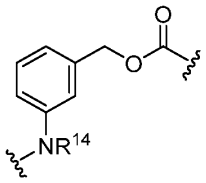
[00390] In certain embodiments, the tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes an acetal group, a disulfide, a hydrazine, or an ester. In some embodiments, the tether group includes an acetal group. In some embodiments, the tether group includes a hydrazine. In some embodiments, the tether group includes a disulfide. In some embodiments, the tether group includes an ester.

[00391] In certain embodiments, a tether group (*e.g.*, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13}) includes a meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), or para-hydroxy-phenyl (PHP).

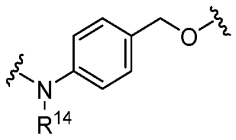
[00392] In some embodiments, a tether group includes a MABO group described by the following structure:



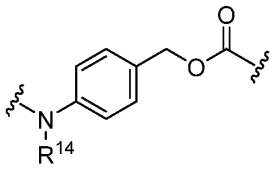
[00393] In some embodiments, a tether group includes a MABC group described by the following structure:



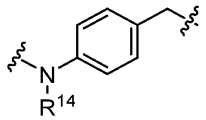
[00394] In some embodiments, a tether group includes a PABO group described by the following structure:



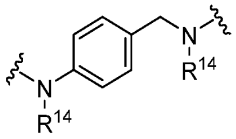
[00395] In some embodiments, a tether group includes a PABC group described by the following structure:



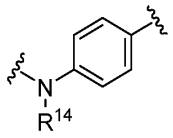
[00396] In some embodiments, a tether group includes a PAB group described by the following structure:



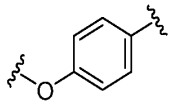
[00397] In some embodiments, a tether group includes a PABA group described by the following structure:



[00398] In some embodiments, a tether group includes a PAP group described by the following structure:



[00399] In some embodiments, a tether group includes a PHP group described by the following structure:



[00400] In certain embodiments, each R^{14} is independently selected from 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, and substituted heterocyclyl.

[00401] In certain embodiments, R^{14} is hydrogen. In certain embodiments, each R^{14} is hydrogen. In certain embodiments, R^{14} is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^{14} is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^{14} is alkynyl or substituted alkynyl. In certain embodiments, R^{14} is alkoxy or substituted alkoxy. In certain embodiments, R^{14} is amino or substituted amino. In certain embodiments, R^{14} is carboxyl or carboxyl ester. In certain embodiments, R^{14} is acyl or acyloxy. In certain embodiments, R^{14} is acyl amino or amino acyl. In certain embodiments, R^{14} is alkylamide or substituted alkylamide. In certain embodiments, R^{14} is sulfonyl. In certain embodiments, R^{14} is thioalkoxy or substituted thioalkoxy. In certain embodiments, R^{14} is aryl or substituted aryl, such as C_{5-8} aryl or C_{5-8} substituted aryl, such as a C_5 aryl or C_5 substituted aryl, or a C_6 aryl or C_6 substituted aryl. In certain embodiments, R^{14} is heteroaryl or substituted heteroaryl, such as C_{5-8} heteroaryl or C_{5-8} substituted heteroaryl, such as a C_5 heteroaryl or C_5 substituted heteroaryl, or a C_6 heteroaryl or C_6 substituted heteroaryl. In certain embodiments, R^{14} is cycloalkyl or substituted cycloalkyl, such as C_{3-8} cycloalkyl or C_{3-8} substituted cycloalkyl, such as a C_{3-6} cycloalkyl or C_{3-6} substituted cycloalkyl, or a C_{3-5} cycloalkyl or C_{3-5} substituted cycloalkyl. In

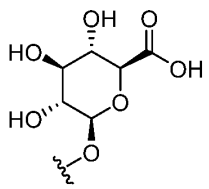
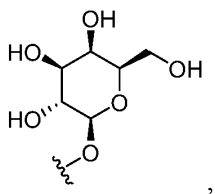
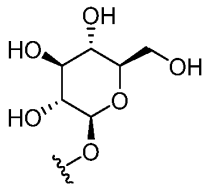
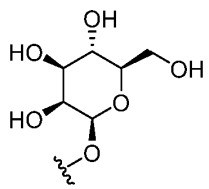
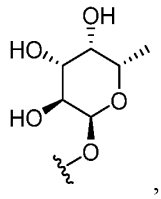
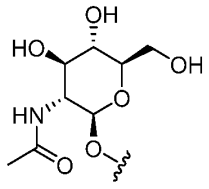
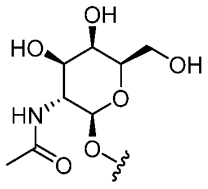
certain embodiments, R^{14} is heterocyclyl or substituted heterocyclyl, such as C_{3-8} heterocyclyl or C_{3-8} substituted heterocyclyl, such as a C_{3-6} heterocyclyl or C_{3-6} substituted heterocyclyl, or a C_{3-5} heterocyclyl or C_{3-5} substituted heterocyclyl.

[00402] In some embodiments of the MABO, MABC, PABO, PABC, PAB, PABA, PAP, and PHP tether structures shown above, the phenyl ring may be substituted with one or more additional groups selected from halogen, 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, and substituted heterocyclyl.

[00403] In certain embodiments, one or more of the tether groups T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and/or T^{13} is each optionally substituted with a glycoside or glycoside derivative. For example, in some instances, T^1, T^2, T^3, T^4, T^5 and T^6 are each optionally substituted with a glycoside. In some instances, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} are each optionally substituted with a glycoside. In certain embodiments, the glycoside or glycoside derivative is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00404] In certain embodiments, the MABO, MABC, PABO, PABC, PAB, PABA, PAP, and PHP tether structures shown above may be substituted with one or more additional groups selected from a glycoside and a glycoside derivative. For example, in some embodiments of the MABO, MABC, PABO, PABC, PAB, PABA, PAP, and PHP tether structures shown above, the phenyl ring may be substituted with one or more additional groups selected from a glycoside and a glycoside derivative. In certain embodiments, the glycoside or glycoside derivative is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc. In some embodiments, the PABC is substituted with a glycoside, for example, a hydrogen of PABC is replaced with a glycoside, such as a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00405] For example, in some embodiments, the glycoside or glycoside derivative is selected from the following structures:


,

,

,

,

,

, and

.

[00406] Regarding the linking functional groups, V^1, V^2, V^3, V^4, V^5, V^6, V^7, V^8, V^9, V^{10}, V^{11}, V^{12}, and V^{13} any convenient linking functional groups may be utilized in the subject linkers. Linking functional groups of interest include, but are not limited to, amino, carbonyl, amido, oxycarbonyl, carboxy, sulfonyl, sulfoxide, sulfonylamino, aminosulfonyl, thio, oxy, phospho, phosphoramidate, thiophosphoraidate, and the like. In some embodiments, V^1, V^2, V^3, V^4, V^5, V^6, V^7, V^8, V^9, V^{10}, V^{11}, V^{12}, and V^{13} are each independently selected from a covalent bond, -CO-, -NR^{15}-, -$NR^{15}(CH_2)_q$-, -$NR^{15}(C_6H_4)$-, -$CONR^{15}$-, -$NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$- and -P(O)OH-, where q is an integer from 1 to 6. In certain embodiments, q is an integer from 1 to 6 (*e.g.*, 1, 2, 3, 4, 5 or 6). In certain embodiments, q is 1. In certain embodiments, q is 2. In certain embodiments, q is 3. In certain embodiments, q is 4. In certain embodiments, q is 5. In certain embodiments, q is 6. In some embodiments, each R^{15} is independently selected from 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, and substituted heterocyclyl.

[00408] In certain embodiments, R^{15} is hydrogen. In certain embodiments, each R^{15} is hydrogen. In certain embodiments, R^{15} is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^{15} is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^{15} is alkynyl or substituted alkynyl. In certain embodiments, R^{15} is alkoxy or substituted alkoxy. In certain embodiments, R^{15} is amino or substituted amino. In certain embodiments, R^{15} is carboxyl or carboxyl ester. In certain

embodiments, R^{15} is acyl or acyloxy. In certain embodiments, R^{15} is acyl amino or amino acyl. In certain embodiments, R^{15} is alkylamide or substituted alkylamide. In certain embodiments, R^{15} is sulfonyl. In certain embodiments, R^{15} is thioalkoxy or substituted thioalkoxy. In certain embodiments, R^{15} is aryl or substituted aryl, such as C_{5-8} aryl or C_{5-8} substituted aryl, such as a C_5 aryl or C_5 substituted aryl, or a C_6 aryl or C_6 substituted aryl. In certain embodiments, R^{15} is heteroaryl or substituted heteroaryl, such as C_{5-8} heteroaryl or C_{5-8} substituted heteroaryl, such as a C_5 heteroaryl or C_5 substituted heteroaryl, or a C_6 heteroaryl or C_6 substituted heteroaryl. In certain embodiments, R^{15} is cycloalkyl or substituted cycloalkyl, such as C_{3-8} cycloalkyl or C_{3-8} substituted cycloalkyl, such as a C_{3-6} cycloalkyl or C_{3-6} substituted cycloalkyl, or a C_{3-5} cycloalkyl or C_{3-5} substituted cycloalkyl. In certain embodiments, R^{15} is heterocyclyl or substituted heterocyclyl, such as C_{3-8} heterocyclyl or C_{3-8} substituted heterocyclyl, such as a C_{3-6} heterocyclyl or C_{3-6} substituted heterocyclyl, or a C_{3-5} heterocyclyl or C_{3-5} substituted heterocyclyl.

[00409] In certain embodiments, each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In these embodiments, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl are as described above for R^{15}.

[00410] As described above, in some embodiments, L^A is a first linker comprising $-(T^1\text{-}V^1)_a\text{-}(T^2\text{-}V^2)_b\text{-}(T^3\text{-}V^3)_c\text{-}(T^4\text{-}V^4)_d\text{-}(T^5\text{-}V^5)_e\text{-}(T^6\text{-}V^6)_f\text{-}$, where a, b, c, d, e and f are each independently 0 or 1, provided at least one of a, b, c, d, e, and f is 1.

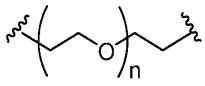
[00411] In some embodiments, in the first linker L^A:

T^1 is selected from a $(C_1\text{-}C_{12})$alkyl and a substituted $(C_1\text{-}C_{12})$alkyl;

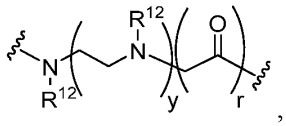
T^2, T^3, T^4, T^5 and T^6 are each independently selected from $(C_1\text{-}C_{12})$alkyl, substituted $(C_1\text{-}C_{12})$alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x\text{-}$,

4-amino-piperidine (4AP), MABO, MABC, PABO, PABC, PAB, PABA, PAP, PHP, an acetal group, a disulfide, a hydrazine, and an ester; and

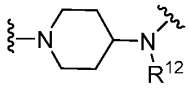
V^1, V^2, V^3, V^4,V^5, and V^6 are each independently selected from a covalent bond, -CO-, -NR^{15}-, -$NR^{15}(CH_2)_q$-, -$NR^{15}(C_6H_4)$-, -$CONR^{15}$-, -$NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$- and -P(O)OH-, wherein q is an integer from 1 to 6; wherein:

$(PEG)_n$ is , where n is an integer from 1 to 30;

EDA is an ethylene diamine moiety having the following structure:

,

where y is an integer from 1 to 6 and r is 0 or 1;

4-amino-piperidine (4AP) is ;

AA is an amino acid residue, where p is an integer from 1 to 20; and

each R^{12} is independently selected from hydrogen, an alkyl, a substituted alkyl, a polyethylene glycol moiety, an aryl, and a substituted aryl, wherein any two adjacent R^{12} groups may be cyclically linked to form a piperazinyl ring;

each R^{13} is independently selected from hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[00412] In some embodiments, L^A comprises:

$$-(T^1\text{-}V^1)_a\text{-}(T^2\text{-}V^2)_b\text{-}(T^3\text{-}V^3)_c\text{-}(T^4\text{-}V^4)_d\text{-}(T^5\text{-}V^5)_e\text{-}(T^6\text{-}V^6)_f\text{-},$$

wherein:

a, b, c, d, e, and f are each independently 0 or 1, provided at least one of a, b, c, d, e, and f is 1;

T^1, T^2, T^3, T^4, T^5 and T^6 are each independently selected from a covalent bond, $(C_1\text{-}C_{12})$alkyl, substituted $(C_1\text{-}C_{12})$alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, -$(CR^{13}OH)_x$-, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-

amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12;

V^1, V^2, V^3, V^4,V^5, and V^6 are each independently selected from the group consisting of a covalent bond, -CO-, -NR^{15}-, -$NR^{15}(CH_2)_q$-, -$NR^{15}(C_6H_4)$-, -$CONR^{15}$-, -$NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$- and -P(O)OH-, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

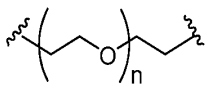
each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[00413] In some embodiments of L^A:

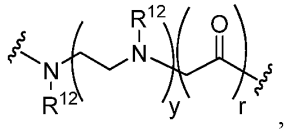
T^1 is selected from a (C_1-C_{12})alkyl and a substituted (C_1-C_{12})alkyl;

T^2, T^3, T^4, T^5 and T^6 are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, -$(CR^{13}OH)_x$-, 4-amino-piperidine (4AP), MABO, MABC, PABO, PABC, PAB, PABA, PAP, PHP, an acetal group, a hydrazine, and an ester; and

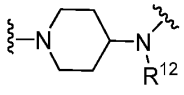
V^1, V^2, V^3, V^4,V^5, and V^6 are each independently selected from the group consisting of a covalent bond, -CO-, -NR^{15}-, -$NR^{15}(CH_2)_q$-, -$NR^{15}(C_6H_4)$-, -$CONR^{15}$-, -$NR^{15}CO$-,
-C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$-, and -P(O)OH-;
wherein:

$(PEG)_n$ is , where n is an integer from 1 to 30;

EDA is an ethylene diamine moiety having the following structure:



where y is an integer from 1 to 6 and r is 0 or 1;

4-amino-piperidine (4AP) is ;

each R^{12} is independently selected from hydrogen, an alkyl, a substituted alkyl, a polyethylene glycol moiety, an aryl, and a substituted aryl, wherein any two adjacent R^{12} groups may be cyclically linked to form a piperazinyl ring;

a, b, c, and d are each 1; and

e and f are 0.

[00414] In some embodiments, T^1, T^2, T^3, T^4, T^5 and T^6 are each optionally substituted with a glycoside.

[00415] In some embodiments, MABO, MABC, PABO, PABC, PAB, PABA, PAP and PHP are each optionally substituted with a glycoside.

[00416] In some embodiments, the glycoside is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00417] In certain embodiments, T^1, T^2, T^3, T^4, T^5 and T^6 and V^1, V^2, V^3, V^4, V^5, and V^6 are selected from the following:

wherein:

T^1 is $(C_1\text{-}C_{12})$alkyl and V^1 is -CO-;

T^2 is $(AA)_p$ and V^2 is absent (*e.g.*, a covalent bond);

T^3 is PABC and V^3 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

d, e, and f are each 0; or

wherein:

T^1 is $(C_1\text{-}C_{12})$alkyl and V^1 is -CONH-;

T^2 is $(PEG)_n$ and V^2 is -CO-;

T^3 is $(AA)_p$ and V^3 is absent (*e.g.*, a covalent bond);

T^4 is PABC and V^4 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

e and f are each 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CO-;

T^2 is an amino acid analog and V^2 is -NH-;

T^3 is $(PEG)_n$ and V^3 is -CO-;

T^4 is $(AA)_p$ and V^4 is absent (*e.g.*, a covalent bond);

T^5 is PABC and V^5 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

f is 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is $(PEG)_n$ and V^2 is -CO-;

T^3 is $(AA)_p$ and V^3 is absent (*e.g.*, a covalent bond);

T^4 is PABC and V^4 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

e and f are each 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is substituted (C_1-C_{12})alkyl and V^2 is -CO-;

T^3 is $(AA)_p$ and V^3 is absent (*e.g.*, a covalent bond);

T^4 is PABC and V^4 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

e and f are each 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is $(PEG)_n$ and V^2 is -CO-;

T^3 is $(AA)_p$ and V^3 is absent (*e.g.*, a covalent bond);

T^4 is PABA and V^4 is -CO-;

T^5 is (C_1-C_{12})alkyl and V^5 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

f is 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CO-;

T^2 is 4AP and V^2 is -CO-;

T^3 is (C_1-C_{12})alkyl and V^3 is -CO-;

T^4 is $(AA)_p$ and V^4 is absent (*e.g.*, a covalent bond);

T^5 is PABC and V^5 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

f is 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CO-;

T^2 is 4AP and V^2 is -CO-;

T^3 is (C_1-C_{12})alkyl and V^3 is -O-;

T^4 is (C_1-C_{12})alkyl and V^4 is -CO-;

T^5 is $(AA)_p$ and V^5 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

T^6 is PABC and V^6 is absent (*e.g.*, a covalent bond); or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CO-;

T^2 is an amino acid analog and V^2 is absent (*e.g.*, a covalent bond);

T^3 is $(AA)_p$ and V^3 is absent (*e.g.*, a covalent bond);

T^4 is PABC and V^4 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

e and f are each 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is $(PEG)_n$ and V^2 is -CONH-;

T^3 is substituted (C_1-C_{12})alkyl and V^3 is -CO-;

T^4 is $(AA)_p$ and V^4 is absent (*e.g.*, a covalent bond);

T^5 is PABC and V^5 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

f is 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CO-;

T^2 is an $(AA)_p$ and V^2 is -NH-;

T^3 is $(PEG)_n$ and V^3 is -CO-;

T^4 is $(AA)_p$ and V^4 is absent (*e.g.*, a covalent bond);

T^5 is PABC and V^5 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

f is 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is $(PEG)_n$ and V^2 is -CO-;

T^3 is $(AA)_p$ and V^3 is absent (*e.g.*, a covalent bond);

T^4 is PAP and V^4 is -C(O)O-;

p is an integer from 1 to 10; and

e and f are each 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is substituted (C_1-C_{12})alkyl and V^2 is -CO-;

T^3 is $(AA)_p$ and V^3 is absent (*e.g.*, a covalent bond);

T^4 is PABC and V^4 is absent (*e.g.*, a covalent bond);

p is an integer from 1 to 10; and

e and f are each 0; or

wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is substituted (C_1-C_{12})alkyl and V^2 is -CO-;

T^3 is PABC and V^3 is absent (*e.g.*, a covalent bond); and

d, e, and f are each 0.

[00418] In certain embodiments, the left-hand side of the above linker structure for the first linker L^A is attached to the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety, and the right-hand side of the above linker structure for the first linker L^A is attached to the first drug or active agent.

[00419] As described above, in some embodiments, L^B is a second linker comprising $-(T^7\text{-}V^7)_g\text{-}(T^8\text{-}V^8)_h\text{-}(T^9\text{-}V^9)_i\text{-}(T^{10}\text{-}V^{10})_j\text{-}(T^{11}\text{-}V^{11})_k\text{-}(T^{12}\text{-}V^{12})_l\text{-}(T^{13}\text{-}V^{13})_m-$, where g, h, i, j, k, l, and m are each independently 0 or 1, provided that at least one of g, h, i, j, k, l, and m is 1.

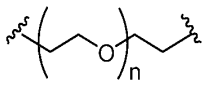
[00420] In some embodiments, in the second linker L^B:

T^7 is selected from a (C_1-C_{12})alkyl and a substituted (C_1-C_{12})alkyl;

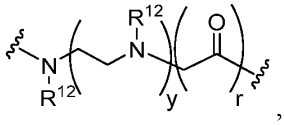
T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} are each independently selected from (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x-$, 4-amino-piperidine (4AP), MABO, MABC, PABO, PABC, PAB, PABA, PAP, PHP, an acetal group, a disulfide, a hydrazine, and an ester; and

V^7, V^8, V^9, V^{10},V^{11}, V^{12}, and V^{13} are each independently selected from a covalent bond, -CO-, -NR^{15}-, -$NR^{15}(CH_2)_q$-, -$NR^{15}(C_6H_4)$-, -$CONR^{15}$-, -$NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$- and -P(O)OH-, wherein q is an integer from 1 to 6;

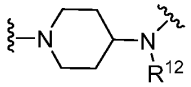
wherein:

$(PEG)_n$ is , where n is an integer from 1 to 30;

EDA is an ethylene diamine moiety having the following structure:

,

where y is an integer from 1 to 6 and r is 0 or 1;

4-amino-piperidine (4AP) is ;

AA is an amino acid residue, where p is an integer from 1 to 20; and

each R^{12} is independently selected from hydrogen, an alkyl, a substituted alkyl, a polyethylene glycol moiety, an aryl, and a substituted aryl, wherein any two adjacent R^{12} groups may be cyclically linked to form a piperazinyl ring;

each R^{13} is independently selected from hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[00421] Any convenient tether groups may be utilized for T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13}. For example, any of the tether groups described above in relation to T^1, T^2, T^3, T^4, T^5 and T^6 may be used for the tether groups T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13}.

[00422] Any convenient linking functional groups may be utilized for V^7, V^8, V^9, V^{10}, V^{11}, V^{12}, and V^{13}. For example, any of the linking functional groups described above in relation to V^1, V^2, V^3, V^4, V^5, and V^6 may be used for the linking functional groups V^7, V^8, V^9, V^{10}, V^{11}, V^{12}, and V^{13}.

[00423] In certain embodiments, each R^{13} is independently selected from hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl. In these embodiments, alkyl, substituted alkyl, aryl, and substituted aryl are as described above for R^{13}.

[00424] In certain embodiments, each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In these embodiments, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl are as described above for R^{15}. In these embodiments, various possible substituents are as described above for R^{15}.

[00425] In certain embodiments of the second linker L^B, one or more of the tether groups T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} is each optionally substituted with a glycoside or glycoside derivative. In certain embodiments, the glycoside or glycoside derivative is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00426] In certain embodiments of the second linker L^B, the MABO, MABC, PABO, PABC, PAB, PABA, PAP, and PHP tether structures shown above may be substituted with one or more additional groups selected from a glycoside and a glycoside derivative. For example, in some embodiments of the MABO, MABC, PABO, PABC, PAB, PABA, PAP, and PHP tether structures shown above, the phenyl ring may be substituted with one or more additional groups selected from a glycoside and a glycoside derivative. In certain embodiments, the glycoside or glycoside derivative is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00427] In some embodiments, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} are each optionally substituted with a glycoside.

[00428] In some embodiments, MABO, MABC, PABO, PABC, PAB, PABA, PAP and PHP are each optionally substituted with a glycoside.

[00429] In some embodiments, the glycoside is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00430] In some embodiments of L^B:

g, h, i, j, and k are each 1;

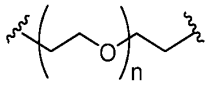
l and m are each 0;

T^7 is a covalent bond;

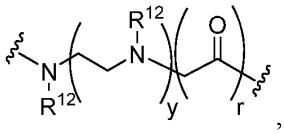
T^8, T^9, T^{10}, T^{11} and T^{12} are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x-$, 4-amino-piperidine (4AP), MABO, MABC, PABO, PABC, PAB, PABA, PAP, PHP, an acetal group, a hydrazine, and an ester; and

V^7, V^8, V^9, V^{10},V^{11} and V^{12} are each independently selected from the group consisting of a covalent bond, -CO-, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$, and -P(O)OH-;

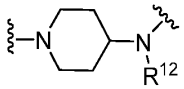
wherein:

$(PEG)_n$ is , where n is an integer from 1 to 30;

EDA is an ethylene diamine moiety having the following structure:

,

where y is an integer from 1 to 6 and r is 0 or 1;

4-amino-piperidine (4AP) is ; and

each R^{12} is independently selected from hydrogen, an alkyl, a substituted alkyl, a polyethylene glycol moiety, an aryl, and a substituted aryl, wherein any two adjacent R^{12} groups may be cyclically linked to form a piperazinyl ring.

[00431] In some embodiments, T^1, T^2, T^3, T^4, T^5, T^6, T^7, T^8, T^9, T^{10}, T^{11}, and T^{12} are each optionally substituted with a glycoside.

[00432] In some embodiments, MABO, MABC, PABO, PABC, PAB, PABA, PAP and PHP are each optionally substituted with a glycoside.

[00433] In some embodiments, the glycoside is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00434] In certain embodiments, T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} and V^7, V^8, V^9, V^{10}, V^{11}, V^{12}, and V^{13} are selected from the following:

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is -CO-;

T^9 is $(AA)_p$ and V^9 is absent (*e.g.*, a covalent bond);

T^{10} is PABC and V^{10} is absent (*e.g.*, a covalent bond); and

k, l, and m are each 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is $(C_1\text{-}C_{12})$alkyl and V^8 is -CONH-;

T^9 is $(PEG)_n$ and V^9 is -CO-;

T^{10} is $(AA)_p$ and V^{10} is absent (*e.g.*, a covalent bond); and

T^{11} is PABC and V^{11} is absent (*e.g.*, a covalent bond); and

l and m are each 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is $(C_1\text{-}C_{12})$alkyl and V^8 is -CO-;

T^9 is an amino acid analog and V^9 is -NH-;

T^{10} is $(PEG)_n$ and V^{10} is -CO-;

T^{11} is $(AA)_p$ and V^{11} is absent (*e.g.*, a covalent bond);

T^{12} is PABC and V^{12} is absent (*e.g.*, a covalent bond); and

m is 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is $(C_1\text{-}C_{12})$alkyl and V^8 is -CONH-;

T^9 is $(PEG)_n$ and V^9 is -CO-;

T^{10} is $(AA)_p$ and V^{10} is absent (*e.g.*, a covalent bond);

T^{11} is PABC and V^{11} is absent (*e.g.*, a covalent bond); and

l and m are each 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is $(C_1\text{-}C_{12})$alkyl and V^8 is -CONH-;

T^9 is substituted $(C_1\text{-}C_{12})$alkyl and V^9 is -CO-;

T^{10} is $(AA)_p$ and V^{10} is absent (*e.g.*, a covalent bond);

T^{11} is PABC and V^{11} is absent (*e.g.*, a covalent bond); and

l and m are each 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is -CONH-;

T^9 is $(PEG)_n$ and V^9 is -CO-;

T^{10} is $(AA)_p$ and V^{10} is absent (*e.g.*, a covalent bond);

T^{11} is PABA and V^{11} is -CO-;

T^{12} is (C_1-C_{12})alkyl and V^{12} is absent (*e.g.*, a covalent bond); and

m is 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is -CO-;

T^9 is 4AP and V^9 is -CO-;

T^{10} is (C_1-C_{12})alkyl and V^{10} is -CO-;

T^{11} is $(AA)_p$ and V^{11} is absent (*e.g.*, a covalent bond);

T^{12} is PABC and V^{12} is absent (*e.g.*, a covalent bond); and

m is 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is -CO-;

T^9 is 4AP and V^9 is -CO-;

T^{10} is (C_1-C_{12})alkyl and V^{10} is -O-;

T^{11} is (C_1-C_{12})alkyl and V^{11} is -CO-;

T^{12} is $(AA)_p$ and V^{12} is absent (*e.g.*, a covalent bond); and

T^{13} PABC and V^{13} is absent (*e.g.*, a covalent bond); or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is -CO-;

T^9 is an amino acid analog and V^9 is absent (*e.g.*, a covalent bond);

T^{10} is $(AA)_p$ and V^{10} is absent (*e.g.*, a covalent bond);

T^{11} is PABC and V^{11} is absent (*e.g.*, a covalent bond); and

l and m are each 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is -CONH-;

T^9 is $(PEG)_n$ and V^9 is -CONH-;

T^{10} is substituted (C_1-C_{12})alkyl and V^{10} is -CO-;

T^{11} is $(AA)_p$ and V^{11} is absent (*e.g.*, a covalent bond);

T^{12} is PABC and V^{12} is absent (*e.g.*, a covalent bond); and

m is 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is $(C_1\text{-}C_{12})$alkyl and V^8 is -CO-;

T^9 is $(AA)_p$ and V^9 is -NH-;

T^{10} is $(PEG)_n$ and V^{10} is -CO-;

T^{11} is $(AA)_p$ and V^{11} is absent (*e.g.*, a covalent bond);

T^{12} is PABC and V^{12} is absent (*e.g.*, a covalent bond); and

m is 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is $(C_1\text{-}C_{12})$alkyl and V^8 is -CONH-;

T^9 is $(PEG)_n$ and V^9 is -CO-;

T^{10} is $(AA)_p$ and V^{10} is absent (*e.g.*, a covalent bond);

T^{11} is PAP and V^{11} is -C(O)O-; and

l and m are each 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is $(C_1\text{-}C_{12})$alkyl and V^8 is -CO-;

T^9 is $(AA)_p$ and V^9 is absent (*e.g.*, a covalent bond);

T^{10} is PABC and V^{10} is absent (*e.g.*, a covalent bond);

T^{11} is PAP and V^{11} is -C(O)O-; and

l and m are each 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is $(C_1\text{-}C_{12})$alkyl and V^8 is -CONH-;

T^9 is substituted $(C_1\text{-}C_{12})$alkyl and V^9 is -CO-;

T^{10} is PABC and V^{10} is absent (*e.g.*, a covalent bond); and

k, l, and m are each 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is $(C_1\text{-}C_{12})$alkyl and V^8 is absent (*e.g.*, a covalent bond);

T^9 is heteroaryl and V^9 is absent (*e.g.*, a covalent bond);

T^{10} is (C_1-C_{12})alkyl and V^{10} is -CONH-;

T^{11} is $(PEG)_n$ and V^{11} is -CO-; and

l and m are each 0; or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is absent (*e.g.*, a covalent bond);

T^9 is heteroaryl and V^9 is absent (*e.g.*, a covalent bond);

T^{10} is (C_1-C_{12})alkyl and V^{10} is -CONH-;

T^{11} is substituted (C_1-C_{12})alkyl and V^{11} is -CO-;

T^{12} is $(AA)_p$ and V^{12} is absent (*e.g.*, a covalent bond); and

T^{13} PAB and V^{13} is absent (*e.g.*, a covalent bond); or

wherein:

T^7 is absent (*e.g.*, a covalent bond) and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is absent (*e.g.*, a covalent bond);

T^9 is heteroaryl and V^9 is absent (*e.g.*, a covalent bond);

T^{10} is (C_1-C_{12})alkyl and V^{10} is -CONH-;

T^{11} is substituted (C_1-C_{12})alkyl and V^{11} is -CO-;

T^{12} is $(AA)_p$ and V^{12} is absent (*e.g.*, a covalent bond); and

T^{13} PABC and V^{13} is absent (*e.g.*, a covalent bond).

[00435] In certain embodiments, the left-hand side of the above linker structure for the second linker L^B is attached to the hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety, and the right-hand side of the above linker structure for the second linker L^B is attached to the second drug or active agent.

[00436] In certain embodiments, the conjugate is an antibody-drug conjugate where the ROR antibody, such as the activatable ROR antibody, and the drugs are linked together by linkers as described above. In some instances, the linker (*e.g.*, L^A and/or L^B) is a cleavable linker. A cleavable linker is a linker that includes one or more cleavable moieties, where the cleavable moiety includes one or more bonds that can dissociate under certain conditions, thus separating the cleavable linker into two or more separable portions. For example, the cleavable moiety may include one or more covalent bonds, which under certain conditions, can dissociate or break apart to separate the cleavable linker into two or more portions. As such the linkers that are included in an antibody-drug conjugate can be cleavable linkers,

such that under appropriate conditions, the cleavable linker is cleaved to separate or release the drug from the antibody at a desired target site of action for the drug.

[00437] In some instances, a cleavable linker includes two cleavable moieties, such as a first cleavable moiety and a second cleavable moiety. The cleavable moieties can be configured such that cleavage of both cleavable moieties is needed in order to separate or release the drug from the ROR antibody, such as the activatable ROR antibody, at a desired target site of action for the drug. For example, cleavage of a cleavable linker can be achieved by initially cleaving one of the two cleavable moieties and then cleaving the other of the two cleavable moieties. In certain embodiments, a cleavable linker includes a first cleavable moiety and a second cleavable moiety that hinders cleavage of the first cleavable moiety. By "hinders cleavage" is meant that the presence of an uncleaved second cleavable moiety reduces the likelihood or substantially inhibits the cleavage of the first cleavable moiety, thus substantially reducing the amount or preventing the cleavage of the cleavable linker. For instance, the presence of uncleaved second cleavable moiety can hinder cleavage of the first cleavable moiety. The hinderance of cleavage of the first cleavable moiety by the presence of the second cleavable moiety, in turn, substantially reduces the amount or prevents the release of the drug from the antibody. For example, the premature release of the drug from the antibody can be substantially reduced or prevented until the antibody-drug conjugate is at or near the desired target site of action for the drug.

[00438] In some cases, since the second cleavable moiety hinders cleavage of the first cleavable moiety, cleavage of the cleavable linker can be achieved by initially cleaving the second cleavable moiety and then cleaving the first cleavable moiety. Cleavage of the second cleavable moiety can reduce or eliminate the hinderance on the cleavage of the first cleavable moiety, thus allowing the first cleavable moiety to be cleaved. Cleavage of the first cleavable moiety can result in the cleavable linker dissociating or separating into two or more portions as described above to release the drug from the antibody-drug conjugate. In some instances, cleavage of the first cleavable moiety does not substantially occur in the presence of an uncleaved second cleavable moiety. By substantially is meant that about 10% or less cleavage of the first cleavable moiety occurs in the presence of an uncleaved second cleavable moiety, such as about 9% or less, or about 8% or less, or about 7% or less, or about 6% or less, or about 5% or less, or about 4% or less, or about 3% or less, or about 2% or less, or about 1% or less, or about 0.5% or less, or about 0.1% or less cleavage of the first cleavable moiety occurs in the presence of an uncleaved second cleavable moiety.

[00439] Stated another way, the second cleavable moiety can protect the first cleavable moiety from cleavage. For instance, the presence of uncleaved second cleavable moiety can protect the first cleavable moiety from cleavage, and thus substantially reduce or prevent premature release of the drug from the antibody until the antibody-drug conjugate is at or near the desired target site of action for the drug. As such, cleavage of the second cleavable moiety exposes the first cleavable moiety (*e.g.*, deprotects the first cleavable moiety), thus allowing the first cleavable moiety to be cleaved, which results in cleavage of the cleavable linker, which, in turn, separates or releases the drug from the antibody at a desired target site of action for the drug as described above. In certain instances, cleavage of the second cleavable moiety exposes the first cleavable moiety to subsequent cleavage, but cleavage of the second cleavable moiety does not in and of itself result in cleavage of the cleavable linker (*e.g.*, cleavage of the first cleavable moiety is still needed in order to cleave the cleavable linker).

[00440] The cleavable moieties included in the cleavable linker may each be an enzymatically cleavable moiety. For example, the first cleavable moiety can be a first enzymatically cleavable moiety and the second cleavable moiety can be a second enzymatically cleavable moiety. An enzymatically cleavable moiety is a cleavable moiety that can be separated into two or more portions as described above through the enzymatic action of an enzyme. The enzymatically cleavable moiety can be any cleavable moiety that can be cleaved through the enzymatic action of an enzyme, such as, but not limited to, an ester, a peptide, a glycoside, and the like. In some instances, the enzyme that cleaves the enzymatically cleavable moiety is present at a desired target site of action, such as the desired target site of action of the drug that is to be released from the antibody-drug conjugate. In some cases, the enzyme that cleaves the enzymatically cleavable moiety is not present in a significant amount in other areas, such as in whole blood, plasma, or serum. As such, the cleavage of an enzymatically cleavable moiety can be controlled such that substantial cleavage occurs at the desired site of action, whereas cleavage does not significantly occur in other areas or before the antibody-drug conjugate reaches the desired site of action.

[00441] For example, as described herein, antibody-drug conjugates of the present disclosure can be used for the treatment of cancer, such as for the delivery of a cancer therapeutic drug to a desired site of action where the cancer cells are present. In some cases, enzymes, such as an esterase that cleaves ester bonds or a glycosidase that cleaves glycosidic bonds, can be a biomarker for cancer that is overexpressed in cancer cells. The overexpression, and thus localization, of certain enzymes in cancer can be used in the context

of the enzymatically cleavable moieties included in the cleavable linkers of the antibody-drug conjugates of the present disclosure to specifically release the drug at the desired site of action (*e.g.*, the site of the cancer (and overexpressed enzyme)). Thus, in some embodiments, the enzymatically cleavable moiety is a cleavable moiety (*e.g.*, an ester or a glycoside) that can be cleaved by an enzyme that is overexpressed in cancer cells. For instance, the enzyme can be an esterase. As such, in some instances, the enzymatically cleavable moiety is a cleavable moiety (*e.g.*, an ester) that can be cleaved by an esterase enzyme. In some instances, the enzyme can be a glycosidase. As such, in some instances, the enzymatically cleavable moiety is a cleavable moiety (*e.g.*, a glycoside or glycoside derivative) that can be cleaved by a glycosidase enzyme.

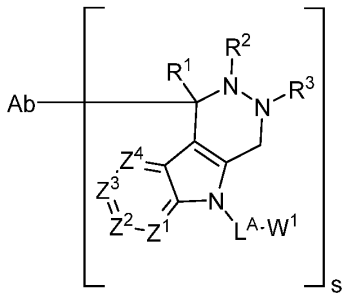
[00442] In certain embodiments, the enzymatically cleavable moiety is an ester bond. For example, the first cleavable moiety described above (*e.g.*, the cleavable moiety protected from premature cleavage by the second cleavable moiety) can include an ester. The presence of uncleaved second cleavable moiety can protect the first cleavable moiety (ester) from cleavage by an esterase enzyme, and thus substantially reduce or prevent premature release of the drug from the antibody until the antibody-drug conjugate is at or near the desired target site of action for the drug. In some instances, a portion of the linker adjacent to the first cleavable moiety is linked to or includes a substituent, where the substituent comprises the second cleavable moiety. In some instances, the second cleavable moiety includes a glycoside or glycoside derivative.

[00443] In some embodiments, the enzymatically cleavable moiety is sugar moiety, such as a glycoside (or glyosyl) or glycoside derivative. In some cases, the glycoside or glycoside derivative can facilitate an increase in the hydrophilicity of the cleavable linker as compared to a cleavable linker that does not include the glycoside or glycoside derivative. The glycoside or glycoside derivative can be any glycoside or glycoside derivative suitable for use in the cleavable linker and that can be cleaved through the enzymatic action of an enzyme. For example, the second cleavable moiety (*e.g.*, the cleavable moiety that protects the first cleavable moiety from premature cleavage) can be a glycoside or glycoside derivative. For instance, in some embodiments, the first cleavable moiety includes an ester, and the second cleavable moiety includes a glycoside or glycoside derivative. In certain embodiments, the second cleavable moiety is a glycoside or glycoside derivative selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc. In some instances, the second cleavable moiety is a glucuronide. In some instances, the second cleavable moiety is a galactoside. In some instances, the second cleavable moiety

is a glucoside. In some instances, the second cleavable moiety is a mannoside. In some instances, the second cleavable moiety is a fucoside. In some instances, the second cleavable moiety is O-GlcNAc. In some instances, the second cleavable moiety is O-GalNAc.

[00444] The glycoside or glycoside derivative can be attached (covalently bonded) to the cleavable linker through a glycosidic bond. The glycosidic bond can link the glycoside or glycoside derivative to the cleavable linker through various types of bonds, such as, but not limited to, an O-glycosidic bond (an O-glycoside), an N-glycosidic bond (a glycosylamine), an S-glycosidic bond (a thioglycoside), or C-glycosidic bond (a C-glycoside or C-glycosyl). In some instances, the glycosidic bond is an O-glycosidic bond (an O-glycoside). In some cases, the glycoside or glycoside derivative can be cleaved from the cleavable linker it is attached to by an enzyme (*e.g.*, through enzymatically mediated hydrolysis of the glycosidic bond). A glycoside or glycoside derivative can be removed or cleaved from the cleavable linker by any convenient enzyme that is able to carry out the cleavage (hydrolysis) of the glycosidic bond that attaches the glycoside or glycoside derivative to the cleavable linker. An example of an enzyme that can be used to mediate the cleavage (hydrolysis) of the glycosidic bond that attaches the glycoside or glycoside derivative to the cleavable linker is a glycosidase, such as a glucuronidase, a galactosidase, a glucosidase, a mannosidase, a fucosidase, and the like. Other suitable enzymes may also be used to mediate the cleavage (hydrolysis) of the glycosidic bond that attaches the glycoside or glycoside derivative to the cleavable linker. In some cases, the enzyme used to mediate the cleavage (hydrolysis) of the glycosidic bond that attaches the glycoside or glycoside derivative to the cleavable linker is found at or near the desired site of action for the drug of the antibody-drug conjugate. For instance, the enzyme can be a lysosomal enzyme, such as a lysosomal glycosidase, found in cells at or near the desired site of action for the drug of the antibody-drug conjugate. In some cases, the enzyme is an enzyme found at or near the target site where the enzyme that mediates cleavage of the first cleavable moiety is found.

[00445] In some embodiments, an ROR-ADC, such as an activatable ROR-ADC, is represented by Formula (I):



(I)

wherein:

Ab represents an antibody that binds to ROR, such as an activatable antibody that binds to ROR when the ROR-ADC is activatable;

Z^1, Z^2, and Z^4 are each independently CR^4;

Z^3 is C-L^B-W^2;

R^1, R^2, R^3 and R^4 are each selected from hydrogen and (C_1-C_{12})alkyl;

L^A is a first linker wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is substituted (C_1-C_{12})alkyl and V^2 is -CO-;

T^3 is $(AA)_p$ where p is an integer from 1-20 and V^3 is a covalent bond;

T^4 is PABC and V^4 is a covalent bond;

a, b, c, and d are each 1;

e and f are each 0; and

L^B is a second linker wherein:

T^7 is a covalent bond and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is -CONH-;

T^9 is substituted (C_1-C_{12})alkyl and V^9 is -CO-;

T^{10} is $(AA)_p$ where p is an integer from 1-20 and V^{10} is a covalent bond;

T^{11} is PABC and V^{11} is a covalent bond; and

h, i, j, and k are each 1; and

l and m are each 0;

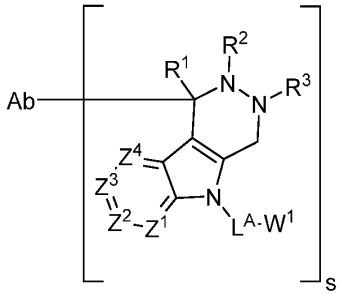
s is an integer from 1 to 10;

W^1 is a first drug; and

W^2 is a second drug.

[00446] In some embodiments, one or both of W^1 and W^2 are camptothecin analogues, for example, belotecan.

[00447] In some embodiments, an ROR-ADC, *e.g.*, activatable ROR-ADC, is represented by Formula (I):



(I)

wherein:

Ab represents an antibody, *e.g.*, activatable antibody, that binds to ROR;

Z^1, Z^2, and Z^4 are each independently CR^4;

Z^3 is C-L^B-W^2;

R^1, R^2, R^3 and R^4 are each selected from hydrogen and (C_1-C_{12})alkyl;

L^A is a linker wherein:

T^1 is (C_1-C_6)alkyl and V^1 is -CONH-;

T^2 is (C_1-C_6)alkylene substituted with -NHCO(PEG)$_k$, wherein k is an integer from 2 to 10 and V^2 is -CO-;

T^3 is $(AA)_2$ and V^3 is a covalent bond;

T^4 is PABC substituted with a glycoside and V^4 is a covalent bond;

a, b, c, and d are each 1; and

e and f are each 0; and

L^B is a linker wherein:

T^7 is a covalent bond and V^7 is -NHCO-;

T^8 is (C_1-C_6)alkyl and V^8 is -CONH-;

T^9 is (C_1-C_6)alkylene substituted with -NHCO(PEG)$_k$, wherein k is an integer from 2 to 10 and V^9 is -CO-;

T^{10} is $(AA)_2$ and V^{10} is a covalent bond;

T^{11} is PABC substituted with a glycoside and V^{11} is a covalent bond;

h, i, j, and k are each 1; and

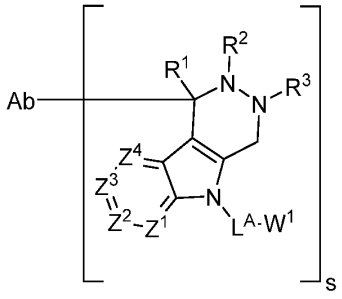
l and m are each 0;

s is an integer from 1 to 10;

W^1 is a first drug; and

W^2 is a second drug.

[00448] In some embodiments, an ROR-ADC, such as an activatable ROR-ADC is represented by Formula (I):



(I)

wherein:

Ab represents an antibody that binds to ROR, such as an activatable antibody that binds to ROR when the ROR-ADC is activatable;

Z^1, Z^2, and Z^4 are each independently CR^4;

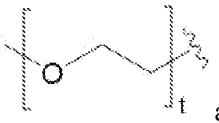
Z^3 is C-L^B-W^2;

R^1, R^2, R^3 and R^4 are each selected from hydrogen and (C_1-C_{12})alkyl;

L^A is a linker wherein:

T^1 is (C_1-C_6)alkyl and V^1 is -CONH-;

T^2 is (C_1-C_6)alkylene substituted with -NHCO(PEG)$_t$, wherein (PEG)$_t$ is



and t is an integer from 2 to 10, optionally 8, and V^2 is -CO-;

T^3 is $(AA)_2$ and V^3 is a covalent bond;

T^4 is PABC substituted with a glycoside and V^4 is a covalent bond;

a, b, c, and d are each 1; and

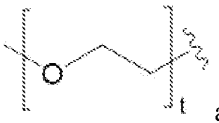
e and f are each 0; and

L^B is a linker wherein:

T^7 is a covalent bond and V^7 is -NHCO-;

T^8 is (C_1-C_6)alkyl and V^8 is -CONH-;

T^9 is (C_1-C_6)alkylene substituted with -NHCO(PEG)$_t$, wherein (PEG)$_t$ is



and t is an integer from 2 to 10, optionally 8, and V^9 is -CO-;

T^{10} is $(AA)_2$ and V^{10} is a covalent bond;

T^{11} is PABC substituted with a glycoside and V^{11} is a covalent bond;

h, i, j, and k are each 1; and

l and m are each 0;

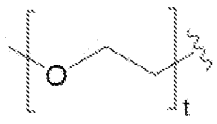
s is an integer from 1 to 10;

W^1 is a first drug; and

W^2 is a second drug.

[00449] In some embodiments, the PABC of one or both of T^4 and T^{11} is substituted with a glucuronide. In some embodiments, one or both of T^1 and T^8 is ethyl. In some embodiments, one or both of T^2 and T^9 is C_5 alkylene substituted with -NHCO$(PEG)_k$, wherein k is an integer from 5 to 10. In some embodiments, one or both of W^1 and W^2 are camptothecin analogues, for example, belotecan.

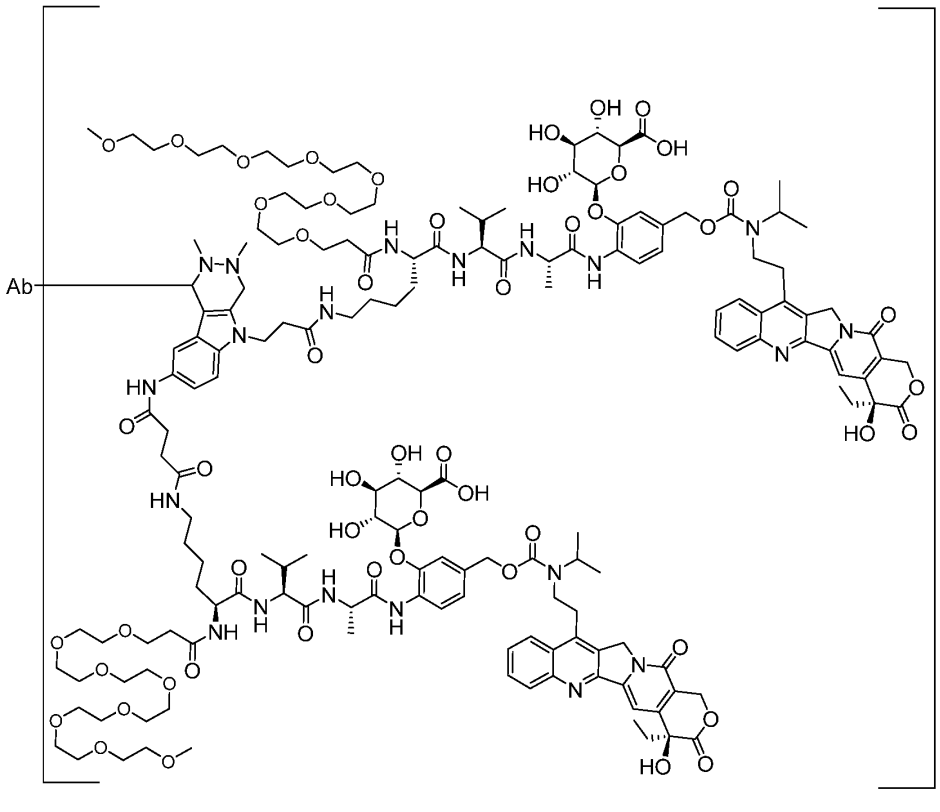
[00450] In some embodiments, the PABC of one or both of T^4 and T^{11} is substituted with a glucuronide. In some embodiments, one or both of T^1 and T^8 is ethyl. In some embodiments, one or both of T^2 and T^9 is C_5 alkylene substituted with -NHCO$(PEG)_t$, wherein $(PEG)_t$ is



and t is an integer from 5 to 10, optionally. In some embodiments, one or both of W^1 and W^2 are camptothecin analogues, for example, belotecan.

[00451] In some embodiments, s is an integer from 1 to 4. In further embodiments, s is 4.

[00452] In some embodiments, an ROR-ADC is represented by Formula (II):



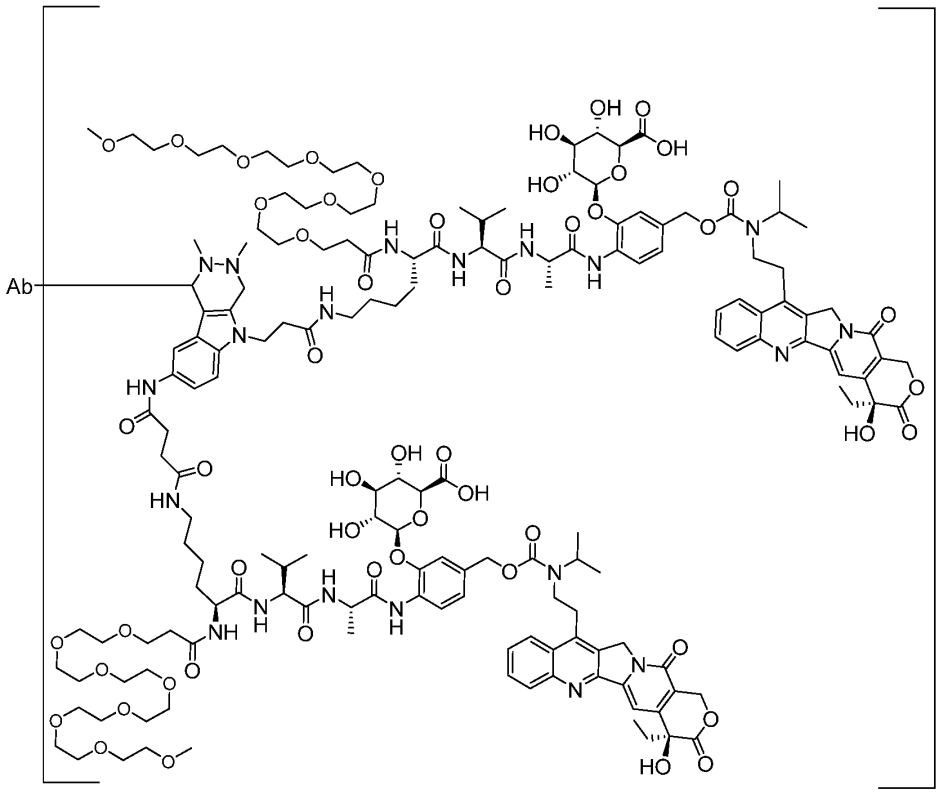
, (II),

wherein:

Ab represents an antibody that binds to ROR; and

s is an integer from 1 to 10.

[00453] In some embodiments, an activatable ROR-ADC is represented by Formula (II):



, (II),

wherein:

Ab represents an activatable antibody that binds to ROR; and

s is an integer from 1 to 10.

[00454] In some embodiments, s is an integer from 1 to 4.

[00455] In further embodiments, s is 4.

[00456] Any of the chemical entities, linkers and conjugation moieties set forth in the structures above may be adapted for use in the subject compounds and conjugates.

[00457] Additional disclosure related to hydrazinyl-indolyl and hydrazinyl-pyrrolo-pyridinyl compounds and methods for producing a conjugate is found in U.S. Patent No. 9,310,374, U.S. Patent No. 9,493,413, U.S. Patent NO. 11,564,989, and International Publication Number WO 2022/187370, the disclosures of each of which are incorporated herein by reference.

7.3 TYROSINE-PROTEIN KINASE MEMBRANE RECEPTOR (ROR) ANTIGENS

[00458] The ROR antibodies, such as the activatable ROR antibodies (*e.g.*, after activation), described herein bind specifically to one or more ROR antigens, such as ROR1, or ROR2, or both ROR1 and ROR2.

[00459] ROR1 and ROR2 proteins typically consist of at least four protein domains: three extracellular domains -- Ig-like, FZ, and Kringle domains -- as well as the intracellular Protein Kinase domain. In some embodiments, the ROR binding agent, such as the activatable ROR binding agent (*e.g.*, after activation), has antigen binding sites that specifically bind to the extracellular portion of the ROR antigen. In certain embodiments, the ROR binding agent, such as the activatable ROR binding agent (*e.g.*, after activation), has antigen binding sites that specifically bind to the Ig-like domain. In other embodiments, the ROR binding agent, such as the activatable ROR binding agent (*e.g.*, after activation), has antigen binding sites that specifically bind to the FZ domain. In still other embodiments, the ROR binding agent, such as the activatable ROR binding agent (*e.g.*, after activation), has antigen binding sites that specifically bind to the Kringle domain. In particular embodiments, the ROR binding agent, such as the activatable ROR binding agent (*e.g.*, after activation), has antigen binding sites that specifically bind to at least a portion of a single ROR domain. In particular embodiments, the ROR binding agent, such as the activatable ROR binding agent (*e.g.*, after activation), has antigen binding sites that specifically bind to at least a portion of more than one ROR domain, such as the junction between a first and a second ROR domain. The ROR domains can refer to ROR1 domains or ROR2 domains.

[00460] In specific embodiments, the ROR antigen is human. UniProt accession #Q01973 describes a canonical human ROR1 protein, including its sequences and domain features, and is hereby incorporated by reference in its entirety. SEQ ID NO:40 provides the full-length ROR1 protein sequence (*see* **Table 10**). With reference to the full-length sequence from the N-terminus to C-terminus, the Ig-Like domain is defined as amino acids 42-147, the FZ domain as amino acids 165-299, and the Kringle domain as amino acids 312-391. UniProt accession #Q01974 describes a canonical human ROR2 protein, including its sequences and domain features, and is hereby incorporated by reference in its entirety. SEQ ID NO:41 provides the full-length ROR2 protein sequence (*see* **Table 10**). With reference to the full-length sequence from the N-terminus to C-terminus, the Ig-Like domain is defined as amino acids 55-145, the FZ domain as amino acids 169-303, and the Kringle domain as amino acids 316-394.

[00461] Various tumors can demonstrate cell-surface expression of ROR antigens, as described in greater detail in Gentile, *et al.* (*Cancer Res*; 71(8) April 15, 2011), Rebagay, *et al.* (*Front. Oncol.*, 18 April 2012), Zhang, *et al.* (*American Journal of Pathology,* Vol. 181, No. 6, December 2012), Henry, *et al.* (*Oncotarget*, Vol. 6, No. 37 2015), Zhang, *et al.* (*PLoS ONE* 7(3): e31127.), and Bainbridge, *et al.* (*PLoS ONE* 9(7): e102695.), each herein

incorporated by reference in their entirety. In addition, ROR expression may not be expressed, or only demonstrate limited expression, in normal, *e.g.*, non-cancerous, tissue as described in Balakrishnan *et al.* (*Clin Cancer Res*. 2017 Jun 15; 23(12): 3061–3071), herein incorporated in its entirety. Thus, ROR antigens can be used as a tumor-specific marker in certain tumors. Examples of tumors and cancers with demonstrated ROR expression include, but are not limited to, pancreatic cancer, ovarian cancer, breast cancer, lung cancer, gastric cancer, melanoma, Ewing sarcoma, chronic lymphocytic leukemia, mantle cell lymphoma, and B-ALL, as described in Gohil *et al.* (*Oncoimmunology*. 2017; 6(7): e1326437.), herein incorporated in its entirety. Other cancers include, but are not limited to, hematological cancer, prostate cancer, colon cancer, renal cancer, and uterine cancer.

7.4 TYROSINE-PROTEIN KINASE MEMBRANE RECEPTOR (ROR) ANTIBODIES

[00462] As noted above, a subject conjugate comprises an antibody (Ab) that binds to ROR, such as an activatable antibody (Ab) that binds to ROR. The amino acid sequence of the antibody can be modified to include a 2-formylglycine (fGly) residue. As used herein, amino acids may be referred to by their standard name, their standard three letter abbreviation and/or their standard one letter abbreviation, such as: Alanine or Ala or A; Cysteine or Cys or C; Aspartic acid or Asp or D; Glutamic acid or Glu or E; Phenylalanine or Phe or F; Glycine or Gly or G; Histidine or His or H; Isoleucine or Ile or I; Lysine or Lys or K; Leucine or Leu or L; Methionine or Met or M; Asparagine or Asn or N; Proline or Pro or P; Glutamine or Gln or Q; Arginine or Arg or R; Serine or Ser or S; Threonine or Thr or T; Valine or Val or V; Tryptophan or Trp or W; and Tyrosine or Tyr or Y.

[00463] In various aspects, the present disclosure also relates to binding polypeptides (*e.g.*, antibodies) that bind to ROR (*e.g.*, human ROR), including antibodies comprising any of the anti-ROR antibodies described herein, antigen binding fragments of the anti-ROR antibodies, and/or derivatives of the anti-ROR antibodies.

[00464] In various aspects, the present disclosure also relates to activatable binding polypeptides (*e.g.*, activatable antibodies) that bind to ROR (*e.g.*, human ROR), including activatable antibodies comprising any of the anti-ROR antibodies described herein, antigen binding fragments of the activatable anti-ROR antibodies, and/or derivatives of the activatable anti-ROR antibodies. In some embodiments, the activatable anti-ROR antibodies described herein may have improved safety profiles. For example, the anti-ROR antibodies described herein may have better safety margin, for example not binding to pancreas (*e.g.*, human pancreatic islets) when in inactive form, or have weaker binding to pancreas (*e.g.*,

human pancreatic islets) when in inactive form relative to when in active form. The binding to pancreas (*e.g.*, human pancreatic islets) may be measured by any suitable method, for example, IHZ, immunohistochemistry (IHC), or immunofluorescence (IF) staining.

[00465] In some embodiments, the ROR antibody of the present disclosure comprises a heavy chain variable region comprising one, two, or three of the heavy chain variable region CDRs described herein, and a light chain variable region comprising one, two, or three of the light chain variable region CDRs described herein (*e.g.*, one, two, or three of the heavy chain variable region CDR sequences, and/or one, two, or three of the light chain variable region CDR sequences as shown in **Table 1**, including all six CDRs of any of the exemplary antibodies as shown in **Table 1**). In some embodiments, the ROR antibody of the present disclosure comprises a heavy chain variable region comprising any of the heavy chain variable region sequences described herein, and a light chain variable region comprising any of the light chain variable region sequences described herein (*e.g.*, a heavy chain variable region sequence and/or a light chain variable region sequence as shown in **Table 1**).

[00466] In some embodiments, the ROR antibody of the present disclosure comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH), wherein the VH and VL forms a binding domain that binds to ROR. In some embodiments, the VH and VL are covalently linked, *e.g.*, in an scFv. In some embodiments, the VH and VL are not covalently linked. In some embodiments, the VH and VL form a Fab fragment. In some embodiments, the VH is linked to an antibody heavy chain constant region, and the VL is linked to an antibody light chain constant region.

[00467] In some embodiments, an activatable antibody of the present disclosure comprises: (a) a masking peptide comprising a masking moiety (MM) and a cleavable moiety (CM); and (b) a target binding moiety (TBM). In some embodiments, the CM is any of the cleavable moieties described herein. In some embodiments, the TBM is any of the target binding moieties described herein (*e.g.*, a target binding moiety (TBM) comprising an antibody light chain variable region and/or an antibody heavy chain variable region, such as a VH and/or VL of any of the anti-ROR antibodies described herein). In some embodiments, the MM interferes with and/or inhibits the binding of the activatable antibody to its target (*e.g.*, human ROR) when the CM is not cleaved. In some embodiments, the activatable antibody is capable of binding to its target (*e.g.*, human ROR) when the CM is cleaved. In some embodiments, MM and CM are disclosed as in International Publication Number WO 2019/149282, which is incorporated by reference in its entirety.

[00468] In some embodiments, an activatable antibody of the present disclosure comprises: (a) a masking moiety (MM); (b) a cleavable moiety (CM); and (c) a target binding moiety (TBM). In some embodiments, the MM is any of the masking moieties described herein. In some embodiments, the CM is any of the cleavable moieties described herein. In some embodiments, the TBM is any of the target binding moieties described herein (*e.g.*, a target binding moiety (TBM) comprising an antibody light chain variable region and/or an antibody heavy chain variable region, such as a VH and/or VL of any of the anti-ROR antibodies described herein). In some embodiments, the MM interferes with and/or inhibits the binding of the activatable antibody to its target (*e.g.*, human ROR) when the CM is not cleaved. In some embodiments, the activatable antibody is capable of binding to its target (*e.g.*, human ROR) when the CM is cleaved.

[00469] In some embodiments, MM and CM are disclosed as in International Publication Number WO 2019/149282, which is incorporated by reference in its entirety.

[00470] In some embodiments, a polypeptide comprising, for example from its N terminus to its C terminus, an MM and a CM is referred to herein as a masking peptide.

[00471] In some embodiments, the activatable antibody comprises: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), where the MM is any of the masking moieties described herein, the CM is any of the cleavable moieties described herein, and where the TBM comprises an antibody light chain variable region (VL); and (b) an antibody heavy chain variable region (VH).

[00472] In some embodiments, the activatable antibody comprises: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), where the MM is any of the masking moieties described herein, the CM is any of the cleavable moieties described herein, and where the TBM comprises an antibody heavy chain variable region (VH); and (b) an antibody light chain variable region (VL).

[00473] In some embodiments, the activatable antibody comprises: a polypeptide comprising, from N-terminus to C-terminus, a masking moiety (MM), a cleavable moiety (CM), and a target binding moiety (TBM), where the MM is any of the masking moieties described herein, the CM is any of the cleavable moieties described herein, and where the TBM comprises an antibody heavy chain variable region (VH) and an antibody light chain variable region (VL).

[00474] The term "activatable binding polypeptide", "ABP", or "activatable antibody" includes a polypeptide that comprises a target binding moiety (TBM), a cleavable moiety (CM), and a masking moiety (MM). In some embodiments, the TBM comprises an amino acid sequence that binds to a target, such as ROR. In some embodiments, the TBM comprises an antigen binding domain (ABD) of an antibody or antibody fragment thereof (*e.g.*, any of the antibodies or antigen binding fragments described herein). In some embodiments, the antigen binding domain comprises a heavy chain variable region comprising one, two, or three of the heavy chain variable region CDRs described herein, and a light chain variable region comprising one, two, or three of the light chain variable region CDRs described herein (*e.g.*, one, two, or three of the heavy chain variable region CDR sequences, and/or one, two, or three of the light chain variable region CDR sequences as shown in **Table 1**, including all six CDRs of any of the exemplary antibodies as shown in **Table 1**). In some embodiments, the antigen binding domain comprises a heavy chain variable region comprising any of the heavy chain variable region sequences described herein, and a light chain variable region comprising any of the light chain variable region sequences described herein (*e.g.*, a heavy chain variable region sequence and/or a light chain variable region sequence as shown in **Table 1**). In some embodiments, the TBM (*e.g.*, comprising an ABD) comprises an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH), wherein the VH and VL forms a binding domain that binds to the target in the absence of the MM. In some embodiments, the VH and VL are covalently linked, *e.g.*, in an scFv. In some embodiments, the VH and VL are not covalently linked. In some embodiments, the VH and VL form a Fab fragment. In some embodiments, the VH is linked to an antibody heavy chain constant region, and the VL is linked to an antibody light chain constant region.

[00475] In some embodiments, the activatable antibody comprises a polypeptide comprising the structure, from N-terminus to C-terminus, of: masking moiety (MM)-cleavable moiety (CM)-VL, and the activatable antibody further comprises a second polypeptide comprising a VH (*e.g.*, a Fab fragment). In some embodiments, the activatable antibody comprises a polypeptide comprising the structure, from N-terminus to C-terminus, of: masking moiety (MM)-cleavable moiety (CM)-VL-VH (*e.g.*, an scFv). In some embodiments, the activatable antibody comprises a polypeptide comprising the structure, from N-terminus to C-terminus, of: masking moiety (MM)-cleavable moiety (CM)-VH, and the activatable antibody further comprises a second polypeptide comprising a VL (*e.g.*, a Fab fragment). In some embodiments, the activatable antibody comprises a polypeptide comprising the structure,

from N-terminus to C-terminus, of: masking moiety (MM)-cleavable moiety (CM)-VH-VL (*e.g.*, an scFv).

[00476] The CM generally includes an amino acid sequence that is cleavable, for example, serves as the substrate for an enzyme and/or a cysteine-cysteine pair capable of forming a reducible disulfide bond. As such, when the terms "cleavage", "cleavable", "cleaved" and the like are used in connection with a CM, the terms encompass enzymatic cleavage, *e.g.*, by a protease (for example, MMP9), as well as disruption of a disulfide bond between a cysteine-cysteine pair via reduction of the disulfide bond that can result from exposure to a reducing agent.

[00477] The MM generally refers to an amino acid sequence that, when the CM of the activatable antibody is intact (*e.g.*, uncleaved by a corresponding enzyme, and/or containing an unreduced cysteine-cysteine disulfide bond), the MM interferes with or inhibits binding of the TBM to its target. In some embodiments, the MM interferes with or inhibits binding of the TBM to its target so efficiently that binding of the TBM to its target is extremely low and/or below the limit of detection (*e.g.*, binding cannot be detected in an ELISA or flow cytometry assay). The amino acid sequence of the CM may overlap with or be included within the MM. It should be noted that for sake of convenience "ABP" or "activatable antibody" are used herein to refer to an ABP or activatable antibody in both their uncleaved (or "native") state, as well as in their cleaved state, depending on the context. It will be apparent to the ordinarily skilled artisan that in some embodiments a cleaved ABP may lack an MM due to cleavage of the CM, *e.g.*, by a protease, resulting in release of at least the MM (*e.g.*, where the MM is not joined to the ABP by a covalent bond (*e.g.*, a disulfide bond between cysteine residues)). Exemplary ABPs are described in more detail below.

[00478] In some embodiments, the masking moiety (MM) interferes with, obstructs, reduces the ability of, prevents, inhibits, or competes with the target binding moiety for binding to its target (*e.g.*, an "inactive activatable antibody). In some embodiments, the masking moiety (MM) interferes with, obstructs, reduces, prevents, inhibits, or competes with the target binding moiety for binding to its target only when the polypeptide has not been activated (*e.g.*, activated by a change in pH (increased or decreased), activated by a temperature shift (increased or decreased), activated after being contacted with a second molecule (such as a small molecule or a protein ligand), etc.). In some embodiments, activation induces cleavage of the polypeptide within the cleavage moiety. In some embodiments, activation induces conformation changes in the polypeptide (*e.g.*, displacement of the masking moiety (MM)), leading to the masking moiety no longer preventing the activatable antibody from binding to

its target. In some embodiments, the masking moiety (MM) interferes with, obstructs, reduces the ability of, prevents, inhibits, or competes with the target binding moiety for binding to its target only when the cleavable moiety (CM) has not been cleaved by one or more proteases that cleave within the cleavable moiety (CM). In some embodiments, the masking moiety (MM) has a masking efficiency of at least about 2.0 (*e.g.*, at least about 2.0, at least about 3.0, at least about 4.0, at least about 5.0, at least about 6.0, at least about 7.0, at least about 8.0, at least about 9.0, at least about 10, at least about 25, at least about 50, at least about 75, at least about 100, at least about 150, at least about 200, at least about 300, at least about 400, at least about 500, etc.) prior to activation. In some embodiments, masking efficiency is measured as the difference in affinity of an activatable antibody comprising the masking moiety (MM) for binding its target (before activation) relative to the affinity of a polypeptide lacking the masking moiety for binding its target (*e.g.*, the difference in affinity for a target antigen (such as ROR) of an activatable antibody comprising a masking moiety (MM) (before activation) relative to a parental antibody lacking the masking moiety (MM), or the difference in affinity for a target antigen (such as ROR) of an activatable antibody comprising a masking moiety (MM) (before activation) relative to the affinity for the target antigen of the activatable antibody after activation). In some embodiments, the masking efficiency is measured by dividing the EC_{50} for binding of an activatable antibody comprising a masking moiety (MM) (before activation) by the EC_{50} of the parental antibody (*e.g.*, by measuring EC_{50} by ELISA). In some embodiments, masking efficiency is measured as the difference in affinity of an activatable antibody comprising the masking moiety (MM) for binding its target before activation relative to the affinity of the activatable antibody comprising the masking moiety (MM) for binding its target after activation (*e.g.*, the difference in affinity for a target antigen (such as ROR) of an activatable antibody before activation relative to the activatable antibody after activation). In some embodiments, the masking moiety (MM) binds to the target binding moiety (TBM) and prevents the activatable antibody from binding to its target (*e.g.*, an "inactive" activatable antibody). In some embodiments, the masking moiety (MM) has a dissociation constant for binding to the target binding moiety (TBM) that is greater than the dissociation constant of the target binding moiety (TBM) for its target.

[00479] In some embodiments, the masking moiety (MM) does not interfere with, obstruct, reduce the ability of, prevent, inhibit, or compete with the target binding moiety (TBM) for binding to its target after the activatable antibody has been activated (*e.g.*, activated by treatment with one or more proteases that cleave within the cleavable moiety (CM), activated by a change in pH (increased or decreased), activated by a temperature shift (increased or

decreased), activated after being contacted with a second molecule (such as an enzyme or a protein ligand), etc.). In some embodiments, the masking moiety (MM) does not interfere with, obstruct, reduce the ability of, prevent, inhibit, or compete with the target binding moiety (TBM) for binding its target after the cleavable moiety (CM) has been cleaved by one or more proteases that cleave within the cleavable moiety (CM). In some embodiments, the masking moiety (MM) has a masking efficiency of at most about 1.75 (*e.g.*, at most about 1.75, at most about 1.5, at most about 1.4, at most about 1.3, at most about 1.2, at most about 1.1, at most about 1.0, at most about 0.9, at most about 0.8, at most about 0.7, at most about 0.6, or at most about 0.5, etc.) after activation (*e.g.*, the relative affinity of the activatable antibody after activation as compared to the affinity of a parental antibody).

[00480] In some embodiments, activatable antibodies of the present disclosure are context-dependent (*e.g.*, are activated (are only capable of binding their targets) in certain contexts (such as in the protease-rich tumor microenvironment)). In some embodiments, the activatable antibodies of the present disclosure provide improved safety over more traditional, non-activatable antibodies (*e.g.*, show reduced toxicity, do not induce significant alterations to the weights of many organs, do not alter liver histopathology, hematology, and/or blood biochemistry, etc.). In some embodiments, activatable antibodies of the present disclosure have improved pharmacokinetic properties as compared to more traditional, non-activatable antibodies (*e.g.*, have longer in vivo half-lives).

[00481] In some embodiments, the present disclosure relates to activatable antibodies that bind to human ROR when in active form (*e.g.*, the activatable antibodies are active after cleavage in the cleavable moiety (*e.g.*, with one or more proteases), but inactive prior to cleavage in the cleavable moiety (*e.g.*, with one or more proteases)). Also provided herein are one or more activatable antibodies that compete or cross-compete for binding to human ROR with one or more of the ROR-targeting activatable antibodies and/or anti-ROR antibodies described herein.

[00482] In some embodiments, the activatable antibodies bind to human, cynomolgus monkey, mouse, rat, and/or dog ROR with a K_D of about 500 nM or more when in inactive form. In some embodiments, the activatable antibodies bind to human, cynomolgus monkey, mouse, rat, and/or dog ROR with a K_D of about 500 nM or less when in active form (*e.g.*, about 500 nM or less, about 450 nM or less, about 400 nM or less, about 350 nM or less, about 300 nM or less, about 250 nM or less, about 200 nM or less, about 150 nM or less, about 100 nM or less, about 90 nM or less, about 80 nM or less, about 70 nM or less, about 60 nM or less, about 50 nM or less, about 40 nM or less, about 30 nM or less, about 25 nM or

less, about 20 nM or less, about 10 nM or less, about 9 nM or less, about 8 nM or less, about 7 nM or less, about 6 nM or less, about 5 nM or less, about 4 nM or less, about 3 nM or less, about 2 nM or less, about 1 nM or less, about 0.5 nM or less, about 0.1 nM or less, etc.) In some embodiments, the activatable antibodies bind to human, cynomolgus monkey, mouse, rat, and/or dog ROR with a K_D of about 10 nM or less when in active form. In some embodiments, the activatable antibodies bind to human ROR with a K_D of about 5 nM or less when in active form. In some embodiments, the activatable antibodies bind to human, cynomolgus monkey, mouse, rat, and/or dog ROR with a K_D of about 2 nM or less when in active form. In some embodiments, the activatable antibodies bind to human ROR with a K_D of about 1 nM or less when in active form. In some embodiments, the activatable antibodies bind to human and cynomolgus monkey ROR1 IgG like domain with a KD of about 2 nM or less (*e.g.*, about 1.5 nM or less, about 1.2 nM or less, or about 1.1 nM or less) when in active form. In some embodiments, the activatable antibodies bind to human and cynomolgus monkey ROR2 IgG like domain with a KD of about 2 nM or less (*e.g.*, about 1.5 nM or less, about 1.2 nM or less, about 1.1 nM or less, about 1.0 nM or less, about 0.9 nM or less, or about 0.8 nM or less) when in active form. In some embodiments, the activatable antibodies bind to mouse ROR1 IgG like domain with a KD of about 2 nM or less (*e.g.*, about 1.5 nM or less, about 1.2 nM or less, about 1.1 nM or less, about 1.0 nM or less, about 0.9 nM or less, or about 0.8 nM or less) when in active form. In some embodiments, the activatable antibodies bind to mouse ROR2 IgG like domain with a KD of about 100 nM or less (*e.g.*, about 90 nM or less, about 80 nM or less, about 70 nM or less, about 60 nM or less, about 50 nM or less, or about 40 nM or less) when in active form. In some embodiments, the activatable antibodies bind to rat ROR1 IgG like domain with a KD of about 2 nM or less (*e.g.*, about 1.5 nM or less, about 1.2 nM or less, about 1.1 nM or less, about 1.0 nM or less, about 0.9 nM or less, or about 0.8 nM or less) when in active form. Methods of measuring the K_D of an activatable antibody may be carried out using any method known in the art, including for example, by surface plasmon resonance, an ELISA, isothermal titration calorimetry, a filter binding assay, an EMSA, etc. In some embodiments, the K_D is measured by an ELISA. In some embodiments, the K_D is measured by biolayer interferometry (BLI). In other embodiments, the K_D is measured by surface plasmon resonance (SPR) (see *e.g.*, the Examples).

[00483] In some embodiments, the activatable antibodies remain intact in its inactive form in circulation about 24 hours, 48 hours, or 96 hours after *in vivo* administration.

[00484] In some embodiments, the activatable antibodies are capable of inhibiting tumor cell growth and/or proliferation. In some embodiments, the tumor cell growth and/or proliferation is inhibited by at least about 5% (*e.g.*, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 99%) when contacted with the activatable antibodies relative to corresponding tumor cells not contacted with the activatable antibodies (or relative to corresponding tumor cells contacted with an isotype control antibody). In some embodiments, the activatable antibodies are capable of reducing tumor volume in a subject when the subject is administered the activatable antibodies. In some embodiments, the activatable antibodies are capable of reducing tumor volume in a subject by at least about 5% (*e.g.*, at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 99%) relative to the initial tumor volume in the subject (*e.g.*, prior to administration of the activatable antibodies; as compared to a corresponding tumor in a subject administered an isotype control antibody). Methods of monitoring tumor cell growth/proliferation, tumor volume, and/or tumor inhibition are known in the art.

[00485] In some embodiments, the activatable antibodies have therapeutic effect on a cancer. In some embodiments, the activatable antibodies reduce one or more signs or symptoms of a cancer. In some embodiments, a subject suffering from a cancer goes into partial or complete remission when administered the activatable antibodies.

7.4.1 Masking moieties (MMs)

[00486] In some embodiments, the present disclosure relates to activatable antibodies comprising a masking moiety (MM). In some embodiments, the masking moiety (MM) comprises an amino acid sequence according to Formula (MM-I): $X_mCX_nCX_o$ (SEQ ID NO:49), where m is from 2-10, n is from 3-10, and o is from 1-10, where each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y. In some embodiments, X is not W, M, and/or C. In some embodiments, each X in X_m of formula (MM-I) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P and/or each X in X_n of formula (MM-I) is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P. In some embodiments, the MM comprises a polypeptide encoded by a polynucleotide sequence according to Formula (II):

$(NNK)_mTGY(NNK)_nTGY(NNK)_o$ (SEQ ID NO:50), wherein each N is independently A, G, T, or C, wherein each K is independently T or G, and wherein each Y is independently T or C. In some embodiments, m is 2, n is 8 and o is 2. In some embodiments, m is 6, n is 8 and o is 2.

[00487] In some embodiments, the masking moiety (MM) comprises an amino acid sequence according to Formula (III): $Z_mCZ_nCZ_o$ (SEQ ID NO:51), where m is from 2-10, n is from 3-10, and o is from 1-10, and each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P. In some embodiments, m is 6, n is 8 and o is 2.

[00488] In some embodiments, m is from 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-10, 5-9, 5-8, 5-7, 5-6, 6-10, 6-9, 6-8, 6-7, 7-10, 7-9, 7-8, 8-10, 8-9, or 9-10. In some embodiments, m is from 6-8. In some embodiments, m is 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, m is 6.

[00489] In some embodiments, n is from 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-10, 5-9, 5-8, 5-7, 5-6, 6-10, 6-9, 6-8, 6-7, 7-10, 7-9, 7-8, 8-10, 8-9, or 9-10. In some embodiments, n is from 6-8. In some embodiments, n is 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, n is 6. In some embodiments, n is 8.

[00490] In some embodiments, o is from 1-10, 1-9, 1-8, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-10, 2-9, 2-8, 2-7, 2-6, 2-5, 2-4, 2-3, 3-10, 3-9, 3-8, 3-7, 3-6, 3-5, 3-4, 4-10, 4-9, 4-8, 4-7, 4-6, 4-5, 5-10, 5-9, 5-8, 5-7, 5-6, 6-10, 6-9, 6-8, 6-7, 7-10, 7-9, 7-8, 8-10, 8-9, or 9-10. In some embodiments, o is from 1-2. In some embodiments, o is 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In some embodiments, o is 2.

[00491] In some embodiments, the masking moiety (MM) comprises an amino acid sequence according to Formula (IV): $(X_6)C(X_8)C(X_2)$ (SEQ ID NO:52), where each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y.

[00492] In some embodiments, the masking moiety (MM) comprises an amino acid sequence according to Formula (V): $(Z_6)C(Z_8)C(Z_2)$ (SEQ ID NO:53), where each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P.

[00493] In some embodiments, an activatable antibody comprises a masking moiety (MM) comprising a sequence selected from the group consisting of $X_mCYYFDPPSHCXX$ (SEQ ID NO:54), X_mC PFFFPAALCXX (SEQ ID NO:55), and $X_mCHFADFVPYCXX$ (SEQ ID

NO:56), where m is from 2-10, and where each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y.

[00494] In some embodiments, an activatable antibody comprises a masking moiety (MM) comprising the sequence ADCPFFFPAALCSQ (SEQ ID NO:57), EVGSYADCPFFFPAALCSQ (SEQ ID NO:58), HDHHFDCYYFDPPSHCPA (SEQ ID NO:59), EVGSYHDHHFDCYYFDPPSHCPA (SEQ ID NO:60), ADPFSVCHFADFVPYCYY (SEQ ID NO:61), or EVGSYADPFSVCHFADFVPYCYY (SEQ ID NO:62).

[00495] In some embodiments, the masking moiety (MM) comprises an amino acid sequence selected from ADCPFFFPAALCSQ (SEQ ID NO:57), EVGSYADCPFFFPAALCSQ (SEQ ID NO:58), HDHHFDCYYFDPPSHCPA (SEQ ID NO:59), EVGSYHDHHFDCYYFDPPSHCPA (SEQ ID NO:60), ADPFSVCHFADFVPYCYY (SEQ ID NO:61), or EVGSYADPFSVCHFADFVPYCYY (SEQ ID NO:62).

[00496] In some embodiments, any of the masking moieties (MMs) described herein may further comprise one or more additional amino acid sequences (*e.g.*, one or more polypeptide tags). Examples of suitable additional amino acid sequence may include, without limitation, purification tags (such as his-tags, flag-tags, maltose binding protein and glutathione-S-transferase tags), detection tags (such as tags that may be detected photometrically (*e.g.*, red or green fluorescent protein, etc.)), tags that have a detectable enzymatic activity (*e.g.*, alkaline phosphatase, etc.), tags containing secretory sequences, leader sequences, and/or stabilizing sequences, protease cleavage sites (*e.g.*, furin cleavage sites, TEV cleavage sites, Thrombin cleavage sites), and the like. In some embodiments, the one or more additional amino acid sequences are at the N-terminus of the masking moiety (MM). In some embodiments, the additional amino acid sequence comprises or consists of the sequence EVGSY (SEQ ID NO:63).

[00497] In some embodiments, the masking moiety binds to the target binding moiety (TBM) and inhibits the activatable antibody from binding to its target before activation (*e.g.*, before treatment with one or more proteases that cleave within the cleavable moiety (CM), before undergoing a (local) change in pH (increased or decreased), before a temperature shift (increased or decreased), before being contacted with a second molecule (such as a small molecule or a protein ligand), etc.), but does not bind to the TBM and/or inhibit the activatable antibody from binding to its target after activation (*e.g.*, after treatment with one or more proteases that cleave within the cleavable moiety (CM), after undergoing a (local)

change in pH (increased or decreased), after a temperature shift (increased or decreased), after being contacted with a second molecule (such as a small molecule or a protein ligand), etc.). In some embodiments, the masking moiety (MM) inhibits binding of an activatable antibody to its target when the CM is not cleaved but does not inhibit binding of the activatable antibody to its target when the CM is cleaved. In some embodiments, the masking moiety (MM) has a dissociation constant for binding to the TBM that is greater (*e.g.*, at least about 1.5-fold greater, at least about 2-fold greater, at least about 2.5-fold greater, at least about 3-fold greater, at least about 3.5-fold greater, at least about 4-fold greater, at least about 4.5-fold greater, at least about 5-fold greater, at least about 10-fold greater, at least about 100-fold greater, at least about 500-fold greater, etc.) than the dissociation constant of the activatable antibody for its target (when in active form).

7.4.2 Cleavable moieties (CMs)

[00498] In some embodiments, the present disclosure relates to activatable antibodies comprising a cleavable moiety (CM). In some embodiments, the cleavable moiety (CM) is cleaved and/or disrupted by treatment with one or more proteases that cleave within the cleavable moiety (CM), by a change in pH (increased or decreased), by a temperature shift (increased or decreased), and/or by contact with a second molecule (such as a small molecule or a protein ligand), etc.)

[00499] In some embodiments, the cleavable moiety (CM) comprises at least a first cleavage site (CS1) (*e.g.*, a first protease cleavage site). In some embodiments, the first cleavage site is a first protease cleavage site. Any suitable protease cleavage site recognized and/or cleaved by any protease (*e.g.*, a protease that is known to be co-localized with a target of an activatable antibody comprising the CM) known in the art may be used, including, for example, a protease cleavage site recognized and/or cleaved by urokinase-type plasminogen activator (uPA); matrix metalloproteinases (*e.g.*, MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-10, MMP-11, MMP-12, MMP-13, MMP-14, MMP-15, MMP-16, MMP-17, MMP-19, MMP-20, MMP-23, MMP-24, MMP-26, and/or MMP-27); Tobacco Etch Virus (TEV) protease; plasmin; Thrombin; PSA; PSMA; ADAMS/ADAMTS (*e.g.*, ADAM 8, ADAM 9, ADAM10, ADAM12, ADAM15, ADAM17/TACE, ADAMDEC1, ADAMTS1, ADAMTS4, and/or ADAMTS5); caspases (*e.g.*, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, and/or Caspase-14); aspartate proteases (*e.g.*, RACE and/or Renin) ; aspartic cathepsins (*e.g.*, Cathepsin D and/or Cathepsin E); cysteine cathepsins (*e.g.*,

Cathepsin B, Cathepsin C, Cathepsin K, Cathepsin L, Cathepsin S, Cathepsin V/L2, and/or Cathepsin X/Z/P); cysteine proteinases (*e.g.*, Cruzipain, Legumain, and/or Otubain-2); KLKs (*e.g.*, KLK4, KLK5, KLK6, KLK7, KLK8, KLK10,KLK11, KLK13, and/or KLK14); metalloproteinases (*e.g.*, Meprin, Neprilysin, PSMA, and/or BMP-1); serine proteases (*e.g.*, activated protein C, Cathepsin A, Cathepsin G, Chymase, and/or coagulation factor proteases (such as FVIIa, FIXa, FXa, FXIa, FXIIa)); elastase; granzyme B; guanidinobenzoatase; HtrA1; human neutrophil elastase; lactoferrin; marapsin; NS3/4A; PACE4; tPA; tryptase; type II transmembrane serine proteases (TTSPs) (*e.g.*, DESC1, DPP-4, FAP, Hepsin, Matriptase-2, MT-SP1/Matriptase, TMPRSS2, TMPRSS3 and/or TMPRSS4); etc. In some embodiments, the first protease cleavage site is a cleavage site for a protease selected from uPA, MMP-1, MMP-2, MMP-3, MMP-8, MMP-9, MMP-14, TEV protease, plasmin, Thrombin, Factor X, PSA, PSMA, Cathepsin D, Cathepsin K, Cathepsin S, ADAM10, ADAM12, ADAMTS, Caspase-1, Caspase-2, Caspase-3, Caspase-4, Caspase-5, Caspase-6, Caspase-7, Caspase-8, Caspase-9, Caspase-10, Caspase-11, Caspase-12, Caspase-13, Caspase-14, and TACE. In some embodiments, the first protease cleavage site is a cleavage site for a protease selected from uPA, MMP-2, MMP-9, and/or TEV protease. In some embodiments, the protease cleavage comprises an amino acid sequence selected from SGRSA (SEQ ID NO:64), PLGLAG (SEQ ID NO:45), and ENLYFQG (SEQ ID NO:65).

[00500] In some embodiments, the cleavable moiety (CM) further comprises a first linker (L1). In some embodiments, the first linker (L1) is N-terminal to the first cleavage site (CS1) (*e.g.*, a first protease cleavage site). In some embodiments, the cleavable moiety (CM) comprises a structure, from N-terminus to C-terminus, of: L1-(CS1).

[00501] Any suitable linker (*e.g.*, a flexible linker) known in the art may be used, including, for example: glycine polymers $(G)_n$, where n is an integer of at least 1 (*e.g.*, at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, etc.); glycine-serine polymers $(GS)_n$, where n is an integer of at least 1 (*e.g.*, at least one, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, etc.) such as GGGGS (SEQ ID NO:66), SGGS (SEQ ID NO:67), GGSG (SEQ ID NO:68), GGSGG (SEQ ID NO:69), GSGSG (SEQ ID NO:70), GSGGG (SEQ ID NO:71), GGGSG (SEQ ID NO:72), and/or GSSSG (SEQ ID NO:73)); glycine-alanine polymers; alanine-serine polymers; and the like. Linker sequences may be of any length, such as from about 1 amino acid (*e.g.*, glycine or serine) to about 20 amino acids (*e.g.*, 20 amino acid glycine polymers or glycine-serine polymers), about 1 amino acid to about 15 amino acids, about 3 amino acids to about 12 amino acids, about 4 amino acids to about 10 amino acids, about 5 amino acids to

about 9 amino acids, about 6 amino acids to about 8 amino acids, etc. In some embodiments, the linker is any of about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 amino acids in length.

[00502] In some embodiments, the cleavable moiety (CM) further comprises at least a second linker (*e.g.*, at least a second, at least a third, at least a fourth, at least a fifth, etc.). In some embodiments, the cleavable moiety (CM) further comprises a second linker (L2). The second linker (L2) may be any suitable linker described above. In some embodiments, the first (L1) and second (L2) linkers are the same. In some embodiments, the first (L1) and second (L2) linkers are different. In some embodiments, the at least second linker (L2) is C-terminal to the second cleavage site (CS1). In some embodiments, the cleavable moiety (CM) comprises a structure, from N-terminus to C-terminus, of: L1- (CS1) -L2. In further embodiments, the CM comprises GGGPLGLAGSGGS (SEQ ID NO:74).

7.4.3 Exemplary MM-CM sequences

[00503] In some embodiments, an activatable antibody of the present disclosure comprises a masking peptide, from N-terminus to C-terminus, of: (MM) - L1- (CS1) -L2. In some embodiments, a polypeptide of the present disclosure comprises the structure, from N-terminus to C-terminus, of: (MM) - L1- (CS1) -L2-(TBM).

[00504] In some embodiments, an activatable antibody comprises an amino acid sequence according to Formula (VI), EVGSY(X_2)C(X_8)C(X_2) GGGPLGLAGSGGS (SEQ ID NO:75), where each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y. In further embodiments, the masking peptide comprises EVGSYADCPFFFPAALCSQGGGPLGLAGSGGS (SEQ ID NO:29).

[00505] In some embodiments, an activatable antibody comprises an amino acid sequence according to Formula (VII), EVGSY(X_6)C(X_8)C(X_2) GGGPLGLAGSGGS (SEQ ID NO:76), where each X is independently an amino acid selected from the group consisting of A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, and Y. In some embodiments, an activatable antibody comprises an amino acid sequence according to Formula (VIII), EVGSY(Z_6)C(Z_8)C(Z_2) GGGPLGLAGSGGS (SEQ ID NO:77), where each Z is independently an amino acid selected from the group consisting of D, A, Y, S, T, N, I, L, F, V, H, and P. In further embodiments, the masking peptide comprises EVGSYHDHHFDCYYFDPPSHCPAGGGPLGLAGSGGS (SEQ ID NO:30). In further embodiments, the masking peptide comprises EVGSYADPFSVCHFADFVPYCYYGGGPLGLAGSGGS (SEQ ID NO:31).

[00506] In some embodiments, an activatable binding polypeptide (e.g., activatable antibody) of the present disclosure comprises: (a) a masking moiety (MM), (b) a cleavable moiety, and (c) a target binding moiety. In some embodiments, the masking moiety (MM) binds to the target binding moiety (TBM) of the activatable antibody and reduces or inhibits binding of the activatable binding moiety to ROR (*e.g.*, human ROR), as compared to the binding of a corresponding binding polypeptide lacking the masking moiety to ROR (*e.g.*, human ROR) and/or as compared to the binding of a parental antibody to ROR (*e.g.*, human ROR).

[00507] In some embodiments, an "activatable" binding polypeptides refers to a binding polypeptide that exhibits a first level of binding to ROR when in an inhibited, masked, and/or uncleaved state, and exhibits a second level of binding to ROR in an uninhibited, unmasked, and/or cleaved state, where the second level of ROR binding is greater than the first level of ROR binding. In some embodiments, access to ROR by the activatable binding polypeptide is greater after cleavage within the cleavable moiety (*e.g.*, by one or more proteases).

[00508] In some embodiments, an activatable antibody of the present disclosure is generally considered to be an "activatable" binding polypeptide when binding affinity of the polypeptide to ROR (*e.g.*, human ROR) increases by at least about 2-fold (*e.g.*, at least about 2-fold, at least about 2.5-fold, at least about 3, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5-fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, at least about 10-fold, at least about 25-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 250-fold, at least about 500-fold, at least about 750-fold, or at least about 1000-fold, or more) after activation of the activatable antibody as compared to prior to activation of the activatable antibody (*e.g.*, after activation by treatment with one or more proteases that cleave within the cleavable moiety (CM), after activation by a change in pH (increased or decreased), after activation by a temperature shift (increased or decreased), after activation by being contacted with a second molecule (such as a small molecule), etc.). In some embodiments, an activatable antibody of the present disclosure is generally considered "activatable" if the EC_{50} of the activatable antibody decreases by at least about 2-fold (*e.g.*, at least about 2-fold, at least about 2.5-fold, at least about 3, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5-fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, at least about 10-

fold, at least about 25-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 250-fold, at least about 500-fold, at least about 750-fold, or at least about 1000-fold, or more) after "activation" (*e.g.*, as measured by an ELISA or FACS assay; see the examples below). In some embodiments, an activatable antibody of the present disclosure is generally considered "activatable" if the EC_{50} of the polypeptide decreases by at least about 2-fold after treatment with a protease that cleaves within the cleavable moiety (CM).

[00509] In some embodiments, when the masking moiety (MM) is bound to the target binding moiety (TBM) of the activatable antibody, the K_D of the activatable antibody for ROR is about 2 (*e.g.*, about 2, about 2.5, about 3, about 3.5 about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, about 10, about 25, about 50, about 75, about 100, about 250, about 500, about 750, or about 1000 or more) times greater than when the masking moiety (MM) is not bound to the target binding moiety (TBM) (*e.g.*, after "activation" of the activatable antibody (such as after protease treatment to cleave within the cleavable moiety (CM))) and/or than the K_D of the parental antibody for ROR.

[00510] In some embodiments, when the masking moiety is bound to the target binding moiety of the activatable antibody, the K_D of the activatable antibody for ROR is reduced by at least about 25% (*e.g.*, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 99%) relative to when the masking moiety is not bound to the target binding moiety (*e.g.*, after "activation" of the activatable antibody (such as after protease treatment to cleave within the cleavable moiety (CM))) and/or relative to the K_D of the parental antibody for ROR.

[00511] In some embodiments, the masking moiety sterically hinders binding of the activatable antibody to ROR and/or allosterically hinders binding of the activatable antibody to ROR. In some embodiments, the masking moiety does not comprise an amino acid sequence of a natural binding partner of the activatable antibody and/or parental antibody.

[00512] In some embodiments, the dissociation constant of the masking moiety for the target binding moiety is greater than the dissociation constant for the activatable antibody for ROR (when activated). In some embodiments, the dissociation constant of the masking moiety for the target binding moiety is about 2 (*e.g.*, about 2, about 2.5, about 3, about 3.5 about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, about 10, about 25, about 50, about 75, about 100, about 250, about 500, about 750, or about 1000 or more) times greater than the dissociation constant for the activatable

antibody for ROR (when activated). In some embodiments, the dissociation constant of the masking moiety for the target binding moiety is about equal to the dissociation constant for the activatable antibody for ROR (when activated).

[00513] In some embodiments, the activatable ROR antibodies comprise: a masking peptide, an antibody light chain variable (VL) region, and an antibody heavy chain variable (VH) region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29, 30 or 31 (*see* **Table 2**), and wherein the antibody light chain variable region and the antibody heavy chain variable region comprise the CDRs described herein (*e.g.*, **Table 1**).

[00514] In some embodiments, the activatable ROR antibodies comprise: (a) a polypeptide comprising a masking peptide and an antibody light chain variable region; and (b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29, 30 or 31 (*see* **Table 2**), and wherein the antibody light chain variable region and the antibody heavy chain variable region comprise the CDRs described herein (*e.g.*, **Table 1**).

[00515] In some embodiments, the activatable ROR antibodies comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain variable region; and (b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29, 30 or 31 (*see* **Table 2**), and wherein the antibody light chain variable region and the antibody heavy chain variable region comprise the CDRs described herein (*e.g.*, **Table 1**).

[00516] In some embodiments, the activatable ROR antibodies comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain variable region; and (b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29, 30 or 31 (*see* **Table 2**), and wherein the antibody light chain variable region and the antibody heavy chain variable region are as described herein (*e.g.*, **Table 1**). In certain embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:35, 37 or 39 (*see* **Tables 4-9**). In certain embodiments, the polypeptide is as described herein (*e.g.*, any one of **Tables 4-9**) or comprises the amino acid sequence of SEQ ID NO:34, 36 or 38 (*see* **Tables 4-9**).

[00517] In some embodiments, the activatable ROR antibodies comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain; and (b) an antibody heavy chain; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29, 30 or 31 (*see* **Table 2**), and wherein the antibody light chain and the antibody heavy chain are as described herein (*e.g.*, **Tables 3-9**) or comprise the antibody

light chain and the antibody heavy chain amino acid sequences described herein (*e.g.*, **Tables 3-9**). In certain embodiments, the polypeptide is as described herein (*e.g.*, any one of **Tables 4-9**) or comprises the amino acid sequence of SEQ ID NO:34, 36 or 38 (*see* **Tables 4-9**).

[00518] In some embodiments, the activatable ROR antibodies bind to the same epitope of ROR (*e.g.*, human ROR) as an antibody comprising the CDRs described herein (*e.g.*, **Table 1**) and comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain variable region; and (b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29, 30 or 31 (*see* **Table 2**).

[00519] In some embodiments, the activatable ROR antibodies bind to the same epitope of ROR (*e.g.*, human ROR) as an antibody comprising a heavy chain variable region and a light chain variable region described herein (*e.g.*, **Table 1**) and comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain variable region; and (b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29, 30 or 31 (*see* **Table 2**).

[00520] In one embodiment, the activatable ROR antibodies provided herein comprise: a masking peptide, an antibody light chain variable region, and an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29.

[00521] In one embodiment, the activatable ROR antibodies provided herein comprise: a masking peptide, an antibody light chain variable region, and an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:30.

[00522] In one embodiment, the activatable ROR antibodies provided herein comprise: a masking peptide, an antibody light chain variable region, and an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:31.

[00523] In one embodiment, the activatable ROR antibodies provided herein comprise: (a) a polypeptide comprising a masking peptide and an antibody light chain variable region; and (b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29.

[00524] In one embodiment, the activatable ROR antibodies provided herein comprise: (a) a polypeptide comprising a masking peptide and an antibody light chain variable region; and

(b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:30.

[00525] In one embodiment, the activatable ROR antibodies provided herein comprise: (a) a polypeptide comprising a masking peptide and an antibody light chain variable region; and (b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:31.

[00526] In one embodiment, the activatable ROR antibodies provided herein comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain variable region; and (b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29.

[00527] In one embodiment, the activatable ROR antibodies provided herein comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain variable region; and (b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:30.

[00528] In one embodiment, the activatable ROR antibodies provided herein comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain variable region; and (b) an antibody heavy chain variable region; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:31.

[00529] In one embodiment, the activatable ROR antibodies provided herein comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain; and (b) an antibody heavy chain; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29.

[00530] In one embodiment, the activatable ROR antibodies provided herein comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain; and (b) an antibody heavy chain; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:30.

[00531] In one embodiment, the activatable ROR antibodies provided herein comprise: (a) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide and an antibody light chain; and (b) an antibody heavy chain; wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:31.

[00532] In other embodiments, a masking peptide described herein comprises, from N-terminus to C-terminus, a masking moiety (MM) and a cleavable moiety (CM), and optionally further comprises one, two, or more linkers.

[00533] The CM generally includes an amino acid sequence that is cleavable, for example, serves as the substrate for an enzyme and/or a cysteine-cysteine pair capable of forming a reducible disulfide bond. As such, when the terms "cleavage," "cleavable," "cleaved" and the like are used in connection with a CM, the terms encompass enzymatic cleavage, *e.g.*, by a protease, as well as disruption of a disulfide bond between a cysteine-cysteine pair via reduction of the disulfide bond that can result from exposure to a reducing agent.

[00534] The MM refers to an amino acid sequence that, when the CM of the activatable antibody is intact (*e.g.,* uncleaved by a corresponding enzyme, and/or containing an unreduced cysteine-cysteine disulfide bond), the MM interferes with or inhibits binding of the antibody light chain variable region and/or heavy chain variable region (as the case may be) to its target. In some embodiments, the MM interferes with or inhibits binding of the antibody light chain variable region and/or heavy chain variable region (as the case may be) to its target so efficiently that binding of the antibody light chain variable region and/or heavy chain variable region (as the case may be) to its target is extremely low and/or below the limit of detection (*e.g.,* binding cannot be detected in an ELISA or flow cytometry assay). The amino acid sequence of the CM may overlap with or be included within the MM.

[00535] In some embodiments, the masking moiety sterically hinders binding of the activatable antibody to its target and/or allosterically hinders binding of the activatable antibody to its target. In some embodiments, the masking moiety does not comprise an amino acid sequence of a natural binding partner of activatable antibody.

[00536] In some embodiments, an antibody of the present disclosure is generally considered to be an "activatable" antibody when binding affinity of the antibody to its target increases (*e.g.,* by at least about 2-fold, at least about 2.5-fold, at least about 3, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5-fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, at least about 10-fold, at least about 25-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 250-fold, at least about 500-fold, at least about 750-fold, or at least about 1000-fold, or more) after activation of the antibody as compared to prior to activation of the binding antibody (*e.g.,* after activation by treatment with one or more proteases that cleave within the cleavable moiety (CM), after activation by a change in pH (increased or decreased), after activation by a temperature shift (increased or decreased), after activation by being contacted with a second molecule (such as a small molecule or a protein ligand), *etc.*). In some embodiments, an antibody of the present disclosure is generally considered

"activatable" if the EC_{50} of the antibody decreases (*e.g.,* by at least about 2-fold, at least about 2.5-fold, at least about 3, at least about 3.5-fold, at least about 4-fold, at least about 4.5-fold, at least about 5-fold, at least about 5.5-fold, at least about 6-fold, at least about 6.5-fold, at least about 7-fold, at least about 7.5-fold, at least about 8-fold, at least about 8.5-fold, at least about 9-fold, at least about 9.5-fold, at least about 10-fold, at least about 25-fold, at least about 50-fold, at least about 75-fold, at least about 100-fold, at least about 250-fold, at least about 500-fold, at least about 750-fold, or at least about 1000-fold, or more) after "activation" (*e.g.,* as measured by an ELISA or FACS assay). In some embodiments, an antibody of the present disclosure is generally considered "activatable" if the EC_{50} of the antibody decreases (*e.g.*, by at least about 2-fold) after treatment with a protease that cleaves within the cleavable moiety (*e.g.,* as measured by an ELISA or FACS assay).

[00537] In some embodiments, when the masking moiety is bound to the antibody light chain variable region and/or heavy chain variable region (as the case may be) of an activatable antibody, the K_D of the activatable antibody for its target is greater (*e.g.,* about 2, about 2.5, about 3, about 3.5 about 4, about 4.5, about 5, about 5.5, about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, about 10, about 25, about 50, about 75, about 100, about 250, about 500, about 750, or about 1000 or more times greater) than when the masking moiety is not bound to the antibody light chain variable region and/or heavy chain variable region (as the case may be) (*e.g.,* after "activation" of the activatable antibody (such as after protease treatment to cleave within the cleavable moiety)) and/or than the K_D of the parental antibody for the target. Methods of measuring affinity are known in the art.

[00538] In some embodiments, when the masking moiety is bound to the antibody light chain variable region and/or heavy chain variable region (as the case may be) of an activatable antibody, the K_D of the activatable antibody for its target is reduced (*e.g.,* by at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 99%) relative to when the masking moiety is not bound to the antibody light chain variable region and/or heavy chain variable region (as the case may be) (*e.g.,* after "activation" of the activatable binding polypeptide (such as after protease treatment to cleave within the cleavable moiety)) and/or relative to the K_D of the parental antibody for the target. Methods of measuring affinity are known in the art.

[00539] In some embodiments, the dissociation constant of the masking moiety for the antibody light chain variable region and/or heavy chain variable region (as the case may be) is greater (*e.g.,* about 2, about 2.5, about 3, about 3.5 about 4, about 4.5, about 5, about 5.5,

about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, about 10, about 25, about 50, about 75, about 100, about 250, about 500, about 750, or about 1000 or more times greater) than the dissociation constant for the activatable antibody for the target (when in activated form). In some embodiments, the dissociation constant of the masking moiety for the antibody light chain variable region and/or heavy chain variable region (as the case may be) is about equal to the dissociation constant for the activatable antibody for the target (when in activated form). In some embodiments, the masking moiety binds to the antibody light chain variable region and/or heavy chain variable region (as the case may be), and prevents the activatable antibody from binding to its target only when the activatable antibody has not been activated (*e.g.,* not activated by treatment with one or more proteases that cleave within the cleavable moiety (CM), not activated by a change in pH (increased or decreased), not activated by a temperature shift (increased or decreased), not activated after being contacted with a second molecule (such as a small molecule or a protein ligand), *etc.*). In some embodiments, activation induces cleavage of the activatable antibody within the cleavage moiety. In some embodiments, activation induces conformation changes in the activatable antibody (*e.g.,* displacement of the masking moiety), leading to the masking moiety no longer preventing the antibody from binding to its target.

[00540] In some embodiments, the ROR antibodies, such as the activatable ROR antibodies, described herein comprise a VH region, VL region, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 of any one of the antibodies described herein, such as the amino acid sequence of a VH region, VL region, VH CDR1, VH CDR2, VH CDR3, VL CDR1, VL CDR2, and/or VL CDR3 depicted in **Table 1**. Accordingly, in some embodiments, an ROR antibody, such as an activatable ROR antibody (*e.g.*, an activatable antibody) described herein comprises any one, any two, and/or all three heavy chain CDRs and/or any one, any two, and/or all three light chain CDRs depicted in **Table 1**. In some embodiments, an ROR antibody, such as an activatable ROR antibody (*e.g.*, an activatable antibody) described herein comprises any one, any two, and/or all three heavy chain CDRs and any one, any two, and/or all three light chain CDRs depicted in **Table 1**.

[00541] In some embodiments, an ROR antibody, such as an activatable ROR antibody, comprises a VH region, which comprises a VH CDR1, a VH CDR2, and/or a VH CDR3, and/or a VL region, which comprises a VL CDR1, a VL CDR2, and/or a VL CDR3, of any one of the antibodies described herein (*see*, *e.g.*, **Table 1**). Accordingly, in some embodiments, an ROR antibody, such as an activatable ROR antibody (*e.g.*, an activatable

antibody), described herein comprises any one, any two, and/or all three heavy chain CDRs and/or any one, any two, and/or all three light chain CDRs from **Table 1**.

[00542] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises (i) a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:25 and/or (ii) a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a VL comprising the amino acid sequence of SEQ ID NO:26.

[00543] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:25 and a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a VL comprising the amino acid sequence of SEQ ID NO:26. CDR sequences can be determined according to well-known numbering systems or a combination thereof. In some embodiments, the CDRs are according to IMGT numbering. In some embodiments, the CDRs are according to Kabat numbering. In some embodiments, the CDRs are according to AbM numbering. In other embodiments, the CDRs are according to Chothia numbering. In other embodiments, the CDRs are according to Contact numbering. In some embodiments, the CDR sequences are determined according to a combination of any two or more of the above-mentioned numbering systems, for example, a combination of Kabat and Chothia. Various exemplary CDR numbering systems are described and illustrated above in Section **7.1**.

[00544] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises (a) a VH region comprising a VH CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 7, 12, 13, 18, and 27; a VH CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 8, 14, 19, and 24; and a VH CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 9, 15, 20, and 28; and/or (b) a VL region comprising a VL CDR1 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 10, 16, and 21; a VL CDR2 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 11, and 22; and a VL CDR3 having an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 17, and 23.

[00545] In some embodiments, the ROR antibody, such as the activatable ROR antibody, provided herein (*e.g.*, an activatable antibody) comprises a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:1, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:2, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:3; and a VL region comprising a VL CDR1 comprising the amino acid

sequence of SEQ ID NO:4, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:6.

[00546] In some embodiments, the ROR antibody, such as the activatable ROR antibody, provided herein (*e.g.*, an activatable antibody) comprises a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:7, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:8, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:9; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:10, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:6.

[00547] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:12, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:2, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:3; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:4, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:6.

[00548] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:13, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:14, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:15; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:16, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:17.

[00549] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:18, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:19, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:20; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:21, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:22, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:23.

[00550] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:1, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:24, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:3; and a VL region comprising a

VL CDR1 comprising the amino acid sequence of SEQ ID NO:4, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:6.

[00551] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:27, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:24, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:28; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:4, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:17.

[00552] In some embodiments, the ROR antibody, such as the activatable antibody, further comprises one or more framework regions of SEQ ID NOs:25 and/or 26. In some embodiments, the ROR antibody, such as the activatable antibody, or fragment thereof 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 SEQ ID NO:25 or 26. In some embodiments, the ROR antibody, such as the activatable antibody, provided herein is a humanized antibody. Framework regions described herein are determined based upon the boundaries of the CDR numbering system. In other words, if the CDRs are determined by, *e.g.*, Kabat, IMGT, or Chothia, then the framework regions are the amino acid residues surrounding the CDRs in the variable region in the format, from the N-terminus to C-terminus: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. For example, FR1 is defined as the amino acid residues N-terminal to the CDR1 amino acid residues as defined by, *e.g.*, the Kabat numbering system, the IMGT numbering system, or the Chothia numbering system, FR2 is defined as the amino acid residues between CDR1 and CDR2 amino acid residues as defined by, *e.g.*, the Kabat numbering system, the IMGT numbering system, or the Chothia numbering system, FR3 is defined as the amino acid residues between CDR2 and CDR3 amino acid residues as defined by, *e.g.*, the Kabat numbering system, the IMGT numbering system, or the Chothia numbering system, and FR4 is defined as the amino acid residues C-terminal to the CDR3 amino acid residues as defined by, *e.g.*, the Kabat numbering system, the IMGT numbering system, or the Chothia numbering system.

[00553] In some embodiments, ROR antibodies, such as activatable ROR antibodies, including activatable human ROR antibodies, described herein comprise a VH region or VH domain. Additionally or alternatively, in some embodiments, activatable ROR antibodies (such as monospecific or bispecific antibodies), including activatable human ROR antibodies,

described herein comprise a polypeptide comprising, from N-terminus to C-terminus, a masking peptide described herein (*e.g.*, **Table 2**), and a VL region or VL domain. In some embodiments, activatable ROR antibodies (such as monospecific or bispecific antibodies), including activatable human ROR antibodies, described herein have a combination of (i) a VH domain or VH region; and (ii) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide described herein (*e.g.*, **Table 2**), and a VL domain or VL region.

[00554] In some embodiments, the ROR antibody, such as the activatable ROR antibody, provided herein comprises a VH comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, the activatable ROR antibody comprises a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the SEQ ID NO:29, 30 or 31, and a VL comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the activatable ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the SEQ ID NO:29, 30 or 31, and a VL comprising the amino acid sequence of SEQ ID NO:26.

[00555] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, the activatable ROR antibody comprises a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:29 and a VL comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the activatable ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:29 and a VL comprising the amino acid sequence of SEQ ID NO:26.

[00556] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, the activatable ROR antibody comprises a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:30 and a VL comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the activatable ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:30 and a VL comprising the amino acid sequence of SEQ ID NO:26.

[00557] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, the activatable ROR antibody comprises a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:31 and a VL comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the activatable ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:31 and a VL comprising the amino acid sequence of SEQ ID NO:26.

[00558] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, the activatable ROR antibody comprises a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:29 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:34. In some embodiments, the activatable ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:29 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:34.

[00559] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, the activatable ROR antibody comprises a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:30 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:36. In some embodiments, the activatable ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:30 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:36.

[00560] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, the activatable ROR antibody comprises a polypeptide comprising, from N-

terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:31 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:38. In some embodiments, the activatable ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:31 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:38.

[00561] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, the activatable ROR antibody comprises a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:29 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:35. In some embodiments, the activatable ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:29 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:35.

[00562] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, the activatable ROR antibody comprises a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:30 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:37. In some embodiments, the activatable ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:30 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:37.

[00563] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises a VH comprising the amino acid sequence of SEQ ID NO:25. In some embodiments, the activatable ROR antibody comprises a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID

NO:31 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:39. In some embodiments, the activatable ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a polypeptide comprising, from N-terminus to C-terminus, a masking peptide comprising the amino acid sequence of SEQ ID NO:31 and a VL comprising the amino acid sequence of SEQ ID NO:26, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:39.

[00564] In some embodiments, the ROR antibody comprises a VH comprising the amino acid sequence of SEQ ID NO:25 and a VL comprising the amino acid sequence of SEQ ID NO:26.

[00565] In certain embodiments, the ROR antibody, such as the activatable ROR antibody, provided herein comprises amino acid sequences with certain percent identity (such as at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or as at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or higher) relative to any antibody or fragment thereof provided herein, for example, a CDR, VH or VL in **Table 1**, or any full-length antibody chain as disclosed herein (*e.g.*, the amino acid sequences depicted in **Tables 3-9**). In some embodiments, the ROR antibody, such as the activatable ROR antibody, provided herein comprises CDRs of any antibody or fragment thereof provided herein, for example in **Table 1**. In further embodiments, the ROR antibody, such as the activatable ROR antibody, provided herein comprises amino acid sequences with certain percent identity (such as at least about 80%, or at least about 81%, or at least about 82%, or at least about 83%, or at least about 84%, or at least about 85%, or at least about 86%, or at least about 87%, or at least about 88%, or at least about 89%, or as at least about 90%, or at least about 91%, or at least about 92%, or at least about 93%, or at least about 94%, or at least about 95%, or at least about 96%, or at least about 97%, or at least about 98%, or at least about 99%, or higher) relative to any antibody or fragment thereof provided herein, for example, a VH or VL in **Table 1**, or any full-length antibody chain as disclosed herein (*e.g.*, the amino acid sequences depicted in **Tables 3-9**).

[00566] The determination of percent identity between two sequences (*e.g.*, amino acid sequences or nucleic acid sequences) can be accomplished using a mathematical algorithm. A non-limiting example of a mathematical algorithm utilized for the comparison of two

sequences is the algorithm of Karlin and Altschul, Proc. Natl. Acad. Sci. U.S.A. 87:2264 2268 (1990), modified as in Karlin and Altschul, Proc. Natl. Acad. Sci. U.S.A. 90:5873 5877 (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.*, J. Mol. Biol. 215:403 (1990). BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, *e.g.*, for score=100, word length=12 to obtain nucleotide sequences homologous to a nucleic acid molecule described herein. BLAST protein searches can be performed with the XBLAST program parameters set, *e.g.*, to score 50, word length=3 to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul *et al.*, Nucleic Acids Res. 25:3389 3402 (1997). In some embodiments, the percent identity between two sequences is calculated by dividing the number of residue(s) varied (excluding or including conservative amino acid substitution(s) or degenerate nucleotide substitution(s)) between the two sequences in the alignment with the residue number of any one of the following: (i) full length of the shorter sequence, (ii) full length of the longer sequence, (iii) mean length of the two sequences, (iv) total length of the non-gap portion of the alignment, (v) length of the alignment excluding overhangs, or (vi) length of the alignment including overhangs. Overhangs as used herein with respect to a sequence alignment refer to either or both ends of the alignment where residues of one sequence are considered as aligning to no residues (*e.g.*, gap) in the other sequence. Alternatively, PSI BLAST can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI Blast programs, the default parameters of the respective programs (*e.g.*, of XBLAST and NBLAST) can be used (see, *e.g.*, National Center for Biotechnology Information (NCBI) on the worldwide web, ncbi.nlm.nih.gov). Another non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS 4:11-17 (1998). Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

[00567] In some embodiments, the antibody, such as the activatable antibody, provided herein contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative

to the reference sequence, but the antibody, such as activatable antibody after activation, comprising that sequence retains the ability to bind to ROR. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in a reference amino acid sequence. In some embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (*e.g.*, in the FRs and/or constant regions).

[00568] In some embodiments, the position of one or more CDRs along the VH (*e.g.,* CDR1, CDR2, or CDR3) and/or VL (*e.g.,* CDR1, CDR2, or CDR3) region of an ROR antibody, such as an activatable ROR antibody (*e.g.*, an activatable antibody), including an activatable human ROR antibody, described herein may vary by one, two, three, four, five, or six amino acid positions so long as binding to ROR (*e.g.,* human ROR) 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** may 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, so long as binding to ROR (*e.g.,* human ROR) 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 CDRs along the VH (*e.g.,* CDR1, CDR2, or CDR3) and/or VL (*e.g.,* CDR1, CDR2, or CDR3) region of an ROR antibody, such as an activatable ROR antibody (*e.g.*, an activatable antibody), including an activatable human ROR antibody, described herein may vary (*e.g.,* be shorter or longer) by one, two, three, four, five, or more amino acids, so long as binding to ROR (*e.g.,* human ROR) 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 described herein may be one, two, three, four, five or more amino acids shorter than one or more of the CDRs described by SEQ ID NOS:1-24 and 27-28, so long as binding to ROR (*e.g.,* human ROR) 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 described herein may be one, two, three, four, five or more amino acids longer than one or more of the CDRs described by SEQ ID NOS: 1-24 and 27-28, so long as binding to ROR (*e.g.,* human ROR) 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 amino terminus of a VH and/or VL CDR1, CDR2, and/or CDR3 described herein may be extended or shortened by one, two, three, four, five or more amino acids compared to one or

more of the CDRs described by SEQ ID NOS: 1-24 and 27-28, so long as binding to ROR (*e.g.,* human ROR) 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 described herein may be extended or shortened by one, two, three, four, five or more amino acids compared to one or more of the CDRs described by SEQ ID NOS: 1-24 and 27-28, so long as binding to ROR (*e.g.,* human ROR) 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 ROR (*e.g.,* human ROR) is maintained.

[00569] In other embodiments, the ROR antibody, such as an activatable ROR antibodies (*e.g.*, activatable antibodies) after activation, including activatable human ROR antibodies after activation, presented herein that bind to ROR, further comprise conservative sequence modifications. With respect to polypeptides that are ROR antibodies, such as activatable ROR antibodies (*e.g.*, activatable antibodies), such as activatable human ROR antibodies, conservative sequence modifications include conservative amino acid substitutions that include ones in which the 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. Thus, in some embodiments, a predicted nonessential amino acid residue in an ROR antibody, such as an activatable ROR antibody, is 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 the ROR antibodies, such as the activatable ROR antibodies (*e.g.*, activatable antibodies), including activatable human ROR antibodies, by 50%, or 55%, or 60%, or 65%, or 70%, or 75%, or 80%, or 85%, or 90%, or 95%, or 98%, or 99%. In some embodiments, the amino acid sequence modifications refer to at most 1, 2, 3, 4, 5, or 6 amino acid substitutions to the CDRs, such as those described in **Table 1**. Thus, for example, each such CDR may contain up to 5 conservative amino acid substitutions, for example up to (not more than) 4 conservative amino acid substitutions, for example up to (not more than) 3 conservative amino acid substitutions, for example up to (not more than) 2 conservative amino acid substitutions, or no more than 1 conservative amino

acid substitution. In some embodiments, an ROR antibody, such as an activatable ROR antibody (*e.g.*, an activatable antibody), including an activatable human ROR antibody, contains one or more, including six, CDRs having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% identity to the CDRs of the antibody depicted in **Table 1**.

[00570] In some embodiments, an ROR antibody, such as an activatable ROR antibody (*e.g.*, an activatable antibody), including an activatable human ROR antibody, contains a VH and a VL comprising CDRs identical to those of the antibody depicted in **Table 1**. In some embodiments, the amino acid sequence modifications do not include any modification within an 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). Additionally or alternatively, the amino acid sequence modifications are in the framework, constant region, and/or fragment crystallizable region (Fc).

[00571] In some embodiments, the ROR antibody, such as an activatable antibody, or fragment provided herein comprises a VH domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:25, and/or a VL domain having at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence of SEQ ID NO:26, and the binding of the ROR antibody, such as the activatable antibody after activation, or fragment thereof to ROR (*e.g.,* human ROR) 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%).

[00572] In some embodiments, functional epitopes can be mapped, *e.g.*, by combinatorial alanine scanning, to identify amino acids in the ROR protein that are necessary for interaction with ROR antibodies, such as activatable ROR antibodies (such as activatable antibodies) provided herein. In some embodiments, conformational and crystal structure of ROR antibodies (such as activatable ROR antibodies) bound to ROR may be employed to identify the epitopes. In some embodiments, the present disclosure provides an antibody that specifically binds to the same epitope as any of the ROR antibodies (such as activatable antibodies or fragments thereof) provided herein.

[00573] For example, in some embodiments, the ROR antibody, such as the activatable ROR antibody after activation, binds to the same epitope as an anti-ROR antibody comprising a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in a VH comprising the amino acid

sequence of SEQ ID NO:25 and a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a VL comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the ROR antibody, such as the activatable ROR antibody after activation, binds to the same epitope as an anti-ROR antibody comprising a VH comprising the amino acid sequence of SEQ ID NO:25, and a VL comprising the amino acid sequence of SEQ ID NO:26.

[00574] In some embodiments, the ROR antibody provided herein further comprises an Fc or a variant thereof. In some embodiments, the Fc comprises an amino acid sequence as set forth in SEQ ID NO: 43. In other embodiments, the Fc variant is a silent Fc (sFc). In further embodiments, the silent Fc comprises an alanine (Ala, A) residue at position Leu234 (L234) according to the EU numbering system and an alanine (Ala, A) residue at position Leu235 (L235) according to the EU numbering system. In some embodiments, the silent Fc comprises an alanine (Ala, A) residue at position Leu234 (L234) according to the EU numbering system and an alanine (Ala, A) residue at position Leu235 (L235) according to the EU numbering system, and a lysine (Lys, K) residue at position Pro329 (P329) according to the EU numbering system (also referred to herein as "LALAPK" or "L234A/L235A/P329K"). In some embodiments, the silent Fc comprises an amino acid sequence as set forth in SEQ ID NO: 44. Additionally or alternatively, a variant Fc region has a reduced potential immunogenicity. In further embodiments, a variant Fc region comprises a glutamic acid (Glu, E) residue at position Asp356 (D356) according to the EU numbering system, a glutamic acid (Glu, E) residue at position Glu357 (E357) according to the EU numbering system, and a methionine (Met, M) residue position Leu358 (L358) according to the EU numbering system (also referred to herein as "EEM" or "D356E/E357E/L358M"). Other suitable Fc can be found, for example in US20230071196 and US20220389055, each of which is incorporated herein by reference in its entirety.

[00575] In some embodiments, the ROR antibody, such as the activatable ROR antibody after activation, specifically binds to ROR competitively with any one of the anti-ROR antibodies or fragments thereof described herein.

[00576] In some embodiments, the ROR antibody, such as the activatable ROR antibody after activation, specifically binds to ROR competitively with an anti-ROR antibody comprising a VH CDR1, a VH CDR2, and a VH CDR3 as set forth in a VH comprising the amino acid sequence of SEQ ID NO:25 and a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a VL comprising the amino acid sequence of SEQ ID NO:26. In some embodiments, the ROR antibody, such as the activatable ROR antibody after activation, specifically binds to ROR competitively with an anti-ROR antibody comprising a VH comprising the amino

acid sequence of SEQ ID NO:25, and a VL comprising the amino acid sequence of SEQ ID NO:26.

[00577] In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises six CDRs as listed in one column of **Table 1**. In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises three CDRs of the heavy chain variable region as set forth in SEQ ID NO:25 and three CDRs of the light chain variable regions as set forth in SEQ ID NO:26. In some embodiments, the ROR antibody, such as the activatable ROR antibody, comprises the heavy chain variable region as set forth in SEQ ID NO:25 and the light chain variable regions as set forth in SEQ ID NO:26.

[00578] In some embodiments, the ROR antibodies, such as the activatable antibodies, are superior in developability based on a known assay in the art, for example, various chromatographic methods, including size exclusion chromatography (SEC), hydrophobic interaction chromatography (HIC), and standup monolayer adsorption chromatography (SMAC). In some embodiments, the antibodies are superior in developability based on measurement of monomer percentage, solubility, and/or antibody aggregation or precipitation.

[00579] In some embodiments, the ROR antibody is an IgG, for example IgG1, IgG2, IgG3, or IgG4. In further embodiments, the ROR antibody is an IgG1. Additionally or alternatively, the ROR antibody comprises a kappa (κ) light chain (*e.g.*, a kappa (κ) antibody). In other embodiments, the ROR antibody comprises a lambda (λ) light chain (*e.g.*, a lambda (λ) antibody). In some embodiments, the ROR antibody is an IgG1 kappa antibody.

[00580] In some embodiments, ROR antibodies, such as the activatable ROR antibodies (such as monospecific or bispecific antibodies), including activatable human ROR antibodies, described herein comprise a heavy chain having a combination of (i) a VH described herein, such as in **Table 1**; and (ii) one or more heavy chain constant domains (*e.g.*, CH1, Hinge, CH2, and/or CH3). In further embodiments, the carboxyl terminus (C-terminus) of the VH is conjugated directly or indirectly to the amino terminus (N-terminus) of the one or more heavy chain constant domains. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:32. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:42. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:42,

except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution.

[00581] In some embodiments, activatable ROR antibodies (such as monospecific or bispecific antibodies), including activatable human ROR antibodies, described herein comprise a polypeptide comprising, from N-terminus to C-terminus, a masking peptide described herein (*e.g.*, **Table 2**) and a light chain having a combination of (i) a VL domain described herein, such as in **Table 1**; and (ii) a light chain constant domain (CL). In further embodiments, the C terminus of the VL is conjugated directly or indirectly to the N-terminus of the CL. In further embodiments, the masking peptide is conjugated directly or indirectly to the N-terminus of the light chain. In a specific embodiment, the light chain comprises the amino acid sequence of SEQ ID NO:33. In a specific embodiment, the polypeptide comprises the amino acid sequence of SEQ ID NO:34. In a specific embodiment, the polypeptide comprises the amino acid sequence of SEQ ID NO:36. In a specific embodiment, the polypeptide comprises the amino acid sequence of SEQ ID NO:38.

[00582] In specific embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO:32. In specific embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution. In specific embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO:42. In specific embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO:42, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution.

[00583] In some embodiments, activatable ROR antibodies (such as monospecific or bispecific antibodies), including activatable human ROR antibodies, described herein comprise (a) a heavy chain having a combination of (i) a VH described herein, such as in **Table 1**, and (ii) one or more heavy chain constant domains (*e.g.*, CH1, Hinge, CH2, and/or CH3); and (b) a polypeptide comprising, from N-terminus to C-terminus, a masking peptide described herein (*e.g.*, **Table 2**) and a light chain having a combination of (i) a VL described herein, such as in **Table 1**, and (ii) a light chain constant domain (CL). In further embodiments, the C-terminus of the VH is conjugated directly or indirectly to the N-terminus of the one or more heavy chain constant domains. In further embodiments, the C terminus of the VL is conjugated directly or indirectly to the N-terminus of the CL. In further embodiments, the masking peptide is conjugated directly or indirectly to the N-terminus of the light chain. In specific embodiments, the heavy chain comprises the amino acid sequence

of SEQ ID NO:32. In specific embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution. In specific embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO:42. In specific embodiments, the heavy chain comprises the amino acid sequence of SEQ ID NO:42, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution. In specific embodiment, the light chain comprises the amino acid sequence of SEQ ID NO:33. In specific embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:34. In specific embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:36. In specific embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:38. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, and the light chain comprises the amino acid sequence of SEQ ID NO:33. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, and the polypeptide comprises the amino acid sequence of SEQ ID NO:34. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, and the polypeptide comprises the amino acid sequence of SEQ ID NO:36. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, and the polypeptide comprises the amino acid sequence of SEQ ID NO:38. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution, and the light chain comprises the amino acid sequence of SEQ ID NO:33. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution, and the polypeptide comprises the amino acid sequence of SEQ ID NO:34. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution, and the polypeptide comprises the amino acid sequence of SEQ ID NO:36. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:32, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution, and the polypeptide comprises the amino acid sequence of SEQ ID NO:38. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:42, and the light chain comprises the amino acid sequence of SEQ ID NO:33. In a specific embodiment, the heavy chain

comprises the amino acid sequence of SEQ ID NO:42, and the polypeptide comprises the amino acid sequence of SEQ ID NO:34. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:42, and the polypeptide comprises the amino acid sequence of SEQ ID NO:36. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:42, and the polypeptide comprises the amino acid sequence of SEQ ID NO:38. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:42, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution, and the light chain comprises the amino acid sequence of SEQ ID NO:33. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:42, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution, and the polypeptide comprises the amino acid sequence of SEQ ID NO:34. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:42, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution, and the polypeptide comprises the amino acid sequence of SEQ ID NO:36. In a specific embodiment, the heavy chain comprises the amino acid sequence of SEQ ID NO:42, except for (i) a D356E substitution and/or an L358M substitution, and/or (ii) a K213R substitution or a K214R substitution, and the polypeptide comprises the amino acid sequence of SEQ ID NO:38.

[00584] In some embodiments, CDRs of an ROR antibody as used herein are disclosed in US Patent Application Publication No. US20210155692A1, which is incorporated by reference in its entirety. In some embodiments, an ROR1 antibody as used herein is disclosed in US Patent Application Publication No. US20210155692A1, which is incorporated by reference in its entirety.

[00585] In some embodiments, provided herein is an ROR antibody, such as an activatable ROR antibody, comprising any one of the anti-ROR antibodies described herein. In some embodiments, the activatable ROR antibody is an activatable antibody comprising two heavy chains and two light chains. In some embodiments, the activatable ROR antibody is an activatable antibody comprising two heavy chains comprising a same VH region and two light chains comprising a same VL region.

[00586] In some embodiments, the ROR antibody is an antibody comprising two heavy chains and two light chains. In some embodiments, the ROR antibody is an antibody comprising two heavy chains comprising a same VH region and two light chains comprising a same VL region.

[00587] In some embodiments, the ROR antibody, such as the activatable ROR antibody, is a monoclonal antibody, including a mouse, chimeric, humanized, or human antibody. In some embodiments, the ROR antibody, such as the activatable anti-ROR antibody, is an antibody fragment, *e.g.*, an scFv. In some embodiments, the ROR antibody, such as the activatable ROR antibody, is a fusion protein comprising the activatable anti-ROR antibody provided herein. In other embodiments, the ROR antibody, such as the activatable ROR antibody, is a multispecific activatable antibody comprising the anti-ROR antibody or fragment thereof provided herein.

[00588] In some embodiments, an ROR-ADC as disclosed herein, such as an activatable ROR-ADC as disclosed herein, comprises an ROR antibody. In some embodiments, the ROR antibody comprises a VH region comprising an amino acid sequence as set forth in SEQ ID NO:25 and a VL region comprising an amino acid sequence as set forth in SEQ ID NO:32. In some embodiments, the ROR antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO:32 or a variant thereof (such as a variant engineered to conjugated to a linker-payload as disclosed herein) and a light chain comprising an amino acid sequence as set forth in SEQ ID NO:33. In some embodiments, the ROR antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO:169 or a variant thereof (such as a variant engineered to conjugated to a linker-payload as disclosed herein) and a light chain comprising an amino acid sequence as set forth in SEQ ID NO:33.

[00589] In some embodiments, an ROR-ADC as disclosed herein, such as an activatable ROR-ADC as disclosed herein, comprises an ROR antibody. In some embodiments, the ROR antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO:34. In some embodiments, the ROR antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO:36. In some embodiments, the ROR antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO:38.

[00590] In some embodiments, an ROR-ADC as disclosed herein, such as an activatable ROR-ADC as disclosed herein, comprises an ROR antibody. In some embodiments, the ROR antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO:171 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO:34. In some embodiments, the ROR antibody comprises a heavy chain comprising an amino acid

sequence as set forth in SEQ ID NO:171 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO:36. In some embodiments, the ROR antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO:171 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO:38.

[00591] Also provided herein are ROR-ADCs, each comprising an activated activatable ROR antibody. In some embodiments, the activatable ROR antibodies are as disclosed herein. In further embodiments, the masking peptide of the activatable ROR antibodies (in an inactivated form) comprises a masking moiety (MM) and a cleavable moiety (CM), wherein the CM is located between the MM and a light chain variable region. In yet further embodiments, the masking peptide of the activatable ROR antibodies comprises, from N-terminus to C-terminus, an MM, a CM, and a light chain variable region. In some embodiments, the CM comprises, from N-terminus to C-terminus, a first optional linker, a cleavage site, and a second optional linker. In further embodiments, the activated form of the activatable ROR antibodies has been activated from the inactivated form by contacting the inactivated form with a protease (for example, MMP9), cleaving the cleavage site. Accordingly, in some embodiments, the activated activatable ROR antibodies (*e.g.*, the activatable ROR antibodies in an activated form) comprise (a) a polypeptide comprising, from N-terminus to C-terminus, amino acid residues (if any) of the cleavage site after the protease cleavage (such as, zero, or one, or two, or three, or four, or more C-terminal amino acid(s) of the cleavage site), the second optional linker, and the light chain variable region and (b) an antibody heavy chain variable region; wherein the masking peptide before protease cleavage comprises the amino acid sequence of SEQ ID NO:29, 30 or 31 (*see* **Table 2**), and wherein the antibody light chain variable region and the antibody heavy chain variable region are described herein (for example, comprise the CDRs described herein (*e.g.*, **Table 1**)).

Table 1: Antibody CDRs, VH and VL

		Exemplary	IMGT	Kabat	Chothia	Contact	AbM	CDR Set 1
VH CDR Seq.	**VH CDR1**	GFTFSSYFIH (SEQ ID NO:1)	GFTFSSYF (SEQ ID NO:7)	SYFIH (SEQ ID NO:12)	GFTFSSY (SEQ ID NO:13)	SSYFIH (SEQ ID NO:18)	GFTFSSYFIH (SEQ ID NO:1)	FSSYFI (SEQ ID NO:27)
	VH CDR2	GIYPSEGYTSY ADSVKG (SEQ ID NO:2)	IYPSEGYT (SEQ ID NO:8)	GIYPSEGYTSY ADSVKG (SEQ ID NO:2)	PSEG (SEQ ID NO:14)	WVAGIYPSEG YTS (SEQ ID NO:19)	GIYPSEGYTS (SEQ ID NO:24)	GIYPSEGYTS (SEQ ID NO:24)
	VH CDR3	YYVSGMDY (SEQ ID NO:3)	ARYYVSGMD Y (SEQ ID NO:9)	YYVSGMDY (SEQ ID NO:3)	YVSGMD (SEQ ID NO:15)	ARYYVSGMD (SEQ ID NO:20)	YYVSGMDY (SEQ ID NO:3)	YYVSGM (SEQ ID NO:28)
VL CDR Seq.	**VL CDR1**	RASQSVSSAV A (SEQ ID NO:4)	QSVSSA (SEQ ID NO:10)	RASQSVSSAV A (SEQ ID NO:4)	SQSVSSA (SEQ ID NO:16)	SSAVAWY (SEQ ID NO:21)	RASQSVSSAV A (SEQ ID NO:4)	RASQSVSSAV A (SEQ ID NO:4)
	VL CDR2	SASSLQS (SEQ ID NO:5)	SAS (SEQ ID NO:11)	SASSLQS (SEQ ID NO:5)	SAS (SEQ ID NO:11)	LLIYSASSLQ (SEQ ID NO:22)	SASSLQS (SEQ ID NO:5)	SASSLQS (SEQ ID NO:5)
	VL CDR3	QQYYYYPGT (SEQ ID NO:6)	QQYYYYPGT (SEQ ID NO:6)	QQYYYYPGT (SEQ ID NO:6)	YYYYPG (SEQ ID NO:17)	QQYYYYPG (SEQ ID NO:23)	QQYYYYPGT (SEQ ID NO:6)	YYYYPG (SEQ ID NO:17)
VH Sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYTSYADSVKGRFTISADTSKNTAYLQMNSLRA EDTAVYYCARYYVSGMDYWGQGTLVTVSS (SEQ ID NO:25)								
VL Sequence: DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPEDFATYYCQQ YYYYPGTFGQGTKVEIK (SEQ ID NO:26)								

Table 2: Masking Peptide

Masking peptide 1 Sequence: EVGSYADCPFFFPAALCSQGGGPLGLAGSGGS (SEQ ID NO:29) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9))
Masking peptide 2 Sequence: EVGSYHDHHFDCYYFDPPSHCPAGGGPLGLAGSGGS (SEQ ID NO:30) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site MMP-2 and MMP-9)
Masking peptide 3 Sequence: EVGSYADPFSVCHFADFVPYCYYGGGPLGLAGSGGS (SEQ ID NO:31) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)

Table 3: Parental Antibody P0

Heavy Chain Sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP E***LL***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***P***APIEKTISKAKGQPREPQ VYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:32) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329. The position numberings here reflect the amino acid numberings in SEQ ID NO:32 as well as according to the EU numbering system)
Heavy Chain Sequence (sFc- L234A/L235A/P329K): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP E***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***K***APIEKTISKAKGQPREP QVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:169) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329. The position numberings here reflect the amino acid numberings in SEQ ID NO:169 as well as according to the EU numbering system)
Light Chain Sequence: DIQMTQSPSSLSASVGDRVTITCRASQSVSSAVAWYQQKPGKAPKLLIYSASSLQSGVP SRFSGSRSGTDFTLTISSLQPEDFATYYCQQYYYYPGTFGQGTKVEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLS STLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO:33)

Table 4: Antibody M1

Heavy Chain Sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP E***LL***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***P***APIEKTISKAKGQPREPQ VYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:32) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329. The position numberings here reflect the amino acid numberings in SEQ ID NO:32 as well as according to the EU numbering system)
Heavy Chain Sequence (sFc- L234A/L235A/P329K): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP E***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***K***APIEKTISKAKGQPREP QVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:169) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329. The position numberings here reflect the amino acid numberings in SEQ ID NO:169 as well as according to the EU numbering system)
Polypeptide (Masking Peptide and Light Chain) Sequence: EVGSYADCPFFFPAALCSQGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTITCRASQ SVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPEDFATYY CQQYYYYPGTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC (SEQ ID NO:34) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)
Masking Peptide and VL Sequence: EVGSYADCPFFFPAALCSQGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTITCRASQ SVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPEDFATYY CQQYYYYPGTFGQGTKVEIK (SEQ ID NO:35) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)

Table 5: Antibody M2

Heavy Chain Sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP E***LL***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT

KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***P***APIEKTISKAKGQPREPQ VYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:32) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329. The position numberings here reflect the amino acid numberings in SEQ ID NO:32 as well as according to the EU numbering system)
Heavy Chain Sequence (sFc- L234A/L235A/P329K): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP E***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***K***APIEKTISKAKGQPREP QVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:169) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329. The position numberings here reflect the amino acid numberings in SEQ ID NO:169 as well as according to the EU numbering system)
Polypeptide (Masking Peptide and Light Chain) Sequence: EVGSYHDHHFDCYYFDPPSHCPAGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTIT CRASQSVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPED FATYYCQQYYYYPGTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC (SEQ ID NO:36) (underlined: protease recognition site for MMP-2 and MMP-9) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)
Masking Peptide and VL Sequence: EVGSYHDHHFDCYYFDPPSHCPAGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTIT CRASQSVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPED FATYYCQQYYYYPGTFGQGTKVEIK (SEQ ID NO:37) (underlined: protease recognition site for MMP-2 and MMP-9) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)

Table 6: Antibody M3

Heavy Chain Sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP E***LL***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***P***APIEKTISKAKGQPREPQ VYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:32) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329. The position numberings here reflect the amino acid numberings in SEQ ID NO:32 as well as according to the EU numbering system)

Heavy Chain Sequence (sFc- L234A/L235A/P329K): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAP E***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKT KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***K***APIEKTISKAKGQPREP QVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFF LYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK (SEQ ID NO:169) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329. The position numberings here reflect the amino acid numberings in SEQ ID NO:169 as well as according to the EU numbering system)
Polypeptide (Masking Peptide and Light Chain) Sequence: EVGSYADPFSVCHFADFVPYCYYGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTIT CRASQSVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPED FATYYCQQYYYYPGTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC (SEQ ID NO:38) (underlined: protease recognition site for MMP-2 and MMP-9) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)
Masking Peptide and VL Sequence: EVGSYADPFSVCHFADFVPYCYYGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTIT CRASQSVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPED FATYYCQQYYYYPGTFGQGTKVEIK (SEQ ID NO:39) (underlined: protease recognition site for MMP-2 and MMP-9) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)

Table 7: Antibody M1 (with sulfatase motifs)

Heavy Chain Sequence (with unconverted sulfatase motifs): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA**LCTPSR**GVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPE***LL***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***P***APIEKTISKAKGQP REPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGS**LCTPSR**GS (SEQ ID NO:42) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif. The position numberings here reflect the amino acid numberings according to the EU numbering system.)
Heavy Chain Sequence (with unconverted sulfatase motifs and sFc- L234A/L235A/P329K): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA**LCTPSR**GVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN

AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***K***APIEKTISKAKGQP REPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGS**LCTPSR**GS (SEQ ID NO:170) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif. The position numberings here reflect the amino acid numberings according to the EU numbering system.)
Heavy Chain Sequence (with fGly'-containing sulfatase motif): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL(fGly')TPSRG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPE***LL***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***P***APIEKTISKAK GQPREPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(fGly')TPSR GS (SEQ ID NO:48) (fGly' indicates the amino acid residue (formylglycine residue) coupled to the drug through a linker in an ADC as disclosed herein.) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif. The position numberings here reflect the amino acid numberings according to the EU numbering system.)
Heavy Chain Sequence (with fGly'-containing sulfatase motif and sFc-L234A/L235A/P329K): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL(fGly')TPSRG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***K***APIEKTISKAK GQPREPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(fGly')TPSR GS (SEQ ID NO:171) (fGly' indicates the amino acid residue (formylglycine residue) coupled to the drug through a linker in an ADC as disclosed herein.) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif. The position numberings here reflect the amino acid numberings according to the EU numbering system.)
Polypeptide (Masking Peptide and Light Chain) Sequence: EVGSYADCPFFFPAALCSQGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTITCRASQ SVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPEDFATYY CQQYYYYPGTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLS SPVTKSFNRGEC (SEQ ID NO:34) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)
Masking Peptide and VL Sequence:

EVGSYADCPFFFPAALCSQGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTITCRASQ SVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPEDFATYY CQQYYYYPGTFGQGTKVEIK (SEQ ID NO:35) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)

Table 8: Antibody M2 (with sulfatase motifs)

Heavy Chain Sequence (with unconverted sulfatase motifs): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA**LCTPSR**GVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGS**LCTPSR**GS (SEQ ID NO:42) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif)
Heavy Chain Sequence (with unconverted sulfatase motifs and sFc- L234A/L235A/P329K): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA**LCTPSR**GVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***K***APIEKTISKAKGQP REPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGS**LCTPSR**GS (SEQ ID NO:170) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif. The position numberings here reflect the amino acid numberings according to the EU numbering system.)
Heavy Chain Sequence (with fGly'-containing sulfatase motif): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL(fGly')TPSRG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPE***LL***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***P***APIEKTISKAK GQPREPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(fGly')TPSR GS (SEQ ID NO:48) (fGly' indicates the amino acid residue (formylglycine residue) coupled to the drug through a linker in an ADC as disclosed herein.) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif. The position numberings here reflect the amino acid numberings according to the EU numbering system.)

Heavy Chain Sequence (with fGly'-containing sulfatase motif and sFc-L234A/L235A/P329K): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL(fGly')TPSRG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***K***APIEKTISKAK GQPREPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(fGly')TPSR GS (SEQ ID NO:171) (fGly' indicates the amino acid residue (formylglycine residue) coupled to the drug through a linker in an ADC as disclosed herein.) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif. The position numberings here reflect the amino acid numberings according to the EU numbering system.)
Polypeptide (Masking Peptide and Light Chain) Sequence: EVGSYHDHHFDCYYFDPPSHCPAGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTIT CRASQSVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPED FATYYCQQYYYYPGTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC (SEQ ID NO:36) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)
Masking Peptide and VL Sequence: EVGSYHDHHFDCYYFDPPSHCPAGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTIT CRASQSVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPED FATYYCQQYYYYPGTFGQGTKVEIK (SEQ ID NO:37) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)

Table 9: Antibody M3 (with sulfatase motifs)

Heavy Chain Sequence (with unconverted sulfatase motifs): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA**LCTPSR**GVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGS**LCTPSR**GS (SEQ ID NO:42) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif)
Heavy Chain Sequence (with unconverted sulfatase motifs and sFc- L234A/L235A/P329K): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGA**LCTPSR**GVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPP

CPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***K***APIEKTISKAKGQP REPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGS**LCTPSR**GS (SEQ ID NO:170) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif. The position numberings here reflect the amino acid numberings according to the EU numbering system.)
Heavy Chain Sequence (with fGly'-containing sulfatase motif): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL(fGly')TPSRG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPE***LL***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***P***APIEKTISKAK GQPREPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(fGly')TPSR GS (SEQ ID NO:48) (fGly' indicates the amino acid residue (formylglycine residue) coupled to the drug through a linker in an ADC as disclosed herein.) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif. The position numberings here reflect the amino acid numberings according to the EU numbering system.)
Heavy Chain Sequence (with fGly'-containing sulfatase motif and sFc-L234A/L235A/P329K): EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGYT SYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTLVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL(fGly')TPSRG VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPE***AA***GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKAL***K***APIEKTISKAK GQPREPQVYTLPPSR*DEL*TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVL DSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL(fGly')TPSR GS (SEQ ID NO:171) (fGly' indicates the amino acid residue (formylglycine residue) coupled to the drug through a linker in an ADC as disclosed herein.) (underlined: positions 213-215; underlined and italic: positions 356-358; bold, italic and underlined: positions 234, 235, and 329; underlined and bold: LCTPSR (SEQ ID NO:100), engineered sulfatase motif. The position numberings here reflect the amino acid numberings according to the EU numbering system.)
Polypeptide (Masking Peptide and Light Chain) Sequence: EVGSYADPFSVCHFADFVPYCYYGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTIT CRASQSVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPED FATYYCQQYYYYPGTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYP REAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTH QGLSSPVTKSFNRGEC (SEQ ID NO:38) (underlined: protease recognition site for MMP-2 and MMP-9) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)

Masking Peptide and VL Sequence: EVGSYADPFSVCHFADFVPYCYYGGGPLGLAGSGGSDIQMTQSPSSLSASVGDRVTIT CRASQSVSSAVAWYQQKPGKAPKLLIYSASSLQSGVPSRFSGSRSGTDFTLTISSLQPED FATYYCQQYYYYPGTFGQGTKVEIK (SEQ ID NO:39) (underlined: protease recognition site for MMP-2 and MMP-9) (underlined: PLGLAG (SEQ ID NO:45), protease recognition site for MMP-2 and MMP-9)

Table 10: Human ROR1 and ROR2 Sequences

Human ROR1 (UniProt accession #Q01973) Sequence: MHRPRRRGTRPPLLALLAALLLAARGAAAQETELSVSAELVPTSSWNISSELNKDSYL TLDEPMNNITTSLGQTAELHCKVSGNPPPTIRWFKNDAPVVQEPRRLSFRSTIYGSRLRI RNLDTTDTGYFQCVATNGKEVVSSTGVLFVKFGPPPTASPGYSDEYEEDGFCQPYRGI ACARFIGNRTVYMESLHMQGEIENQITAAFTMIGTSSHLSDKCSQFAIPSLCHYAFPYC DETSSVPKPRDLCRDECEILENVLCQTEYIFARSNPMILMRLKLPNCEDLPQPESPEAA NCIRIGIPMADPINKNHKCYNSTGVDYRGTVSVTKSGRQCQPWNSQYPHTHTFTALRF PELNGGHSYCRNPGNQKEAPWCFTLDENFKSDLCDIPACDSKDSKEKNKMEILYILVP SVAIPLAIALLFFFICVCRNNQKSSSAPVQRQPKHVRGQNVEMSMLNAYKPKSKAKEL PLSAVRFMEELGECAFGKIYKGHLYLPGMDHAQLVAIKTLKDYNNPQQWTEFQQEAS LMAELHHPNIVCLLGAVTQEQPVCMLFEYINQGDLHEFLIMRSPHSDVGCSSDEDGTV KSSLDHGDFLHIAIQIAAGMEYLSSHFFVHKDLAARNILIGEQLHVKISDLGLSREIYSA DYYRVQSKSLLPIRWMPPEAIMYGKFSSDSDIWSFGVVLWEIFSFGLQPYYGFSNQEVI EMVRKRQLLPCSEDCPPRMYSLMTECWNEIPSRRPRFKDIHVRLRSWEGLSSHTSSTT PSGGNATTQTTSLSASPVSNLSNPRYPNYMFPSQGITPQGQIAGFIGPPIPQNQRFIPING YPIPPGYAAFPAAHYQPTGPPRVIQHCPPPKSRSPSSASGSTSTGHVTSLPSSGSNQEANI PLLPHMSIPNHPGGMGITVFGNKSQKPYKIDSKQASLLGDANIHGHTESMISAEL (SEQ ID NO:40)
Human ROR2 (UniProt accession #Q01974) Sequence: MARGSALPRRPLLCIPAVWAAAALLLSVSRTSGEVEVLDPNDPLGPLDGQDGPIPTLK GYFLNFLEPVNNITIVQGQTAILHCKVAGNPPPNVRWLKNDAPVVQEPRRIIIRKTEYG SRLRIQDLDTTDTGYYQCVATNGMKTITATGVLFVRLGPTHSPNHNFQDDYHEDGFC QPYRGIACARFIGNRTIYVDSLQMQGEIENRITAAFTMIGTSTHLSDQCSQFAIPSFCHF VFPLCDARSRTPKPRELCRDECEVLESDLCRQEYTIARSNPLILMRLQLPKCEALPMPE SPDAANCMRIGIPAERLGRYHQCYNGSGMDYRGTASTTKSGHQCQPWALQHPHSHH LSSTDFPELGGGHAYCRNPGGQMEGPWCFTQNKNVRMELCDVPSCSPRDSSKMGILY ILVPSIAIPLVIACLFFLVCMCRNKQKASASTPQRRQLMASPSQDMEMPLINQHKQAKL KEISLSAVRFMEELGEDRFGKVYKGHLFGPAPGEQTQAVAIKTLKDKAEGPLREEFRH EAMLRARLQHPNVVCLLGVVTKDQPLSMIFSYCSHGDLHEFLVMRSPHSDVGSTDDD RTVKSALEPPDFVHLVAQIAAGMEYLSSHHVVHKDLATRNVLVYDKLNVKISDLGLF REVYAADYYKLLGNSLLPIRWMAPEAIMYGKFSIDSDIWSYGVVLWEVFSYGLQPYC GYSNQDVVEMIRNRQVLPCPDDCPAWVYALMIECWNEFPSRRPRFKDIHSRLRAWG NLSNYNSSAQTSGASNTTQTSSLSTSPVSNVSNARYVGPKQKAPPFPQPQFIPMKGQIR PMVPPPQLYVPVNGYQPVPAYGAYLPNFYPVQIPMQMAPQQVPPQMVPKPSSHHSGS GSTSTGYVTTAPSNTSMADRAALLSEGADDTQNAPEDGAQSTVQEAEEEEEGSVPET ELLGDCDTLQVDEAQVQLEA (SEQ ID NO:41)

7.5 CONJUGATION

[00592] In certain embodiments, the amino acid sequence of an ROR antibody, such as an activatable ROR antibody, is modified to include a sulfatase motif that contains a serine or cysteine residue that is capable of being converted (oxidized) to a 2-formylglycine (fGly) residue by action of a formylglycine generating enzyme (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.

7.5.1 Sulfatase motifs

[00593] 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.

[00594] In certain embodiments, ROR antibodies, such as activatable ROR 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 ROR antibody, such as the activatable ROR antibody. In certain embodiments, the ROR antibody, such as the activatable ROR 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 ROR antibody, such as the activatable ROR antibody. Where an amino acid sequence native to the ROR antibody, such as the activatable ROR 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.

[00595] 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.

[00596] 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.

[00597] In some embodiments, the ROR antibody, such as the activatable ROR antibody, as disclosed herein comprises a sulfatase motif.

[00598] In certain embodiments, the sulfatase motif used may be described by the formula:

$X^1Z^{10}X^2Z^{20}X^3Z^{30}$ (V)

wherein:

Z^{10} is cysteine or serine (which can also be represented by (C/S));

Z^{20} is either a proline or alanine residue (which can also be represented by (P/A));

Z^{30} 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 ROR 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.

[00599] 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 the formula $X^1Z^{10}X^2Z^{20}X^3Z^{30}$,

wherein:

Z^{10} is cysteine or serine;

Z^{20} is a proline or alanine residue;

Z^{30} 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 ROR antibody, such as the activatable ROR antibody, X^1 is present;

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

[00600] 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.

[00601] For example, where the FGE is a eukaryotic FGE (*e.g.*, a mammalian FGE, including a human FGE), the sulfatase motif can be of the formula:

$X^1CX^2PX^3Z^{30}$ (VI)

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 ROR antibody, such as the activatable ROR 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^{30} 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.

[00602] Specific examples of sulfatase motifs include LCTPSR (SEQ ID NO:100), MCTPSR (SEQ ID NO:101), VCTPSR (SEQ ID NO:102), LCSPSR (SEQ ID NO:103), LCAPSR (SEQ ID NO:104), LCVPSR (SEQ ID NO:105), LCGPSR (SEQ ID NO:106), ICTPAR (SEQ ID NO:107), LCTPSK (SEQ ID NO:108), MCTPSK (SEQ ID NO:109), VCTPSK (SEQ ID NO:110), LCSPSK (SEQ ID NO:111), LCAPSK (SEQ ID NO:112), LCVPSK (SEQ ID NO:113), LCGPSK (SEQ ID NO:114), LCTPSA (SEQ ID NO:115), ICTPAA (SEQ ID NO:116), MCTPSA (SEQ ID NO:117), VCTPSA (SEQ ID NO:118), LCSPSA (SEQ ID NO:119), LCAPSA (SEQ ID NO:120), LCVPSA (SEQ ID NO:121), and LCGPSA (SEQ ID NO:122).

7.5.2 fGly-containing Sequences

[00603] In some embodiments, the ROR antibody as disclosed herein, such as the activatable ROR antibody as disclosed herein, comprises a fGly-containing sulfatase motif.

[00604] 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 of the formula:

$X^1(fGly)X^2Z^{20}X^3Z^{30}$ (VII)

wherein:

fGly is the formylglycine residue;

Z^{20} is either a proline or alanine residue (which can also be represented by (P/A));

Z^{30} 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 ROR 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.

[00605] Specific examples of fGly-containing sulfatase motifs include L(fGly)TPSR (SEQ ID NO:123), M(fGly)TPSR (SEQ ID NO:124), V(fGly)TPSR (SEQ ID NO:125), L(fGly)SPSR (SEQ ID NO:126), L(fGly)APSR (SEQ ID NO:127), L(fGly)VPSR (SEQ ID NO:128), L(fGly)GPSR (SEQ ID NO:129), I(fGly)TPAR (SEQ ID NO:130), L(fGly)TPSK (SEQ ID NO:131), M(fGly)TPSK (SEQ ID NO:132), V(fGly)TPSK (SEQ ID NO:133), L(fGly)SPSK (SEQ ID NO:134), L(fGly)APSK (SEQ ID NO:135), L(fGly)VPSK (SEQ ID NO:136), L(fGly)GPSK (SEQ ID NO:137), L(fGly)TPSA (SEQ ID NO:138), I(fGly)TPAA (SEQ ID NO:139), M(fGly)TPSA (SEQ ID NO:140), V(fGly)TPSA (SEQ ID NO:141), L(fGly)SPSA (SEQ ID NO:142), L(fGly)APSA (SEQ ID NO:143), L(fGly)VPSA (SEQ ID NO:144), and L(fGly)GPSA (SEQ ID NO:145).

[00606] In some embodiments, the ROR antibody Ab of Formula (I) or (II) as disclosed herein comprises a fGly'-containing sulfatase motif. In further embodiment, the ROR antibody Ab of Formula (I) or (II) as disclosed herein comprises a light chain (such as SEQ ID NO:33, 34, 36 or 38) and a heavy chain (such as a variant thereof of SEQ ID NO:32 further comprising one or more sequences of Formula (VIII), for example SEQ ID NO:48 or 171.

[00607] In some embodiments, the ROR antibody Ab of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)) comprises a fGly'-containing sulfatase motif. In further embodiment, the ROR antibody Ab of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)) comprises a light chain (such as SEQ ID NO:33, 34, 36 or 38) and a heavy chain (such as a variant thereof of SEQ ID NO:32 further comprising one or more sequences of Formula (VIII), for example SEQ ID NO:48 or 171.

[00608] As described above, to produce the conjugate, the ROR antibody, such as the activatable ROR 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 the formula:

$$X^1(fGly')X^2Z^{20}X^3Z^{30} \qquad \text{(VIII)}$$

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^{20} is either a proline or alanine residue (which can also be represented by (P/A));

Z^{30} 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 ROR 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.

[00609] Specific examples of fGly'-containing sulfatase motifs include L(fGly')TPSR (SEQ ID NO:146), M(fGly')TPSR (SEQ ID NO:147), V(fGly')TPSR (SEQ ID NO:148), L(fGly')SPSR (SEQ ID NO:149), L(fGly')APSR (SEQ ID NO:150), L(fGly')VPSR (SEQ ID NO:151), L(fGly')GPSR (SEQ ID NO:152), I(fGly')TPAR (SEQ ID NO:153), L(fGly')TPSK (SEQ ID NO:154), M(fGly')TPSK (SEQ ID NO:155), V(fGly')TPSK (SEQ ID NO:156), L(fGly')SPSK (SEQ ID NO:157), L(fGly')APSK (SEQ ID NO:158), L(fGly')VPSK (SEQ ID NO:159), L(fGly')GPSK (SEQ ID NO:160), L(fGly')TPSA (SEQ ID NO:161), I(fGly')TPAA (SEQ ID NO:162), M(fGly')TPSA (SEQ ID NO:163), V(fGly')TPSA (SEQ ID NO:164), L(fGly')SPSA (SEQ ID NO:165), L(fGly')APSA (SEQ ID NO:166), L(fGly')VPSA (SEQ ID NO:167), and L(fGly')GPSA (SEQ ID NO:168).

[00610] 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."

[00611] 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.

[00612] 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 to 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.

[00613] 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.

[00614] 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.

[00615] 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.

[00616] A sulfatase motif can be provided within or adjacent 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.

[00617] 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.

[00618] 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.).

[00619] As further non-limiting examples, a subject antibody conjugate can bind an antigen present on a cell infected with a virus (*e.g.*, where the antigen is encoded by the virus; where the antigen is expressed on a cell type that is infected by a virus; etc.), and the conjugated moiety can be a drug, such as a viral fusion inhibitor. For example, a subject antibody conjugate can bind an antigen present on a cell infected with a virus, and the conjugated moiety can be a drug, such as a viral fusion inhibitor.

[00620] In some embodiments, the ROR antibody, such as the activatable antibody that binds to ROR, comprises a heavy chain which has been modified to include unconverted sulfatase motifs (*e.g.*, any one of SEQ ID NOs:100-122) in the CH1 region and in the CT region, and therefore comprises the amino acid sequence of:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGY
TSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALCTPSRGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHT
CPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK
AKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP
PVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSLCTPS
RGS (SEQ ID NO:42), wherein each underline portion comprises the unconverted sulfatase motif of SEQ ID NO:100 but, in any embodiment, may be independently replaced with any one of SEQ ID NOs:101-122.

[00621] In further embodiments, the antibody further comprises a light chain comprising an amino acid sequence of any one of SEQ ID NO: 33, 34, 36, or 38. Additionally or alternatively, there may or may not be an additional K at the C terminal of the heavy chain.

As it would be understood by one of skill in the art, the C-terminal K residue of the heavy chain can be removed, for example, during production of the antibody. Additionally or alternatively, either or both of the sulfatase motifs are converted and conjugated to a linker-payload in an ROR-ADC as disclosed herein.

[00622] In some embodiments, the ROR antibody, such as the activatable antibody that binds to ROR, comprises a heavy chain which has been modified to include unconverted sulfatase motifs (*e.g.*, any one of SEQ ID NOs:100-122) in the CH1 region and in the CT region, and therefore comprises the amino acid sequence of: SEQ ID NO:170, wherein each underline portion comprises the unconverted sulfatase motif of SEQ ID NO:100 but, in any embodiment, may be independently replaced with any one of SEQ ID NOs:101-122.

[00623] In further embodiments, the antibody further comprises a light chain comprising an amino acid sequence of any one of SEQ ID NO: 33, 34, 36, or 38. Additionally or alternatively, there may or may not be an additional K at the C terminal of the heavy chain. As it would be understood by one of skill in the art, the C-terminal K residue of the heavy chain can be removed, for example, during production of the antibody. Additionally or alternatively, either or both of the sulfatase motifs are converted and conjugated to a linker-payload in an ROR-ADC as disclosed herein.

[00624] In some embodiments, the ROR antibody, such as the activatable antibody that binds to ROR, comprises a heavy chain which has been modified to include formylglycine residues in the CH1 and CT regions and therefore comprises the amino acid sequence of: EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGY TSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTL VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL(fGly)TPS RGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL (fGly)TPSRGS (SEQ ID NO:47), wherein fGly indicates are a formylglycine residue, wherein each underline portion comprises the fGly-containing sulfatase motif of SEQ ID NO:123 but, in any embodiment, may be independently replaced with any one of SEQ ID NOs:124-145.

[00625] In further embodiments, the antibody further comprises a light chain comprising an amino acid sequence of any one of SEQ ID NO: 33, 34, 36, or 38. Additionally or

alternatively, there may or may not be an additional K at the C terminal of the heavy chain. As it would be understood by one of skill in the art, the C-terminal K residue of the heavy chain can be removed, for example, during production of the antibody. Additionally or alternatively, either or both of the sulfatase motifs are conjugated to a linker-payload in an ROR-ADC as disclosed herein.

[00626] In some embodiments, the ROR antibody, such as the activatable antibody that binds to ROR, comprises a heavy chain which has been modified to include formylglycine residues in the CH1 and CT regions and therefore comprises the amino acid sequence of:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGY
TSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL(fGly)TPS
RGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY
VDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALKAPIE
KTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN
YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGS
L(fGly)TPSRGS (SEQ ID NO:172), wherein fGly indicates a formylglycine residue, wherein each underline portion comprises the fGly-containing sulfatase motif of SEQ ID NO:123 but, in any embodiment, may be independently replaced with any one of SEQ ID NOs:124-145.

[00627] In further embodiments, the antibody further comprises a light chain comprising an amino acid sequence of any one of SEQ ID NO: 33, 34, 36, or 38. Additionally or alternatively, there may or may not be an additional K at the C terminal of the heavy chain. As it would be understood by one of skill in the art, the C-terminal K residue of the heavy chain can be removed, for example, during production of the antibody. Additionally or alternatively, either or both of the sulfatase motifs are conjugated to a linker-payload in an ROR-ADC as disclosed herein.

[00628] In some embodiments, an ROR antibody, such as an activatable ROR antibody, in an ROR-ADC as disclosed herein comprises a heavy chain comprising the amino acid sequence of:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYFIHWVRQAPGKGLEWVAGIYPSEGY
TSYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARYYVSGMDYWGQGTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL(fGly')TP
SRGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCD
KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV

DGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGSL (fGly')TPSRGS (SEQ ID NO:48), wherein each (fGly') is an amino acid (formylglycine residue) having its side chain replaced by a linker-drug as disclosed herein, for example, in the ADC of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)). In SEQ ID NO:48, each underline portion comprises the fGly'-containing sulfatase motif of SEQ ID NO:146 but, in any embodiment, may be independently replaced with any one of SEQ ID NOs:147-168.

[00629] In further embodiments, the antibody further comprises a light chain comprising an amino acid sequence of any one of SEQ ID NO: 33, 34, 36, or 38. Additionally or alternatively, there may or may not be an additional K at the C terminal of the heavy chain. As it would be understood by one of skill in the art, the C-terminal K residue of the heavy chain can be removed, for example, during production of the antibody. Additionally or alternatively, either or both of the sulfatase motifs are conjugated to a linker-payload in an ROR-ADC as disclosed herein.

[00630] In some embodiments, an ROR antibody, such as an activatable ROR antibody, in an ROR-ADC as disclosed herein comprises a heavy chain having the amino acid sequence of SEQ ID NO:171, wherein each (fGly') is an amino acid (formylglycine residue) having its side chain replaced by a linker-drug as disclosed herein, for example, in the ADC of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)). In SEQ ID NO:48, each underline portion comprises the fGly'-containing sulfatase motif of SEQ ID NO:146 but, in any embodiment, may be independently replaced with any one of SEQ ID NOs:147-168.

[00631] In further embodiments, the antibody further comprises a light chain comprising an amino acid sequence of any one of SEQ ID NO: 33, 34, 36, or 38. Additionally or alternatively, there may or may not be an additional K at the C terminal of the heavy chain. As it would be understood by one of skill in the art, the C-terminal K residue of the heavy chain is removed, for example, during production of the antibody. Additionally or alternatively, either or both of the sulfatase motifs are conjugated to a linker-payload in an ROR-ADC as disclosed herein.

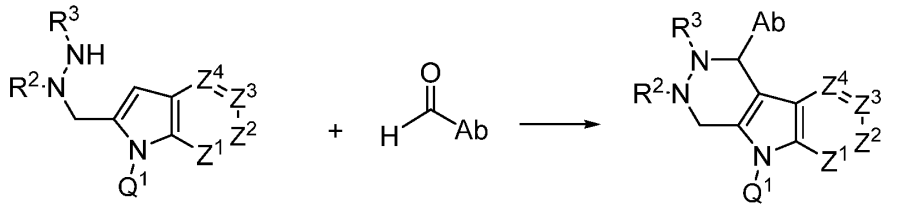
[00632] Other suitable sulfatase motif and their location in an antibody can also be used, such as International Publication Nos. WO2012097333, WO2017189432, and WO2018169953, each of which is incorporated herein by reference in its entirety.

[00633] In some embodiments, an ROR-ADC is prepared from an ROR antibody, wherein the antibody comprises (i) a heavy chain that is inserted with one or more fGly-containing sulfatase motifs, such as a heavy chain comprising an amino acid sequence as set forth in SEQ ID No: 47 or 172, and (ii) a light chain, such as the one comprising an amino acid sequence as set forth in any one of SEQ ID NO: 33, 34, 36, or 38.

[00634] In some embodiments, any ROR-ADC as disclosed herein comprises an ROR antibody conjugated to a linker-payload as disclosed herein (such as (Ia) or (IIa)), wherein the antibody comprises (i) a heavy chain that is inserted with one or more fGly'-containing sulfatase motifs, such as a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 48 or 171, and (ii) a light chain, such as the one comprising an amino acid sequence as set forth in any one of SEQ ID NO: 33, 34, 36, or 38.

[00635] In some embodiments, Ab of any ADC formula as disclosed herein (such as Formula (I), (II), (XIV-3), (XIV-4), (XIV-5), (XIV-6), or (XIV-7)) comprises (i) a heavy chain that is inserted with one or more fGly'-containing sulfatase motifs, such as a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 48 or 171, and (ii) a light chain, such as the one comprising an amino acid sequence as set forth in any one of SEQ ID NO: 33, 34, 36, or 38.

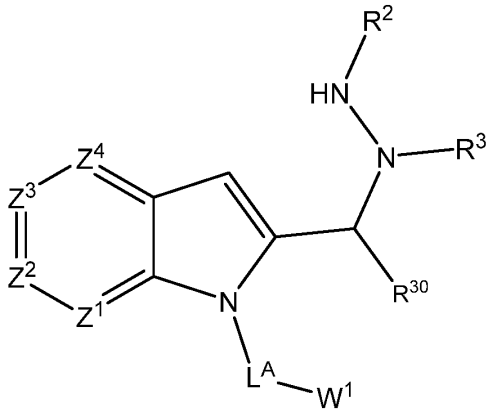
[00636] A general scheme for coupling an antibody to a pyridazine-pyrrolo coupling moiety is shown below.



[00637] 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 as shown above. An ROR antibody, such as an activatable ROR antibody, can include a 2-formylglycine residue (fGly) that is reacted with azaHIPS coupling moiety, thus conjugating the two together. To generate an ROR antibody, such as an activatable ROR-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.*, Z^1, Z^2, Z^3, Z^4, or Q^1). 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. Z^1, Z^2, Z^3, and Z^4 can be as described herein, such as for Formula (I). Q^1 may be $-L^A-W^1$, as described herein, such as for Formula (I).

[00638] Formula (Ia) below represents a hydrazinyl-pyrrolo coupling moiety that can be used to link an ROR antibody and a drug in an ROR-ADC.



(Ia).

Accordingly, provided is an ADC produced by conjugating an ROR antibody as disclosed herein to one or more of a linker-payload represented by Formula (Ia). In some embodiments, one or more of the components (such as R^2, R^3, Z^1, Z^2, Z^3, Z^4, L^A, or W^1) of Formula (Ia) are each as defined herein, such as for Formula (I). In further embodiments, R^{30} is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In some embodiments of Formula (Ia),

Z^1, Z^2, Z^3 and Z^4 are each independently selected from CR^4, N and $C-L^B-W^2$, wherein at least one Z^1, Z^2, Z^3 and Z^4 is $C-L^B-W^2$;

R^{30} is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

R^2 and R^3 are each independently selected from 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, and substituted heterocyclyl, or R^2 and R^3 are optionally cyclically linked to form a 5 or 6-membered heterocyclyl;

each R^4 is independently selected from hydrogen, halogen, 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, and substituted heterocyclyl;

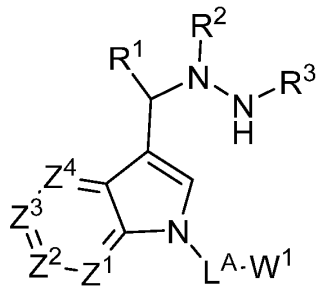
L^A is a first linker as described herein;

L^B is a second linker as described herein;

W^1 is a first drug as described herein; and

W^2 is a second drug as described herein.

[00639] Formula (X) below represents a hydrazinyl-pyrrolo coupling moiety that can be used to link an ROR antibody, such as an activatable ROR antibody, and a drug in a ROR-ADC:



(X)

wherein:

Z^1, Z^2, Z^3 and Z^4 are each independently selected from CR^4, N and C-L^B-W^2, wherein at least one Z^1, Z^2, Z^3 and Z^4 is C-L^B-W^2;

R^1 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

R^2 and R^3 are each independently selected from 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, and substituted heterocyclyl, or R^2 and R^3 are optionally cyclically linked to form a 5 or 6-membered heterocyclyl;

each R^4 is independently selected from hydrogen, halogen, 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, and substituted heterocyclyl;

L^A is a first linker as described herein;

L^B is a second linker as described herein;

W^1 is a first drug as described herein; and

W^2 is a second drug as described herein.

[00640] In some embodiments, provided is an ADC of Formula (I),

wherein

R^1 is hydrogen;

Z^1 is X^1-Y^1, wherein X^1 represents an atom in the ring of Formula (I) and is selected from the group consisting of C, N, O and S;

Z^2 is C-Y^2;

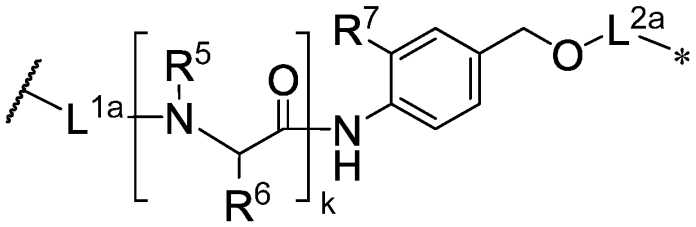
Y^1 and Y^2 are each independently selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl substituted heterocyclyl, and absent when adjacent to N; or Y^1 and Y^2 are cyclically linked;

Z^3 is C-L^B-W^2;

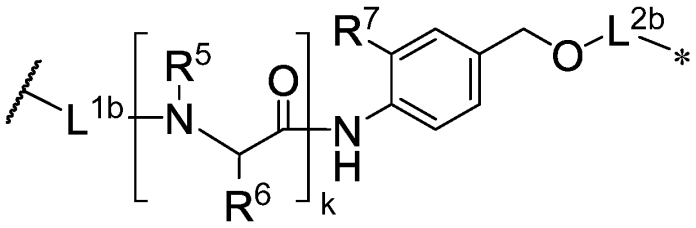
Z^4 is CH;

L^A is linker (L-3-a); and

L^B is linker (L-3-b).



(L-3-a),



(L-3-b).

In some embodiments of linker (L-3-a) and linker (L-3-b),

each ~~~ represents the point of attachment to a pyridazine-pyrrolo coupling moiety as described herein (*e.g.*, in any of Formulas as disclosed herein);

* in linker (L-3-a) represents the point of attachment to W^1;

* in linker (L-3-b) represents the point of attachment to W^2;

each R^5 is independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl;

each R^6 is independently alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl;

each R^7 is a cleavable moiety;

each k is an integer from 1 to 10;

L^{1a} comprises $-(T^1\text{-}V^1)_a\text{-}(T^2\text{-}V^2)_b\text{-}(T^3\text{-}V^3)_c\text{-}(T^4\text{-}V^4)_d\text{-}$,

L^{1b} comprises $-(T^1\text{-}V^1)_a\text{-}(T^2\text{-}V^2)_b\text{-}(T^3\text{-}V^3)_c\text{-}(T^4\text{-}V^4)_d\text{-}$,

L^{2a} comprises $-(T^5\text{-}V^5)_e\text{-}(T^6\text{-}V^6)_f\text{-}(T^7\text{-}V^7)_g\text{-}(T^8\text{-}V^8)_h\text{-}$;

L^{2b} comprises $-(T^5\text{-}V^5)_e\text{-}(T^6\text{-}V^6)_f\text{-}(T^7\text{-}V^7)_g\text{-}(T^8\text{-}V^8)_h\text{-}$;

T^1, T^2, T^3, T^4, T^5, T^6, T^7, and T^8 are each independently a covalent bond, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_m\text{-}$, P4A-R^{12}, acetal, a hydrazine, a disulfide, or an ester;

V^1, V^2, V^3, V^4, V^5, V^6, V^7, and V^8 are each independently a covalent bond, -CO-, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$, or -P(O)OH-;

each of a, b, c, d, e, f, g, and h is independently 1 or 0;

each m is independently an integer from 1 to 12;

each n is independently an integer from 1 to 30;

each p is independently an integer from 1 to 20;

each q is independently an integer from 1 to 6;

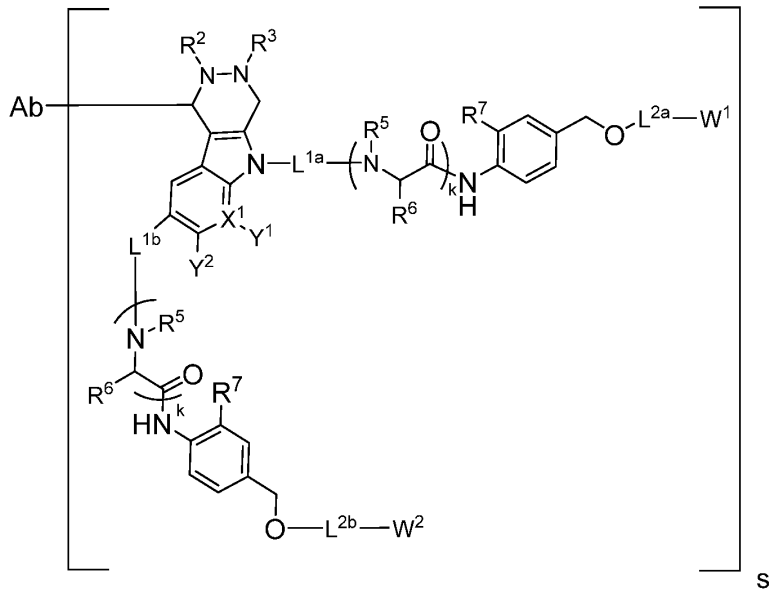
each w is independently an integer from 1 to 20;

each R^{12} is independently hydrogen, alkyl, substituted alkyl, a polyethylene glycol moiety (*e.g.*, a polyethylene glycol or a modified polyethylene glycol), 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;

each R^{13} is independently hydrogen, alkyl, substituted alkyl, aryl, or substituted aryl; and

each R^{15} is independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl. In some embodiments, each substituent is as defined herein, such as with respect to Formula (I).

[00641] Accordingly, in some embodiments, the ROR-ADC is represented by Formula (XIV-3):



(XIV-3)

wherein:

X^1 is selected from the group consisting of C, N, O and S;

Y^1 and Y^2 are each independently selected from the group consisting of hydrogen, halogen, 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 substituted heterocyclyl, and absent when adjacent to N; or Y^1 and Y^2 are cyclically linked;

each R^5 is independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl;

each R^6 is independently alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl;

each R^7 is a cleavable moiety;

L^{1a} comprises $-(T^1\text{-}V^1)_a\text{-}(T^2\text{-}V^2)_b\text{-}$,

L^{2a} comprises $-(T^5\text{-}V^5)_e\text{-}(T^6\text{-}V^6)_f\text{-}$;

L^{1b} comprises $-(T^7\text{-}V^7)_g\text{-}(T^8\text{-}V^9)_h\text{-}$,

L^{2b} comprises $-(T^{12}\text{-}V^{12})_l\text{-}(T^{13}\text{-}V^{13})_m\text{-}$;

T^1, T^2, T^5, and T^6 are each independently a covalent bond, C_1-C_{12} alkyl, substituted C_1-C_{12} alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, EDA, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_v-$, 4AP, acetal, a hydrazine, a disulfide, or an ester;

V^1, V^2, V^5, and V^6 are each independently a covalent bond, -CO-, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$, or -P(O)OH-;

T^7, T^8, T^{12}, and T^{13} are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x-$, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12;

V^7, V^8, V^{12}, and V^{13} are each independently selected from the group consisting of a covalent bond, -CO-, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$ and -P(O)OH-;

each of a, b, e, g, h, l, and m is independently 1 or 0;

each k is an integer from 1 to 10;

each p is independently an integer from 1 to 20;

each q is independently an integer from 1 to 6;

each v is independently an integer from 1 to 12;

each z is an integer from 1 to 10;

each R^{12} is independently hydrogen, alkyl, substituted alkyl, a polyethylene glycol moiety (*e.g.*, a polyethylene glycol or a modified polyethylene glycol), 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;

each R^{13} is independently hydrogen, alkyl, substituted alkyl, aryl, or substituted aryl; and

each R^{15} is independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl.In some embodiments, each substituent is as defined herein, such as with respect to Formula (I).

[00642] In some embodiments, provided is an ADC of Formula (I),

wherein

R^1 is hydrogen;

Z^1 is X^1-Y^1, wherein X^1 represents an atom in the ring of Formula (I) and is selected from the group consisting of C, N, O and S;

Z^2 is C-Y^2;

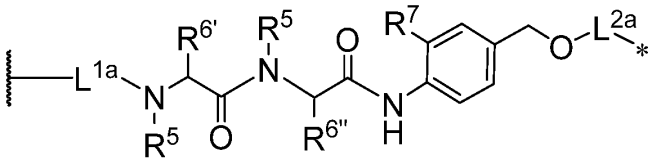
Y^1 and Y^2 are each independently selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl substituted heterocyclyl, and absent when adjacent to N; or Y^1 and Y^2 are cyclically linked;

Z^4 is CH;

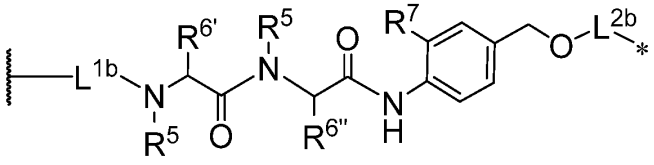
Z^3 is C-L^B-W^2;

L^A is linker (L-4-a); and

L^B is linker (L-4-b).



(L-4-a),



(L-4-b),

wherein:

each ~~~ represents the point of attachment to a pyridazine-pyrrolo coupling moiety as described herein (*e.g.*, any of Formulas as disclosed herein);

* in linker (L-4-a) represents the point of attachment to W^1;

* in linker (L-4-b) represents the point of attachment to W^2;

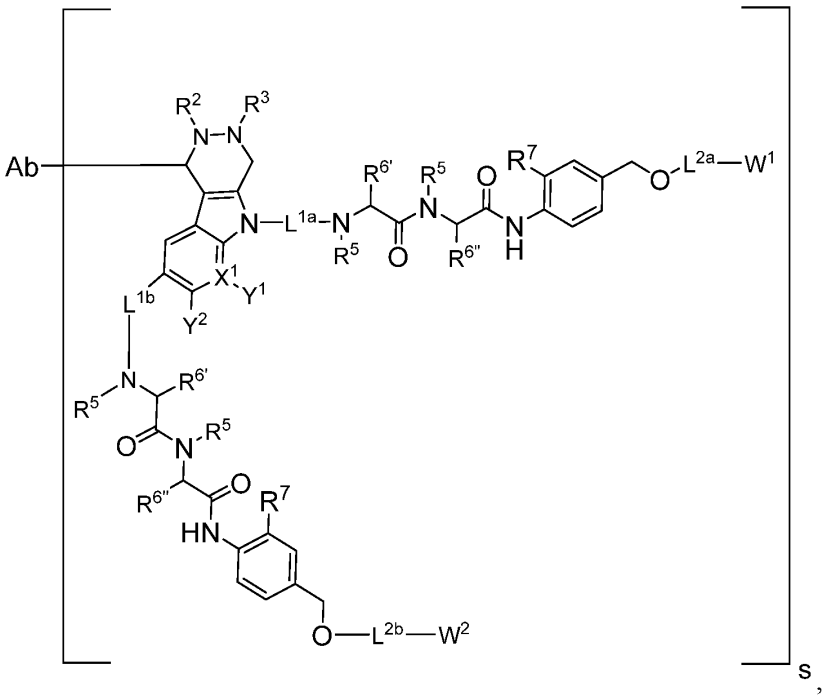
each R^5 is independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl;

each $R^{6'}$ and $R^{6''}$ are each independently alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl;

each R^7 is a cleavable moiety; and

L^{1a}, L^{2a}, L^{1b}, and L^{2b} are as defined herein, such as for linker (L-3-a) or (L-3-b). In some embodiments, each substituent is as defined herein, such as with respect to Formula (I).

[00643] Accordingly, in some embodiments, the ROR-ADC is represented by Formula (XIV-4):



(XIV-4)

wherein each substituent is as defined with respect to Formula (XIV-3). In some embodiments, R^7 is an enzymatically cleavable moiety comprising a sugar moiety, such as a

glycoside or glycosyl. In some embodiments, each substituent is as defined herein, such as with respect to Formula (I).

[00644] In some embodiments, provided is an ADC of Formula (I),

wherein

R^1 is hydrogen;

Z^1 is X^1-Y^1, wherein X^1 represents an atom in the ring of Formula (I) and is selected from the group consisting of C, N, O and S;

Z^2 is C-Y^2;

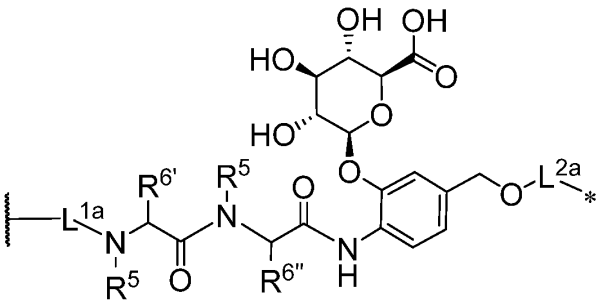
Y^1 and Y^2 are each independently selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl substituted heterocyclyl, and absent when adjacent to N; or Y^1 and Y^2 are cyclically linked;

Z^4 is CH;

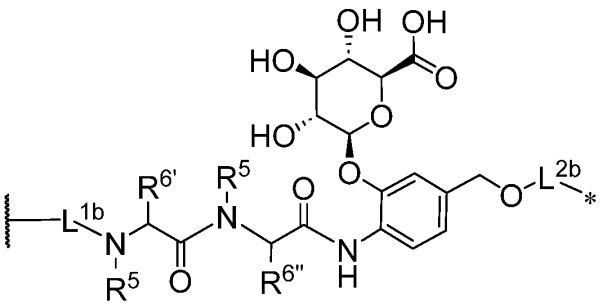
Z^3 is C-L^B-W^2;

L^A is linker (L-5-a); and

L^B is linker (L-5-b):



(L-5-a),



(L-5-b).

wherein:

each ~~~ represents the point of attachment to a pyridazine-pyrrolo coupling moiety as described herein (*e.g.*, any of Formulas as disclosed herein);

* in linker (L-5-a) represents the point of attachment to W^1;

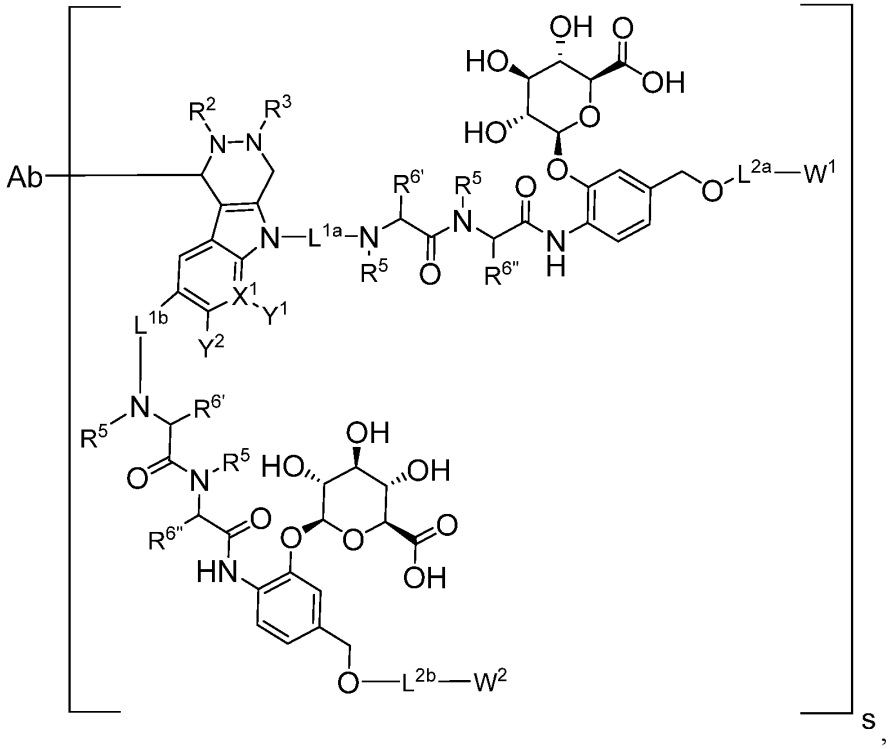
* in linker (L-5-b) represents the point of attachment to W^2;

each R^5 is independently hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl;

$R^{6'}$ and $R^{6''}$ are independently alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl; and

L^{1a}, L^{2a}, L^{1b}, and L^{2b} are as defined herein, such as for linker (L-3-a) or (L-3-b). In some embodiments, each substituent is as defined herein, such as with respect to Formula (I).

[00645] Accordingly, in some embodiments, the ROR-ADC is represented by Formula (XIV-5):



(XIV-5)

wherein each substituent is as defined with respect to Formula (XIV-3). In some embodiments, each substituent is as defined herein, such as with respect to Formula (I).

[00646] In some embodiments, provided is an ADC of Formula (I),

wherein

R^1 is hydrogen;

Z^2 is C-Y^2;

Z^1 is X^1-Y^1, wherein X^1 represents an atom in the ring of Formula (I) and is selected from the group consisting of C, N, O and S;

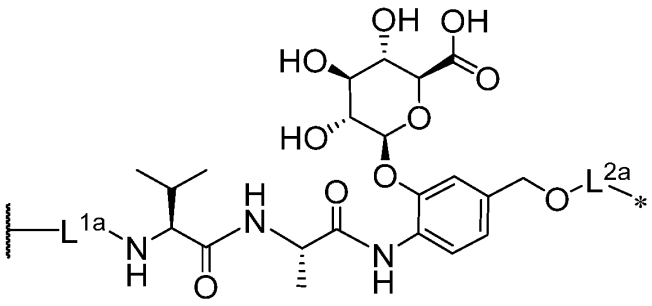
Y^1 and Y^2 are each independently selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl substituted heterocyclyl, and absent when adjacent to N; or Y^1 and Y^2 are cyclically linked;

Z^4 is CH;

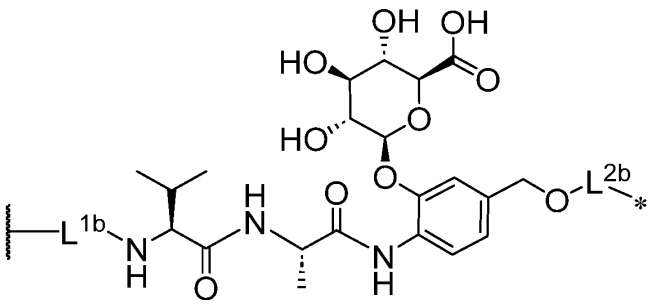
Z^3 is C-L^B-W^2;

L^A is linker (L-6-a); and

L^B is linker (L-6-b):



(L-6-a),



(L-6-b),

wherein:

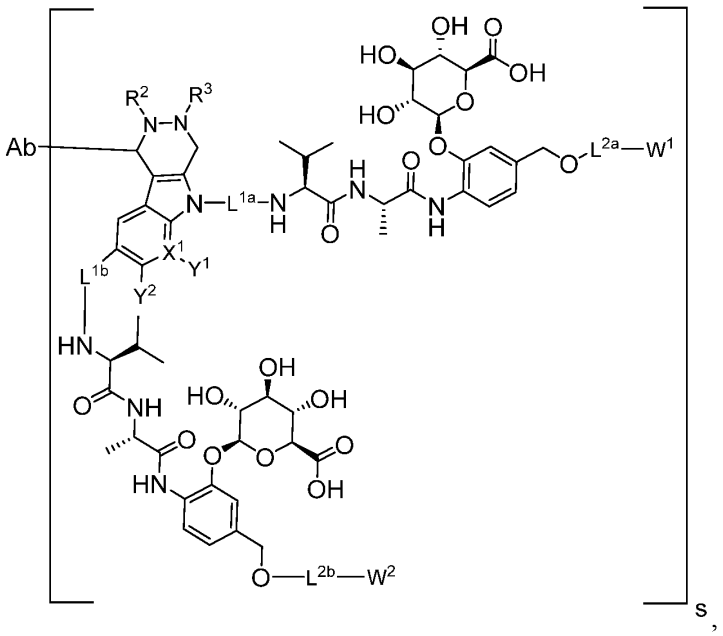
each ~~~ represents the point of attachment to a pyridazine-pyrrolo coupling moiety (*e.g.*, any of Formulas as disclosed herein);

* in linker (L-6-a) represents the point of attachment to W^1;

* in linker (L-6-b) represents the point of attachment to W^2; and

L^{1a}, L^{2a}, L^{1b}, and L^{2b} are as defined herein, such as for linker (L-3-a) or (L-3-b). In some embodiments, each substituent is as defined herein, such as with respect to Formula (I).

[00647] Accordingly, in some embodiments, the ROR-ADC is represented by Formula (XIV-6):



(XIV-6)

wherein each substituent is as defined with respect to Formula (XIV-3). In some embodiments, each substituent is as defined herein, such as with respect to Formula (I).

[00648] In some embodiments, provided is an ADC of Formula (I),

wherein

R^1 is hydrogen;

Z^1 is X^1-Y^1, wherein X^1 represents an atom in the ring of Formula (I) and is selected from the group consisting of C, N, O and S;

Z^2 is C-Y^2;

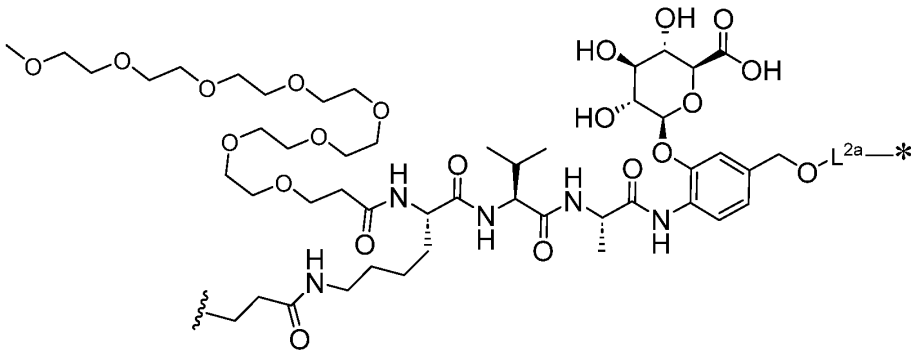
Y^1 and Y^2 are each independently selected from the group consisting of hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl substituted heterocyclyl, and absent when adjacent to N; or Y^1 and Y^2 are cyclically linked;

Z^4 is CH;

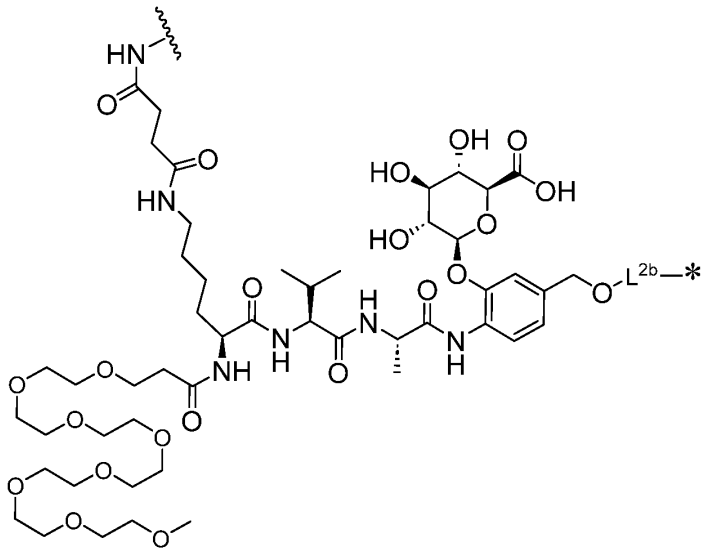
Z^3 is C-L^B-W^2;

L^A is linker (L-7-a); and

L^B is linker (L-7-b):



(L-7-a),



(L-7-b),

wherein:

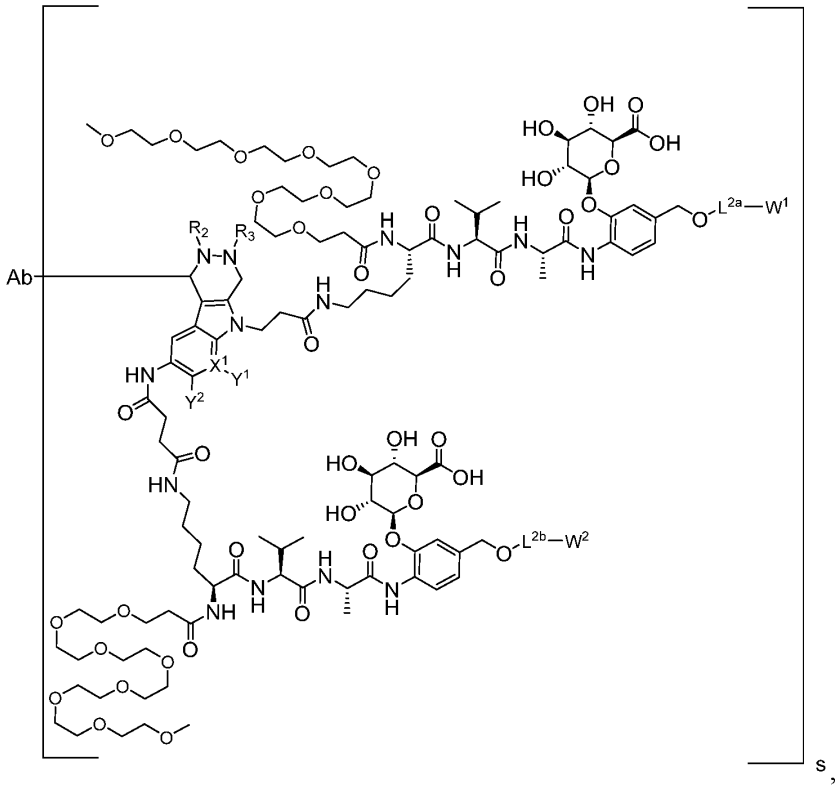
each ~~~ represents the point of attachment to a pyridazine-pyrrolo coupling moiety (*e.g.*, any of Formulas as disclosed herein);

* in linker (L-7-a) represents the point of attachment to W^1;

* in linker (L-7-b) represents the point of attachment to W^2; and

L^{2a} and L^{2b} are as defined herein, such as for linker (L-3-a) or (L-3-b). In some embodiments, each substituent is as defined herein, such as with respect to Formula (I).

[00649] Accordingly, in some embodiments, the ROR-ADC is represented by Formula (XIV-7):



(XIV-7)

wherein each substituent is as defined with respect to Formula (XIV-3). In some embodiments, each substituent is as defined herein, such as with respect to Formula (I).

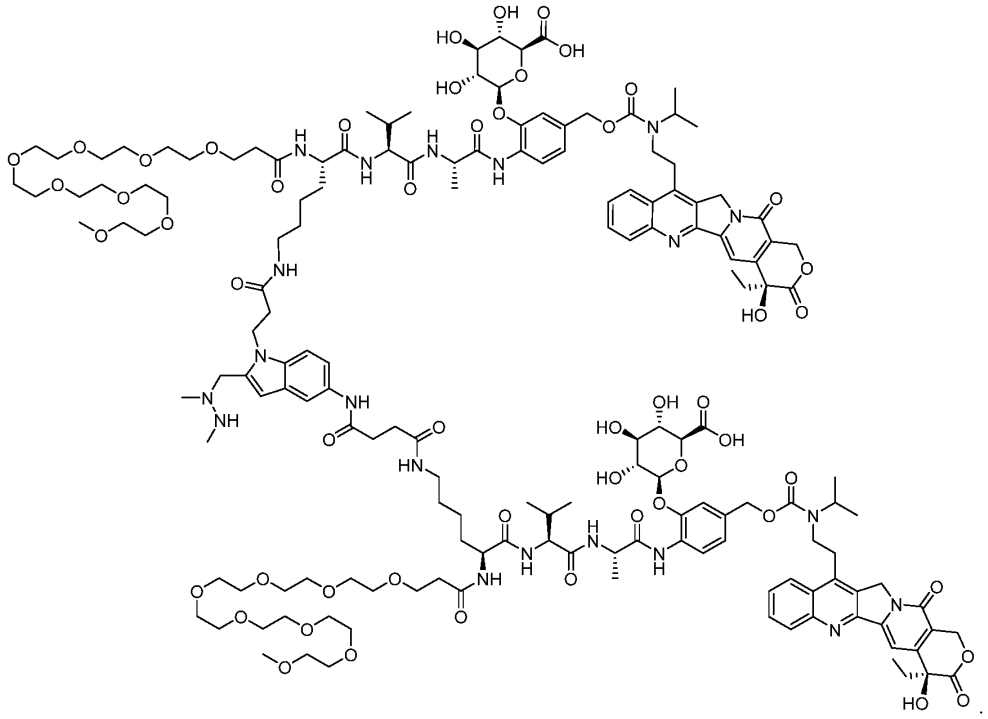
[00650] In some embodiments of any Formula as disclosed herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), or (XIV-7)), L^{2a} is -CO-. Additionally or alternatively, L^{2b} is -CO-. In some embodiments, both L^{2a} and L^{2b} are -CO-.

[00651] In certain embodiments, at least one of W^1 and W^2 (*e.g.*, in Formula (I), Formula (XIV-3), (XIV-4), (XIV-5), (XIV-6), or (XIV-7)) is belotecan. In some embodiments, both W^1 and W^2 are belotecan.

[00652] In some embodiments of any Formula as disclosed herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), or (XIV-7)), W^1 comprises a camptothecin or an analog thereof, such as belotecan. In further embodiments, W^1 comprises belotecan.

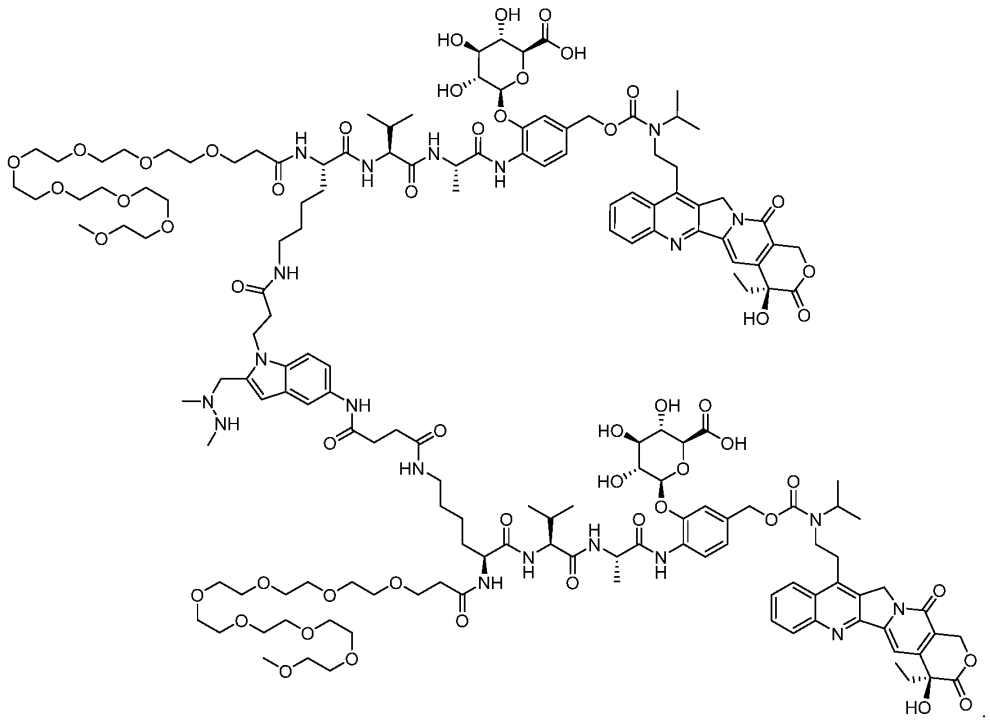
[00653] In some embodiments of any Formula as disclosed herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), or (XIV-7)), W^2 comprises a camptothecin or an analog thereof, such as belotecan. In yet further embodiments, W^2 comprises belotecan. Additionally or alternatively, W^1 and W^2 are the same. In other embodiments, W^1 and W^2 are different.

[00654] For example, an activatable ROR-ADC of Formula (II) may be prepared by conjugating one or more linker-payloads of Formula (IIa), shown below, to an activatable ROR antibody:



(IIa)

[00655] For example, an ROR-ADC of Formula (II) may be prepared by conjugating one or more linker-payloads of Formula (IIa), shown below, to an ROR antibody:

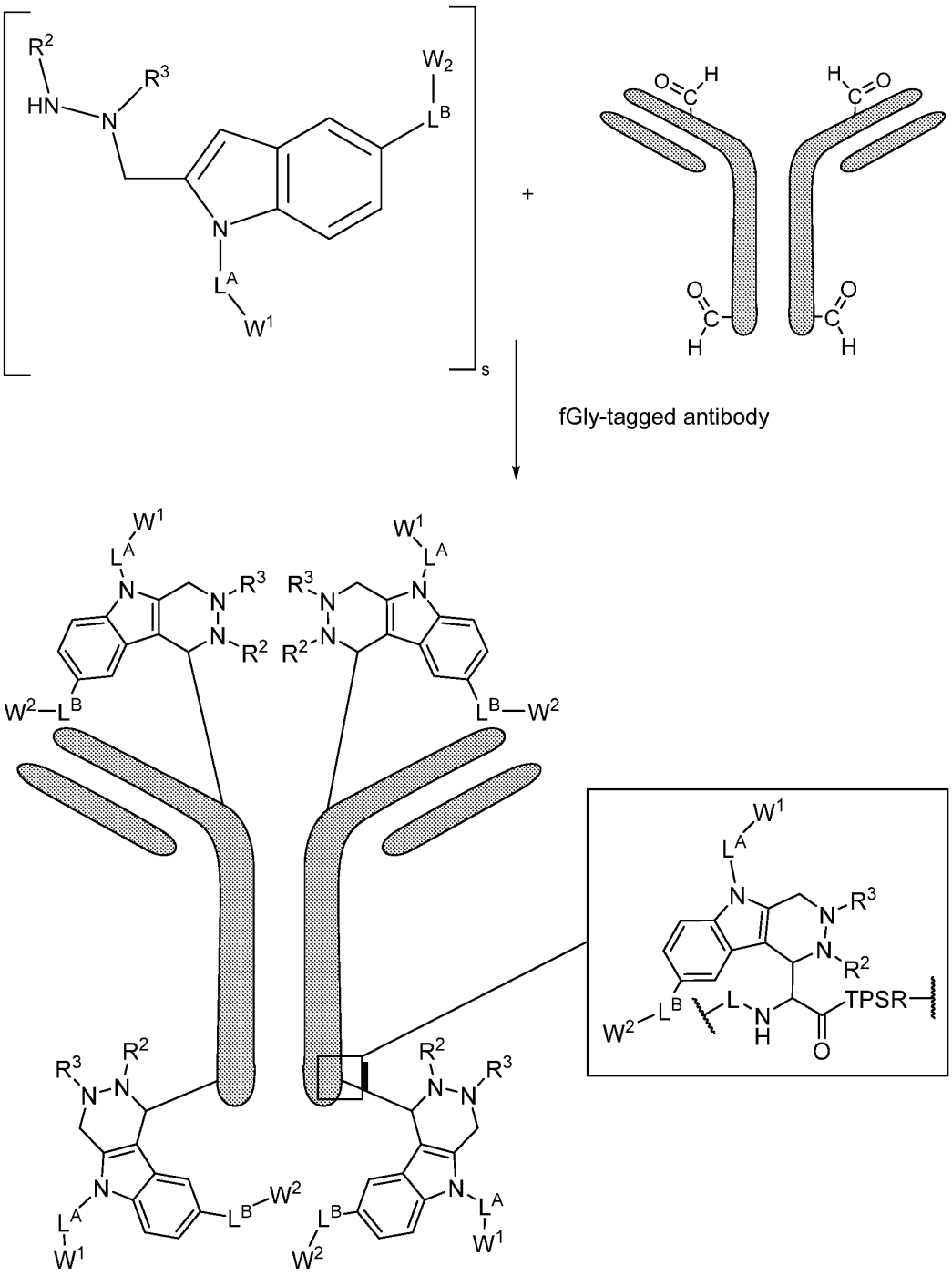


(IIa)

[00656] In some embodiments of any Formula as disclosed herein (such as Formula (I), (II), (XIV-3), (XIV-4), (XIV-5), (XIV-6), or (XIV-7)), s is an integer from 1 to 10, for example 1 to 8. In some embodiments, s is 2. In some embodiments, s is 4.

[00657] In some embodiments of any Formula as disclosed herein (such as Formula (I), (II), (XIV-3), (XIV-4), (XIV-5), (XIV-6), or (XIV-7)), Ab is an ROR antibody as disclosed herein. For example, see the descriptions and embodiments relating to an ROR antibody detailed below in **Sections 7.3**, **7.4, and 7.5**.

[00658] An exemplary scheme for making an ROR-ADC, such as an activatable ROR-ADC, is shown in **Scheme A**:



Scheme A
Site-specifically conjugated ADC (DAR 8)

[00659] In **Scheme A**, the linker-drug is conjugated to an ROR antibody, such as an activatable ROR antibody. The ROR antibody, such as the activatable ROR antibody, has a recognition motif, for example, a L(C/S)TPSR (SEQ ID NO:99) recognition motif in each of the CH1 constant region and c-terminus (CT) of the heavy chain (*e.g.*, SEQ ID NO:42, 170, 48, 171, 172, or 47 as described above). The cysteine residue of a L(C/S)TPSR (SEQ ID NO:99) recognition motif can be converted to a formylglycine (fGly) and subsequently conjugated to an azaHIPs moiety through a Pictet-Spengler reaction (fGly').

[00660] In some embodiments, the ROR1 antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 48 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 33. In some embodiments, the ROR1 antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 171 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 33. In some embodiments, the ROR1 antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 48 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 34. In some embodiments, the ROR1 antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 171 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 34. In some embodiments, the ROR1 antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 48 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 36. In some embodiments, the ROR1 antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 171 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 36. In some embodiments, the ROR1 antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 48 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 38. In some embodiments, the ROR1 antibody comprises a heavy chain comprising an amino acid sequence as set forth in SEQ ID NO: 171 and a light chain comprising an amino acid sequence as set forth in SEQ ID NO: 38.

[00661] In some embodiments, an ROR-ADC, such as an activatable ROR-ADC, includes one or more linker-drug conjugated to each heavy chain constant region of an ROR antibody, such as an activatable ROR antibody, via a pyridazine-pyrrolo coupling moiety and can be characterized by a stoichiometric ratio of antibody to drug (DAR) of about 1 to about 20, for example, a DAR of about 2 to about 8, about 1 to about 4, about 2 to about 4, about 3 to about 4, about 4, about 1 to about 8, about 2 to about 8, about 3 to about 8, about 4 to about 8,

about 5 to about 8, about 6 to about 8, about 6.5 to about 8, about 6 to about 7, about 6.5 to about 7.5, about 7 to about 8, about 6.5, about 7, about 7.5, or about 8**.**

[00662] In some embodiments, the ROR-ADC is **ADC-11**, wherein the ROR-ADC is of Formula (II), s is 4, the antibody Ab is **M1** as described herein and the conjugation sites of the pyridazine-pyrrolo moiety to the antibody are in the CH1 and CT regions of each heavy chain of the antibody, as shown in **Scheme A**. Accordingly, the DAR of **ADC-11** is 8. In some embodiments, the antibody Ab of **ADC-11** comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:34.

[00663] In some embodiments, the ROR-ADC is **ADC-13**, wherein the ROR-ADC is of Formula (II), s is 4, the antibody Ab is **M2** as described herein and the conjugation sites of the pyridazine-pyrrolo moiety to the antibody are in the CH1 and CT regions of each heavy chain of the antibody, as shown in **Scheme A**. Accordingly, the DAR of **ADC-13** is 8. In some embodiments, the antibody Ab of **ADC-13** comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:36.

[00664] In some embodiments, the ROR-ADC is **ADC-M3**, wherein the ROR-ADC is of Formula (II), s is 4, the antibody Ab is **M3** as described herein and the conjugation sites of the pyridazine-pyrrolo moiety to the antibody are in the CH1 and CT regions of each heavy chain of the antibody, as shown in **Scheme A**. Accordingly, the DAR of **ADC-M3** is 8. In some embodiments, the antibody Ab of **ADC-M3** comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:38.

[00665] In some embodiments, the ROR-ADC is **ADC-11-sFc**, wherein the ROR-ADC is of Formula (II), s is 4, the antibody Ab is **M1** as described herein having a silent Fc and the conjugation sites of the pyridazine-pyrrolo moiety to the antibody are in the CH1 and CT regions of each heavy chain of the antibody, as shown in **Scheme A**. Accordingly, the DAR of **ADC-11-sFc** is 8. In some embodiments, the antibody Ab of **ADC-11-sFc** comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:171 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:34.

[00666] In some embodiments, the ROR-ADC is **ADC-13-sFc**, wherein the ROR-ADC is of Formula (II), s is 4, the antibody Ab is **M2** as described herein having a silent Fc and the conjugation sites of the pyridazine-pyrrolo moiety to the antibody are in the CH1 and CT regions of each heavy chain of the antibody, as shown in **Scheme A**. Accordingly, the DAR

of **ADC-13-sFc** is 8. In some embodiments, the antibody Ab of **ADC-13-sFc** comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:171 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:36.

[00667] In some embodiments, the ROR-ADC is **ADC-M3-sFc**, wherein the ROR-ADC is of Formula (II), s is 4, the antibody Ab is **M3** as described herein having a silent Fc and the conjugation sites of the pyridazine-pyrrolo moiety to the antibody are in the CH1 and CT regions of each heavy chain of the antibody, as shown in **Scheme A**. Accordingly, the DAR of **ADC-M3-sFc** is 8. In some embodiments, the antibody Ab of **ADC-M3-sFc** comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:171 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:38.

[00668] In some embodiment, the ROR-ADCs, including activatable ROR-ADC, are described below in **Table 11**.

Table 11: Exemplary Activatable ROR-ADCs

ADC No.	Antibody	Linker-Payload	Conjugation Sites	DAR
ADC-00	P0	IIa	CH1/CT	8
ADC-11	M1	IIa	CH1/CT	8
ADC-13	M2	IIa	CH1/CT	8
ADC-M3	M3	IIa	CH1/CT	8

7.6 DRUGS FOR CONJUGATION

[00669] As noted above, a conjugate or a compound of the present disclosure can include as substituents W^1 and W^2 a drug or active agent. Any of a number of drugs are suitable for use or can be modified to be rendered suitable for use, as a reactive partner to conjugate to an antibody. Examples of drugs include small molecule drugs and peptide drugs.

[00670] "Small molecule drug" as used herein refers to a compound, *e.g.*, an organic compound, which exhibits a pharmaceutical activity of interest and which is generally of a molecular weight of 800 Da or less, or 2000 Da or less, but can encompass molecules of up to 5 kDa and can be as large as 10 kDa. A small inorganic molecule refers to a molecule containing no carbon atoms, while a small organic molecule refers to a compound containing at least one carbon atom.

[00671] For example, the drug or active agent can be a topoisomerase inhibitor (*e.g.*, a topoisomerase I inhibitor), such as a camptothecin, or an analog or derivative thereof, or a pharmaceutically active camptothecin moiety and/or a portion thereof. A topoisomerase inhibitor (*e.g.*, camptothecin, or analog or derivative thereof) conjugated to the ROR antibody, such as the activatable ROR antibody, can be any of a variety of topoisomerase

inhibitors, for example camptothecin or camptothecin moieties such as, but not limited to, camptothecin and analogs and derivatives thereof as described herein. Examples of drugs that find use in the conjugates and compounds described herein include, but are not limited to, a topoisomerase inhibitor, for example camptothecin or a camptothecin derivative, such as SN-38, Belotecan, Exatecan, 9-aminocamptothecin (9-AC), topotecan, *des*-Me-topotecan, derivatives thereof, and the like. Additional examples of topoisomerase inhibitors that find use in the present disclosure are described in PCT/US2022/012325, the disclosure of which is incorporated herein by reference.

[00672] In other embodiments, the drug or active agent can be a maytansine. "Maytansine," "maytansine moiety," "maytansine active agent moiety" and "maytansinoid" refer to a maytansine and analogs and derivatives thereof, and pharmaceutically active maytansine moieties and/or portions thereof. A maytansine conjugated to the ROR antibody, such as the activatable ROR antibody, can be any of a variety of maytansinoid moieties such as, but not limited to, maytansine and analogs and derivatives thereof as described herein (*e.g.*, deacylmaytansine).

[00673] In other instances, the drug or active agent can be an auristatin, or an analog or derivative thereof, or a pharmaceutically active auristatin moiety and/or a portion thereof. An auristatin conjugated to the ROR antibody, such as the activatable ROR antibody, can be any of a variety of auristatin moieties such as, but not limited to, an auristatin and analogs and derivatives thereof as described herein. Examples of drugs that find use in the conjugates and compounds described herein include but are not limited to an auristatin or an auristatin derivative, such as monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), derivatives thereof, and the like.

[00674] In other cases, the drug or active agent can be a duocarmycin, or an analog or derivative thereof, or a pharmaceutically active duocarmycin moiety and/or a portion thereof. A duocarmycin conjugated to the ROR antibody, such as the activatable ROR antibody, can be any of a variety of duocarmycin moieties such as, but not limited to, a duocarmycin and analogs and derivatives thereof as described herein. Examples of drugs that find use in the conjugates and compounds described herein include but are not limited to a duocarmycin or a duocarmycin derivative, such as duocarmycin A, duocarmycin B1, duocarmycin B2, duocarmycin C1, duocarmycin C2, duocarmycin D, duocarmycin SA, and CC-1065, derivatives thereof, and the like. In some embodiments, the duocarmycin is a duocarmycin analog, such as, but not limited to, adozelesin, bizelesin, or carzelesin.

[00675] In certain embodiments, the drug is selected from a cytotoxin, a kinase inhibitor, a selective estrogen receptor modulator, an immunostimulatory agent, a toll-like receptor (TLR) agonist, an oligonucleotide, an aptamer, a cytokine, a steroid, and a peptide.

[00676] For example, a cytotoxin can include any compound that leads to cell death (*e.g.*, necrosis or apoptosis) or a decrease in cell viability.

[00677] Kinase inhibitors can include, but are not limited to, Adavosertib, Afatinib, Axitinib, Bosutinib, Cetuximab, Cobimetinib, Crizotinib, Cabozantinib, Dacomitinib, Dasatinib, Entrectinib, Erdafitinib, Erlotinib, Fostamatinib, Gefitinib, Ibrutinib, Imatinib, Lapatinib, Lenvatinib, Mubritinib, Nilotinib, Pazopanib, Pegaptanib, Ruxolitinib, Sorafenib, Sunitinib, Tucatinib, Vandetanib, Vemurafenib, and the like.

[00678] For example, selective estrogen receptor modulators include, but are not limited to, Endoxifen, Tamoxifen, Afimoxifene, Toremifene, and the like.

[00679] Immunostimulatory agents can include, but are not limited to, vaccines (*e.g.*, bacterial or viral vaccines), colony stimulating factors, interferons, interleukins, and the like. TLR agonists include, but are not limited to, imiquimod, resiquimod, and the like.

[00680] Oligonucleotide dugs include, but are not limited to, fomivirsen, pegaptanib, mipomersen, eteplirsen, defibrotide, nusinersen, golodirsen, viltolarsen, volanesorsen, inotersen, tofersen, tominersen, and the like.

[00681] Aptamer drugs include, but are not limited to, pegaptanib, AS1411, REG1, ARC1779, NU172, ARC1905, E10030, NOX-A12, NOX-E36, and the like.

[00682] Cytokines include, but are not limited to, Albinterferon Alfa-2B, Aldesleukin, ALT-801, Anakinra, Ancestim, Avotermin, Balugrastim, Bempegaldesleukin, Binetrakin, Cintredekin Besudotox, CTCE-0214, Darbepoetin alfa, Denileukin diftitox, Dulanermin, Edodekin alfa, Emfilermin, Epoetin delta, Erythropoietin, Human interleukin-2, Interferon alfa, Interferon alfa-2c, Interferon alfa-n1, Interferon alfa-n3, Interferon alfacon-1, Interferon beta-1a, Interferon beta-1b, Interferon gamma-1b, Interferon Kappa, Interleukin-1 alpha, Interleukin-10, Interleukin-7, Lenograstim, Leridistim, Lipegfilgrastim, Lorukafusp alfa, Maxy-G34, Methoxy polyethylene glycol-epoetin beta, Molgramostim, Muplestim, Nagrestipen, Oprelvekin, Pegfilgrastim, Pegilodecakin, Peginterferon alfa-2a, Peginterferon alfa-2b, Peginterferon beta-1a, Peginterferon lambda-1a, Recombinant CD40-ligand, Regramostim, Romiplostim, Sargramostim, Thrombopoietin, Tucotuzumab celmoleukin, Viral Macrophage-Inflammatory Protein, and the like.

[00683] Steroid drugs include, but are not limited to, prednisolone, betamethasone, dexamethasone, hydrocortisone, methylprednisolone, deflazacort, and the like.

[00684] "Peptide drug" as used herein refers to amino acid-containing polymeric compounds and is meant to encompass naturally occurring and non-naturally occurring peptides, oligopeptides, cyclic peptides, polypeptides, and proteins, as well as peptide mimetics. The peptide drugs may be obtained by chemical synthesis or be produced from a genetically encoded source (*e.g.*, recombinant source). Peptide drugs can range in molecular weight and can be from 200 Da to 10 kDa or greater in molecular weight. Suitable peptides include, but are not limited to, cytotoxic peptides; angiogenic peptides; anti-angiogenic peptides; peptides that activate B cells; peptides that activate T cells; anti-viral peptides; peptides that inhibit viral fusion; peptides that increase production of one or more lymphocyte populations; anti-microbial peptides; growth factors; growth hormone-releasing factors; vasoactive peptides; anti-inflammatory peptides; peptides that regulate glucose metabolism; an anti-thrombotic peptide; an anti-nociceptive peptide; a vasodilator peptide; a platelet aggregation inhibitor; an analgesic; and the like.

[00685] Additional examples of drugs that find use in the conjugates and compounds described herein include, but are not limited to Tubulysin M, Calicheamicin, a STAT3 inhibitor, alpha-Amanitin, an aurora kinase inhibitor, belotecan, and an anthracycline.

[00686] Other examples of drugs include small molecule drugs, such as a cancer chemotherapeutic agent. For example, where a tumor cell expresses or overexpresses an ROR antigen, the ROR antibody, such as the activatable ROR antibody, can be produced as described herein to include a modified amino acid, which can be subsequently conjugated to a cancer chemotherapeutic agent. Cancer chemotherapeutic agents include non-peptidic (*e.g.*, non-proteinaceous) compounds that reduce proliferation of cancer cells and encompass cytotoxic agents and cytostatic agents. Non-limiting examples of chemotherapeutic agents include alkylating agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones. Peptidic compounds can also be used.

[00687] Suitable cancer chemotherapeutic agents include dolastatin and active analogs and derivatives thereof; and auristatin and active analogs and derivatives thereof (*e.g.*, Monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), and the like). See, *e.g.*, WO 96/33212, WO 96/14856, and U.S. 6,323,315. For example, dolastatin 10 or auristatin PE can be included in an antibody-drug conjugate of the present disclosure. Suitable cancer chemotherapeutic agents also include maytansinoids and active analogs and derivatives thereof (see, *e.g.*, EP 1391213; and Liu et al (1996) *Proc. Natl. Acad. Sci. USA* 93:8618-8623); duocarmycins and active analogs and derivatives thereof (*e.g.*, including the synthetic analogues, KW-2189 and CB 1-TM1); and

benzodiazepines and active analogs and derivatives thereof (*e.g.*, pyrrolobenzodiazepine (PBD).

[00688] Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (Cytoxan™), melphalan (L-sarcolysin), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, uracil mustard, chlormethine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.

[00689] Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, 10-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatine, and gemcitabine.

[00690] Suitable natural products and their derivatives, (*e.g.*, vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (Taxol®), docetaxel (Taxotere®), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, *e.g.* vincristine, vinblastine, vinorelbine, vindesine, etc.; podophyllotoxins, *e.g.* etoposide, teniposide, etc.; antibiotics, *e.g.* anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, etc.; phenoxizone biscyclopeptides, *e.g.* dactinomycin; basic glycopeptides, *e.g.* bleomycin; anthraquinone glycosides, *e.g.* plicamycin (mithramycin); anthracenediones, *e.g.* mitoxantrone; azirinopyrrolo indolediones, *e.g.* mitomycin; macrocyclic immunosuppressants, *e.g.* cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, etc.; and the like.

[00691] Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrazole, letrazole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

[00692] Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (*e.g.*, NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (Taxol®), Taxol® derivatives, docetaxel (Taxotere®), thiocolchicine (NSC 361792), trityl cysterin, vinblastine

sulfate, vincristine sulfate, natural and synthetic epothilones including but not limited to, eopthilone A, epothilone B, discodermolide; estramustine, nocodazole, and the like.

[00693] Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, *e.g.* prednisone, dexamethasone, etc.; estrogens and pregestins, *e.g.* hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; etc.; and adrenocortical suppressants, *e.g.* aminoglutethimide; 17α-ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, Flutamide (Drogenil), Toremifene (Fareston), and Zoladex®. Estrogens stimulate proliferation and differentiation; therefore, compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids may inhibit T cell proliferation.

[00694] Other suitable chemotherapeutic agents include metal complexes, *e.g.,* cisplatin (cis-DDP), carboplatin, etc.; ureas, *e.g.,* hydroxyurea; and hydrazines, *e.g.,* N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur; etc. Other anti-proliferative agents of interest include immunosuppressants, *e.g.,* mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); Iressa® (ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)quinazoline); etc.

[00695] Taxanes are suitable for use. "Taxanes" include paclitaxel, as well as any active taxane derivative or pro-drug. "Paclitaxel" (which should be understood herein to include analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL , TAXOTERE™ (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3'N-desbenzoyl-3'N-t-butoxycarbonyl analogs of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076; U.S. Pat. Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; and EP 590,267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from *Taxus brevifolia*; or T-1912 from *Taxus yannanensis*).

[00696] Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (*e.g.*, Taxotere™ docetaxel, as noted

above) and paclitaxel conjugates (*e.g.*, paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylose).

[00697] Also included within the term "taxane" are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but not limited to, galactose and mannose derivatives described in International Patent Application No. WO 99/18113; piperazino and other derivatives described in WO 99/14209; taxane derivatives described in WO 99/09021, WO 98/22451, and U.S. Patent No. 5,869,680; 6-thio derivatives described in WO 98/28288; sulfenamide derivatives described in U.S. Patent No. 5,821,263; and taxol derivative described in U.S. Patent No. 5,415,869. It further includes prodrugs of paclitaxel including, but not limited to, those described in WO 98/58927; WO 98/13059; and U.S. Patent No. 5,824,701.

[00698] Biological response modifiers suitable for use include, but are not limited to, (1) inhibitors of tyrosine kinase (RTK) activity; (2) inhibitors of serine/threonine kinase activity; (3) tumor-associated antigen antagonists, such as antibodies that bind specifically to a tumor antigen; (4) apoptosis receptor agonists; (5) interleukin-2; (6) IFN-α; (7) IFN-γ; (8) colony-stimulating factors; and (9) inhibitors of angiogenesis.

[00699] Examples of drugs include small molecule drugs, such as a cancer chemotherapeutic agent. For example, where a tumor cell expresses or overexpresses an ROR antigen, the ROR antibody, such as the activatable ROR antibody, can be produced as described herein to include a modified amino acid, which can be subsequently conjugated to a cancer chemotherapeutic agent, such as a microtubule affecting agent. In certain embodiments, the drug is a microtubule affecting agent that has antiproliferative activity, such as a maytansinoid.

[00700] Embodiments of the present disclosure include conjugates where an antibody is conjugated to two or more drug moieties, such as 3 drug moieties, 4 drug moieties, 5 drug moieties, 6 drug moieties, 7 drug moieties, 8 drug moieties, 9 drug moieties, 10 drug moieties, 11 drug moieties, 12 drug moieties, 13 drug moieties, 14 drug moieties, 15 drug moieties, 16 drug moieties, 17 drug moieties, 18 drug moieties, 19 drug moieties, or 20 or more drug moieties. The drug moieties may be conjugated to the antibody at one or more sites in the antibody, as described herein. In certain embodiments, the conjugates have an average drug-to-antibody ratio (DAR) (molar ratio) in the range of from 0.1 to 20, or from 0.5 to 20, or from 1 to 20, such as from 1 to 19, or from 1 to 18, or from 1 to 17, or from 1 to 16, or from 1 to 15, or from 1 to 14, or from 1 to 13, or from 1 to 12, or from 1 to 11, or from 1 to 10, or from 1 to 9, or from 1 to 8, or from 1 to 7, or from 1 to 6, or from 1 to 5, or from 1

to 4, or from 1 to 3, or from 1 to 2. In certain embodiments, the conjugates have an average DAR from 1 to 10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, the conjugates have an average DAR of 1 to 10. In certain embodiments, the conjugates have an average DAR of 1 to 5 (*e.g.*, 4). In certain embodiments, the conjugates have an average DAR of 5 to 10 (*e.g.*, 8). By average is meant the arithmetic mean.

[00701] In certain embodiments, the two drugs or active agents attached to the branched linker are the same drug or active agent. For example, a first branch of a branched linker may be attached to a drug, or an active agent and a second branch of the branched linker may be attached to the same drug or the same active agent as the first branch. In other embodiments, the two drugs or active agents attached to the branched linker are different drugs or active agents. For example, a first branch of a branched linker may be attached to a first drug, or a first active agent and a second branch of the branched linker may be attached to a second drug or a second active agent different from the first drug or the first active agent attached to the first branch.

[00702] In some embodiments, where two different drugs or active agents are attached to the branched linker, the drugs or active agents may be selected from drugs and active agents that have a synergistic therapeutic effect. For example, in some instances, the use of two different drugs or active agents attached to the branched linker may provide a lower therapeutically effective concentration at which both payloads act, thereby increasing overall potency of the ADC.

[00703] In some embodiments, where two different drugs or active agents are attached to the branched linker, the drugs or active agents may be selected from drugs and active agents that provide an enhanced therapeutic benefit as compared to the use of the drugs or active agents separately, For example, the drugs or active agents may provide an increased effect on drug delivery of the ADC (*e.g.*, some payloads, such as the iRGD peptide, can increase extravasation into tissues and augment tumor penetration).

[00704] In some embodiments, where two different drugs or active agents are attached to the branched linker, the drugs or active agents may be selected from drugs and active agents that use different mechanisms of action. In some cases, this may provide a decrease in tumor drug resistance by targeting multiple pathways. Examples of payload combinations can include, but are not limited to, cytotoxic drugs, immunomodulatory molecules to activate or inhibit immune cell populations, cytokines, hormones, chelating agents loaded with radioisotopes, and the like.

[00705] In some embodiments, where two different drugs or active agents are attached to the branched linker, the two different drugs or active agents are a topoisomerase inhibitor (*e.g.*, belotecan) as described herein and an auristatin (*e.g.*, MMAE) as described herein. In some embodiments, where two different drugs or active agents are attached to the branched linker, the two different drugs or active agents are a topoisomerase inhibitor (*e.g.*, belotecan) as described herein and an iRGD peptide as described herein. In some embodiments, where two different drugs or active agents are attached to the branched linker, the two different drugs or active agents are an auristatin (*e.g.*, MMAE) as described herein and an iRGD peptide as described herein. In some embodiments, where two different drugs or active agents are attached to the branched linker, the two different drugs or active agents are an auristatin (*e.g.*, MMAE) as described herein and a kinase inhibitor (*e.g.*, Sorafenib, Lapatinib, Gefitinib, and the like) as described herein. In some embodiments, where two different drugs or active agents are attached to the branched linker, the two different drugs or active agents are a topoisomerase inhibitor (*e.g.*, belotecan) as described herein and a kinase inhibitor (*e.g.*, Sorafenib, Lapatinib, Gefitinib, and the like) as described herein. In some embodiments, where two different drugs or active agents are attached to the branched linker, the two different drugs or active agents are an auristatin (*e.g.*, MMAE) as described herein and a selective estrogen receptor modulator (*e.g.*, Endoxifen) as described herein. In some embodiments, where two different drugs or active agents are attached to the branched linker, the two different drugs or active agents are a topoisomerase inhibitor (*e.g.*, belotecan) as described herein and a selective estrogen receptor modulator (*e.g.*, Endoxifen) as described herein.

[00706] Drugs to be conjugated to an ROR antibody, such as an activatable ROR antibody, may be modified to incorporate a reactive partner for reaction with the antibody. Where the drug is a peptide drug, the reactive moiety (*e.g.*, aminooxy or hydrazide can be positioned at an N-terminal region, the N-terminus, a C-terminal region, the C-terminus, or at a position internal to the peptide. For example, an example of a method involves synthesizing a peptide drug having an aminooxy group. In this example, the peptide is synthesized from a Boc-protected precursor. An amino group of a peptide can react with a compound comprising a carboxylic acid group and oxy-N-Boc group. As an example, the amino group of the peptide reacts with 3-(2,5-dioxopyrrolidin-1-yloxy)propanoic acid. Other variations on the compound comprising a carboxylic acid group and oxy-N-protecting group can include different number of carbons in the alkylene linker and substituents on the alkylene linker. The reaction between the amino group of the peptide and the compound comprising a carboxylic acid

group and oxy-N-protecting group occurs through standard peptide coupling chemistry. Examples of peptide coupling reagents that can be used include, but not limited to, DCC (dicyclohexylcarbodiimide), DIC (diisopropylcarbodiimide), di-p-toluoylcarbodiimide, BDP (1-benzotriazole diethylphosphate-1-cyclohexyl-3-(2-morpholinylethyl)carbodiimide), EDC (1-(3-dimethylaminopropyl-3-ethyl-carbodiimide hydrochloride), cyanuric fluoride, cyanuric chloride, TFFH (tetramethyl fluoroformamidinium hexafluorophosphophosphate), DPPA (diphenylphosphorazidate), BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate), HBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate), TBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate), TSTU (O-(N-succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate), HATU (N-[(dimethylamino)-1-H-1,2,3-triazolo[4,5,6]-pyridin-1-ylmethylene]- -N-methylmethanaminium hexafluorophosphate N-oxide), BOP-Cl (bis(2-oxo-3-oxazolidinyl)phosphinic chloride), PyBOP ((1-H-1,2,3-benzotriazol-1-yloxy)-tris(pyrrolidino)phosphonium tetrafluorophopsphate), BrOP (bromotris(dimethylamino)phosphonium hexafluorophosphate), DEPBT (3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one) PyBrOP (bromotris(pyrrolidino)phosphonium hexafluorophosphate). As a non-limiting example, HOBt and DIC can be used as peptide coupling reagents.

[00707] Deprotection to expose the amino-oxy functionality is performed on the peptide comprising an N-protecting group. Deprotection of the N-oxysuccinimide group, for example, occurs according to standard deprotection conditions for a cyclic amide group. Deprotecting conditions can be found in Greene and Wuts, Protective Groups in Organic Chemistry, 3rd Ed., 1999, John Wiley & Sons, NY and Harrison et al. Certain deprotection conditions include a hydrazine reagent, amino reagent, or sodium borohydride. Deprotection of a Boc protecting group can occur with TFA. Other reagents for deprotection include, but are not limited to, hydrazine, methylhydrazine, phenylhydrazine, sodium borohydride, and methylamine. The product and intermediates can be purified by conventional means, such as HPLC purification.

[00708] The ordinarily skilled artisan will appreciate that factors such as pH and steric hindrance (*e.g.*, the accessibility of the amino acid residue to reaction with a reactive partner of interest) are of importance, modifying reaction conditions to provide for optimal conjugation conditions is well within the skill of the ordinary artisan, and is routine in the art. Where conjugation is conducted with an ROR antibody, such as an activatable ROR antibody present in or on a living cell, the conditions are selected so as to be physiologically

compatible. For example, the pH can be dropped temporarily for a time sufficient to allow for the reaction to occur but within a period tolerated by the cell (*e.g.*, from about 30 min to 1 hour). Physiological conditions for conducting modification of antibodies on a cell surface can be similar to those used in a ketone-azide reaction in modification of cells bearing cell-surface azides (see, *e.g.*, U.S. 6,570,040).

[00709] Small molecule compounds containing, or modified to contain, an α-nucleophilic group that serves as a reactive partner with a compound or conjugate disclosed herein are also contemplated for use as drugs in the antibody-drug conjugates of the present disclosure. General methods are known in the art for chemical synthetic schemes and conditions useful for synthesizing a compound of interest (see, *e.g.*, Smith and March, March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, Fifth Edition, Wiley-Interscience, 2001; or Vogel, A Textbook of Practical Organic Chemistry, Including Qualitative Organic Analysis, Fourth Edition, New York: Longman, 1978).

7.7 FORMULATIONS & COMPOSITIONS

[00710] ROR-ADCs as disclosed herein, such as activatable ROR-ADCs. can be included in a pharmaceutical composition for administration, *e.g.*, to a subject for treating a disease, disorder, or condition. A pharmaceutical composition comprises ROR-ADCs which, on average, can exhibit a drug-to-antibody ratio ("DAR") of about 0.1 to about 20, or from 0.5 to 20, or from 1 to 20. In other words, each antibody is conjugated to one or two linker-drug conjugates. Methods to determine DAR are well known to the skilled person and include methods using Reverse Phase Chromatography, or HPLC-MS.

[00711] For example, in any embodiment, a pharmaceutical composition comprising an ROR-ADC exhibits a DAR of about 0.1 to about 20, or from 0.5 to 20, or from 1 to 20, such as from 1 to 19, or from 1 to 18, or from 1 to 17, or from 1 to 16, or from 1 to 15, or from 1 to 14, or from 1 to 13, or from 1 to 12, or from 1 to 11, or from 1 to 10, or from 1 to 9, or from 1 to 8, or from 1 to 7, or from 1 to 6, or from 1 to 5, or from 1 to 4, or from 1 to 3, or from 1 to 2, or from 2 to 20, or from 2 to 19, or from 2 to 18, or from 2 to 17, or from 2 to 16, or from 2 to 15, or from 2 to 14, or from 2 to 13, or from 2 to 12, or from 2 to 11, or from 2 to 10, or from 2 to 9, or from 2 to 8, or from 2 to 7, or from 2 to 6, or from 2 to 5, or from 2 to 4, or from 2 to 3, or from 3 to 20, or from 3 to 19, or from 3 to 18, or from 3 to 17, or from 3 to 16, or from 3 to 15, or from 3 to 14, or from 3 to 13, or from 3 to 12, or from 3 to 11, or from 3 to 10, or from 3 to 9, or from 3 to 8, or from 3 to 7, or from 3 to 6, or from 3 to 5, or from 3 to 4, or from 4 to 20, or from 4 to 19, or from 4 to 18, or from 4 to 17, or from 4 to 16, or from 4 to

15, or from 4 to 14, or from 4 to 13, or from 4 to 12, or from 4 to 11, or from 4 to 10, or from 4 to 9, or from 4 to 8, or from 4 to 7, or from 4 to 6, or from 4 to 5, or from 5 to 20, or from 5 to 19, or from 5 to 18, or from 5 to 17, or from 5 to 16, or from 5 to 15, or from 5 to 14, or from 5 to 13, or from 5 to 12, or from 5 to 11, or from 5 to 10, or from 5 to 9, or from 5 to 8, or from 5 to 7, or from 5 to 6, or from 6 to 20, or from 6 to 19, or from 6 to 18, or from 6 to 17, or from 6 to 16, or from 6 to 15, or from 6 to 14, or from 6 to 13, or from 6 to 12, or from 6 to 11, or from 6 to 10, or from 6 to 9, or from 6 to 8, or from 6 to 7, or from 7 to 20, or from 7 to 19, or from 7 to 18, or from 7 to 17, or from 7 to 16, or from 7 to 15, or from 7 to 14, or from 7 to 13, or from 7 to 12, or from 7 to 11, or from 7 to 10, or from 7 to 9, or from 7 to 8. In certain embodiments, the composition exhibits a DAR from 1 to 10, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10. In certain embodiments, the composition exhibits a DAR of 1 to 10. In certain embodiments, the composition exhibits a DAR of 1 to 5 (*e.g.*, 3 or 3.5 or 4). In certain embodiments, the composition exhibits a DAR of 5 to 10 (*e.g.*, 6, or 6.5, or 7, or 7.5 or 8). In some embodiments, a pharmaceutical composition comprises an ROR-ADC comprising an ROR antibody as described herein, including **P0**, **M1**, **M2**, or **M3** (*see, e.g.*, **Tables 1-9**), and a pharmaceutically acceptable excipient. In some embodiments, a pharmaceutical composition comprises ROR-ADC, such as **ADC-00**, **ADC-11**, **ADC-13**, **or ADC-M3** (*see, e.g.*, **Table 11**), and a pharmaceutically acceptable excipient.

[00712] In some embodiments, a pharmaceutical composition comprises ROR-ADC, such as **ADC-00**, **ADC-11**, **ADC-13**, **ADC-M3, ADC-11-sFc**, **ADC-13-sFc**, **or ADC-M3-sFc** (*see, e.g.*, **Table 11**), and a pharmaceutically acceptable excipient.

[00713] In some embodiments, provided herein is a pharmaceutical composition comprising an ROR-ADC of any formula as disclosed herein (such as Formula (I), (II), (XIV-3), (XIV-4), (XIV-5), (XIV-6), or (XIV-7)) and a pharmaceutically acceptable excipient. In some embodiments, provided herein is a pharmaceutical composition comprising a population of ROR-ADCs, wherein the ROR-ADCs are of a same formula as disclosed herein (such as any one of Formula (I), (II), (XIV-3), (XIV-4), (XIV-5), (XIV-6), or (XIV-7)), yet, their s are different. Accordingly, the pharmaceutical composition may exhibit various DAR, such as about 1 to about 10, about 1 to about 8, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 1.0, about 1.1, about 1.2, about 1.3, about 1.4, about 1.5, about 1.6, about 1.7, about 1.8, about 1.9, about 2.0, about 2.1, about 2.2, about 2.3, about 2.4, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3.0, about 3.1, about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5, about 4.6, about 4.7, about 4.8,

about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, about 5.5, about 5.6, about 5.7, about 5.8, about 5.9, about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, about 8.0, about 8.1, about 8.2, about 8.3, about 8.4, about 8.5, about 8.6, about 8.7, about 8.8, about 8.9, about 9.0, about 9.1, about 9.2, about 9.3, about 9.4, about 9.5, about 9.6, about 9.7, about 9.8, about 9.9, about 10.0.

[00714] An ROR-ADC, such as an activatable ROR-ADC, can be formulated in any of a variety of different ways. An ROR-ADC, such as an activatable ROR-ADC, of the present disclosure can be provided in any suitable form, *e.g.*, in the form of a pharmaceutically acceptable salt, and can be formulated for any suitable route of administration, *e.g.*, oral, topical, or parenteral administration. Where an ROR-ADC, such as an activatable ROR-ADC, is provided as a liquid injectable (such as in those embodiments where they it can be administered intravenously or directly into a tissue), an ROR-ADC, such as an activatable ROR-ADC, can be provided as a ready-to-use dosage form, or as a storage-stable powder for reconstitution or liquid composed of pharmaceutically acceptable excipients and excipients.

[00715] Methods for formulating an ROR-ADC, such as an activatable ROR-ADC, can be adapted from those available in the art. For example, ROR-ADCs, such as activatable ROR-ADCs, can be provided in a pharmaceutical composition comprising an effective amount of an ROR-ADC, such as an activatable ROR-ADC, and a pharmaceutically acceptable excipient (*e.g.*, saline). The pharmaceutical composition can optionally include other additives (*e.g.*, buffers, stabilizers, preservatives, and the like). In some embodiments, the formulations are suitable for administration to a mammal, such as those that are suitable for administration to a human.

[00716] Also provided herein are pharmaceutical compositions that contain an effective amount of an ROR-ADC, such as an activatable ROR-ADC, described herein and a pharmaceutically acceptable excipient. In some embodiments, the ROR-ADC, such as activatable ROR-ADC, comprises an ROR antibody as described herein, including **P0**, **M1**, **M2**, or **M3**, as described in any one of **Tables 1-9**. In some embodiments, a pharmaceutical composition comprises an effective amount of an ROR-ADC, such as an activatable ROR-ADC, of Formula (I) or Formula (II) and a pharmaceutically acceptable excipient. In some embodiments, a pharmaceutical composition comprises an effective amount of **ADC-00**, **ADC-11**, **ADC-13**, **or ADC-M3** (*see, e.g.*, **Table 11**), and a pharmaceutically acceptable excipient.

[00717] In some embodiments, a pharmaceutical composition comprises an effective amount of an ROR-ADC, such as an activatable ROR-ADC, of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)) and a pharmaceutically acceptable excipient. In some embodiments, a pharmaceutical composition comprises an effective amount of **ADC-00**, **ADC-11**, **ADC-13**, **ADC-M3, ADC-11-sFc**, **ADC-13-sFc**, **or ADC-M3-sFc** (*see, e.g.*, **Table 11**), and a pharmaceutically acceptable excipient.

[00718] The pharmaceutically acceptable excipient can be one or more compatible 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.

[00719] Pharmaceutical compositions of the invention can additionally contain 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. The pharmaceutical compositions can also optionally contain suitable preservatives, such as benzalkonium chloride, chlorobutanol, parabens, and thimerosal. Pharmaceutical compositions of the invention can be presented in unit dosage form and can be prepared by any suitable method, many of which are well known in the art of pharmacy. Such methods include the step of bringing the antibody or antigen-binding fragment of the invention into association with an excipient that constitutes one or more accessory ingredients. In general, the pharmaceutical composition is prepared by uniformly and intimately bringing the active agent into association with a liquid excipient, a finely divided solid excipient, or both, and then, if necessary, shaping the product.

[00720] A composition suitable for parenteral administration conveniently comprises a sterile aqueous preparation of the composition, which preferably is isotonic with the blood of the recipient. This aqueous preparation can be formulated of known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also can be 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. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally 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 injectables. Excipient formulations suitable for oral, subcutaneous, intravenous, intramuscular, and the like, administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

[00721] Preparation of pharmaceutical compositions of the invention and their various routes of administration can be carried out in accordance with methods well known in the art. The delivery systems useful in the context of the invention include time-released, delayed release, and sustained release systems such that the delivery of the composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. The composition can be used in conjunction with other therapeutic agents or therapies. Such systems can avoid repeated administrations of the composition, thereby increasing convenience to the subject and the physician, and can be particularly suitable for some compositions of the invention.

[00722] Many types of release delivery systems are available and known to those of ordinary skill in the art. Suitable release delivery systems include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are lipids such as sterols such as cholesterol, cholesterol esters, and fatty acids or neutral fats such as mono-di-and triglycerides; hydrogel release systems; sylastic systems; peptide-based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include but are not limited to: (a) erosional systems in which the active composition is contained in a form within a matrix such as those described in U.S. Patents 4,452,775, 4,667,014, 4,748,034, and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patents 3,832,253 and 3,854,480. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

[00723] Generally, an ROR-ADC, such as an activatable ROR-ADC, or pharmaceutical composition is suitably packaged, *e.g.,* in a vial, pouch, ampoule, and/or any container appropriate for a therapeutic method. Components can be provided as concentrates (including

lyophilized compositions), which can be further diluted prior to use, or they can be provided at the concentration of use. For use of an ROR-ADC, such as an activatable ROR-ADC, *in vivo*, single dosages can be provided in sterilized containers having the desired amount and concentration of components.

7.8 METHODS OF TREATMENT

[00724] Also provided herein are methods of treating, preventing, or alleviating an ROR-mediated disease, disorder, or condition, including one or more symptoms of the ROR-mediated disease, disorder, or condition with an ROR-ADC comprising an ROR antibody, and a drug conjugated directly or indirectly thereto. Also provided herein are methods of killing tumor cells with an ROR-ADC comprising an ROR antibody, and a drug conjugated directly or indirectly thereto.

[00725] Also provided herein are methods of treating, preventing, or alleviating an ROR-mediated disease, disorder, or condition, including one or more symptoms of the ROR-mediated disease, disorder, or condition with an activatable ROR-ADC comprising an activatable ROR antibody, and a drug conjugated directly or indirectly thereto. Also provided herein are methods of killing tumor cells with an activatable ROR-ADC comprising an activatable ROR antibody, and a drug conjugated directly or indirectly thereto.

[00726] The ROR antibody, such as the activatable antibody that binds to ROR, can include any as described herein, such as **P0**, **M1**, **M2**, or **M3**, as described in any one of **Tables 1-9**. In some embodiments, a method of killing a tumor cell comprises contacting the tumor cell with an ROR-ADC of Formula (I), such as an activatable ROR-ADC of Formula (I). In some embodiments, a method of killing a tumor cell comprises contacting the tumor cell with an ROR-ADC, such as an activatable ROR-ADC comprising a camptothecin analogue (*e.g.*, belotecan), or any derivative thereof. In some embodiments, a method of killing a tumor cell comprises contacting the tumor cell with an ROR-ADC, such as an activatable ROR-ADC, of Formula (I) or (II). In some embodiments, a method of killing a tumor cell comprises contacting the tumor cell with an ROR-ADC, such as an activatable ROR-ADC, of **P0**, **M1**, **M2**, or **M3**. In some embodiments, the contacting is *in vivo* or *in vitro*. In some embodiments, a method of killing a tumor cell comprises contacting the tumor cell with **ADC-00**, **ADC-11**, **ADC-13**, **or ADC-M3** (*see, e.g.*, **Table 11**).

[00727] In some embodiments, a method of killing a tumor cell comprises contacting the tumor cell with an ROR-ADC, such as an activatable ROR-ADC, of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)). In some embodiments, the contacting is *in vivo* or *in vitro*. In some embodiments, a method of killing

a tumor cell comprises contacting the tumor cell with **ADC-00**, **ADC-11**, **ADC-13**, **ADC-M3, ADC-11-sFc**, **ADC-13-sFc**, **or ADC-M3-sFc** (*see, e.g.*, **Table 11**).

[00728] In any embodiment, the method of contacting the tumor cell with an ROR-ADC, such as an activatable ROR-ADC, comprises contacting the tumor cell with a composition comprising the ROR-ADC, such as the activatable ROR-ADC, and one or more pharmaceutically acceptable excipients. In some embodiments, the ROR-ADC, such as the activatable ROR-ADC, in the composition is characterized by a DAR of about 1 to about 20, about 1 to about 10, about 2 to about 4, about 3 to about 4, about 5 to about 8, about 6 to about 8, about 6 to about 7, or about 7 to about 8.

[00729] Also provided herein is a method of treating a cancer in a subject in need thereof comprising administering an effective amount of an ROR-ADC, to the subject, wherein the ROR-ADC comprises an ROR antibody and a drug conjugated thereto via a linker, as described herein. In some embodiments, the ROR antibody is **P0**, **M1**, **M2**, or **M3**, as described herein, such as in any one of **Tables 1-9**. In some embodiments, the ROR-ADC is **ADC-00**, **ADC-11**, **ADC-13**, **or ADC-M3** (*see, e.g.*, **Table 11**).

[00730] In some embodiments, the ROR-ADC is ADC-00, ADC-11, ADC-13, ADC-M3, ADC-11-sFc, ADC-13-sFc, or ADC-M3-sFc (*see, e.g.*, Table 11).

[00731] Also provided herein is a method of treating a cancer in a subject in need thereof comprising administering an effective amount of an activatable ROR-ADC, to the subject, wherein the activatable ROR-ADC comprises an activatable ROR antibody and a drug conjugated thereto via a linker, as described herein. In some embodiments, the activatable ROR antibody is **M1**, **M2**, or **M3**, as described herein, such as in any one of **Tables 1-9**. In some embodiments, the activatable ROR-ADC is **ADC-11**, **ADC-13**, **or ADC-M3** (*see, e.g.*, **Table 11**).

[00732] In some embodiments, the activatable ROR-ADC is **ADC-11**, **ADC-13**, **ADC-M3, ADC-11-sFc**, **ADC-13-sFc**, **or ADC-M3-sFc** (*see, e.g.*, **Table 11**).

[00733] In some embodiments, a method of treating cancer in a subject in need thereof comprises administering to the subject an effective amount of an ROR-ADC of Formula (I), such as an activatable ROR-ADC of Formula (I).

[00734] In some embodiments, a method of treating cancer in a subject in need thereof comprises administering to the subject an effective amount of an ROR-ADC of Formula (I), such as an activatable ROR-ADC of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)).

[00735] In any embodiment, the method of treating cancer in a subject in need thereof comprises administering to the subject a pharmaceutical composition comprising the ROR-ADC, such as the activatable ROR-ADC, and a pharmaceutically acceptable excipient. In some embodiments, the ROR-ADC, such as the activatable ROR-ADC, in the pharmaceutical composition is characterized by a DAR of about 1 to about 20, about 1 to about 10, about 2 to about 4, about 3 to about 4, about 5 to about 8, about 6 to about 8, about 6 to about 7, or about 7 to about 8.

[00736] In some embodiments, the method of treating cancer in a subject in need thereof is effective to enhance the removal of the cancer cells, for example, tumor cells, in the subject.

[00737] "Enhancing" the removal of cancer or tumor cells does not require a 100% enhancement of removal. Any enhancement in the rate of removal is contemplated. Similarly, "modulating" tumor growth refers to reducing the size of the tumor, slowing tumor growth, or inhibiting an increase in the size of an existing tumor. Complete abolition of a tumor is not required; any decrease in tumor size or slowing of tumor growth constitutes a beneficial biological effect in a subject. In this regard, tumor cell removal can be enhanced by, for example, at least about 5%, at least about 10% or at least about 20% compared to levels of removal observed in the absence of the method (*e.g.*, in a biologically matched control subject or specimen that is not exposed to the agent of the method). The effect is detected by, for example, a reduction in tumor size, a decrease or maintenance of the levels of tumor markers, or reduction or maintenance of a tumor cell population. In some embodiments, removal of tumor cells is enhanced by, for example, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more (about 100%) compared to the removal of tumor cells in the absence of an ROR-ADC, such as activatable ROR-ADC of the method.

[00738] The present disclosure also provides a method of modulating (*e.g.*, inhibiting, reducing, preventing) tumor growth in a subject in need thereof, the method comprising administering to the subject an ROR-ADC comprising an ROR antibody and a drug linked thereto via a linker, as described herein, in an amount effective to modulate the tumor growth in the subject. In some embodiments, a method of modulating tumor growth in a subject in need thereof comprising administering an effective amount of an ROR-ADC comprising an ROR antibody and a drug conjugated thereto via a linker, as described herein. In some embodiments, the antibody that binds to ROR is **P0**, **M1**, **M2**, or **M3**, as described in any one of **Tables 1-9**. In some embodiments, a method of modulating tumor growth in a subject in need thereof comprises administering to the subject an effective amount of an ROR-ADC of

Formula (I) or Formula (II). In some embodiments, a method of modulating tumor growth in a subject in need thereof comprises administering to the subject an effective amount of **ADC-00**, **ADC-11**, **ADC-13**, **or ADC-M3** (*see, e.g.*, **Table 11**).

[00739] In some embodiments, a method of modulating tumor growth in a subject in need thereof comprises administering to the subject an effective amount of an ROR-ADC of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)). In some embodiments, a method of modulating tumor growth in a subject in need thereof comprises administering to the subject an effective amount of **ADC-00**, **ADC-11**, **ADC-13**, **ADC-M3, ADC-11-sFc**, **ADC-13-sFc**, **or ADC-M3-sFc** (*see, e.g.*, **Table 11**).

[00740] The present disclosure also provides a method of modulating (*e.g.*, inhibiting, reducing, preventing) tumor growth in a subject in need thereof, the method comprising administering to the subject an activatable ROR-ADC comprising an activatable ROR antibody and a drug linked thereto via a linker, as described herein, in an amount effective to modulate the tumor growth in the subject. In some embodiments, a method of modulating tumor growth in a subject in need thereof comprising administering an effective amount of an activatable ROR-ADC comprising an activatable ROR antibody and a drug conjugated thereto via a linker, as described herein. In some embodiments, the activatable antibody that binds to ROR is **M1**, **M2**, or **M3**, as described in any one of **Tables 1-9**. In some embodiments, a method of modulating tumor growth in a subject in need thereof comprises administering to the subject an effective amount of an activatable ROR-ADC of Formula (I) or Formula (II). In some embodiments, a method of modulating tumor growth in a subject in need thereof comprises administering to the subject an effective amount of **ADC-11**, **ADC-13**, **or ADC-M3** (*see, e.g.*, **Table 11**).

[00741] In some embodiments, a method of modulating tumor growth in a subject in need thereof comprises administering to the subject an effective amount of an activatable ROR-ADC of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)). In some embodiments, a method of modulating tumor growth in a subject in need thereof comprises administering to the subject an effective amount of **ADC-11**, **ADC-13**, **ADC-M3, ADC-11-sFc**, **ADC-13-sFc**, **or ADC-M3-sFc** (*see, e.g.*, **Table 11**).

[00742] In any embodiment, the method of modulating tumor growth in a subject in need thereof can comprise administering to the subject a pharmaceutical composition comprising the ROR-ADC, such as the activatable ROR-ADC, and one or more pharmaceutically acceptable excipients. In some embodiments, the ROR-ADC, such as the activatable ROR-ADC in the pharmaceutical composition is characterized by a DAR of about 1 to about 20,

about 1 to about 10, about 2 to about 4, about 3 to about 4, about 5 to about 8, about 6 to about 8, about 6 to about 7, or about 7 to about 8.

[00743] In some embodiments, the cancer and tumor cells described herein that may be treated and/or killed by the methods described herein express an ROR antigen, for example, as on a surface of the cancer or tumor cell. In some embodiments, a tumor or cancer cell may overexpress an ROR antigen. As used herein, the term "overexpress" means to transcribe and translate more genetic product than normal (such as in normal cells), the process of which is often a characteristic of cancer cells.

[00744] Therefore, the present disclosure also provides a method of alleviating or reducing side effects associated with cancer comprising administering an effective amount of an ROR-ADC to a subject in need thereof. In some embodiments, a method of alleviating or reducing side effects associated with cancer comprises administering an effective amount of an ROR-ADC comprising an activatable ROR antibody, and a drug conjugated thereto via a linker, as described herein. In some embodiments, the antibody that binds to ROR is **M1**, **M2**, or **M3**, as described in any one of **Tables 1-9**. In some embodiments, a method of alleviating or reducing side effects associated with cancer comprises administering to the subject an effective amount of an ROR-ADC of Formula (I) or (II). In some embodiments, a method of alleviating or reducing side effects associated with cancer comprises administering to the subject an effective amount of **ADC-00**, **ADC-11**, **ADC-13**, **or ADC-M3** (*see, e.g.*, **Table 11**).

[00745] In some embodiments, a method of alleviating or reducing side effects associated with cancer comprises administering to the subject an effective amount of an ROR-ADC of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)). In some embodiments, a method of alleviating or reducing side effects associated with cancer comprises administering to the subject an effective amount of **ADC-00**, **ADC-11**, **ADC-13**, **ADC-M3, ADC-11-sFc**, **ADC-13-sFc**, **or ADC-M3-sFc** (*see, e.g.*, **Table 11**).

[00746] In certain embodiments, the present disclosure also provides a method of alleviating or reducing side effects associated with cancer comprising administering an effective amount of an ROR-ADC, such as an activatable ROR-ADC, to a subject in need thereof. In some embodiments, a method of alleviating or reducing side effects associated with cancer comprises administering an effective amount of an ROR-ADC, such as an activatable ROR-ADC comprising an activatable ROR antibody, and a drug conjugated thereto via a linker, as described herein. In some embodiments, the activatable antibody that binds to ROR is **M1**,

M2, or **M3**, as described in any one of **Tables 1-9**. In some embodiments, a method of alleviating or reducing side effects associated with cancer comprises administering to the subject an effective amount of an activatable ROR-ADC of Formula (I) or (II). In some embodiments, a method of alleviating or reducing side effects associated with cancer comprises administering to the subject an effective amount of **ADC-00**, **ADC-11**, **ADC-13**, **or ADC-M3** (*see, e.g.*, **Table 11**).

[00747] In some embodiments, a method of alleviating or reducing side effects associated with cancer comprises administering to the subject an effective amount of an activatable ROR-ADC of any formula as described herein (such as Formula (I), (XIV-3), (XIV-4), (XIV-5), (XIV-6), (XIV-7), or (II)). In some embodiments, a method of alleviating or reducing side effects associated with cancer comprises administering to the subject an effective amount of **ADC-00**, **ADC-11**, **ADC-13**, **ADC-M3, ADC-11-sFc**, **ADC-13-sFc**, **or ADC-M3-sFc** (*see, e.g.*, **Table 11**).

[00748] In any embodiment, the method of alleviating or reducing side effects associated with cancer in a subject in need thereof comprises administering to the subject a pharmaceutical composition comprising an ROR-ADC as disclosed herein, such as an activatable ROR-ADC as disclosed herein and one or more pharmaceutically acceptable excipients. In some embodiments, the ROR-ADC, such as the activatable ROR-ADC, in the pharmaceutical composition is characterized by a DAR of about 1 to about 20, about 1 to about 10, about 2 to about 4, about 3 to about 4, about 5 to about 8, about 6 to about 8, about 6 to about 7, or about 7 to about 8.

[00749] Additionally, an ROR-ADC as disclosed herein, such as an activatable ROR-ADC comprising an activatable ROR antibody, can be used to treating, preventing, or alleviating an ROR-mediated disease, disorder, or condition, including one or more symptoms of the ROR-mediated disease, disorder, or condition with an ROR-ADC as disclosed herein, such as an activatable ROR-ADC comprising an activatable ROR antibody.

[00750] In another aspect, provided herein is a method of detecting ROR antigen in a subject having or suspected of having a disease or condition, the method comprising: (a) administering to the subject any antibody above; and (b) detecting the presence or the level of ROR antigen in the subject.

[00751] Also provided is a method for treating a subject with cancer. The method comprises administering a therapeutically effective amount of the ADC as disclosed herein or the pharmaceutical composition as disclosed herein to the subject. In some embodiments, the cancer is an ROR antigen expressing cancer. In further embodiments, the cancer expresses

ROR1. Additionally or alternatively, the cancer expresses ROR2. In yet further embodiments, the cancer expresses both ROR1 and ROR2.

[00752] In some embodiments, the disease or condition is cancer. The cancer may be a cancer from the bladder, blood (myeloid leukemia [acute and chronic], acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), bone, bone marrow, brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma [pinealoma], glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), breast, colon, esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), gastrointestinal, gum, head, kidney (adenocarcinoma, Wilm's tumor [nephroblastoma], lymphoma, leukemia, renal cell carcinoma), liver, lung, nasopharynx, neck, ovary, prostate (adenocarcinoma, sarcoma, castrate resistant prostate cancer), skin, stomach (carcinoma, lymphoma, leiomyosarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma), tongue, or uterus. In some embodiments, the cancer may be a neoplasm, malignant; carcinoma; carcinoma, undifferentiated; giant and spindle cell carcinoma; small cell carcinoma; papillary carcinoma; squamous cell carcinoma; lymphoepithelial carcinoma; basal cell carcinoma; pilomatrix carcinoma; transitional cell carcinoma; papillary transitional cell carcinoma; adenocarcinoma; gastrinoma, malignant; cholangiocarcinoma; hepatocellular carcinoma; combined hepatocellular carcinoma and cholangiocarcinoma; trabecular adenocarcinoma; adenoid cystic carcinoma; adenocarcinoma in adenomatous polyp; adenocarcinoma, familial polyposis coli; solid carcinoma; carcinoid tumor, malignant; branchiolo-alveolar adenocarcinoma; papillary adenocarcinoma; chromophobe carcinoma; acidophil carcinoma; oxyphilic adenocarcinoma; basophil carcinoma; clear cell adenocarcinoma; granular cell carcinoma; follicular adenocarcinoma; papillary and follicular adenocarcinoma; nonencapsulating sclerosing carcinoma; adrenal cortical carcinoma; endometroid carcinoma; skin appendage carcinoma; apocrine adenocarcinoma; sebaceous adenocarcinoma; ceruminous adenocarcinoma; mucoepidermoid carcinoma; cystadenocarcinoma; papillary cystadenocarcinoma; papillary serous cystadenocarcinoma; mucinous cystadenocarcinoma; mucinous adenocarcinoma; signet ring cell carcinoma; infiltrating duct carcinoma; medullary carcinoma; lobular carcinoma; inflammatory carcinoma; Paget's disease, mammary; acinar cell carcinoma; adenosquamous carcinoma; adenocarcinoma w/squamous metaplasia; thymoma, malignant; ovarian stromal tumor, malignant; thecoma, malignant; granulosa cell tumor, malignant; androblastoma, malignant;

Sertoli cell carcinoma; Leydig cell tumor, malignant; lipid cell tumor, malignant; paraganglioma, malignant; extra-mammary paraganglioma, malignant; pheochromocytoma; glomangiosarcoma; malignant melanoma; amelanotic melanoma; superficial spreading melanoma; malignant melanoma in giant pigmented nevus; epithelioid cell melanoma; blue nevus, malignant; sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma); fibrosarcoma; fibrous histiocytoma, malignant; myxosarcoma; liposarcoma; leiomyosarcoma; rhabdomyosarcoma; embryonal rhabdomyosarcoma; alveolar rhabdomyosarcoma; stromal sarcoma; mixed tumor, malignant; mullerian mixed tumor; nephroblastoma; hepatoblastoma; carcinosarcoma; mesenchymoma, malignant; Brenner tumor, malignant; phyllodes tumor, malignant; synovial sarcoma; mesothelioma, malignant; dysgerminoma; embryonal carcinoma; teratoma, malignant; struma ovarii, malignant; choriocarcinoma; mesonephroma, malignant; hemangiosarcoma; hemangioendothelioma, malignant; Kaposi's sarcoma; hemangiopericytoma, malignant; lymphangiosarcoma; osteosarcoma; juxtacortical osteosarcoma; chondrosarcoma; chondroblastoma, malignant; mesenchymal chondrosarcoma; giant cell tumor of bone; Ewing's sarcoma; odontogenic tumor, malignant; ameloblastic odontosarcoma; ameloblastoma, malignant; ameloblastic fibrosarcoma; pinealoma, malignant; chordoma; glioma, malignant; ependymoma; astrocytoma; protoplasmic astrocytoma; fibrillary astrocytoma; astroblastoma; glioblastoma; oligodendroglioma; oligodendroblastoma; primitive neuroectodermal; cerebellar sarcoma; ganglioneuroblastoma; neuroblastoma; retinoblastoma; olfactory neurogenic tumor; meningioma, malignant; neurofibrosarcoma; neurilemmoma, malignant; granular cell tumor, malignant; malignant lymphoma (reticulum cell sarcoma); Hodgkin's disease; Hodgkin's; paragranuloma; malignant lymphoma, small lymphocytic; malignant lymphoma, large cell, diffuse; malignant lymphoma, follicular; mycosis fungoides; other specified non-Hodgkin's lymphomas; malignant histiocytosis; multiple myeloma; mast cell sarcoma; immunoproliferative small intestinal disease; leukemia; lymphoid leukemia; plasma cell leukemia; erythroleukemia; lymphosarcoma cell leukemia; myeloid leukemia; basophilic leukemia; eosinophilic leukemia; monocytic leukemia; mast cell leukemia; megakaryoblastic leukemia; myeloid sarcoma; hairy cell leukemia; myxoma; rhabdomyoma; fibroma; squamous cell carcinomas of the head and neck; laryngeal and hypopharyngeal cancer; nasal cavity and paranasal sinus cancer; nasopharyngeal cancer; salivary gland cancer; oral; orppharyngeal cancer; bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma, non-small cell lung cancer); alveolar (bronchiolar) carcinoma; bronchial adenoma; chondromatous hamartoma; colorectal cancer;

gastrointestinal stromal tumors; carcinoids; Turcot Syndrome; gastric cancer; gastroesophageal junction adenocarcinoma; pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma); small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Karposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma); large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma); metastatic breast cancer; ductal carcinoma in situ; invasive ductal carcinoma; tubular carcinoma; mucinous carcinoma; lobular carcinoma in situ; triple negative breast cancer; bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma, urothelial carcinoma); clear cell carcinoma; hepatoma (hepatocellular carcinoma); angiosarcoma; hepatocellular adenoma; hemangioma; osteogenic sarcoma (osteosarcoma); malignant fibrous histiocytoma; malignant giant cell tumor chordoma; osteochrondroma (osteocartilaginous exostoses); benign chondroma; chondromyxofibroma; osteoid osteoma; giant cell tumors; medullary thyroid cancer; differentiated thyroid cancer; papillary thyroid cancer; follicular thyroid cancer; Hürthle cell cancer; anaplastic thyroid cancer; skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans); meninges (meningioma, meningiosarcoma, gliomatosis); spinal cord (neurofibroma, meningioma, glioma, sarcoma); uterus (clear); cervix (cervical carcinoma, pre-tumor cervical dysplasia); ovaries (ovarian carcinoma [serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma], granulosa-thecal cell tumors, Sertoli--Leydig cell tumors, dysgerminoma, malignant teratoma); vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma); vagina (clear cell carcinoma, squamous cell carcinoma); botryoid sarcoma (embryonal rhabdomyosarcoma); fallopian tubes (carcinoma); non-Hodgkin's lymphoma [malignant lymphoma]; Karposi's sarcoma; moles dysplastic nevi; angioma; dermatofibroma; keloids; psoriasis; neuroblastoma; adrenocortical carcinoma; pheochromocytomas; paragangliomas; Merkel cell carcinoma; pancreatic neuroendocrine and carcinoid tumors; neuroendocrine tumors; carcinoid tumors; pancreatic cancers; gastroesophageal; clear cell renal cell carcinoma; and primary peritoneal cancer.

[00753] In some embodiments, the cancer is chronic myelogenous leukemia, colorectal cancer, estrogen receptor positive, progesterone receptor positive and HER2 negative breast cancer, triple negative breast cancer, non-small cell lung cancer, or mantle cell lymphoma.

[00754] An ROR-ADC, such as an activatable ROR-ADC comprising an activatable ROR antibody, of the present disclosure may be administered to a subject per se or in the form of a pharmaceutical composition for the treatment of, *e.g.*, cancer, autoimmunity, transplantation

rejection, post-traumatic immune responses, graft-versus-host disease, ischemia, stroke, and infectious diseases (*e.g.*, by targeting viral antigens, such as gp120 of HIV).

[00755] In another aspect, an ROR-ADC, such as an activatable ROR-ADC, as described herein may be used in a method of treating a subject with cancer in combination with one or more additional therapies. The additional therapies that may be used in combination with an ROR-ADC, such as an activatable ROR-ADC, described herein include but are not limited to: (i) surgery; (ii) radiotherapy; (iii) endocrine therapy; (iv) immunotherapy (including adjuvant therapy and cell therapy such as CAR T-cell therapy); and (v) chemotherapy, including cytotoxic agents and chemotherapeutic agents.

[00756] Any therapy that has an activity against a cancer may be used in combination with an ROR-ADC, such as an activatable ROR-ADC, provided herein. Examples of such agents for cancer treatment can be found, for instance, at www.cancer.gov/about-cancer/treatment/drugs and in publicly available sources such as Cancer Principles and Practice of Oncology by V. T. Devita and S. Hellman (editors), 11th edition (2018), Lippincott Williams & Wilkins Publishers. A person of ordinary skill in the art would be able to discern which combinations of agents would be useful based on the particular characteristics of the drugs and the type of cancer involved.

[00757] In certain embodiments, the additional therapy is a radiotherapy including, for example, gamma radiation, neutron beam radiotherapy, electron beam radiotherapy, proton therapy, brachytherapy, and systemic radioactive isotopes. Radiotherapy may comprise radiation or associated administration of radiopharmaceuticals. The source of radiation may be either external or internal to the subject being treated (radiation treatment may, for example, be in the form of external beam radiation therapy (EBRT) or brachytherapy (BT)). Exemplary radioactive elements include, *e.g.*, radium, cesium-137, iridium-192, americium-241, gold-198, cobalt-57, copper-67, technetium-99, iodide-123, iodide-131, and indium-111.

[00758] In certain embodiments, the additional therapy is an immunotherapy. Immunotherapy (also called biological response modifier therapy, biologic therapy, biotherapy, immune therapy, or biological therapy) is treatment that uses parts of the immune system to fight disease. Immunotherapy can help the immune system recognize cancer cells or enhance a response against cancer cells. Immunotherapies include active and passive immunotherapies. Active immunotherapies, including immunotherapeutic agents, stimulate the body's own immune system (*e.g.*, vaccines) while passive immunotherapies, including immunotherapeutic agents, generally use immune system components created outside of the

body (*e.g.*, antibodies), antibodies conjugated with drugs, toxins, or radionuclides, and targeted therapeutics.

[00759] Exemplary immunotherapeutic agents include immune checkpoint inhibitors. In some embodiments, the immune checkpoint inhibitor used in methods of treatment can totally or partially reduce, inhibit, interfere with, or modulate one or more checkpoint proteins which regulate T-cell activation or function. Numerous checkpoint proteins are known, such as CTLA-4 and its ligands CD80 and CD86; and PD-1 with its ligands PD-Ll and PD-L2 (Pardoll, Nature Reviews Cancer, 2012, 12, 252-264). Immune checkpoint inhibitors include antibodies or are derived from antibodies.

[00760] In certain embodiments, the checkpoint inhibitor is an OX40 (CD134) agonist. In some embodiments, the checkpoint inhibitor is an anti-OX40 antibody. In some embodiments, the anti-OX40 antibody is anti-OX-40. In some embodiments, the anti-OX40 antibody is MEDI6469.

[00761] In certain embodiments, the checkpoint inhibitor is a CD40 agonist. In some embodiments, the checkpoint inhibitor is an anti-CD40 antibody. In some embodiments, the anti-CD40 antibody is CF-870,893.

[00762] In certain embodiments, the checkpoint inhibitor is a CTLA-4 inhibitor. In some embodiments, the CTLA-4 inhibitor is an anti-CTLA-4 antibody. Examples of anti CTLA 4 antibodies include, but are not limited to, those described in US Patent Nos: 5,811,097; 5,811,097; 5,855,887; 6,051,227; 6,207,157; 6,682,736; 6,984,720; and 7,605,238. In some embodiments, the anti-CTLA-4 antibody is tremelimumab (also known as ticilimumab or CP-675,206). In some embodiments, the anti-CTLA-4 antibody is ipilimumab (also known as MDX-010 or MDX-101). Ipilimumab is a fully human monoclonal IgG antibody that binds to CTLA-4. Ipilimumab is marketed under the trade name Yervoy™.

[00763] In certain embodiments, the checkpoint inhibitor is a PD-1/PD-L1 inhibitor. Examples of PD-l/PD-L1 inhibitors include, but are not limited to, those described in US Patent Nos. 7,488,802; 7,943,743; 8,008,449; 8,168,757; 8,217,149, and PCT Patent Application Publication Nos. WO2003042402, WO2008156712, WO2010089411, WO2010036959, WO2011066342, WO2011159877, WO2011082400, and WO2011161699.

[00764] In certain embodiments, the checkpoint inhibitor is a PD-1 inhibitor. In some embodiments, the PD-1 inhibitor is an anti-PD-1 antibody. In some embodiments, the anti-PD-1 antibody is BGB-A317, nivolumab (also known as ONO-4538, BMS-936558, or MDX1106) or pembrolizumab (also known as MK-3475, SCH 900475, or lambrolizumab). In some embodiments, the anti-PD-1 antibody is nivolumab. Nivolumab is a human IgG4

anti-PD-1 monoclonal antibody and is marketed under the trade name Opdivo™. In some embodiments, the anti-PD-1 antibody is pembrolizumab. Pembrolizumab is a humanized monoclonal IgG4 antibody and is marketed under the trade name Keytruda™. In some embodiments, the anti-PD-1 antibody is CT-011, a humanized antibody. CT-011 administered alone has failed to show response in treating acute myeloid leukemia (AML) at relapse. In some embodiments, the anti-PD-1 antibody is AMP-224, a fusion protein. In some embodiments, the PD-1 antibody is BGB-A317. BGB-A317 is a monoclonal antibody in which the ability to bind Fc gamma receptor I is specifically engineered out, and which has a unique binding signature to PD-1 with high affinity and superior target specificity.

[00765] In certain embodiments, the checkpoint inhibitor is a PD-L1 inhibitor. In one embodiment, the PD-L1 inhibitor is an anti-PD-L1 antibody. In some embodiments, the anti-PD-L1 antibody is MEDI4736 (durvalumab). In some embodiments, the anti-PD-L1 antibody is BMS-936559 (also known as MDX-1105-01). In some embodiments, the PD-L1 inhibitor is atezolizumab (also known as MPDL3280A, and Tecentriq®).

[00766] In certain embodiments, the checkpoint inhibitor is a PD-L2 inhibitor. In some embodiments, the PD-L2 inhibitor is an anti-PD-L2 antibody. In some embodiments, the anti-PD-L2 antibody is rHIgM12B7A.

[00767] In certain embodiments, the checkpoint inhibitor is a lymphocyte activation gene-3 (LAG-3) inhibitor. In some embodiments, the LAG-3 inhibitor is IMP321, a soluble Ig fusion protein (Brignone et al., J. Immunol., 2007, 179, 4202-4211). In some embodiments, the LAG-3 inhibitor is BMS-986016.

[00768] In certain embodiments, the checkpoint inhibitor is a B7 inhibitor. In some embodiments, the B7 inhibitor is a B7-H3 inhibitor or a B7-H4 inhibitor. In some embodiments, the B7-H3 inhibitor is MGA271, an anti-B7-H3 antibody (Loo et al., Clin. Cancer Res., 2012, 3834).

[00769] In certain embodiments, the checkpoint inhibitor is a TIM3 (T-cell immunoglobulin domain and mucin domain 3) inhibitor (Fourcade et al., J. Exp. Med., 2010, 207, 2175-86; Sakuishi et al., J. Exp. Med., 2010, 207, 2187-94).

[00770] In certain embodiments, the checkpoint inhibitor is a GITR agonist. In some embodiments, the checkpoint inhibitor is an anti-GITR antibody. In some embodiments, the anti-GITR antibody is TRX518.

[00771] In certain embodiments, the checkpoint inhibitor is a CD137 agonist. In some embodiments, the checkpoint inhibitor is an anti-CD137 antibody. In some embodiments, the

anti-CD137 antibody is urelumab. In some embodiments, the anti-CD137 antibody is PF-05082566.

[00772] In certain embodiments, the checkpoint inhibitor is recombinant human interleukin-15 (rhIL-15).

[00773] In certain embodiments, the checkpoint inhibitor is an IDO inhibitor. In some embodiments, the IDO inhibitor is INCB024360. In some embodiments, the IDO inhibitor is indoximod.

[00774] Other exemplary immunotherapies include adjuvant therapies, including immunotherapeutic agents such as cytokines, chemokines, interferons, interleukins, or lymphokines. Examples include cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte-colony stimulating factor (G-CSF), macrophage inflammatory protein (MIP)-1-alpha, interleukins (including IL-1, IL-2, IL-4, IL-6, IL-7, IL-12, IL-15, IL-18, IL-21, and IL-27), tumor necrosis factors (including TNF-alpha), and interferons (including IFN-alpha, IFN-beta, and IFN-gamma); aluminum hydroxide (alum); Bacille Calmette-Guerin (BCG); Keyhole limpet hemocyanin (KLH); Incomplete Freund's adjuvant (IFA); QS-21; DETOX; Levamisole; and Dinitrophenyl (DNP), and combinations thereof, such as, for example, combinations of, interleukins, for example, IL-2 with other cytokines, such as IFN-alpha.

[00775] Other exemplary immunotherapies include cell therapies, for example, a population of immune cells, such as leukocytes (nucleated white blood cells), comprising (*e.g.*, expressing) a receptor that binds to an antigen of interest. A leukocyte of the present disclosure may be, for example, a neutrophil, eosinophil, basophil, lymphocyte, or a monocyte. In some embodiments, a leukocyte is a lymphocyte. Examples of lymphocytes include T cells, B cells, Natural Killer (NK) cells or NKT cells. In some embodiments, a T-cell is a CD4+ Th (T helper) cell, a CD8+ cytotoxic T cell, a γδT cell or a regulatory (suppressor) T cell. In some embodiments, an immune cell is a dendritic cell. In some embodiments, the cell therapies are CAR-T cell therapies. In some embodiments, a bispecific CAR is comprised of two distinct antigen recognition domains present in tandem on a single transgenic receptor (referred to as a TanCAR; see, *e.g.*, Grada Z et al. Molecular Therapy Nucleic Acids 2013; 2:e105, incorporated herein by reference in its entirety). Thus, methods, in some embodiments, comprise delivering to a tumor a combination comprising an ROR-ADC, such as an activatable ROR-ADC, and an immunotherapeutic agent, wherein the immunotherapeutic agent is an engineered nucleic acid that encodes an antigen, or delivering to a tumor an engineered nucleic acid that induces expression of a self-antigen, and delivering

to the tumor an immune cell expressing a bispecific CAR that binds to two antigens, one of which is encoded by the engineered nucleic acid.

[00776] Other exemplary immunotherapies include immunotherapeutic agents such as cancer vaccines, which can be used to elicit an immune response in a subject against a cancer antigen. An exemplary method involves administering to a subject a RNA vaccine comprising at least one RNA polynucleotide having an open reading frame encoding at least one antigenic polypeptide or an immunogenic fragment thereof, thereby inducing in the subject an immune response specific to the antigenic polypeptide or an immunogenic fragment thereof, in combination with administering an ROR-ADC, such as an activatable ROR-ADC, either in the same composition or a separate composition, administered at the same time, or sequentially dosed, wherein the anti-antigenic polypeptide antibody titer in the subject is increased following vaccination relative to anti-antigenic polypeptide antibody titer in a subject vaccinated with a prophylactically effective dose of a traditional vaccine against the cancer.

[00777] In certain embodiments, the additional therapies include chemotherapy such as one or more cytotoxic agents or one or more chemotherapeutic agents. A cytotoxic agent can inhibit or prevent a cellular function and/or cause cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (*e.g.*, At^{211}, I^{131}, I^{125}, Y^{90}, Re^{186}, Re^{188}, Sm^{153}, Bi^{212}, P^{32}, Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents; growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof.

[00778] In certain embodiments, the additional therapy includes one or more chemotherapeutic agents. Chemotherapeutic agents include chemical compounds useful in the treatment of cancer. Chemotherapeutic agents include (i) anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators; (ii) aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands; (iii) anti-androgens; (iv) protein kinase inhibitors; (v) lipid kinase inhibitors; (vi) antisense oligonucleotides, including those which inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation; (viii) vaccines such as gene therapy vaccines. Chemotherapeutic agents can also include antibodies.

[00779] Exemplary kinase inhibitors include erlotinib (Tarceva®), gefitinib (Iressa®), dasatinib (Sprycel®), nilotinib (Tasigna®), crizotinib (Xalkori®), ruxolitinib (Jakafi®),

vemurafenib (Zelboraf®), vandetanib (Caprelsa®), pazopanib (Votrient®), afatinib, alisertib, amuvatinib, axitinib, baricitinib, bosutinib, brivanib, canertinib, cabozantinib (Cabometyx®), cediranib, ceritinib, crenolanib, dabrafenib, dacomitinib, danusertib, dovitinib, foretinib, ganetespib, ibrutinib, idelalisib, imatinib, iniparib, lapatinib, lenvatinib, linifanib, linsitinib, masitinib, momelotinib, motesanib, neratinib, nintedanib, niraparib, oprozomib, olaparib, palbociclib, pictilisib, pirfenidone, ponatinib, quizartinib, regorafenib, rigosertib, rucaparib, saracatinib, saridegib, sorafenib, sunitinib, tandutinib, tasocitinib, telatinib, tivantinib, tivozanib, tofacitinib, trametinib, veliparib, vismodegib, volasertib, cobimetinib (Cotellic®), XL-147, XL-765, XL-499, XL-880, and others. In some embodiments, an ROR-ADC, such as an activatable ROR-ADC, can be used in combination with a HSP90 inhibitor (*e.g.*, XL888), liver X receptor (LXR) modulators, retinoid-related orphan receptor gamma (RORy) modulators, a CK1 inhibitor, a CK1-α inhibitor, a Wnt pathway inhibitor (*e.g.*, SST-215), or a mineralocorticoid receptor inhibitor, (*e.g.*, esaxerenone or XL-550) for the treatment of cancer.

[00780] Kinase inhibitors can be tyrosine kinase inhibitors, such as the EGFR inhibitors; small molecule HER2 tyrosine kinase inhibitor such as Mubritonib (TAK165, Takeda); CP-724.714, (Axon Medchem BV, an oral selective inhibitor of the ErbB2 receptor tyrosine kinase); dual-HER inhibitors such as EKB-569 (available from Wyeth) which preferentially binds EGFR but inhibits both HER2 and EGFR-overexpressing cells; lapatinib (GSK572016; available from Glaxo-SmithKline), an oral HER2 and EGFR tyrosine kinase inhibitor; PKI-166 (available from Novartis); pan-HER inhibitors such as canertinib (CI-1033; Pharmacia); Raf-1 inhibitors such as antisense agent ISIS-5132 available from ISIS Pharmaceuticals which inhibit Raf-1 signaling; non-HER targeted TK inhibitors such as imatinib mesylate (GLEEVEC®, available from Glaxo SmithKline); multi-targeted tyrosine kinase inhibitors such as sunitinib (SUTENT®, available from Pfizer); VEGF receptor tyrosine kinase inhibitors such as vatalanib (PTK787/ZK222584, available from Novartis/Schering AG); MAPK extracellular regulated kinase 1 inhibitor CI-1040 (available from Pharmacia); quinazolines, such as PD 153035,4-(3-chloroanilino) quinazoline; pyridopyrimidines; pyrimidopyrimidines; pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706; pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d] pyrimidines; curcumin (diferuloyl methane, 4,5-bis (4-fluoroanilino)phthalimide); tyrphostines containing nitrothiophene moieties; antisense molecules (*e.g.*, those that bind to HER-encoding nucleic acid); quinoxalines (U.S. Pat. No. 5,804,396); tryphostins (U.S. Pat. No. 5,804,396); Affinitac (ISIS 3521; Isis/Lilly); PKI166 (Novartis); Semaxinib (Pfizer); INC-1C11 (Imclone), rapamycin

(sirolimus, RAPAMUNE®); or as described in any of the following patent publications: U.S. Pat. No. 5,804,396; WO 1999/09016 (American Cyanamid); WO 1998/43960 (American Cyanamid); WO 1997/38983 (Warner Lambert); WO 1999/06378 (Warner Lambert); WO 1999/06396 (Warner Lambert); WO 1996/30347 (Pfizer, Inc); WO 1996/33978 (Zeneca); WO 1996/3397 (Zeneca) and WO 1996/33980 (Zeneca).

[00781] Combined treatment with the ROR-ADC, such as the activatable ROR-ADC. provided herein and the additional therapy, such as a therapeutic agent, may be simultaneous, separate, or sequential, in any order. For combinations of therapeutic agents such as an ROR-ADC or an activatable ROR-ADC and another therapeutic agent such as an immunotherapeutic agent or a chemotherapeutic agent, simultaneous administration, the therapeutics agents may be administered as one composition or as separate compositions, as appropriate.

5.9 KITS

[00782] Also provided herein are kits comprising an ROR-ADC, such as an activatable ROR-ADC, provided herein, or a composition (*e.g.*, a pharmaceutical composition) provided herein, packaged into suitable packaging material. A kit optionally includes a label or packaging insert including a description of the components or instructions for use *in vitro*, *in vivo*, or *ex vivo*, of the components therein.

[00783] The term "packaging material" refers to a physical structure housing the components of the kit. The packaging material can maintain the components sterilely and can be made of material commonly used for such purposes (*e.g.*, paper, corrugated fiber, glass, plastic, foil, ampoules, vials, tubes, etc.).

[00784] Kits provided herein can include labels or inserts. Labels or inserts include "printed matter," *e.g.*, paper or cardboard, separate or affixed to a component, a kit or packing material (*e.g.*, a box), or attached to, for example, an ampoule, tube, or vial containing a kit component. Labels or inserts can additionally include a computer readable medium, such as a disk (*e.g.*, hard disk, card, memory disk), optical disk such as CD- or DVD-ROM/RAM, DVD, MP3, magnetic tape, or an electrical storage media such as RAM and ROM or hybrids of these such as magnetic/optical storage media, FLASH media, or memory type cards. Labels or inserts can include information identifying manufacturer information, lot numbers, manufacturer location, and date.

[00785] Kits provided herein can additionally include other components. Each component of the kit can be enclosed within an individual container, and all of the various containers can be within a single package. Kits can also be designed for cold storage. A kit can further be

designed to contain antibodies provided herein, or cells that contain nucleic acids encoding the antibodies provided herein. The cells in the kit can be maintained under appropriate storage conditions until ready to use.

[00786] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described herein.

[00787] As used herein, numerical values are often presented in a range format throughout this document. The use of a range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention unless the context clearly indicates otherwise. Accordingly, the use of a range expressly includes all possible subranges, all individual numerical values within that range, and all numerical values or numerical ranges including integers within such ranges and fractions of the values or the integers within ranges unless the context clearly indicates otherwise. This construction applies regardless of the breadth of the range and in all contexts throughout this patent document. Thus, for example, reference to a range of 90-100% includes 91-99%, 92-98%, 93-95%, 91-98%, 91-97%, 91-96%, 91-95%, 91-94%, 91-93%, and so forth. Reference to a range of 90-100% also includes 91%, 92%, 93%, 94%, 95%, 95%, 97%, etc., as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, etc., 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, etc., and so forth.

[00788] In addition, reference to a range of 1-3, 3-5, 5-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, 70-80, 80-90, 90-100, 100-110, 110-120, 120-130, 130-140, 140-150, 150-160, 160-170, 170-180, 180-190, 190-200, 200-225, 225-250 includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc. In a further example, reference to a range of 25-250, 250-500, 500-1,000, 1,000-2,500, 2,500-5,000, 5,000-25,000, 25,000-50,000 includes any numerical value or range within or encompassing such values, *e.g.*, 25, 26, 27, 28, 29…250, 251, 252, 253, 254…500, 501, 502, 503, 504…, etc.

[00789] As also used herein a series of ranges are disclosed throughout this document. The use of a series of ranges includes combinations of the upper and lower ranges to provide another range. This construction applies regardless of the breadth of the range and in all contexts throughout this patent document. Thus, for example, reference to a series of ranges such as 5-10, 10-20, 20-30, 30-40, 40-50, 50-75, 75-100, 100-150, includes ranges such as 5-

20, 5-30, 5-40, 5-50, 5-75, 5-100, 5-150, and 10-30, 10-40, 10-50, 10-75, 10-100, 10-150, and 20-40, 20-50, 20-75, 20-100, 20-150, and so forth.

[00790] It is understood that modifications that do not substantially affect the activity of the various embodiments described herein are also provided within the definition of the subject matter described herein. Accordingly, the following examples are intended to illustrate but not limit the present disclosure.

8. EXAMPLES

[00791] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (*e.g.*, amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. By "average" is meant the arithmetic mean. Standard abbreviations may be used, *e.g.*, bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

General Synthetic Procedures

[00792] Many general references providing commonly known chemical synthetic schemes and conditions useful for synthesizing the disclosed compounds are available (see, *e.g.*, Smith and March, March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure, Fifth Edition, Wiley-Interscience, 2001; or Vogel, A Textbook of Practical Organic Chemistry, Including Qualitative Organic Analysis, Fourth Edition, New York: Longman, 1978).

[00793] Compounds as described herein can be purified by any purification protocol known in the art, including chromatography, such as HPLC, preparative thin layer chromatography, flash column chromatography and ion exchange chromatography. Any suitable stationary phase can be used, including normal and reversed phases as well as ionic resins. In certain embodiments, the disclosed compounds are purified via silica gel and/or alumina chromatography. See, *e.g.*, Introduction to Modern Liquid Chromatography, 2nd Edition, ed. L. R. Snyder and J. J. Kirkland, John Wiley and Sons, 1979; and Thin Layer Chromatography, ed E. Stahl, Springer-Verlag, New York, 1969.

[00794] During any of the processes for preparation of the subject compounds, it may be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules concerned. This may be achieved by means of conventional protecting groups as described in standard works, such as J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London and New York 1973, in T. W. Greene and P. G. M. Wuts, "Protective Groups in Organic Synthesis", Third edition, Wiley, New York 1999, in "The Peptides"; Volume 3 (editors: E. Gross and J. Meienhofer), Academic Press, London and New York 1981, in "Methoden der organischen Chemie", Houben-Weyl, 4th edition, Vol. 15/l, Georg Thieme Verlag, Stuttgart 1974, in H.-D. Jakubke and H. Jescheit, "Aminosauren, Peptide, Proteine", Verlag Chemie, Weinheim, Deerfield Beach, and Basel 1982, and/or in Jochen Lehmann, "Chemie der Kohlenhydrate: Monosaccharide and Derivate", Georg Thieme Verlag, Stuttgart 1974. The protecting groups may be removed at a convenient subsequent stage using methods known from the art.

[00795] The subject compounds can be synthesized via a variety of different synthetic routes using commercially available starting materials and/or starting materials prepared by conventional synthetic methods. A variety of examples of synthetic routes that can be used to synthesize the compounds disclosed herein are described in the schemes below.

[00796] Example 1. Generation of anti-ROR activatable antibodies

[00797] To achieve tumor-specific activation of immune cells by the anti-ROR antibodies, activatable antibodies of **P0** were further developed. Using recombinant DNA technology and as described in US20210207126A1, a masking moiety (MM) and a cleavable moiety (CM) linker containing an enzyme-specific cleavage site were tandemly connected to the N-terminus of the light chain of the parental antibody **P0** to produce activatable antibodies **M1**, **M2** and **M3**.

[00798] Briefly, the antigens as described in **Table E1** below were designed and purchased from Acro Biosystems or produced internally. **P0** was first displayed on the cell surface and confirmed to be functional in binding to its antigens, ROR1 and ROR2. Then the improved peptide libraries as described in US20210207126A1 were directly fused to the N-terminus of the light chain, and a cell library was constructed that displays the fusion protein on the cell surface. The cell library then underwent several rounds of FACS-based screening: firstly, the cell clones that have low binding to antigens were enriched (negative selection), then the enriched cell clones were treated with a protease (*e.g.*, MMP9) to remove the N-terminal peptide, and the clones with high binding to antigens were selected (positive selection). See,

FIG. 1. After several rounds of positive selections, the plasmids were extracted from these clones and the masking peptide sequences were confirmed through DNA sequencing.

Table E1. Prepared Antigens, including extracellular domains (ECDs) of human and mouse ROR1 and ROR2.

Target	Species	Domain	Description
ROR1	Human	30-403	Human Protein, Fc Tag
	Human	39-151	Human (39-151, Ig-like domain) Protein, His Tag
	Mouse	30-403	Mouse Protein, Fc Tag
ROR2	Human	34-403	Human Protein, Fc Tag
	Mouse	34-403	Mouse Protein, Fc Tag

[00799] The obtained antibody clones were then converted into IgG1s. In one embodiment, the heavy and light chains were cloned into the mammalian expression vector pCDNA3.3 (Thermo Fisher Scientific, cat #K830001) separately, and the masking peptides and the invariant cleavage peptide were fused to the N-terminus of the light chain in the same manner as displayed on cell surface. Pairs of plasmids were transiently transfected into HEK293F cells. After six days, the supernatants were harvested, cleared by centrifugation and filtration, and IgGs were purified with standard protein A affinity chromatography (MabSelect SuRe, GE Healthcare). The IgGs were eluted and neutralized, and buffer exchanged into PB buffer (20 mM sodium phosphate, 150 mM NaCl, pH 7.0).

[00800] To better characterize the selected antibody clones, several cell lines, including those as illustrated in **Table E2** below, were obtained. Then, the selected antibody clones, together with the parental antibody **P0**, were assessed for their binding to ROR1 expressed on 293 cells, ROR2 expressed on 293 cells, HT-29 (SIBS, Catalog # HTB-38, cultured in McCoy's 5A (Gibco, Catalog # 16600082) +10% FBS (Gibco, Catalog # 10099-141C)), H226 (SIBS, Catalog # CRL-5826, cultured in RPMI1640 (Gibco, Catalog # 11875119) +10%FBS), and 293F (293F, Sigma, Catalog # A14528, cultured in Dynamis™ medium (Gibco, Catalog # A2661501)) cells using enzyme-linked immunosorbent assay (ELISA) (measuring absorbance at 450 nm) or fluorescence activated cell sorting (FACS) (measuring APC mean fluorescence intensity (MFI)).

Table E2. Prepared cell lines.

Cell Line	Cell Type	Reported ROR1 Copy Number	Reported ROR2 Copy Number
K562	Chronic Myelogenous Leukemia	<7,000	68,000
PANC1	Pancreatic Carcinoma	60,773	<7,000
MDA-MB-231	TNBC	114,262	<7,000
RPMI-8226	B-Lymphoma	111,000	72,000
HOP-92	Lung Adenocarcinoma	227,038	9,000
HT-29	Colorectal Carcinoma	TBD	TBD

[00801] When indicated, the masked antibodies as disclosed herein were activated as follows and then applied to an assay as described herein.

[00802] 100 mM APMA (Sigma, Catalog # 164610-700MG) in DMSO was prepared. APMA (700 mg) was reconstituted with 20 mL DMSO to generate a stock solution of 100 mM. After the powder was completely dissolved, the solution was gently shaken to mix well. No less than 20 μL/vial stock solution was aliquoted and stored at ≤ -20°C. Repeatedly freezing and thawing were avoided.

[00803] MMP-9 was prepared. A vial of lyophilized MMP-9 (Human MMP-9 Protein, His Tag, Acro Biosystems, Catalog # MM9-H5221) was taken from ≤ -20 °C and equilibrated at bench top for at least 15 minutes. 20 μg MMP-9 was reconstituted in 80 μL water to generate a stock solution of 250 μg/mL and left on bench for 30 minutes to 60 minutes. Vortexing was avoided. After the lyophilized powder was completely dissolved, the solution was gently shaken to mix well. Repeatedly freezing and thawing were avoided. Multiple vials might be reconstituted at each preparation and mixed well before using.

[00804] MMP-9 activation system was prepared. 10 mM APMA was prepared. AMPA stock (100 mM) was diluted to a final concentration of 10 mM APMA with water. MMP-9 activation system was then prepared as follows: 20 μL of 250 μg/mL MMP-9 (final concentration 100 μg/mL), 25 μL of TCNB (50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% Brij-35, pH 7.50), and 5 μL of 10 mM AMPA (final concentration 1 mM). The solution was gently shaken to mix well, while avoiding generation of foam and bubbles, and incubate at 37 °C for 24 h. The activation system expired after 7 days at 5±3°C.

[00805] The tested antibodies were then activated. The enzyme digestion system as prepared as follows: the tested antibody at a final concentration of 1 mg/mL, 10 μL of 100 μg/mL activated MMP-9, and TCNB to make the final volume to 100 μL. TCNB buffer was firstly into a microcentrifuge tube, then the tested antibody and activated MMP-9 were added. The final concentration of antibody was 0.5 to 2 mg/mL, and antibody/enzyme (w/w) was 100:1.

The reaction mixture was mixed gently to avoid generation of foam and bubbles, and incubate at 37 °C for 20 to 24 hr.

[00806] **ELISA**: Human ROR1 (human ROR1-Fc, Acro Biosystems, Catalog # R01-H5250) was diluted to 2 μg/mL with coating buffer (15 mM $NaCO_3$, 35 mM $NaHCO_3$, pH 9.6). 50 μL/well diluted human ROR1 was added to a 96-well ELISA plate (Corning, Catalog # 3690). The ELISA plate was covered and incubated at 5±3 °C in a wet box overnight. The ELISA plate was then washed with 0.05% Tween 20 in PBS three times using the BioTEK 405LS microplate washer (Wash cycles: 003; dispense/aspiration volume: 150 μL/well; and flow rate 07). The plate was blotted on a stack of paper towels until no residual fluid was observed. 3% skim milk in PBS was added to the 96-well ELISA plate, 150 μL/well. The ELISA plate was kept in a wet box and incubated at 37 °C for 1 hour.

[00807] The sample concentration was measured with UV-Vis spectrophotometer. The samples were diluted with 3% skim milk in PBS first to 20 μg/mL and then a first round of serial dilutions was performed to arrive at lower concentrations, such as 100 μg/mL and 20000 ng/mL. A second round of serial dilutions were made (for example starting from 20000 ng/mL) in a low-binding 96-well plate to arrive at the following final concentrations: 20000 ng/mL, 6666.667 ng/mL, 2222.222 ng/mL, 740.741 ng/mL, 246.914 ng/mL, 82.305 ng/mL, 27.435 ng/mL, 9.145 ng/mL, 3.048 ng/mL, 1.016 ng/mL, and 0.339 ng/mL. 3% skim milk in PBS was used as a blank.

[00808] The ELISA plate was washed with 0.05% Tween 20 in PBS three times using the BioTEK 405LS microplate washer (Wash cycles: 003; dispense/aspiration volume: 150 μL/well; and flow rate 07). The plate was blotted on a stack of paper towels until no residual fluid was observed.

[00809] The samples and blanks were transferred to the ELISA plate in duplicates (50 μL/well) with multi-channel pipettes. The ELISA plate was kept in a wet box and incubated at 37 °C for 1 hour. After the incubation, the ELISA plate was washed with 0.05% Tween 20 in PBS four times using BioTEK 405LS microplate washer (Wash cycles: 004; dispense/aspiration volume: 150 μL/well; and flow rate 07). The plate was then blotted on a stack of paper towels until no residual fluid was observed.

[00810] HRP Goat-anti-human IgG (Fab Specific) antibody (Sigma-Aldrich, Catalog # A0293-1ML) was diluted to 1000 ng/mL with 3% skim milk in PBS, then added at 50 μL/well to the ELISA plate. The ELISA plate was kept in a wet box and incubated at 37 °C for 1 hour. After the incubation, the ELISA plate was washed with 0.05% Tween 20 in PBS four times using BioTEK 405LS microplate washer (Wash cycles: 004; dispense/aspiration

volume: 150 μL/well; and flow rate 07). The plate was then blotted on a stack of paper towels until no residual fluid was observed.

[00811] 50 μL/well of TMB (KPL, Catalog # 52-00-01) was added to the ELISA plate with a multi-channel electronic pipette. After about 5 minutes, 50 μL/well of 1 M phosphoric acid was added with a multi-channel electronic pipette to quench the reactions. The absorbance of every valid well was read at 450 nm with a microplate reader as soon as the reaction was quenched. The molar concentration of the test sample was calculated. The molar concentration of standard curve was converted to log (M). log (M) as X and absorbance as Y were fit with Asymmetric Sigmoidal, 4 PL by GraphPad.

[00812] **FACS:** For adherent cells (HT-29, NCI-H226, EMT6 (ATCC, Catalog # CRL-2755, cultured in Waymouth's MB 752/1 Medium (Sigma, Catalog # W1625) +15% FBS+2 mM L-glutamine (Gibco, Catalog # 25030081)) engineered to express ROR1 (cultured in Waymouth's MB 752/1 Medium + 15% FBS + 2 mM L-glutamine + 3 μg/mL Puromycin (InvivoGen, Catalog # ant-pr-1)) and EMT6 engineered to express ROR2 (Waymouth's MB 752/1 Medium + 15% FBS + 2 mM L-glutamine + 3 μg/mL Puromycin)), the spent cell culture medium was discarded, and the cells were washed with DPBS (Meilunobio, Catalog # MA0010). The cells were then digested with Trypsin-EDTA (Gibco, Catalog # 25300-062) until the cells were detached from the flask. Cell culture medium was added to stop the digestion, and the medium was dispersed by pipetting over the cell layer surface several times to get cell suspensions. For suspension cells (293F), the cell suspension was mixed gently.

[00813] The cell suspension was mixed gently. 20 μL of cell suspension was added to 1.5 mL counter tube and 1 μL of Solution 18 AO·DAPI staining solution (ChemoMetec, Product Number: 910-3018) was also added. The samples were then mixed well and 12 μL mixed solution was added to the counting plate to calculate the cell number with NucleoCounter. The necessary number of cells were transferred into 50 mL centrifuge tubes. The cell suspension was centrifuged at 200 x g at room temperature for 5 min and the supernatant was discarded. The cells were then washed once with 5 mL of DPBS, and centrifuged at 200 x g at room temperature for 5 min. The supernatant was discarded. Cell pellet was resuspended with 2% FBS/DPBS to adjust the cell density to 2×10^6 cells/mL. The cell suspension was mixed well and 50 μL/well of the cells was transferred into 96-well plates.

[00814] The test antibodies were diluted in 96 well plate with 2% FBS/DPBS to make 7-9 serial dilutions (2× working concentration). 50 μL/well of the serial diluted test antibodies was transferred into the 96-well plate containing 50μL/well of cell suspension, and incubated for 30 min at 4 °C, protected from light. The cells were then washed with 150 μL/well of 2%

FBS/DPBS, and centrifuged at 500 x g at room temperature for 5 min. The supernatant was discarded. The cells were further washed with 250 µL/well 2% FBS/DPBS, and centrifuged at 500 x g at room temperature for 5 min. The supernatant was discarded. 100 µL/well APC Anti Human IgG Fc (1:200 dilution, Biolegend, Catalog # 410712) with 2% FBS/DPBS was added to the plate, and incubated for 30 min at 4 °C, protected from light. The cells were then washed with 2% FBS/DPBS, and centrifuge at 500 x g at room temperature for 5 min. The supernatant was discarded. The cell pellet was resuspended with 100 µL of DPBS for flow cytometry analysis.

[00815] As shown in **FIGs. 2A-2F** and **Table E3** below, the selected activatable ROR antibody clones, including **M1**, **M2**, and **M3**, exhibited little binding to antigens in the presence of masking peptide, indicating all lead candidates exhibit efficient/facile cleavage of masking peptide.

Table E3. Masking efficiency (ME) over **P0** of **M1**, **M2**, and **M3**.

Antibodies	**Masking Efficiency (ME)**				
	ROR1 (ELISA)	**ROR2 (ELISA)**	**HT-29**	**H226**	**293F**
M1	90	40	430	350	380
M2	140	150	820	490	500
M3	50	60	220	200	210
P0	1	1	1	1	1

[00816] Example 2. Developability of anti-ROR activatable antibodies

[00817] Protein concentrations of the produced activatable antibodies were determined by UV-spectrophotometry, and IgG purity was analyzed under denaturing, reducing and non-reducing conditions by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE) and size exclusion-high-performance liquid chromatography (SEC-HPLC).

[00818] SDS-PAGE: The antibody samples were diluted to 1 mg/mL with formulation buffer. Under non-reduced conditions, the antibody samples were further diluted to 0.5 mg/mL with SDS sample buffer (Bio-Rad #1610737). Under reduced conditions, the antibody samples were further diluted to 0.5 mg/mL with SDS sample buffer within 20 mM DTT. All samples were then heated at 70 °C for 5 min. 12% gel and 8% gel was used. 1 µg denaturing sample was loaded into the gel hole and electrophoresis apparatus was used to separate the samples. The gel was destained after staining with Coomassie brilliant blue (CBB) and pictured by the gel camera.

[00819] SEC-HPLC: The following instrument and materials were used, including HPLC: Thermo U3000; Column: Waters XBridge BEH SEC column (200Å, 3.5 µm, 7.8×300 mm); and Mobile Phase: 50 mM phosphate, 300 mM sodium chloride, pH 6.8. The antibody

samples were prepared as followed: if the sample concentration was above 10.0 mg/mL, the sample was diluted to 10.0 mg/mL with sample buffer; and if the sample concentration was below or equal to 10.0 mg/mL, no dilution was performed. The samples were then centrifuged at 13,800 g for 10 min. Before the test, the column was washed with ultrapure water and mobile phase successively, each for 15 column volumes at least. The absorbance and back pressure were monitored until a stable baseline was obtained. The injection sample amount was 40 μg and the flow rate was 0.7 mL/min while the run time was 20 min. The peak intensity was measured by absorbance at 280 nm. The percent of relative peak area of monomer, high molecular weight (HMW), and low molecular weight (LMW) were determined by area normalization method.

[00820] As shown in **Table E4**, all selected candidates exhibited high expression, high purity and monomeric (SEC-HPLC).

Table E4. *In vitro* characterization of **P0**, **M1**, **M2**, and **M3**.

Antibodies	Titer (mg/L)	SEC Purity		SDS-PAGE	Easily Cleaved
		HMW (%)	LMW (%)		
M1	170	1.7	0.5	Normal	Yes
M2	91	3.4	0.0	Normal	Yes
M3	122	3.4	0.1	Normal	Yes
P0	146	2.6	0.5	Normal	NA

[00821] Masked antibody stabilities (Tm) were then evaluated using differential scanning fluorimetry (DSF). Briefly, the tested antibody was diluted to 1 mg/mL and the dye reagent (SYPRO® Orange, Invitrogen #S6650) was diluted to 50 times with formulation buffer. 16 μL sample solution was mixed with 4 μL dye solution in 96-well plate (Roche #4729692001). The sample solutions were then heated from 20 °C to 95 °C at a ramp rate of 0.02 °C/s in the LightCycler 480 system. The DSF data was analyzed and fitted with LCS480 software 1.5.1.62.

[00822] The results are shown in **Table E5** below and **FIGs. 3A-3B**, providing that Tm 1 and Tm 2 of masked antibodies were similar to parental **P0**. These results indicated that the masked antibodies did not have altered overall stabilities.

Table E5. Stabilities of **P0**, **M1**, **M2** and **M3**.

Antibodies	Tm 1	Tm 2
M1	64.7	81.3
M2	64.7	81.1
M3	64.7	81.1
P0	64.8	80.4

[00823] The masked antibodies were also evaluated using reduced and non-reduced capillary electrophoresis sodium dodecyl sulfate (CE-SDS). The following instrument and materials were used: Capillary Electrophoresis: Protein Simple Maurice and Maurice CE-SDS Cartridge (Protein Simple #PS-MC02-SP). The antibody samples were first diluted to 1 mg/mL with SDS sample buffer (Protein Simple #046-567 for non-reducing, Protein Simple #046-012 for reducing). 2 µL 25×Internal Standard (Protein Simple #046-144) was added into 50 µL diluted sample. Under non-reduced conditions, the sample was treated with 2.5 µL 250 mM IAM (Aladdin #I105563) and heated at 65°C for 10 min. Under reduced conditions, the sample was treated with 2.5 µL 14.2 M 2-mercaptoethanol (BME) (Sigma #97622) and heated at 70 °C for 10 min. The prepared samples were then measured (detection: 220 nm; loading sample: 4600V, 20 s; and separation: 5750 V 35 min (non-reduced) and 5750 V 25 min (reduced)).

[00824] Exemplary results are shown in **FIGs. 4A-4D**, indicating the masked antibodies did not show significant differences in CE-SDS patterns (HC and LC) relative to the parental **P0** antibody.

[00825] Using capillary isoelectric focusing (cIEF), isoelectric points (pI) of the masked antibodies were evaluated. The following instrument and materials were used: Capillary Electrophoresis: Protein Simple Maurice; and Maurice cIEF Cartridge (Protein Simple #PS-MC02-C). Briefly, a master mix was prepared according to the total sample number and component amounts needed for each sample (for a total volume of 100 µL, the master mix per sample includes 0.5 µL of pI marker (pI 5.85, Protein Simple #102225); 1 µL of pI marker (pI 10.17, Protein Simple #046-035); 1 µL of Pharmalyte 3-10 (Cytiva #17045601); 3 µL of Pharmalyte 5-8 (Cytiva #17045301); 35 µL of 1% methylcellulose (Protein Simple #101876); 20 µL of 10 M Urea; 2 µL of 500 mM Arginine (Sigma #V900343); and 17.5 µL of Ultrapure water). The antibody sample was diluted to 1.0 mg/mL. 20 µL of the diluted sample was then mixed with 80 µL of the master mix in an Eppendorf tube. The mixture was mixed well, centrifuged briefly, and measured (focusing voltage and duration: focus period 1: 1 minute at 1500 V and focus period 2: 9 minutes at 3000V; and sample injection duration: 55 seconds).

[00826] The results are shown in **FIGs. 5A-5D**, while % main peak was indicated. The results shown that all masked antibody candidates had reduced pI relative to parental **P0**.

[00827] The antibodies were further tested using hydrophobic interaction chromatography (HIC). The following instrument and materials were used: HPLC: Thermo U3000; Column: Thermo MAbPac HIC-10, 5 µm, 1000 Å, 4.6 × 100 mm; Mobile Phase A: 1 M ammonium

sulfate, 100 mM sodium phosphate, pH 7.0; and Mobile Phase B: 100 mM sodium phosphate, pH 7.0. If the concentration of sample was more than 2 mg/mL, the samples were diluted to 1 mg/mL with mobile phase A. If the concentration of sample was less than 2 mg/mL, the samples were diluted half-and-half with mobile phase A. The injection sample amount was 10 µg, and the instrument was set as illustrated in **Table E6** below.

Table E6. HIC setting.

Pump parameter						
No.	Time	Flow (mL/min)	B%	C%	D%	Curve
1	0.000	1.0	0.0	0.0	0.0	5
2	15.000	1.0	100.0	0.0	0.0	5
3	20.000	1.0	100.0	0.0	0.0	5
4	20.001	1.0	0.0	0.0	0.0	5
5	25.000	1.0	0.0	0.0	0.0	5
6	25.000	Stop Run				
Sampler parameter						
Inject Mode		Normal				
Temperature		5 ± 3℃				
Column Oven parameter						
Temperature		25 ± 5℃				
Ready Temp Delta		5℃				
UV detector parameter						
No.	Channel	Wavelength (nm)	Bandwidth (nm)	Refwavelength (nm)		
1	UV_VIS_1	280	4	Off		

[00828] All lead candidates displayed predominantly single peaks, were more hydrophilic than the parental **P0** antibody, and exhibited improved hydrophobicity profiles, while **M3** was more hydrophobic than **M1** and **M2**. See, **FIG. 6** and **Table E7** below.

Table E7. HIC-HPLC results of **P0**, **M1**, **M2** and **M3**.

Antibodies	**RT (min)**
M1	7.8
M2	7.8
M3	11.0
P0	12.4

[00829] Additionally, antibody stabilities were examined under different stress conditions.

[00830] Firstly, the stability of the masked antibodies was evaluated after 0 days, 7 days, and 14 days at an elevated temperature (40°C). Briefly, the samples were diluted to 1.0 mg/mL

with 20 mM Histidine, pH5.5. 120 μL of diluted sample was aliquoted into each tube. The samples were then stored at 40 °C, 75% relative humidity for 7 or 14 days, and analyzed by UV280 absorbance, SEC, and SDS-PAGE.

[00831] As shown in **FIGs. 7A-7D**, a very slight reduction in main peak % was observed, while the slight reduction in main peak % was comparable to parental **P0**.

[00832] Further, the stability of the masked antibodies was analyzed after 3 and 6 cycles of freezing and thawing (F/T or FT). Briefly, the samples were diluted to 1.0 mg/mL with 20 mM Histidine, pH5.5. 120 μL of diluted sample was aliquoted into each tube. The samples were placed at -80°C refrigerator for 70 min, removed, and thawed at room temperature for 70 min. Such freezing and thawing steps were repeated for a total of 3 or 6 cycles (also referred to herein as FT3 or FT6, respectively). The samples were then analyzed by UV280 absorbance, SEC, and SDS-PAGE.

[00833] The results are shown in **FIGs. 8A-8D**, indicating that candidates were all stable after 3 cycles of F/T, additional F/T cycles (6) showed detectable levels of HMW values and a slight instability. Therefore, additional F/T cycles were suggested to be avoided.

[00834] In addition, the masked antibodies were stable to low pH conditions as measured by SEC-HPLC and shown in **FIGs. 9A-9D**; while the masked antibodies were stable to oxidation (2% H_2O_2) as measured by SEC-HPLC and shown in **FIGs. 10A-10D**.

[00835] The masked antibodies were also stable in cyno blood (plasma or serum for 1 week exposure). Briefly, 100 μg/mL antibodies were incubated in cyno plasma or serum at 37°C for 1 week, and the total antibody was then measured by ELISA (anti-Fc capture, anti-Fab detection), while the activated antibody was detected by ELISA based on binding to the ROR1 antigen.

[00836] The samples were prepared as follows. The test antibodies were diluted in monkey plasma or serum at 100 μg/mL, aliquoted and incubated at 37 °C for 3h, 6h, 24h, 48h, 96h, or 168h. After the incubation, each sample was stored at -20 °C until analysis. One vial sample was saved immediately at -20°C for 0h control.

[00837] The plate was then coated as follows. 100 μL coating antibody solution (for total form detection, Goat Anti-Human IgG, 1 μg/mL, SouthernBiotech, Catalog # 2049-01) or coating antigen solution (for cleaved form detection, His-tagged ROR1 domain 1, 1 μg/mL, Acro Biosystems, Catalog # RO1-H5221) was added to a 96-well ELISA plate. The plate was then sealed and incubated at 4 °C overnight. After the incubation, the plate was blocked as follows. The plate was washed for three times with 0.05% PBST. 300 μL blocking buffer

(PBS containing 2% skim milk) was added to each well. The plate was then sealed and incubated at 37 °C for 1 hour.

[00838] Standard (STD) was prepared as follows. Sol 0 solution was prepared by diluting pooled monkey plasma or serum by 100-fold with PBS containing 2% skim milk. The MRD (minimum required dilution) was 1:100. Antibody concentrations were prepared at 2.00, 4.41, 9.70, 21.34, 46.96, 103.31, 227.27, and 500 ng/mL for the coating antibody (Goat Anti-Human IgG) STD (Total form, the standard curve was prepared for each antibody), and at 8.82, 19.40, 42.69, 93.91, 206.61, 454.55, and 1000 ng/mL for the coating antigen (His-tagged ROR1 domain 1) STD (Cleaved/Activated form, parental antibody **P0** was used as the STD for all tested activatable antibodies). A quality control (QC) of 100 ng/mL was prepared for each of the two STDs.

[00839] The samples were diluted with Sol 0 for 800× for the total antibody testing and for 100× for the activated antibody testing and incubated as follows. The plate was washed for three times with 300 µL/well 0.05% PBST. 100 µL sample was added to each well. The plate was sealed and incubated at 37 °C for 1 hour. After the incubation, the plate was washed for three times with 300 µL/well 0.05% PBST. 100 µL detection antibody (HRP goat anti-hIgG (Fab specific), Sigma, Catalog # A0293, diluted by 1:5000 with 2% skim milk) was added to each well. The plate was sealed and incubated at 37 °C for 1 hour. After washing the plate for four times with 300 µL/well 0.05% PBST, 50 µL TMB substrate (Abcam, Catalog # ab171525) was added to each well, and incubated at room temperature for 2-3 minutes until the desired color intensity was reached. 50 µL stop solution (Abcam, Catalog # ab171529) was added to stop the reaction and the OD_{450} and OD_{630} values were measured in a microplate reader. The obtained data was processed by GraphPad Prism 7.0 with an Asymmetric Sigmoidal, 5PL, X is log (concentration) regression mode. OD_{450}-OD_{630} of the testing samples was interpolated into the standard curve and the sample concentrations were calculated.

[00840] The results are shown in **FIGs. 11A-11C**, indicating that activated masked antibody levels remained below the limit of detection (<2 µg/mL) for all candidates and all candidates remained masked in cyno blood.

[00841] Taken together, these results indicated that even without formulation optimization, **M1**, **M2**, and **M3** had excellent developability profiles.

[00842] Example 3. Binding affinities of anti-ROR activatable antibodies

[00843] The binding affinities to human/cynomolgus monkey (cyno), mouse, and rat ROR1 and ROR2 were measured, for example using surface plasmon resonance (SPR) or biolayer interferometry (BLI).

[00844] BLI: Kinetic buffer (1×PBS, 0.02% Tween20 (v/v), 0.1% BSA (w/v), pH 7.4) was applied as the sample dilution buffer. The antibodies and antigens were diluted to 100 nM. The testing temperature of ForteBio Octet RED96 System was set to 25°C. The AHC biosensors (Sartorius Fortebio #18-2060) were dipped into wells containing kinetic buffer for 60 s for baseline phase, into wells containing antibodies for 300 s for the loading phase, and into wells containing kinetic buffer for 120 s for baseline phase. The AHC biosensors were then dipped into wells containing antigens for 300 s for the association phase, before dipping into kinetic buffer for 300 s for the dissociation phase. Finally, the AHC biosensors were dipped into regeneration buffer (10 mM Glycine-HCl, pH 1.5) for 30 s for the regeneration phase. The acquired ForteBio data was processed with Data Acquisition software 7.1. The kinetic data was fitted using the 1:1 Langmuir binding model.

[00845] SPR: Anti-Human IgG (Fc) antibody (Cytiva, catalog# BR-1008-39) was immobilized onto CM5 chips by coupling its amine groups onto carboxylated surfaces of sensor chip following the instruction of the amine coupling kit (Cytiva, catalog# BR-1000-50). The final response of immobilization level should be between 5,000 and 10,000 RU (6,000 was measured for Edelweiss experiments). The test antibodies were diluted with 1×HBS-EP buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA·2Na, 0.005% (v/v) Tween 20, pH 7.4) to 7.5 μg/mL, and injected into the system for 10 s at a flow rate of 10 μL/min for immobilization in the range of 300-500 RU capture level (for example, 415 RU was measured). The antigens were serially diluted to concentrations ranging from 0.625 to 20 nM with 1×HBS-EP buffer, and flowed through the test antibody captured surface for 300 s at flow rate of 30 μL/min. After association, 1×HBS-EP buffer was injected to the surface at flow rate of 30 μL/min for another 1200 s to measure the antigen dissociation rate. The chip-captured test antibody/antigen complex was removed using 10 mM Glycine-HCl, pH 1.5 to regenerate the sensor chip for the next test antibody/antigen association/dissociation measurement cycle. A double reference subtraction method was applied, including reference subtraction using a reference channel with no test antibody captured and blank subtraction using blank sample with zero concentration. The association and dissociation sensorgrams were fitted to a 1:1 binding model using Biacore T200 Evaluation Software.

[00846] The results are shown in **Table E8** below. **P0** mAb demonstrated binding at both ROR1 and ROR2 receptors. In the absence of activated MMP9, masked mAb did not

demonstrate ROR1 or ROR2 receptor binding (NB, no binding). In the presence of activated MMP9, unmasked mAb demonstrated binding that was comparable to non-masked **P0** mAb.

Table E8. Monovalent K_D values (nM) of the tested antibodies.

As Ligand (capture on chip)	**As Analytes (flow)**					
Antibody Test Article	**Recombinant Protein Antigen**					
	ROR1 (IgG-like Domain)			**ROR2 (IgG-like Domain)**		
	Human/Cyno	**Mouse**	**Rat**	**Human/Cyno**	**Mouse**	**Rat**
	Hu v cyno ROR1 IgG-like domain are identical	**Mo v Rat ROR1 IgG-like domain: 1AA difference**		**Hu v cyno ROR2 IgG-like domain are identical**	**Mo v Rat ROR2 IgG-like domain: 1AA difference**	
M1 masked	NB	NB	NB	NB	NB	n/a
M2 masked	NB	NB	NB	NB	NB	n/a
M3 masked	NB	NB	NB	NB	NB	n/a
M1 unmasked	1.15	0.838	0.924	0.695	32.1	n/a
M2 unmasked	1.06	0.704	0.815	0.741	51.7	n/a
M3 unmasked	1.09	0.776	0.784	0.796	48.87	n/a
P0	0.671	0.544	0.556	0.387	28.94	n/a

[00847] Example 4. *In vivo* characterization of anti-ROR activatable antibodies – Study 1

[00848] ROR1-expressing EMT6 cells were implanted into BALB/c mice, and the tumor-bearing mice were then treated with tested articles as detailed in **Table E9** below. The mouse plasma was harvested at 24 hours after the administration and the total IgGs were purified using Protein A beads. The samples were then processed for anti-human-IgG western blotting (WB). The results are shown in **FIG. 12**, indicating that **M2** remained intact in circulation 24 hours after dosing.

Table E9. *In vivo* administration in EMT6-ROR1 tumor-bearing BALB/c mice.

Group No.	**N**	**Test Article**	**Dosage (mg/kg)**	**Dosing Route**	**Frequency**
1	1	P0	3	i.p.	Single dose
2	1	P0	10	i.p.	Single dose
3	1	M2	3	i.p.	Single dose
4	1	M2	10	i.p.	Single dose
5	1	PBS	-	i.p.	Single dose

[00849] Later, a similar but expanded experiment was performed. See the experimental design shown in **Table E10** below. The mouse plasma was harvested at 24, 72, and 96 hours after the administration (N=3 mice per group) and the total IgGs were purified using Protein A beads. The samples were then processed for anti-human-IgG western blotting (WB). 100 ng of **P0** was tested in parallel and served as a control, along with 100 ng of mixed each of the masked antibodies and *in vitro* cleaved antibodies. The results are shown in **FIGs. 13A-13C**, indicating all three masked antibodies remained intact in circulation up to 96 hours after dosing.

Table E10. *In vivo* administration in EMT6-ROR1 tumor-bearing BALB/c mice.

Group No.	N	Test Article	Dosage (mg/kg)	Dosing Route	Frequency
1	9	P0	10	i.p.	Single dose
2	9	M1	10	i.p.	Single dose
3	9	M2	10	i.p.	Single dose
4	9	M3	10	i.p.	Single dose
5	9	Isotype Ctrl	10	i.p.	Single dose

[00850] In summary, the masked antibodies predominantly circulated as an intact/masked antibody following systemic dosing in tumor-bearing mice.

[00851] Example 5. *In vivo* characterization of anti-ROR activatable antibodies – Study 2

[00852] ROR1-expressing EMT6 cells were implanted into BALB/c mice. When the average tumor volume reached about 196 mm^3, the tumor-bearing mice were treated with tested articles as detailed in **Table E11** below. The xenograft tumors were then harvested at 14 hours and 96 hours after dosing for Receptor Occupancy (RO) assay using flow cytometry (N=3 mice per group).

Table E11. *In vivo* administration in EMT6-ROR1 tumor-bearing BALB/c mice.

Group No.	N	Test Article	Dosage (mg/kg)	Dosing Route	Frequency
1	6	P0	10	i.p.	Single dose
2	6	M1	10	i.p.	Single dose
3	6	M2	10	i.p.	Single dose
4	6	M3	10	i.p.	Single dose
5	2	Isotype Ctrl	10	i.p.	Single dose

[00853] The harvested tumor tissue was minced into ~1 mm pieces with a sterile scalpel or scissors and the tissue pieces were transferred into a 15 mL tube. The tissue pieces were then washed with 10 mL PBS for three times. The residual PBS was removed. Pre-warmed 0.25% trypsin was added to the tissue pieces (1 mL of trypsin for every 100mg tissue) and incubated

at 37°C for 10 min. The tube was gently flapped every 5 min during incubation. At the end of trypsin digestion period, the trypsin was neutralized by adding PBS containing 2% FBS. The suspension and tissue were poured into 40 μm cell strainer on top of 50 mL tube. The tissue pieces were grinded gently using the plunger, while adding PBS to flush the grinded tissue pieces from time to time. The leftover tissue pieces were discarded if there were any. The cell suspension was centrifuged at 500 x g for 5 min. The cells were washed with PBS twice. The total cell number and viability were measured using Vi-CELL XR.

[00854] Receptor occupancy was assessed as described as follows. For each tumor sample, $3x10^6$ cells were added into a 1.5 mL Eppendorf tube. The cells were centrifuged, and the supernatant was discarded. The cell pellet was re-suspended with 100 μL PBS containing 1:1000 live/dead dye (Invitrogen, L34955) and incubated in dark at room temperature for 10 min. 300 μL PBS was added to stop the staining. The samples were centrifuged at 500 x g for 5 min and washed one more time with PBS. 300 μL PBS was added to re-suspend the cells, then the cells were equally divided into 3 tubes labeled as **P0**, isotype, cell blank, respectively. **P0** or isotype antibody was added to the corresponding tube to a final concentration of 100 nM for each and incubated at 4°C for 30 min. After the incubation, the cells were washed with PBS twice, and incubated with PBS containing 1:500 APC anti-human IgG(H+L) secondary antibody (Jackson Immuno Research, 109-136-088), and 1:300 anti-mouse CD45 antibody (BD, 557235) in dark at 4°C for 30 min. The cells were further washed with PBS twice, re-suspended in PBS, and then analyzed by flow cytometry.

[00855] As shown in **FIG. 14**, **FIGs. 15A-15B**, and **Table E12**, higher RO levels (40-60%) at the early time point of 14 hours were observed and the RO levels were similar between the parental antibody and the masked antibodies. As shown in **FIG. 14**, **FIGs. 16A-16B**, and **Table E13**, at 96 hours, RO levels decreased, and a more rapid decrease was observed with the parental antibody than with the masked antibodies.

Table E12. RO at 14 hours after dosing.

Test Article	P0			M1			M2			M3			Isotype Control
Group No.	1			2			3			4			5
Animal No.	1-1	1-2	1-3	2-1	2-2	2-3	3-1	3-2	3-3	4-1	4-2	4-3	5-1
Anti-ROR	104122	79584	80042	130639	111226	65290	102635	77155	115691	97944	75023	77459	105228
IgG1	72500	46624	50727	47450	39744	39861	69135	44568	73712	52160	45744	51052	8898
RO%	66.79%	53.37%	58.79%	31.67%	30.14%	54.91%	64.26%	52.26%	60.69%	48.58%	55.72%	61.48%	-

Table E13. RO at 96 hours after dosing.

Test Article	P0			M1			M2			M3			Isotype Control
Group No.	1			2			3			4			5
Animal No.	1-4	1-5	1-6	2-4	2-5	2-6	3-4	3-5	3-6	4-4	4-5	4-6	5-2
Anti-ROR	88290	43072	63123	67703	72623	98372	49866	92876	44103	59678	44827	65005	71505
IgG1	16088	10822	10779	15579	27882	13773	17374	37802	14893	20729	18272	19671	1198
RO%	17.01%	22.81%	15.34%	21.51%	37.28%	12.85%	33.11%	39.86%	31.77%	33.29%	39.00%	28.84%	-

[00856] In summary, the masked antibodies achieved ≥90% calculated intratumoral RO (or a similar level to Parental Ab) when tumor-bearing mice were dosed systemically at 10 mg/kg.

[00857] Example 6. *In vivo* characterization of anti-ROR activatable antibodies – Study 3

[00858] A dual staining IHZ assay was developed to show ROR1 and insulin at the same time. To confirm that the stating pattern in IHZ was not different than that in IF, frozen human pancreas samples, which were deemed to be of good integrity by the pathologist, were fixed and IF staining for ROR1 and insulin. The results are shown in **FIG. 17**. The insulin stating was shown in green fluorescence while the ROR1 stating was shown in red fluorescence. The pancreatic islets were easily identified and both IF and IHZ showed staining of ROR1 inside and outside of the pancreatic islets.

[00859] After validating the IHZ assay protocol, the following frozen samples were tested for ROR1 expression using **P0**, **M1**, **M2**, or **M3**, including HT-29 serving as a positive control, MCF7 serving as a negative control, and several human pancreas samples. All human pancreas samples used here were deemed to be of good integrity by the pathologist and further stained for insulin.

[00860] Representative results are shown in **FIGs. 18A-18D**. As shown in **FIG. 18A**, using the parental **P0**, strong staining of HT-29 (positive control) cells was observed, while very weak staining of MCF7 (negative control) cells was shown. No staining of either cell line was observed using the three tested masked antibodies or the isotype control. As shown in **FIG. 18B**, in human pancreas samples #5 and #3, the parental **P0** antibody showed some scattered cytoplasmic and membranous staining throughout the pancreas and in pancreatic islets. **M1** and **M2** showed very weak scattered staining in the pancreas but no staining in pancreatic islets, while **M3** showed weak scattered staining. As shown in **FIG. 18C**, in human pancreas samples #6 and #7, the parental **P0** antibody showed some scattered cytoplasmic and membranous staining throughout the pancreas and in pancreatic islets. **M1**, **M2**, and **M3** showed very weak scattered staining in the pancreas but no staining in pancreatic islets. As shown in **FIG. 18D**, in human pancreas samples #8 and #9, the parental **P0** antibody showed some scattered cytoplasmic and membranous staining throughout the pancreas and in pancreatic islets. **M1**, **M2**, and **M3** showed weak scattered staining of human pancreas #8 (all 3 masked antibodies gave relatively higher staining in this pancreas vs. the other five pancreases). **M3** showed weak scattered staining.

[00861] Overall, **P0** demonstrated scattered cytoplasmic and membranous staining throughout the pancreas and in pancreatic islets. It appeared to be more intense staining in

pancreatic islets and the staining pattern by **P0** in IHZ resembles that in IHC. The three masked antibodies showed very weak staining in the pancreas and no staining in pancreatic islets. There is a slightly higher staining in human pancreas #8 vs. the other 5 human pancreas. Without wishing to be bound by the theory, Pancreas #8 might be of poorer integrity than the other 5. Also, there was slightly higher staining by **M3** than the other 2 masked antibodies, correlating with levels of *in vitro* masking efficiencies. See **Table E14** below. In summary, the masked antibodies did not show binding to pancreatic islets in IHC (human tissues).

Table E14. *In vitro* masking efficiencies.

HT-29	EC_{50} (nM)	ME	%Max	EC_{50} (nM)	ME	%Max
	CHO-S produced antibody			HEK293 produced antibody		
P0	0.45	1	100	0.34	1	100
M1	92	200	49	150	430	50
M2	220	480	45	280	820	52
M3	51	110	46	76	220	46

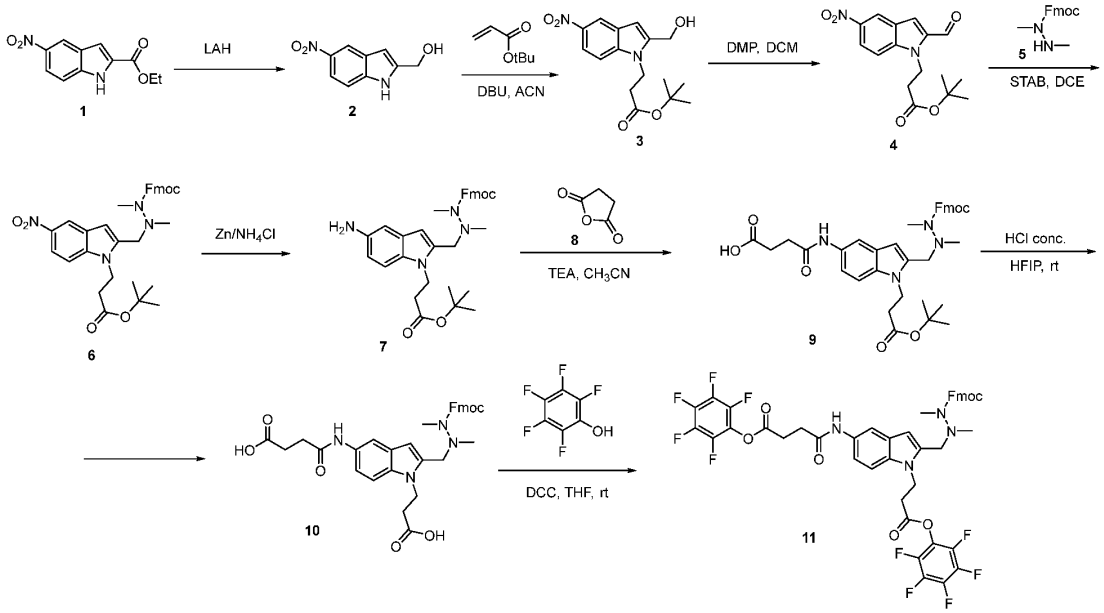
[00862] Example 7. Aldehyde-Tagged Antibodies: Preparation

[00863] Tagged antibodies were 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, an antibody containing a heterologous sulfatase motif was contacted with a formylglycine-generating enzyme to convert a cysteine or serine in the motif to a formylglycine having an aldehyde moiety, thereby generating an "aldehyde-tagged antibody." Any formylglycine-generating enzyme can be used, such as *Mycobacterium tuberculosis* or *Mycobacterium tuberculosis*.

[00864] Example 8. Linker-Payload: Preparation

[00865] Synthetic reagents were purchased from Sigma-Aldrich, Acros, AK Scientific, or other commercial sources and used as received. Anhydrous solvents were obtained from commercial sources in sealed bottles. Compound **12** (previously reported in Chuprakov et al. *Bioconjugate Chem.* **2021**, *32*, 746-754) was obtained commercially from Shanghai Medicilon and used without purification. Belotecan **13** as well as compounds **16** and **17** were obtained from commercial sources and used as received. Column chromatography was performed using a Biotage Isolera or Biotage Selekt chromatography system. Low-resolution mass spectra (LRMS) were acquired on Agilent Technology 6120 Quadrupole LC/MS,

equipped with Agilent 1260 Infinity HPLC system, G1314 variable wavelength detector, and Agilent Poroshell 120 SB C18, 4.6 mm x 50 mm column at room temperature using 10-100% gradient of water and acetonitrile containing 0.1% formic acid. HPLCs were monitored at 254 or 205 nm.



SCHEME 1

[00866] Preparation of (5-nitro-1H-indol-2-yl)methanol (**2**)

[00867] To an oven-dried round-bottom flask were added ethyl 5-nitro-1*H*-indole-2-carboxylate (**1**, 25 g, 107 mmol) and 250 mL of anhydrous THF. The resulting solution was cooled down to 0 °C and treated with lithium aluminum hydride (4.9 g, 129 mmol) in small portions over 30 minutes with vigorous stirring under nitrogen. Reaction mixture was stirred at room temperature for 2 h, poured into 100 mL of water, and extracted with ethyl acetate (400 mL). Organic layer was concentrated under vacuum and purified on silica gel (75% v/v EtOAc/hexane) to afford 14 g (73 mmol, 68% yield) of product **2** as a brown solid. LRMS (ESI): *m/z* 192.9 $[M+H]^+$, calculated for $C_9H_8N_2O_3$ *m/z* 193.1.

[00868] Preparation of tert-butyl 3-(2-(hydroxymethyl)-5-nitro-1H-indol-1-yl)propanoate (**3**)

[00869] In an oven-dried round-bottom flask were combined 5-nitro-1*H*-indol-2-yl)methanol (**2**, 14 g, 73 mmol), 140 mL of acetonitrile, and 12 g (94 mmol) of *tert*-butyl acrylate. The resulting solution was treated with DBU (16.6 g, 109 mmol) at room temperature over 30 minutes. Reaction mixture was stirred at 80 °C for 3 h, then poured into 100 mL of water and concentrated under reduced pressure to remove acetonitrile. Aqueous residue was then extracted with dichloromethane (300 mL). Organic layer was washed with brine, dried over

sodium sulfate, concentrated under vacuum, and purified on silica gel (25% v/v EtOAc-hexane) to give 18 g (56 mmol, 77 % yield) of product **3** as a yellow solid. LRMS (ESI): *m/z* 320.9 $[M+H]^+$, calculated for $C_{16}H_{20}N_2O_5$ *m/z* 321.1.

[00870] Preparation of tert-butyl 3-(2-formyl-5-nitro-1H-indol-1-yl)propanoate (**4**)

[00871] To a stirred solution of compound **3** (18 g, 56 mmol) in 200 mL of anhydrous DCM were added Dess-Martin periodinane (28 g, 66 mmol) in small portions over 15 minutes at room temperature. The resulting mixture was stirred for 1 h at RT and quenched by adding 100 mL of water. Aqueous layer was extracted with DCM (200 mL), combined organic layers were washed with brined and dried over sodium sulfate. After removal of solvents, the residue was purified on silica gel (0-25% v/v EtOAc-hexanes) to give 14 g (41 mmol, 73% yield) of aldehyde **4** as a yellow solid. LRMS (ESI): *m/z* 340.9 $[M+Na]^+$, calculated for $C_{16}H_{18}N_2O_5$ *m/z* 341.1.

[00872] Preparation of (9H-fluoren-9-yl)methyl 1,2-dimethylhydrazine-1-carboxylate (**5**)

[00873] To a solution of 1,2-dimethylhydrazine dihydrochloride (20 g, 150 mmol) in DCM (200 mL) at 25 °C were added triethylamine (45.6 g, 452 mmol) slowly over 10 min. The mixture was stirred at 25°C for 30 min and treated with (9H-fluoren-9-yl)methyl carbonochloridate (Fmoc chloride, 19.4 g, 75 mmol) at room temperature and stirred for 3 h. Reaction mixture was poured into water (300 mL) and extracted with ethyl acetate (200 mL). Combined organic phase was dried over sodium sulfate, concentrated under vacuum, and purified by silica-gel chromatography (eluting with 30% v/v EtOAc-hexanes) to give compound **5** (20 g, 71 mmol, 95% yield) as a yellow low-melting solid. LRMS (ESI): *m/z* 283.2 $[M+H]^+$, calculated for $C_{17}H_{18}N_2O_2$ *m/z* 283.1.

[00874] Preparation of (9H-fluoren-9-yl)methyl 2-((1-(3-(tert-butoxy)-3-oxopropyl)-5-nitro-1H-indol-2-yl)methyl)-1,2-dimethylhydrazine-1-carboxylate (**6**)

[00875] In an oven-dried round-bottom flask were combined aldehyde **4** (14 g, 41 mmol) and hydrazine **5** (11 g, 39 mmol) in 140 mL of anhydrous DCE at ambient temperature. The mixture was stirred for 1 h and then treated with STAB (17 g, 80 mmol) in portions over 30 minutes. Stirring continued for 2 h at room temperature, then reaction mixture was quenched with 100 mL of water, aqueous layer was extracted with DCM (300 mL). Combined organic layer was washed with brine and dried over sodium sulfate. After removal of solvents, the residue was purified on silica gel (0-25% v/v EtOAc-hexane) to obtain 18 g (31 mmol, 80 % yield) of compound **6** as a brown solid. LRMS (ESI): *m/z* 584.9 $[M+H]^+$, calculated for $C_{33}H_{36}N_4O_6$ *m/z* 585.3.

[00876] Preparation of (9H-fluoren-9-yl)methyl 2-((5-amino-1-(3-(tert-butoxy)-3-oxopropyl)-1H-indol-2-yl)methyl)-1,2-dimethylhydrazine-1-carboxylate (**7**)

[00877] A solution of nitro compound **6** (18 g, 31 mmol) in 180 mL of THF was combined with a solution of ammonium chloride (13 g, 243 mmol) in 180 mL of water, and 90 mL of methanol at ambient temperature. The resulting mixture was treated with zinc powder (16 g, 245 mmol) in several portions at room temperature. Reaction mixture was stirred vigorously for 2 hours, solids were filtered off, filtrate was concentrated under vacuum and partitioned between saturated aqueous ammonium chloride (100 mL) and ethyl acetate (100 mL). Aqueous layer was separated and extracted with ethyl acetate (100 mL). Combined organic layer was washed with brine and dried over sodium sulfate. Removal of solvents under vacuum afforded 15 g of crude product **7** as a brown oil which was used further without purification. LRMS (ESI): *m/z* 555.3 $[M+H]^+$, calculated for $C_{33}H_{38}N_4O_4$ *m/z* 555.3.

[00878] Preparation of 4-((2-((2-(((9H-fluoren-9-yl)methoxy)carbonyl)-1,2-dimethylhydrazineyl)methyl)-1-(3-(tert-butoxy)-3-oxopropyl)-1H-indol-5-yl)amino)-4-oxobutanoic acid (**9**)

[00879] To a solution of compound **7** (1.0 g, 1.8 mmol) in MeCN (10 mL) were added succinic anhydride (905 mg, 5.0 equiv., 9 mmol) and triethylamine (1.2 mL, 9 mmol). Reaction mixture was stirred for 3 hours at room temperature and purified by reversed-phase chromatography (C18 column, 0-100% v/v MeCN/H2O with 0.05% TFA). Pure fractions were combined, concentrated under reduced pressure until murky, and lyophilized to give 0.95 g of product **9** (1.5 mmol, 83% yield) as an off-white solid. LRMS (ESI): *m/z* 677.3 $[M+Na]^+$, calculated for $C_{37}H_{42}N_4O_7$ *m/z* 677.3.

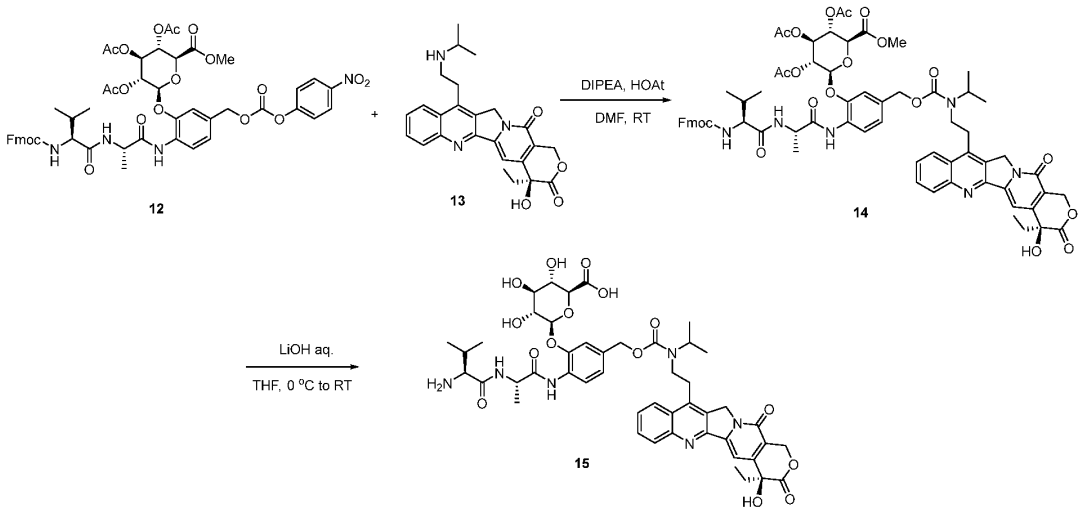
[00880] Preparation of 4-((2-((2-(((9H-fluoren-9-yl)methoxy)carbonyl)-1,2-dimethylhydrazinyl)methyl)-1-(2-carboxyethyl)-1H-indol-5-yl)amino)-4-oxobutanoic acid (**10**)

[00881] Compound **9** (9 g, 14 mmol) was dissolved in 90 mL of hexafluoro isopropanol and treated with 10 mL of concentrated HCl at room temperature. Reaction mixture was stirred for 1 h at room temperature, then solvent was removed in vacuum, and the residue was purified by reversed-phase flash chromatography (C18, 0-100% v/v CH_3CN-H_2O with 0.05% TFA). Fractions containing product were combined and lyophilized to give diacid **10** (6.5 g, 11 mmol, 79% yield) as a tan solid. LRMS (ESI): *m/z* 621.3 $[M+Na]^+$, calculated for $C_{33}H_{34}N_4O_7$ *m/z* 621.2.

[00882] Preparation of (9H-fluoren-9-yl)methyl 1,2-dimethyl-2-((1-(3-oxo-3-(perfluorophenoxy)propyl)-5-(4-oxo-4-(perfluorophenoxy)butanamido)-1H-indol-2-yl)methyl)hydrazine-1-carboxylate (**11**)

[00883] A solution of diacid **10** (2.0 g, 3.34 mmol) in 40 mL of anhydrous THF was combined with 6.2 g (33.4 mmol) of pentafluorophenol. The mixture was stirred and treated with DCC (2.0 g, 10 mmol) in a few small portions at room temperature. Reaction mixture was stirred for 48 hours at RT, then all solids were removed by filtration and washed with THF on filter. Combined filtrates were concentrated under vacuum and purified by silica gel chromatography (0-25-35% gradient of EtOAc-Hexanes) to give 2.4 g of bis-PFP ester **11** (2.6 mmol, 77% yield) as a white foaming solid. LRMS (ESI): *m/z* 931.2 $[M+H]^+$, calculated for $C_{45}H_{32}F_{10}N_4O_7$ *m/z* 931.2.

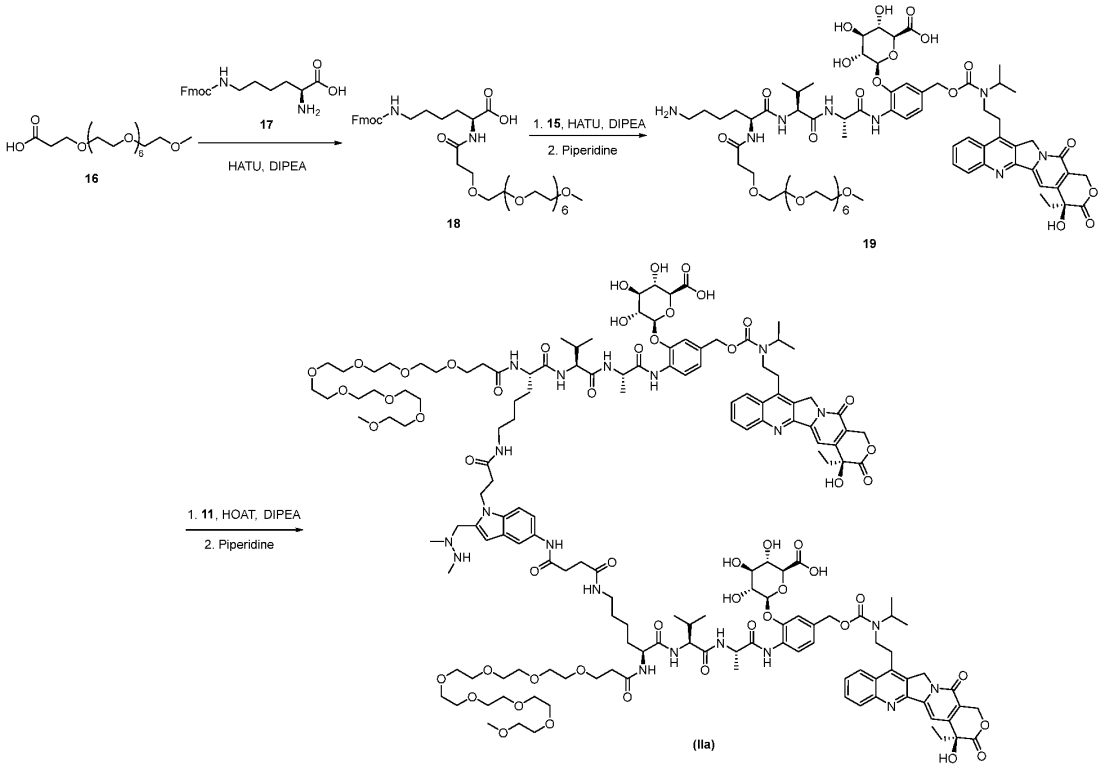
[00884] Preparation of (2S,3S,4S,5R,6S)-6-(2-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-5-((((2-((S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)ethyl)(isopropyl)carbamoyl)oxy)methyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**15**)



[00885] Belotecan hydrochloride **13** (2.35 g, 5.0 mmol) was suspended in a mixture of 30 mL of anhydrous DMF and 1.75 mL of DIPEA (10 mmol). The resulting mixture was stirred and treated with HOAt (0.68 g, 5 mmol), followed by PNP-carbonate **12** (5.1 g, 5 mmol) in small portions at room temperature. Reaction mixture was stirred at RT for 8 h until starting materials were judged fully consumed based on HPLC analysis. The mixture was poured onto 300 mL of ice with vigorous stirring, the resulting yellowish precipitate was collected by filtration, washed with 30 mL of water twice, dried on air overnight to give 6.7 g of crude

coupling product **14** as a light-yellow powder. LRMS (ESI): *m/z* 1307.5 $[M+H]^+$, calculated for $C_{69}H_{74}N_6O_{20}$ *m/z* 1307.5.

[00886] A solution of crude intermediate **14** (6.7 g) in 30 mL of THF was cooled down to 0 °C in an ice bath and treated slowly with 2 M aqueous lithium hydroxide solution (10 mL). Reaction mixture was stirred at 0 °C for 1 h, then another 10 mL of 2 M LiOH solution was added and stirring continued for 15 minutes before warming the reaction mixture to room temperature and adding another 10 mL of 2 M lithium hydroxide and 5 mL of methanol. The resulting mixture was stirred for 1 h at room temperature, then quenched by adding 2 M aqueous HCl solution to pH 2-3 and let stir for 30 minutes. The mixture was transferred to a separatory funnel and washed with MTBE (2x50 mL). Aqueous layer was separated and directly purified by reversed-phase chromatography (C18 column, 0-40% CH_3CN-H_2O with 0.05% TFA). Pure fractions were combined, concentrated under reduced pressure, and lyophilized to give 3.4 g of product **15** (3.6 mmol, 72% yield over 2 steps) as a bright-yellow fluffy powder. LRMS (ESI): *m/z* 945.4 $[M+H]^+$, calculated for $C_{47}H_{56}N_6O_{15}$ *m/z* 945.4.



SCHEME 2

[00887] Preparation of N^6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N^2-(3-(2-(2-methoxyethoxy)ethoxy)propanoyl)-L-lysine (**18**)

[00888] Carboxylic acid **16** (5.0 g, 12 mmol) was dissolved in anhydrous DMF (10 mL) and treated with DIPEA (2.1 mL, 12 mmol), followed by HATU (4.6 g, 12 mmol) at ambient temperature. The resulting mixture was stirred for 30 minutes and then combined with solid H-Lys(Fmoc)-OH **17** (4.5 g, 12 mmol). Reaction mixture was stirred for 1 h at RT and then directly purified by reversed-phase chromatography (C18 column, 0-70% CH_3CN-H_2O with 0.05% TFA). Fractions containing product were combined and solvents were removed in vacuum. The residue was dried under high vacuum overnight to give product **18** (5.5 g, 7.2 mmol, 60% yield) as a clear colorless oil. LRMS (ESI): *m/z* 763.4 $[M+H]^+$, calculated for $C_{39}H_{58}N_2O_{13}$ *m/z* 763.4.

[00889] Preparation of (2S,3S,4S,5R,6S)-6-(2-((28S,31S,34S)-28-(4-aminobutyl)-31-isopropyl-34-methyl-26,29,32-trioxo-2,5,8,11,14,17,20,23-octaoxa-27,30,33-triazapentatriacontan-35-amido)-5-((((2-((S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)ethyl)(isopropyl)carbamoyl)oxy)methyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**19**)

[00890] Carboxylic acid **18** (2.9 g, 3.8 mmol) was combined with DIPEA (1.3 mL, 7.6 mmol) and HATU (1.45 g, 3.8 mmol) in 12 mL of anhydrous DMF at room temperature. The mixture was stirred for 20 minutes before combining with a solution of compound **15** (3.3 g, 3.5 mmol) in 15 mL of DMF. The resulting mixture was stirred at room temperature for 1 hour, then treated with 13 mL of triethylamine and stirred at room temperature for 7 h until reaction was judged complete by LCMS analysis. Reaction mixture was then concentrated under reduced pressure to remove triethylamine and purified by reversed-phase chromatography (C18 column, 0-40% CH_3CN-H_2O with 0.05% TFA). Pure fractions were combined, concentrated under reduced pressure to ~100 mL final volume, and lyophilized to give 3.5 g of product **19** as a bright-yellow light powder (2.4 mmol, 69% yield over 2 steps). LRMS (ESI): *m/z* 1467.7 $[M+H]^+$, calculated for $C_{71}H_{102}N_8O_{25}$ *m/z* 1467.7.

[00891] Preparation of (2S,3S,4S,5R,6S)-6-(2-((28S,31S,34S)-28-(4-(3-(5-((S)-28-(((S)-1-(((S)-1-((2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-4-((((2-((S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)ethyl)(isopropyl)carbamoyl)oxy)methyl)phenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamoyl)-26,34-dioxo-2,5,8,11,14,17,20,23-octaoxa-27,33-diazaheptatriacontan-37-amido)-2-((1,2-dimethylhydrazineyl)methyl)-1H-indol-1-yl)propanamido)butyl)-31-isopropyl-34-methyl-26,29,32-trioxo-2,5,8,11,14,17,20,23-

octaoxa-27,30,33-triazapentatriacontan-35-amido)-5-((((2-((S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-11-yl)ethyl)(isopropyl)carbamoyl)oxy)methyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**IIa**)

[00892] To a stirred solution of compound **19** (3.5 g, 2.4 mmol) in 16 mL of anhydrous DMA were added DIPEA (0.84 mL, 4.8 mmol) and HOAt (0.33 g, 2.4 mmol) at room temperature. The resulting mixture was treated with a separately prepared solution of bis-PFP ester **11** ester (1.0 g, 1.1 mmol in 2 mL of DMA) in a few small portions with 10 minutes intervals between additions. After the addition was complete, reaction mixture was stirred for 15 minutes at room temperature and treated with 2.1 mL of piperidine (22 mmol). After 20 minutes, reaction mixture was directly purified by reversed-phase chromatography (C18 column, 0-40% CH_3CN-H_2O with 0.05% TFA). Pure fractions were combined, concentrated under reduced pressure at 30 °C, and lyophilized to obtain 3.2 g of linker-payload **(IIa)** as a bright-yellow fluffy powder (0.98 mmol, 89% yield of two steps based on **11**). LRMS (ESI): *m/z* 1638.3 $[M+H]^{2+}$, calculated for $C_{160}H_{224}N_{20}O_{53}$ *m/z* 1638.8.

[00893] Example 9. ADC: Preparation

[00894] Aldehyde-tagged antibodies (15 mg/mL) were conjugated to synthesized linker-drugs (17.5 mol. equivalents drug:antibody for DAR8) by reacting the linker-drug and aldehyde-tagged antibody for 72 hours at 37 °C in 67 mM sodium citrate, pH 5.5, 43 mM sodium chloride containing 3.5 or 4.5% DMA.

[00895] 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, filtration area 88 cm^2, NMWCO 30 kDa) and exchanging for 12 diavolumes (600 mL) into 20 mM sodium citrate, pH 5.5, 50 mM NaCl.

[00896] In some embodiments, residual free drug was further removed using SEC-FPLC (HiLoad 26/600 Superdex® 200) followed by concentration using AMICON® 0.5 mL 30 kD molecular weight cut off (MWCO) centrifugal filters (Millipore Sigma, Cat. No. #UFC5030BK). After the FPLC purification, the ROR-ADC samples were measured as having about 1.4% free payload.

[00897] In some embodiments, ion exchange chromatography is further used to purify the ROR-ADC samples.

[00898] To determine the drug-to-antibody ratio (DAR) of the final product, antibody-drug conjugates were examined by analytical hydrophobic interaction chromatography (HIC) or polymeric reverse phase chromatography (PLRP).

[00899] The HIC column (Tosoh Bioscience, Cat. No. 14947) was run with 1.5 M ammonium sulfate and 25 mM sodium phosphate (pH 7.0) as mobile phase A and 25% isopropanol and 18.75 mM sodium phosphate (pH 7.0) as mobile phase B. The PLRP column (Agilent, Cat. No. PL1912-1802) was run with 0.1% trifluoroacetic acid in H_2O as mobile phase A and 0.1% trifluoroacetic acid in CH_3CN for mobile phase B, with the column heated to 80 °C.

[00900] In some embodiments, analytical Hydrophobic Interaction Chromatography (HIC) was performed as described below: Samples were analyzed on an Agilent 1100 Instrument equipped with a 4.6 mm x 35 mm TSK gel butyl-NPR column from Tosoh (#14947) held at 25 °C. Samples were prepared by adding 20 µL of 50 mM NaCl (pH 5.5) and 20 µL of Mobile Phase A to 20 µg of sample and were injected at room temperature. Gradient elution utilized mobile phase A (0.1% trifluoroacetic acid in H_2O) and Mobile Phase B (0.1% trifluoroacetic acid in CH_3CN) in the program reported in the table below at a flow rate of 1.0 mL/minute. Detection was via UV at 215 nm, 252 nm, and 280 nm.

[00901] In some embodiments, analytical Hydrophobic Interaction Chromatography (HIC) (Tosoh #14947) with UV/Vis detection was performed. The reduced form of the final product was analyzed by reverse phase liquid chromatography (RP-HPLC) (Agilent Cat. No. PL1912-1802) with UV/Vis detection to verify DAR according to **Table E15** below.

Table E15. RPLC Gradient Elution Program

Time (min)	Mobile Phase B (%)
0.0	12
5.5	42
7.5	70
8.5	100
9.5	100
10	12
12	12

[00902] In some embodiments, Reverse Phase HPLC was performed as described below: Samples were analyzed on an Agilent 1100 Instrument equipped with a 50 x 2.1 mm PL1912-1802 (8 µm, 1000Å) column from Agilent held at 80 °C. Samples were prepared by adding 10 µL of a mixture of 0.5 mM DTT, 50 µL of 8 M guanidine HCl, 130 mM tris, and 1 mM EDTA (pH 7.6) to a 20 µg sample and adding PBS to a final volume of 100 µL. Samples

were incubated at 37 °C for 30 minutes after preparation. Gradient elution utilized mobile phase A (25 mM Na_3PO_4, 1.5 M $(NH_4)_2SO_4$, pH 7.0) and Mobile Phase B (18.75 mM Na_3PO_4, pH 7.0, 25% isopropyl alcohol) in the program reported in the **Table E16** below at a flow rate of 2.0 mL/minute. Detection was via UV at 215 nm, 252 nm, and 280 nm.

Table E16. RPLC Gradient Elution Program.

Time (min)	Mobile Phase B (%)
0.0	25
8	50
8.1	95
10	95

[00903] To determine aggregation, samples were analyzed using analytical size exclusion chromatography (Tosoh Bioscience, Cat. No. 08541) using a mobile phase of 300 mM NaCl, 25 mM sodium phosphate, and 5% isopropanol (pH 6.8).

[00904] HPLC analysis of ADCs by reduced PLRP was performed to determine DARs of the produced ADCs. In some embodiments, Reverse Phase HPLC was performed as described below: Samples were analyzed on an Agilent 1100 Instrument equipped with a 50 x 2.1 mm PL1912-1802 (8 µm, 1000Å) column from Agilent held at 80 °C. Samples were prepared by adding 10 µL of 0.5 M DTT, 50 µL of 8 M guanidine HCl, 130 mM tris, and 1 mM EDTA (pH 7.6) to a 10 µg sample and adding PBS to a final volume of 100 µL. Samples were incubated at 37 °C for 30 minutes after preparation. Gradient elution utilized mobile phase A (0.1% trifluoroacetic acid in H_2O) and Mobile Phase B (0.1% trifluoroacetic acid in CH_3CN) in the program reported in **Table E17** below at a flow rate of 2.0 mL/minute. Detection was via UV at 215 nm, 252 nm, and 280 nm.

Table E17. RPLC Gradient Elution Program.

Time (min)	Mobile Phase B (%)
0.0	25
16.0	50
16.1	95
18.0	95

[00905] Accordingly, ADC-00 (an ADC represented by formula (II), wherein s is 4 and wherein the antibody Ab comprises a heavy chain of SEQ ID NO:48 and a light chain of SEQ ID NO:33), ADC-11 (an ADC represented by formula (II), wherein s is 4 and wherein the antibody Ab comprises a heavy chain of SEQ ID NO:48 and a light chain of SEQ ID NO:34), and ADC-13 (an ADC represented by formula (II), wherein s is 4 and wherein the antibody

Ab comprises a heavy chain of SEQ ID NO:48 and a light chain of SEQ ID NO:36) were prepared. Additional prepared are corresponding ADCs with a silent Fc (sFc), such as ADC-13-sFc, which is an ADC represented by formula (II), wherein s is 4 and wherein the antibody Ab comprises a heavy chain of SEQ ID NO:171 and a light chain of SEQ ID NO:36).

[00906] Unless otherwise specified, "non-masked ADC" refers to an ROR-ADC not comprising a masking peptide, such as ADC-00; "masked ADC" refers to an ROR-ADC comprising a masking peptide (such as ADC-11, ADC-13, or ADC-13-sFc) with or without MMP-9 activation; and "unmasked ADC" refers to an ROR-ADC originally comprising a masking peptide (such as ADC-11, ADC-13, or ADC-13-sFc) but activated by MMP-9.

[00907] Isotype ADCs (such as an ADC represented by formula (II) having an s of 4 but its ab is an hEWL or KLH isotype antibody having the same Fc of the ROR-ADCs as produced herein) were prepared and served as negative controls. For example, "KLH Isotype ADC" refers to an isotype ADC represented by formula (II) having an s of 4 but its ab is a KLH isotype antibody having the same Fc of ACD-13. Another example is "KLH Isotype ADC-sFc" refers to an isotype ADC represented by formula (II) having an s of 4 but its ab is a KLH isotype antibody having the same Fc of ACD-13-sFc.

[00908] Further, the purity and DAR of the produced ADCs were determined as described above. A representative result is provided in **Table E18** below.

Table E18. Characterization of ADC-13, ADC-13-sFc, KLH Isotype ADC, and KLH Isotype ADC-sFc.

ADC	HMW (%; SEC)	DAR (PLRP)
ADC-13	1.9	7.10
ADC-13-sFc	0.9	7.18
KLH Isotype ADC	1.7	7.45
KLH Isotype ADC-sFc	1.5	7.48

[00909] Example 10. ADC: *In Vitro* Cytotoxicity

[00910] *In vitro* cytotoxicity of the ADC prepared as described above was further assessed.

[00911] hROR1 expressing HEK cells (1500 cells/well in 100 µL of media) were plated in Costar (Corning) 3610 plate (individually packed, sterile, white wall clear bottom, TC treated, VWR# 29444-010) and incubated overnight.

[00912] Dilution series of the tested articles (such as, ADC-11 or ADC-13 or ADC-13-sFc with or without MMP9 activation) were made. Belotecan and ACD-00 (**P0** conjugated to the ADC-11/13 linker payload) with or without MMP9 activation were tested in parallel and

served as controls. The final top concentration was 100 nM for free drugs and 10 nM for ADCs. To get a good sigmoidal curve, the free drug was serially diluted 1:6 and ADCs were serially diluted 1:4. Starting with a top concentration of 600 nM free drug or 60 nM ADCs (6x the final top concentration), each of the samples were serially diluted 5 times (at 1:6 for free drug and 1:4 for ADC). 20 µL of each sample were added to 100 µL cells. The assay plate with each sample were prepared in duplicate. The treated cells were incubated for 5 days. The cell viability reading was taken using Cell Titer-Glo (Promega, cat# G7573) and normalized to the untreated cells.

[00913] In order to activate the masked ADCs, an enzyme digestion system as prepared as follows: 100 µg of the tested antibody/ADC, 10 µL of 100 µg/mL activated MMP-9 (Sigma, Catalog #PF024-5ug), and TCNB (50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% Brij-35, pH 7.50) to make the final volume to 100 µL. TCNB buffer was firstly into a microcentrifuge tube, then the tested antibody and activated MMP-9 were added. The final concentration of antibody was 0.5 to 2 mg/mL, and antibody (or ADC)/enzyme (w/w) was 100:1. The reaction mixture was mixed gently to avoid generation of foam and bubbles and incubated at 37 °C for 10 to 24 hr. Concentrations of the activated ADCs were determined after incubation and frozen until use.

[00914] One set of exemplary results is provided in **FIG. 19A**, indicating that +/- activated MMP9 did not affect the potency of the non-masked **P0** ADCs; masked ADCs potency shifted to right vs non-masked ADCs in absence of activated MMP-9; addition of activated MMP-9 resulted in equivalent potency for non-masked and masked **P0** ADCs; and there was no apparent difference in the potency among +/- activated MMP9 for **M1** or **M2** ADCs.

[00915] Another set of exemplary results is provided in **FIG. 19B** and **FIG. 19C**, showing both ADC-13 and ADC-13-sFc were potent on ROR1 expressing HEK cells (having an ROR1 copy number of 290,332) and ROR2 expressing HEK cells (having an ROR1 copy number of 418,894) after MMP-9 activation. Under the same experimental setting, KLH Isotype ADC-sFc and KLH Isotype ADC were not potent on either cell sample (data not shown).

[00916] Example 11. ADC: Binding Affinities of ADCs -/+ MMP-9 Activation to ROR1 and ROR2

[00917] ELISA was performed to evaluate the binding affinities to ROR1 and ROR2 of ADC-00, ADC-11, and ADC-13 with or without MMP-9 activation.

[00918] In order to activate the masked ADCs, an enzyme digestion system as prepared as follows: 100 µg of the tested antibody or ADC, 10 µL of 100 µg/mL activated MMP-9

(Sigma, Catalog #PF024-5ug), and TCNB (50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl, 0.05% Brij-35, pH 7.50) to make the final volume to 100 µL. TCNB buffer was firstly into a microcentrifuge tube, then the tested antibody/ADC and activated MMP-9 were added. The final concentration of antibody was 0.5 to 2 mg/mL, and antibody/ADC to enzyme ratio (w/w) was 100:1. The reaction mixture was mixed gently to avoid generation of foam and bubbles and incubated at 37 °C for 10 to 24 hr. Concentrations of the activated ADCs were determined after incubation and frozen until use.

[00919] For the ELISA assays, each well of a 96 well maxisorp plate (VWR, Catalog # 62409-024) was coated with 50 µL of antigen (human ROR1-Fc, Acro Biosystems, Catalog # R01-H5250) or ROR2-his) at 2 µg/mL in PBS. The plate was incubated overnight at 4°C and then washed for four times with 0.1% Tween PBS. The wells were blocked with 150 µL PBS casein (Thermo Fisher, Catalog # 37528) for 1- 2 hours at room temperature with shaking, and then washed for four times with 0.1% Tween PBS. The antibody/ADC samples were diluted to 20 µg/mL starting concentration in PBS, and then serially diluted at 1:3 in PBS arriving at additional 10 concentrations. 50 µL of dilutions was added to the 96 well plate and incubated at room temperature 1 hour with shaking. The plate was then washed four times with 0.1% Tween PBS. 50uL goat anti human HRP (Sigma, Catalog # A0293-1ML; dilute to 1000 ng/mL in PBS) was added to wells and incubated at room temperature for 1 hour with shaking. The plate was then washed for four times with 0.1% Tween PBS. 50uL TMB (Thermo Fisher, Catalog # 34028) was added, and the plate was developed until top well turned a dark blue color. 50uL 2N H_2SO_4 (Sigma, Catalog #320501) was added, and the absorbance was read on plate reader (Molecular Devices).

[00920] The results are provided in **FIGs. 20A-20D**, showing that MMP-9-treated masked ADC-11 and ADC-13 ADCs had similar binding profiles to ROR1 and ROR2 as the non-masked ADC-00.

[00921] FACS assays were further performed to evaluate the binding affinities to MDA-MB-231 cells, for example following the protocol described above in **Example 1**. Masked ADC-13 was activated with MMP-9 as described above ("ADC-13 (+MMP9)") or not ("ADC-13 (-MMP9)"). An isotype control ADC and P0 were tested in parallel as controls. The result showed that unmasked ADC-13 (activated with MMP-9) and P0 bound to MDA-MB-231 cells, but the masked ADC-13 without MMP-9 activation or the isotype control ADC did not.

[00922] Example 12. ADC: *In Vivo* Efficacy in MDA-MB-231 TNBC Xenograft Model

[00923] CB.17 female SCID mice were implanted (flank) with 1.0 x 10^7 of MDA-MB-231 tumor cells in 50% MATRIGEL® (Corning Life Sciences) matrix (1:1 cell to Matrigel) on Day -13. When the average tumor volume reached about 200 mm^3 on Day 1, mice were randomized into respective treatment groups (10 mice per group). The mice then received intravenous injections of the tested ADCs (*e.g.*, ADC-00, ADC-11, and ADC-13, at 7.5 mg/kg) on Day 1 and Day 8. PBS vehicle was tested in parallel, serving as a control. Body weight and tumor volume were measured twice per week. The study was ended on Day 45.

[00924] Tumor volumes were measured and %TGI was calculated. The tumor growth inhibition rate TGI_{TV} was calculated by the following equation: $TGI_{TV}\% = [1-(T_i-T_0)/(V_i-V_0)] \times 100\%$. ($T_i$ means tumor volume of the treated groups at day i following treatment; T_0: means tumor volume of the treated groups at day 0; V_i: means tumor volume of the vehicle groups at day i following treatment; V_0: means tumor volume of the vehicle groups at day 0). Further, the %TGI result was analyzed with One Way ANOVA with Dunnett's post hoc test as appropriate, while **** indicated $p < 0.0001$ and NS indicated $p > 0.05$.

[00925] The result is provided in **FIGs. 21A-21B**, showing the masked ADCs demonstrated significant tumor suppression compared to control.

[00926] Example 13. ADC: *In Vivo* Efficacy in JEKO-1 MCL Xenograft Model

[00927] CB.17 female SCID mice were inoculated subcutaneously in the right up flank region with Jeko-1 tumor cells (5×10^6) in 0.1 mL of PBS with Matrigel (1:1) for tumor development. When the tumor volumes reached about 150~250 mm^3 on Day 1, mice were randomized into respective treatment groups (8 mice per group). The mice then received intravenous injections of vehicle or the tested ADCs (*e.g.*, ADC-00, ADC-11, and ADC-13,7.5 mg/kg) on Day 1 and Day 8. PBS vehicle was tested in parallel, serving as a control.

[00928] After tumor cells inoculation, the animals were checked daily for morbidity and mortality. During routine monitoring, the animals were checked for any effects of tumor growth and treatments on behavior such as mobility, food and water consumption, body weight gain/loss (Body weights were measured twice per week after randomization), eye/hair matting and any other abnormalities. Mortality and observed clinical signs were recorded for individual animals in detail.

[00929] Tumor volumes were measured twice per week after randomization in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: "V = (L x W x W)/2, where V is tumor volume, L is tumor length (the longest tumor dimension), and W is tumor width (the longest tumor dimension perpendicular to L). Dosing as well as tumor and body weight measurements were conducted in a Laminar Flow Cabinet.

[00930] Tumor volumes were measured and %TGI was calculated. The tumor growth inhibition rate TGI_{TV} was calculated by the following equation: $TGI_{TV}\% = [1-(T_i-T_0)/(V_i-V_0)] \times 100\%$. ($T_i$: means tumor volume of the treated groups at day i following treatment; T_0: means tumor volume of the treated groups at day 0; V_i: means tumor volume of the vehicle groups at day i following treatment; V_0: means tumor volume of the vehicle groups at day 0). Further, the %TGI result was analyzed with One Way ANOVA with Dunnett's post hoc test as appropriate, while **** indicated $p < 0.0001$.

[00931] The result is provided in **FIGs. 22A-22B**, showing non-masked ADC-00 did not demonstrate efficacy in the JEKO-1 model, while masked ADC-11 and ADC-13 produced significant tumor suppression.

[00932] Example 14. ADC: *In Vivo* Efficacy in Patient-Derived Xenograft (PDX) Models

[00933] CB.17 female SCID mice are implanted (flank) with patient tumor cells in 50% MATRIGEL® (Corning Life Sciences) matrix (1:1 cell to Matrigel). When the average tumor volume reaches about 200 mm^3 on Day 1, mice are randomized into respective treatment groups and receive intravenous injections of vehicle or the tested ADC (ADC-11 and ADC-13, at 10 mg/kg) every three weeks (Q3W). Tumor volumes are measured and %TGI is calculated.

[00934] The tumor growth inhibition rate TGI_{TV} is calculated by the following equation: $TGI_{TV}\% = [1-(T_i-T_0)/(V_i-V_0)] \times 100\%$. ($T_i$: means tumor volume of the treated groups at day i following treatment; T_0: means tumor volume of the treated groups at day 0; V_i: means tumor volume of the vehicle groups at day i following treatment; V_0: means tumor volume of the vehicle groups at day 0). Further, the %TGI result is analyzed with One Way ANOVA with Dunnett's post hoc test as appropriate.

[00935] Additionally, female athymic nude-Foxn1nu mice were used. When sufficient stock animals reached 1000-1500 mm^3, non-small cell lung cancer (NSCLC) tumors (having an H Score of 78) were harvested for re-implantation into pre-study animals. Pre-study animals were implanted unilaterally on the left flank with tumor fragments harvested from stock animals. When the average tumor volume reached 150-300 mm^3, mice were matched by tumor volume into treatment or control groups to be used for dosing (6 mice per group) and dosing initiated on Day 0. The animals received intravenous injections of vehicle or the tested ADCs (10 mg/kg) once per week for 2 weeks (Q7D x 2). Body weights and tumor volumes were measured twice per week until the end of the study. Any animal exhibiting >20% net weight loss for a period lasting 7 days or mice displaying >30% net weight loss when compared to Day 0 was considered moribund and euthanized.

[00936] Beginning on Day 0, tumor dimensions were measured twice weekly by digital caliper, and data including individual and mean estimated tumor volumes (Mean TV ± SEM) was recorded for each group; tumor volume (TV) was calculated using the following formula: $TV = width^2 \, x \, length \, x \, 0.52$.

[00937] The result is provided in **FIG. 26**, showing ADC-11 and ADC-13 significantly inhibited the tumor growth. Additionally, there was no evidence of body weight loss greater than 5% across the study.

[00938] Example 15. ADC: Pharmacokinetics Study in Rats

[00939] Sprague-Dawley rats were dosed intravenously with a single dose of 5 mg/kg of the tested ADCs on Day 0 after 16 hours of fasting. The rats' body weights were taken on Day -3. Day 0, Day 7, Day 14, and Day 21. 100 µL plasma was collected at 30 min (on Day 0), 6 h (on Day 0), 24 h (on Day 1), 48 h (on Day 2), Day 4, Day 7, Day 10, and Day 14, saved in bullet tubes with K^2EDTA and stored at -20 °C until end of the study.

[00940] Total antibody and total ADC concentrations (referred to herein as tAb and tADC respectively) were quantified using MSD GOLD 96-well Small Spot Streptavidin SECTOR Plate (having an assay MRD of 1:100 and a quantitation range of 10 ng/mL (LLOQ) – 10240 ng/mL (ULOQ)). A 1000X dilution linearity was used, while the assay controls included 34 ng/mL (LQC), 800 ng/mL (MQC), 8000 ng/mL (HQC).

[00941] For total antibody measurements, conjugates were captured with an anti-human IgG (Fc specific), F(ab′)2 fragment, highly cross adsorbed-Biotin antibody produced in goat (Sigma Aldrich, SAB3701268) at 1 µg/mL and detected with a Sulfo TAG-labeled goat anti-human antibody (R32AJ) at 0.15 µg/mL. For total belotecan ADC measurements, conjugates were captured with an anti-human IgG (Fc specific), $F(ab')_2$ fragment, highly cross adsorbed-Biotin antibody produced in goat (Sigma Aldrich, SAB3701268) at 1 µg/mL and detected with a Sulfo TAG-labeled anti-Belotecan (Clone 1H11, Catalent) at 1 µg/mL. Pooled Sprague Dawley rat plasma was used as a negative control (NC). The assay buffer used was 1xPBS+1%BSA+0.1%Tween, while the read buffer was 1X MSD Read Buffer T (MSD R92TC-1). Data were analyzed and plotted using GraphPad Prism.

[00942] As shown in **FIG. 23** and **Table E19** below, masked ADCs had comparable exposure while non-masked ADC (ADC-00) had lower exposure following a single dose of 5 mg/kg. The results also indicate total antibody and ADC curves for masked ADCs overlapped, suggesting *in vivo* stability; non-masked ADC had lower exposure than masked ADCs with clearance approximately >3-fold faster and >4-fold shorter half-life; and data suggests that masked ADCs protected the ADCs from clearing via target mediated clearance.

Table E19. Rat PK results.

Analyte		C_{max} (µg/mL)	AUC_{Inf} (day x µg/mL)	CL (mL/day/kg)	$t_{1/2}$ (day)
ADC-11	Mean	110	363	13.8	3.59
	CV%[1]	4.48	8.2	8.0	7.0
ADC-13	Mean	88.9	355	14.2	5.96
	CV%	4.16	13.2	12.4	9.1
ADC-00	Mean	142	99.6	50.2	0.803
	CV%	16.9	2.0	1.9	10.3

[00943] Example 16. ADC: Toxicity and Toxicokinetics (TK) Study in Rats

[00944] Female Sprague-Dawley rats were dosed intravenously with the tested ADC via *i.v.* bolus once per week for four weeks (Q1W x 4) as detailed in **Table E20** below. 100 µL plasma was collected at various time points post-dose, saved in bullet tubes with EDTA and stored at -20 °C until end of the study. In addition, clinical observation, body weights (BW), clinical pathology, and anatomical pathology were also monitored. Toxicokinetic (TK) profiles were analyzed for the 1st and last dose cycles.

Table E20. Rat toxicity study design.

Group No.	Test Article	Dose Level (mg/kg/day)	Dose Volume (mL/kg)*	Dose Concentration (mg/mL)	No. of Females	
					Main Study	TK Study
1	Control	0	10	0	5	3
2	ADC-00	10	10	1	5	3
3		30	10	3	5	3
4		60	10	6	5	3
5	ADC-13	10	10	1	5	3
6		30	10	3	5	3
7		60	10	6	5	3
8	ADC-11	10	10	1	5	3
9		30	10	3	5	3
10		60	10	6	5	3

* Based on the most recent body weight measurement.

[00945] Total antibody and total ADC concentrations (referred to herein as tAb and tADC respectively) were quantified using MSD GOLD 96-well Small Spot Streptavidin SECTOR Plate (having an assay MRD of 1:100 and a quantitation range of 10 ng/mL (LLOQ) – 10240 ng/mL (ULOQ)). A 1000X dilution linearity was used, while the assay controls included 34 ng/mL (LQC), 800 ng/mL (MQC), 8000 ng/mL (HQC).

[00946] For total antibody measurements, conjugates were captured with an anti-human IgG (Fc specific), $F(ab')_2$ fragment, highly cross adsorbed-Biotin antibody produced in goat

(Sigma Aldrich, SAB3701268) at 1 μg/mL and detected with a Sulfo TAG-labeled goat anti-human antibody (R32AJ) at 0.15 μg/mL. For total belotecan ADC measurements, conjugates were captured with an anti-human IgG (Fc specific), $F(ab')_2$ fragment, highly cross adsorbed-Biotin antibody produced in goat (Sigma Aldrich, SAB3701268) at 1 μg/mL and detected with a Sulfo TAG-labeled anti-Belotecan (Clone 1H11, Catalent) at 1 μg/mL. Pooled Sprague Dawley rat plasma was used as a negative control (NC). The assay buffer used was 1xPBS+1%BSA+0.1%Tween, while the read buffer was 1X MSD Read Buffer T (MSD R92TC-1). Data were analyzed and plotted using GraphPad Prism.

[00947] Briefly, the results show that the non-masked ADC-00 had a projected maximum tolerated dose (MTD) of 30 mg/kg, while both ADC-11 and ADC-13 had a projected MTD of ≥ 60 mg/kg.

[00948] As further shown in **FIG. 24**, masked-ADCs had comparable exposure, while non-masked ADC had lower exposure following repeat dosing; and total antibody and ADC were overlapped, suggesting *in vivo* stability.

[00949] The results shown in the above examples were further summarized in **FIG. 25**.

*****

[00950] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made, and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit, and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

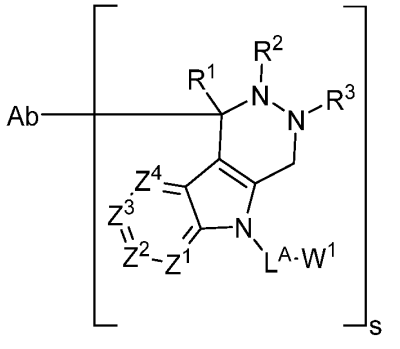
CLAIMS

What is claimed is:

1. An antibody-drug conjugate (ADC) of Formula (I) comprising:

a. an activatable antibody that binds to tyrosine-protein kinase membrane receptor (ROR); and

b. two or more drugs conjugated to a pyridazine-pyrrolo coupling moiety, each via a linker



(I)

wherein:

Ab represents an antibody that binds to ROR, optionally an activatable antibody that binds to ROR, further optionally, wherein the activatable antibody comprises (a) a masking peptide comprising a masking moiety (MM) and a cleavable moiety (CM); and (b) a target binding moiety (TBM);

Z^1, Z^2, and Z^4 are each independently CR^4;

Z^3 is C-L^B-W^2;

R^1, R^2, R^3, and R^4 are each selected from hydrogen and alkyl;

L^A is a first linker comprising:

$$-(T^1\text{-}V^1)_a\text{-}(T^2\text{-}V^2)_b\text{-}(T^3\text{-}V^3)_c\text{-}(T^4\text{-}V^4)_d\text{-}(T^5\text{-}V^5)_e\text{-}(T^6\text{-}V^6)_f\text{-},$$

wherein:

a, b, c, d, e, and f are each independently 0 or 1, provided at least one of a, b, c, d, e, and f are 1;

T^1, T^2, T^3, T^4, T^5 and T^6 are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x-$, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-

benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12;

V^1, V^2, V^3, V^4, V^5, and V^6 are each independently selected from the group consisting of a covalent bond, -CO-, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$ and -P(O)OH-, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

L^B is a second linker comprising:

$$-(T^7\text{-}V^7)_g\text{-}(T^8\text{-}V^8)_h\text{-}(T^9\text{-}V^9)_i\text{-}(T^{10}\text{-}V^{10})_j\text{-}(T^{11}\text{-}V^{11})_k\text{-}(T^{12}\text{-}V^{12})_l\text{-}(T^{13}\text{-}V^{13})_m\text{-},$$

wherein:

g, h, i, j, k, l, and m are each independently 0 or 1, provided that at least one of g, h, i, j, k, l, and m is 1;

T^7, T^8, T^9, T^{10}, T^{11}, T^{12}, and T^{13} are each independently selected from a covalent bond, $(C_1\text{-}C_{12})$alkyl, substituted $(C_1\text{-}C_{12})$alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x-$, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each x is an integer from 1 to 12;

V^7, V^8, V^9, V^{10}, V^{11}, V^{12}, and V^{13} are each independently selected from the group consisting of a covalent bond, -CO-, -NR^{15}-, -$NR^{15}(CH_2)_q$-, -$NR^{15}(C_6H_4)$-, -$CONR^{15}$-, -$NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$- and -P(O)OH-, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

s is an integer from 1 to 10, optionally 4;

W^1 is a first drug; and

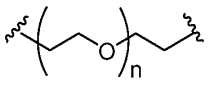
W^2 is a second drug.

2. The ADC of claim 1, wherein:

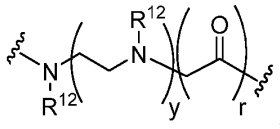
T^1 is selected from a (C_1-C_{12})alkyl and a substituted (C_1-C_{12})alkyl;

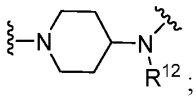
T^2, T^3, T^4, T^5 and T^6 are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, -$(CR^{13}OH)_x$-, 4-amino-piperidine (4AP), MABO, MABC, PABO, PABC, PAB, PABA, PAP, PHP, an acetal group, a hydrazine, and an ester; and

V^1, V^2, V^3, V^4, V^5, and V^6 are each independently selected from the group consisting of a covalent bond, -CO-, -NR^{15}-, -$NR^{15}(CH_2)_q$-, -$NR^{15}(C_6H_4)$-, -$CONR^{15}$-, -$NR^{15}CO$-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO_2-, -SO_2NR^{15}-, -$NR^{15}SO_2$-, and -P(O)OH-;

$(PEG)_n$ is ;

EDA is an ethylene diamine moiety having the following structure:

;

4-amino-piperidine (4AP) is ;

each R^{12} is independently selected from hydrogen, an alkyl, a substituted alkyl, a polyethylene glycol moiety, an aryl, and a substituted aryl, wherein any two adjacent R^{12} groups may be cyclically linked to form a piperazinyl ring;

q is an integer from 1 to 6;

r is 0 or 1; and

y is an integer from 1 to 6.

3. The ADC of claims 1 or 2, wherein:

T^1 is (C_1-C_{12})alkyl and V^1 is -CONH-;

T^2 is substituted (C_1-C_{12})alkyl and V^2 is -CO-;

T^3 is $(AA)_p$ and V^3 is absent;

T^4 is PABC and V^4 is absent;

p is an integer from 1 to 10; and

a, b, c, and d are each 1; and

e and f are each 0.

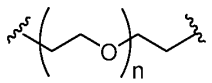
4. The ADC of any one of claims 1-3, wherein:

T^7 is a covalent bond;

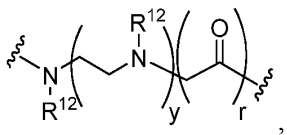
T^8, T^9, T^{10}, T^{11} and T^{12} are each independently selected from a covalent bond, (C_1-C_{12})alkyl, substituted (C_1-C_{12})alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x-$, 4-amino-piperidine (4AP), MABO, MABC, PABO, PABC, PAB, PABA, PAP, PHP, an acetal group, a hydrazine, and an ester; and

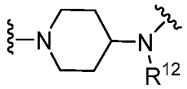
V^7, V^8, V^9, V^{10},V^{11} and V^{12} are each independently selected from the group consisting of a covalent bond, -CO-, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$, and -P(O)OH-;

wherein:

$(PEG)_n$ is , where n is an integer from 1 to 30;

EDA is an ethylene diamine moiety having the following structure:

, where y is an integer from 1 to 6 and r is 0 or 1;

4-amino-piperidine (4AP) is  ; and

each R^{12} is independently selected from hydrogen, an alkyl, a substituted alkyl, a polyethylene glycol moiety, an aryl, and a substituted aryl, wherein any two adjacent R^{12} groups may be cyclically linked to form a piperazinyl ring;

g, h, i, j, and k are each 1; and

l and m are each 0.

5. The ADC of any one of claims 1-4, wherein:

T^7 is absent and V^7 is -NHCO-;

T^8 is (C_1-C_{12})alkyl and V^8 is -CONH-;

T^9 is substituted (C_1-C_{12})alkyl and V^9 is -CO-;

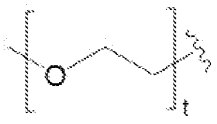
T^{10} is $(AA)_p$ and V^{10} is absent;

T^{11} is PABC and V^{11} is absent;

p is an integer from 1 to 10; and

g, h, i, j, and k are each 1; and

l and m are each 0.

6. The ADC of any one of claims 1-5 wherein one or both of T^2 and T^9 is (C_1-C_6)alkylene substituted with -NHCO$(PEG)_t$, wherein $(PEG)_t$ is  and t is an integer from 2 to 10, optionally 8.

7. The ADC of any one of claims 1-6, wherein one or both T^3 and T^{10} has p of 2.

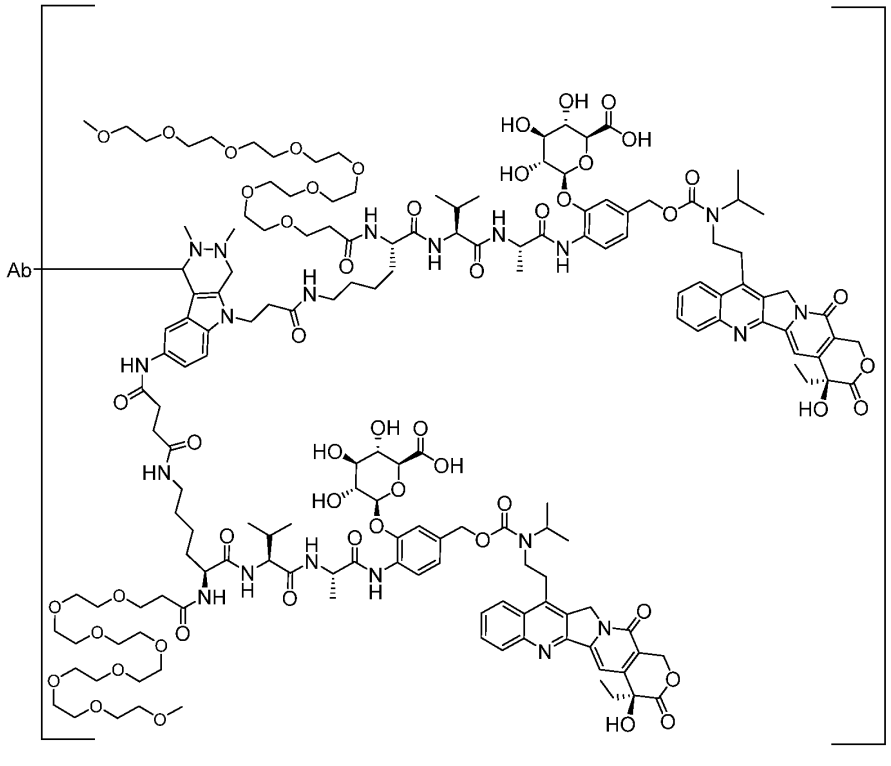
8. The ADC of any one of claims 1-7, wherein s is 4.

9. The ADC of any one of claims 1-8, wherein MABO, MABC, PABO, PABC, PAB, PABA, PAP and PHP are each optionally substituted with a glycoside, optionally wherein the glycoside is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

10. The ADC of any one of claims 1-9, wherein one or both of W^1 and W^2 is a camptothecin analog, optionally wherein the camptothecin analog is belotecan.

11. The ADC of any one of claim 1-10, wherein each of W^1 and W^2 is belotecan.

12. An ADC represented by Formula (II):



(II),

wherein:

Ab represents an antibody that binds to ROR, optionally an activatable antibody that binds to ROR, further optionally, wherein the activatable antibody comprises (a) a masking peptide comprising a masking moiety (MM) and a cleavable moiety (CM); and (b) a target binding moiety (TBM); and

s is an integer from 1 to 10, optionally 4.

13. The ADC of any one of claims 1-12, wherein TBM of the activatable antibody comprises an antibody light chain variable (VL) region, and an antibody heavy chain variable (VH) region, and wherein the VH region comprises a VH complementarity determining region 1 (CDR1), a VH complementarity determining region 2 (CDR2), and a VH complementarity determining region 3 (CDR3) as set forth in a VH comprising the amino acid sequence of SEQ ID NO:25, and the VL region comprises a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a VL comprising the amino acid sequence of SEQ ID NO:26; and wherein the activatable antibody is capable of binding to ROR when the CM is cleaved.

14. The ADC of any one of claims 1-13, wherein Ab comprises:

a masking peptide, an antibody light chain variable (VL) region, and an antibody heavy chain variable (VH) region;

wherein the masking peptide comprises the amino acid sequence of any one of SEQ ID NO:29, 30 or 31, and

wherein the VH region comprises a VH complementarity determining region 1 (CDR1), a VH complementarity determining region 2 (CDR2), and a VH complementarity determining region 3 (CDR3) as set forth in a VH comprising the amino acid sequence of SEQ ID NO:25, and the VL region comprises a VL CDR1, a VL CDR2, and a VL CDR3 as set forth in a VL comprising the amino acid sequence of SEQ ID NO:26; and

optionally wherein the activatable antibody or fragment thereof comprises a polypeptide comprising, from N-terminus to C-terminus, the masking peptide and the antibody VL region.

15. The ADC of any one of claims 1-14, wherein Ab comprises:

a VH region comprising:

(1) a VH CDR1comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 7, 12, 13, 18, and 27;

(2) a VH CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 8, 14, 19, and 24; and

(3) a VH CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 9, 15, 20, and 28; and

a VL region comprising:

(1) a VL CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 10, 16, and 21;

(2) a VL CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 5, 11, and 22; and

(3) a VL CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 6, 17, and 23.

16. The ADC of any one of claims 1-15, wherein Ab comprises:

(i) a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:1, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:2, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:3; and

a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:4, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:6;

(ii) a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:7, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:8, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:9; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:10, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:6;

(iii) a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:12, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:2, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:3; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:4, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:6;

(iv) a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:13, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:14, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:15; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:16, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:11, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:17;

(v) a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:18, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:19, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:20; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:21, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:22, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:23;

(vi) a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:1, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:24, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:3; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:4, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:6; or

(vii) a VH region comprising a VH CDR1 comprising the amino acid sequence of SEQ ID NO:27, a VH CDR2 comprising the amino acid sequence of SEQ ID NO:24, and a VH CDR3 comprising the amino acid sequence of SEQ ID NO:28; and a VL region comprising a VL CDR1 comprising the amino acid sequence of SEQ ID NO:4, a VL CDR2 comprising the amino acid sequence of SEQ ID NO:5, and a VL CDR3 comprising the amino acid sequence of SEQ ID NO:17.

17. The ADC of any one of claims 1-16, wherein Ab further comprises a framework 1 (FR1), a framework 2 (FR2), a framework 3 (FR3) and/or a framework 4 (FR4) sequence.

18. The ADC of any one of claims 1-17, wherein Ab further comprises human framework sequences, optionally an FR1, an FR), an FR3 and/or an FR) sequence as set forth in SEQ ID NO:25 or 26.

19. The ADC of any one of claims 14-18, wherein the VH comprises the amino acid sequence of SEQ ID NO:25 and the VL comprises the amino acid sequence of SEQ ID NO:26.

20. The ADC of any one of claims 14-19, wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:29.

21. The ADC of claim 20, wherein Ab comprises the polypeptide, and the polypeptide comprises the amino acid sequence of SEQ ID NO:34 or 35.

22. The ADC of any one of claims 14-19, wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:30.

23. The ADC of claim 22, wherein Ab comprises the polypeptide, and wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:36 or 37.

24. The ADC of any one of claims 14-19, wherein the masking peptide comprises the amino acid sequence of SEQ ID NO:31.

25. The ADC of claim 24, wherein Ab comprises the polypeptide, and wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:38 or 39.

26. The ADC of any one of claims 1-25, wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:32 or a variant thereof engineered to conjugate to the linker payload, or SEQ ID NO:42 or a variant thereof conjugated to the linker payload.

27. The ADC of any one of claims 1-26, wherein the antibody Ab comprises a sequence of Formula (VIII)

$$X^1(fGly')X^2Z^{20}X^3Z^{30} \qquad \text{(VIII)}$$

wherein:

fGly' is the amino acid residue coupled to the drug through a linker;

Z^{20} is either a proline (P) or alanine (A) residue;

Z^{30} 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, can be any amino acid residue, with the proviso that when the sequence of Formula (VIII) is at the N-terminus of the antibody Ab, X^1 is present; and

X^2 and X^3 independently can be any amino acid residue,

optionally wherein,

the sequence of Formula (VIII) is selected from the group consisting of: L(fGly')TPSR (SEQ ID NO:146), M(fGly')TPSR (SEQ ID NO:147), V(fGly')TPSR (SEQ ID NO:148), L(fGly')SPSR (SEQ ID NO:149), L(fGly')APSR (SEQ ID NO:150), L(fGly')VPSR (SEQ ID NO:151), L(fGly')GPSR (SEQ ID NO:152), I(fGly')TPAR (SEQ ID NO:153), L(fGly')TPSK (SEQ ID NO:154), M(fGly')TPSK (SEQ ID NO:155), V(fGly')TPSK (SEQ ID NO:156), L(fGly')SPSK (SEQ ID NO:157), L(fGly')APSK (SEQ ID NO:158), L(fGly')VPSK (SEQ ID NO:159), L(fGly')GPSK (SEQ ID NO:160), L(fGly')TPSA (SEQ ID NO:161), I(fGly')TPAA (SEQ ID NO:162), M(fGly')TPSA (SEQ ID NO:163), V(fGly')TPSA (SEQ ID NO:164), L(fGly')SPSA (SEQ ID NO:165), L(fGly')APSA (SEQ ID NO:166), L(fGly')VPSA (SEQ ID NO:167), and L(fGly')GPSA (SEQ ID NO:168);

further optionally wherein the sequence of Formula (VIII) comprises L(fGly')TPSR (SEQ ID NO:146).

28. The ADC of any one of claims 1-27, wherein Ab is an IgG1 antibody, optionally an IgG1 kappa antibody.

29. The ADC of any one of claims 1-28, wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:32 or 169 or a variant thereof further comprising one or more sequences of Formula (VIII) and a light chain comprising the amino acid sequence of any one of SEQ ID NOs: 34, 36, or 38.

30. The ADC of any one of claims 1-29, wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:48.

31. The ADC of any one of claims 1-29, wherein Ab comprises a heavy chain comprising the amino acid sequence of SEQ ID NO:171.

32. The ADC of any one of claims 1-31, wherein Ab comprises a light chain comprising the amino acid sequence of any one of SEQ ID NOs: 34, 36, or 38.

33. The ADC of any one of claims 1-32, wherein Ab comprises any one of the following (i) – (vi):

(i) a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:34;

(ii) a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:36;

(iii) a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:38;

(iv) a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:171 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:34;

(v) a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:171 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:36; or

(vi) a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:171 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:38.

34. The ADC of any one of claims 1-33, wherein Ab is a monoclonal antibody.

35. The ADC of any one of claims 1-34, wherein Ab is a humanized, human, or chimeric antibody.

36. The ADC of any one of claims 1-27, where Ab is a Fab, Fab', $F(ab')_2$, Fv, scFv, $(scFv)_2$, single chain antibody molecule, dual variable region antibody, single variable region antibody, linear antibody, V region, or a multispecific antibody formed from antibody fragments.

37. The ADC of any one of claims 1-35, wherein the Ab is a multispecific antibody, optionally wherein the multispecific antibody is a bispecific antibody.

38. The ADC of any one of claims 1-37, wherein Ab binds to both ROR1 and ROR2, optionally, wherein Ab binds to both human ROR1 and human ROR2, optionally wherein Ab comprises a silent Fc (sFc), further optionally wherein Ab comprises an sFc comprising an amino acid sequence as set forth in SEQ ID NO:44.

39. A pharmaceutical composition comprising the ADC of any one of claims 1-38 and a pharmaceutically acceptable excipient.

40. The pharmaceutical composition of claim 39, characterized by an ADC drug-to-antibody ratio (DAR) of about 1 to about 20.

41. The pharmaceutical composition of claim 40, wherein the DAR is about 2 to about 8.

42. The pharmaceutical composition of claim 40 or 41, wherein the DAR is about 8.

43. An activated activatable ADC, produced by activating the ADC of any one of claims 1-38.

44. An activated activatable ADC, which is the ADC of any one of claims 1-38 having the activatable antibody Ab activated.

45. The ADC of claim 43 or 44, wherein the ADC or Ab was activated by treating the ADC comprising the activatable antibody Ab with one or more proteases, wherein the Ab comprises a masking peptide, and wherein the one or more proteases cleave within the masking peptide, optionally, wherein the one or more proteases comprises MMP-9.

46. The ADC of any one of claims 43-45, wherein Ab comprises a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:48 or 171 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:33.

47. The ADC of any one of claims 43-46, wherein Ab comprises:

(i) a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:48 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:33; or

(ii) a heavy chain comprising the amino acid sequence as set forth in SEQ ID NO:171 and a light chain comprising the amino acid sequence as set forth in SEQ ID NO:33.

48. A method for treating a subject with cancer, the method comprising administering a therapeutically effective amount of the ADC of any one of claims 1-38 and 43-47 or the pharmaceutical composition of any one of claims 39-42 to the subject.

49. The method of claim 48, wherein the cancer is an ROR antigen expressing cancer.

50. The method of claim 48 or 49, wherein the cancer expresses an ROR1 antigen.

51. The method of claim 48 or 49, wherein the cancer expresses and ROR2 antigen.

52. The method of claim 48 or 49, wherein the cancer expresses an ROR1 antigen and an ROR2 antigen.

53. The method of any of claims 48-52, wherein the cancer is selected from the group consisting of: pancreatic cancer, ovarian cancer, breast cancer, lung cancer, gastric cancer, melanoma, Ewing sarcoma, chronic lymphocytic leukemia, mantle cell lymphoma, B-ALL, hematological cancer, prostate cancer, colon cancer, renal cancer, thyroid cancer, liver cancer, urothelial carcinoma, melanoma, endometrial cancer, clear cell renal cell carcinoma, clear cell carcinoma, and uterine cancer, optionally wherein the cancer is chronic myelogenous leukemia, colorectal cancer, estrogen receptor positive, progesterone receptor

positive and HER2 negative breast cancer, triple negative breast cancer, or non-small cell lung cancer.