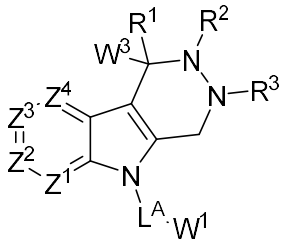
CLAIMS

1. A conjugate of formula (I):



(I)

wherein:

Z^1, Z^2, Z^3 and Z^4 are each independently selected from CR^4, N and 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;

W^1 is a first drug;

W^2 is a second drug; and

W^3 is an anti-Nectin-4 antibody.

2. The conjugate of Claim 1, wherein Z^1 is CR^4 or N.

3. The conjugate of Claim 1, wherein Z^3 is CR^4 or $C\text{-}L^B\text{-}W^2$.

4. The conjugate of any of Claims 1-3, wherein 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;

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, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_x\text{-}$, 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, optionally wherein T^1, T^2, T^3, T^4, T^5 and T^6 are each optionally substituted with a glycoside, or optionally wherein MABO, MABC, PABO, PABC, PAB, PABA, PAP and PHP are each optionally substituted with a glycoside, further optionally wherein the glycoside is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc;

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-, $-NR^{15}\text{-}$, $-NR^{15}(CH_2)_q\text{-}$, $-NR^{15}(C_6H_4)\text{-}$, $-CONR^{15}\text{-}$, $-NR^{15}CO\text{-}$, -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
optionally,

wherein:

T^1 is $(C_1\text{-}C_{12})$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 and V^4 is absent;

T^5 is PABC and V^5 is absent; and

f is 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 and V^3 is absent;

T^4 is PABC and V^4 is absent; and

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

T^3 is AA and V^3 is absent;

T^4 is PABC and V^4 is absent; and

e and f are each 0.

5. The conjugate of any one of Claims 1-4, wherein 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, 1 and m are each independently 0 or 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, optionally wherein T^7, T^8, T^9, T^{10}, T^{11}, T^{12} and T^{13} are each optionally substituted with a glycoside, or optionally wherein MABO, MABC, PABO, PABC, PAB, PABA, PAP and PHP are each optionally substituted with a glycoside, further optionally wherein the glycoside is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc;

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

optionally,

wherein:

T^7 is absent 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 and V^{10} is absent; and

T^{11} is PABC and V^{11} is absent; and

l and m are each 0; or

wherein:

T^7 is absent and V^7 is -NHCO-;

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

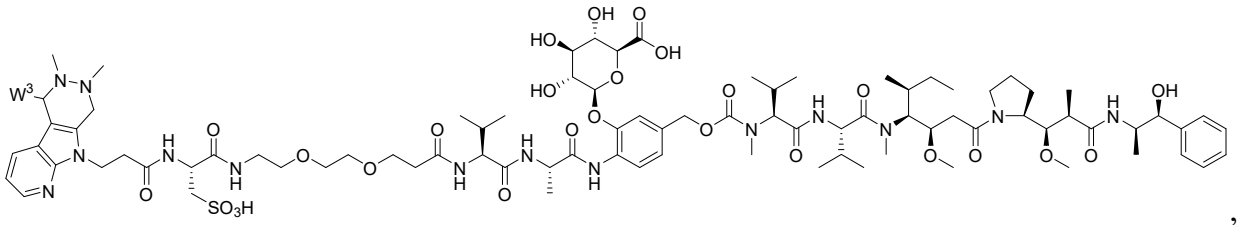
T^9 is substituted $(C_1\text{-}C_{12})$alkyl and V^9 is -CO-;

T^{10} is AA and V^{10} is absent;

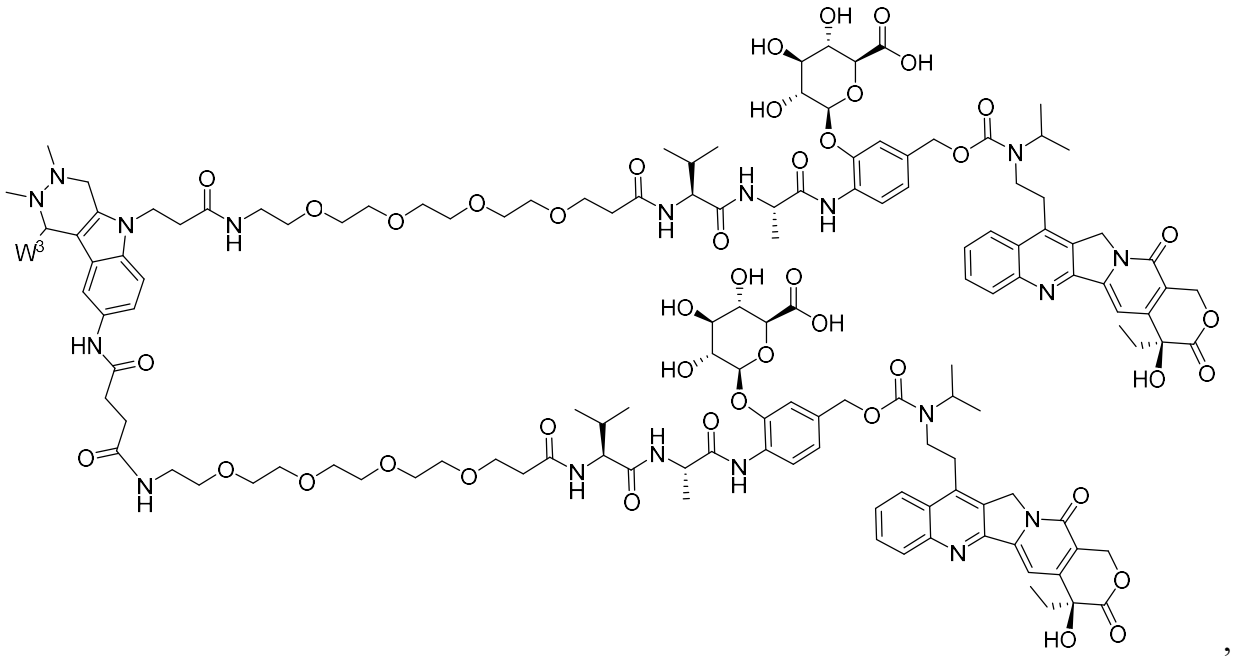
T^{11} is PABC and V^{11} is absent; and

l and m are each 0.

6. The conjugate of any one of Claims 1-5, wherein the conjugate is selected from:

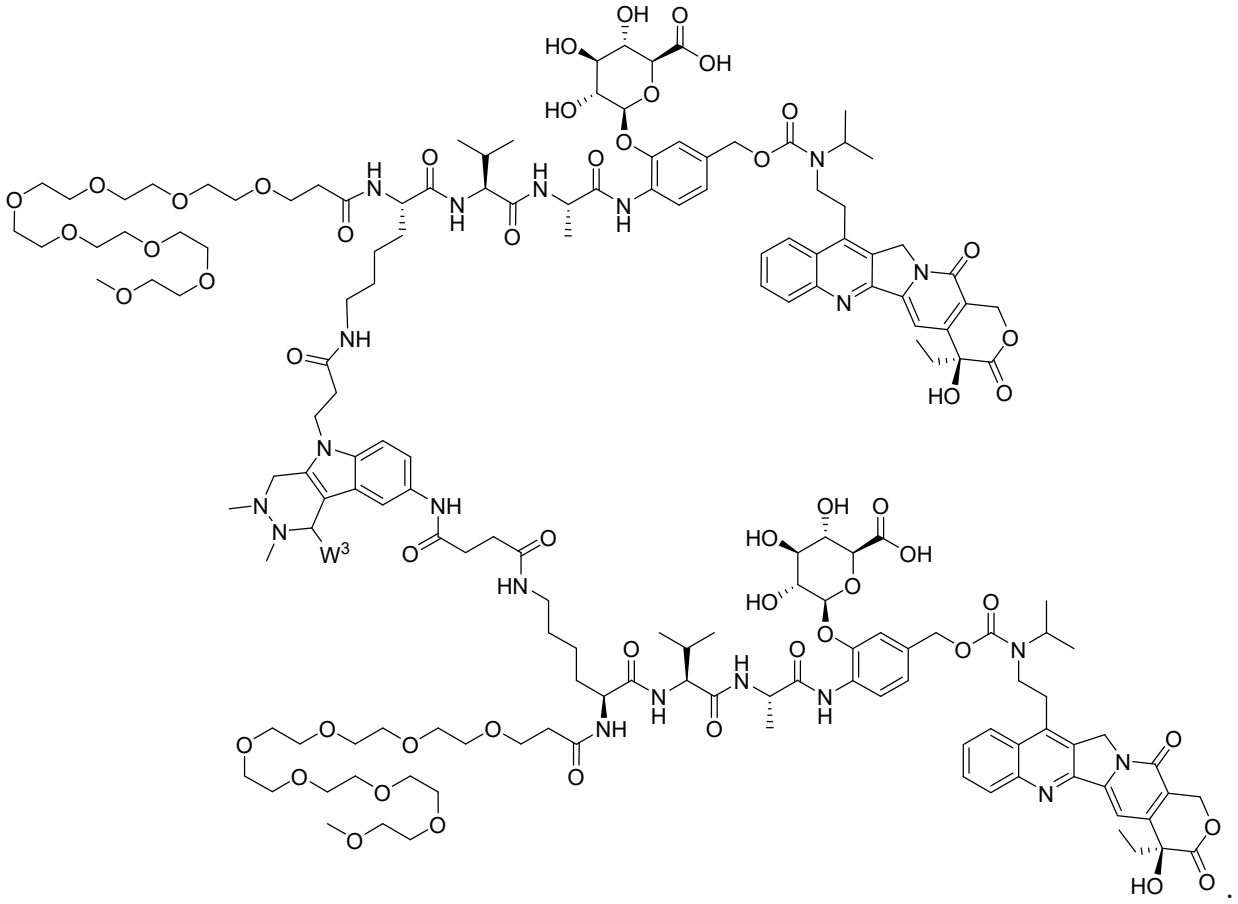


,



,

and



7. The conjugate of any one of Claims 1 to 6, wherein the anti-Nectin-4 antibody is an IgG1 antibody, optionally an IgG1 kappa antibody.

8. The conjugate of any one of Claims 1 to 7, wherein the anti-Nectin-4 antibody comprises a sequence of the formula (II):

X^1(fGly')$X^2Z^2X^3Z^3$ (SEQ ID NO: 128) (II),

wherein

X^1 is present or absent and, when present, can be any amino acid, with the proviso that when the sequence is at the N-terminus of the conjugate, X^1 is present;

fGly' is an amino acid residue coupled to the first drug or the second drug through the first linker or the second linker, respectively;

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

Z^2 is either a proline or alanine residue; and

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

optionally,

wherein the sequence is L(fGly')TPSR (SEQ ID NO: 246),

or optionally,

wherein

Z^3 is selected from R, K, H, A, G, L, V, I, and P;

X^1 is selected from L, M, S, and V; and

X^2 and X^3 are each independently selected from S, T, A, V, G, and C.

9. The conjugate of Claim 8, wherein the sequence is positioned at a C-terminus of a heavy chain constant region of the anti-Nectin-4 antibody,

optionally,

wherein the heavy chain constant region comprises a sequence of the formula (II):

X^1(fGly')$X^2Z^2X^3Z^3$ (SEQ ID NO: 128) (II),

wherein

X^1 is present or absent and, when present, can be any amino acid, with the proviso that when the sequence is at the N-terminus of the conjugate, X^1 is present;

fGly' is an amino acid residue coupled to the first drug or the second drug through the first linker or the second linker, respectively

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

Z^2 is either a proline or alanine residue;

Z^3 is a basic amino acid or an aliphatic amino acid, and

wherein the sequence is C-terminal to the amino acid sequence SLSLSPG (SEQ ID NO: 247),

further optionally,

wherein the heavy chain constant region comprises the sequence SPGSL(fGly')TPSRGS (SEQ ID NO: 130),

optionally,

wherein

Z^3 is selected from R, K, H, A, G, L, V, I, and P;

X^1 is selected from L, M, S, and V; and

X^2 and X^3 are each independently selected from S, T, A, V, G, and C.

10. The conjugate of Claim 9, wherein the heavy chain constant region of the anti-Nectin-4 antibody comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence set forth in SEQ ID NO: 70 and comprises the fGly' residue instead of C in the sequence LCTPSR (SEQ ID NO: 104).

11. The conjugate of Claim 8, wherein the heavy chain constant region of the anti-Nectin-4 antibody comprises an amino acid sequence at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to the amino acid sequence set forth in any one of SEQ ID NOs: 71-86 and comprises the fGly' residue instead of C in the sequence LCTPSR (SEQ ID NO: 104).

12. The conjugate of Claim 8, wherein the fGly' residue is positioned in a light chain constant region of the anti-Nectin-4 antibody,

optionally

wherein the light chain constant region comprises a sequence of the formula (II):

X^1(fGly')$X^2Z^2X^3Z^3$ (SEQ ID NO: 128) (II),

wherein

X^1 is present or absent and, when present, can be any amino acid, with the proviso that when the sequence is at the N-terminus of the conjugate, X^1 is present;

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

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

Z^2 is either a proline or alanine residue;

Z^3 is a basic amino acid or an aliphatic amino acid, and

wherein the sequence is C-terminal to the amino acid sequence KVDNAL (SEQ ID NO: 132), and/or is N-terminal to the sequence QSGNSQ (SE ID NO: 133),

further optionally,

wherein the light chain constant region comprises the sequence KVDNAL(fGly')TPSRQSGNSQ (SEQ ID NO: 134),

or further optionally,

wherein

Z^3 is selected from R, K, H, A, G, L, V, I, and P;

X^1 is selected from L, M, S, and V; and

X^2 and X^3 are each independently selected from S, T, A, V, G, and C.

13. The conjugate of Claim 8, wherein the fGly' residue is positioned in a heavy chain CH1 region of the anti-Nectin-4 antibody,

optionally,

wherein the heavy chain CH1 region comprises a sequence of the formula (II):

X^1(fGly')$X^2Z^2X^3Z^3$ (SEQ ID NO: 128) (II),

wherein

X^1 is present or absent and, when present, can be any amino acid, with the proviso that when the sequence is at the N-terminus of the conjugate, X^1 is present;

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

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

Z^2 is either a proline or alanine residue;

Z^3 is a basic amino acid or an aliphatic amino acid, and

wherein the sequence is C-terminal to the amino acid sequence SWNSGA (SEQ ID NO: 135) and/or is N-terminal to the amino acid sequence GVHTFP (SEQ ID NO: 136),

further optionally,

wherein the heavy chain CH1 region comprises the sequence SWNSGAL(fGly')TPSRGVHTFP (SEQ ID NO: 137),

or further optionally,

wherein

Z^3 is selected from R, K, H, A, G, L, V, I, and P;

X^1 is selected from L, M, S, and V; and

X^2 and X^3 are each independently selected from S, T, A, V, G, and C.

14. The conjugate of Claim 8, wherein the fGly' residue is positioned in a heavy chain CH2 region of the anti-Nectin-4 antibody, or wherein the fGly' residue is positioned in a heavy chain CH3 region of the anti-Nectin-4 antibody.

15. The conjugate of any one of Claims 1 to 14, wherein the anti-Nectin-4 antibody competes for binding to Nectin-4 with an anti-Nectin-4 antibody comprising:

a variable heavy chain (VH) chain comprising heavy chain CDRs 1-3 (HCDRs 1-3) of a VH chain having a sequence selected from SEQ ID NOs: 1 to 17; and

a variable light chain (VL) chain comprising light chain CDRs 1-3 (LCDRs 1-3) of a VL chain having a sequence selected from SEQ ID NOs: 18 to 31.

16. The conjugate of any one of Claims 1 to 14, wherein the anti-Nectin-4 antibody comprises:

a VH chain comprising heavy chain CDRs 1-3 (HCDRs 1-3) of a VH chain having a sequence selected from SEQ ID NOs: 1 to 17; and

a VL chain comprising light chain CDRs 1-3 (LCDRs 1-3) of a VL chain having a sequence selected from SEQ ID NOs: 18 to 31.

17. The conjugate of Claim 16, wherein the anti-Nectin-4 antibody comprises:

(a) a VH chain comprising a sequence selected from SEQ ID NOs: 1 to 17, and

a VL chain comprising a sequence selected from SEQ ID NOs: 18 to 31; or

(b) a VH chain comprising HCDRs 1-3 of a VH chain having a sequence selected from SEQ ID NOs: 1 to 6, and

a VL chain comprising LCDRs 1-3 of a VL chain having a sequence selected from SEQ ID NOs: 18 to 23; or

(c) a VH chain comprising a sequence selected from SEQ ID NOs: 1 to 6, and

a VL chain comprising a sequence selected from SEQ ID NOs: 18 to 23; or

(d) a VH chain comprising HCDRs 1-3 of a VH chain having a sequence selected from SEQ ID NOs: 7 to 13, and

a VL chain comprising LCDRs 1-3 of a VL chain having a sequence selected from SEQ ID NOs: 24 to 27; or

(e) a VH chain comprising a sequence selected from SEQ ID NOs: 7 to 13, and

a VL chain comprising a sequence selected from SEQ ID NOs: 24 to 27; or

(f) a VH chain comprising HCDRs 1-3 of a VH chain having a sequence selected from SEQ ID NOs: 14 to 17, and

a VL chain comprising LCDRs 1-3 of a VL chain having a sequence selected from SEQ ID NOs: 28 to 31; or

(g) a VH chain comprising a sequence selected from SEQ ID NOs: 14 to 17, and

a VL chain comprising a sequence selected from SEQ ID NOs: 28 to 31; or

(h) the VH chain of an anti-Nectin-4 antibody comprising the HCDRs 1-3 of a VH chain having a sequence selected from SEQ ID NOs: 1 to 17 and an amino acid sequence having 80% or greater, 85% or greater, 90% or greater, 95% or greater, 99% or greater, or 100% sequence identity to the amino acid sequence set forth in a sequence selected from SEQ ID NOs:1 to 17, wherein any amino acid differences between the VH chain of an anti-Nectin-4 antibody and a sequence selected from SEQ ID NOs: 1 to 17 is in the regions outside of the CDRs, and

the VL chain of an anti-Nectin-4 antibody comprises the LCDRs 1-3 of a VL chain having a sequence selected from SEQ ID NOs: 18 to 31 and comprises an amino acid sequence having 80% or greater, 85% or greater, 90% or greater, 95% or greater, 99% or greater, or 100% sequence identity to the amino acid sequence set forth in a sequence selected from SEQ ID NOs: 18 to 31, wherein any amino acid differences between the VL chain of an anti-Nectin-4 antibody and a sequence selected from SEQ ID NOs: 18 to 31 is within the regions outside of the CDRs; alternatively or optionally wherein the anti-Nectin-4 antibody comprises: a heavy chain constant region having the amino acid sequence set forth in any one of SEQ ID NOs: 70 to 86, wherein the C present in the sequence LCTPSR in the constant region is replaced by fGly.

18. A pharmaceutical composition comprising:

a conjugate of any one of Claims 1 to 17; and

a pharmaceutically-acceptable excipient.

19. An anti-Nectin-4 antibody, comprising:

a variable heavy chain (VH) chain comprising heavy chain CDRs 1-3 (HCDRs 1-3) of a VH chain having a sequence selected from SEQ ID NOs: 1 to 17; and

a variable light chain (VL) chain comprising light chain CDRs 1-3 (LCDRs 1-3) of a VL chain having a sequence selected from SEQ ID NOs: 18 to 31.

20. The anti-Nectin-4 antibody of Claim 19, comprising:

(a) a VH chain comprising a sequence selected from SEQ ID NOs: 1 to 17, and

a VL chain comprising a sequence selected from SEQ ID NOs: 18 to 31; or

(b) a VH chain comprising HCDRs 1-3 of a VH chain having a sequence selected from SEQ ID NOs: 1 to 6, and

a VL chain comprising LCDRs 1-3 of a VL chain having a sequence selected from SEQ ID NOs: 18 to 23; or

(c) a VH chain comprising a sequence selected from SEQ ID NOs: 1 to 6, and

a VL chain comprising a sequence selected from SEQ ID NOs: 18 to 23; or

(d) a VH chain comprising HCDRs 1-3 of a VH chain having a sequence selected from SEQ ID NOs: 7 to 13, and

a VL chain comprising LCDRs 1-3 of a VL chain having a sequence selected from SEQ ID NOs: 24 to 27; or

(e) a VH chain comprising a sequence selected from SEQ ID NOs: 7 to 13, and

a VL chain comprising a sequence selected from SEQ ID NOs: 24 to 27; or

(f) a VH chain comprising HCDRs 1-3 of a VH chain having a sequence selected from SEQ ID NOs: 14 to 17, and

a VL chain comprising LCDRs 1-3 of a VL chain having a sequence selected from SEQ ID NOs: 28 to 31; or

(g) a VH chain comprising a sequence selected from SEQ ID NOs: 14 to 17, and

a VL chain comprising a sequence selected from SEQ ID NOs: 28 to 31; or

(h) the VH chain of an anti-Nectin-4 antibody comprising the HCDRs 1-3 of a VH chain having a sequence selected from SEQ ID NOs: 1 to 17 and an amino acid sequence having 80% or greater, 85% or greater, 90% or greater, 95% or greater, 99% or greater, or 100% sequence identity to the amino acid sequence set forth in a sequence selected from SEQ ID NOs: 1 to 17, wherein any amino acid differences between the VH chain of an anti-Nectin-4 antibody and a sequence selected from SEQ ID NOs: 1 to 17 is in the regions outside of the CDRs, and

the VL chain of an anti-Nectin-4 antibody comprises the LCDRs 1-3 of a VL chain having a sequence selected from SEQ ID NOs: 18 to 31 and comprises an amino acid sequence having 80% or greater, 85% or greater, 90% or greater, 95% or greater, 99% or greater, or 100% sequence identity to the amino acid sequence set forth in a sequence selected from SEQ ID NOs: 18 to 31, wherein any amino acid differences between the VL chain of an anti-Nectin-4 antibody and a sequence selected from SEQ ID NOs: 18 to 31 is within the regions outside of the CDRs;

alternatively or optionally wherein the anti-Nectin-4 antibody comprises a heavy chain constant region having the amino acid sequence set forth in any one of SEQ ID NOs: 70 to 86, wherein the C present in the sequence LCTPSR in the constant region is replaced by fGly.

21. A pharmaceutical composition comprising:

an antibody of Claim 19 or 20; and

a pharmaceutically-acceptable excipient.

22. Use of the conjugate of any one of claims 1-17 or the antibody of Claim 19 or 20 or the pharmaceutical composition of Claim 18 or 21, in the manufacture of a medicament for the treatment of cancer in a subject, optionally,

wherein the cancer is ovarian cancer, ductal breast carcinoma, lung adenocarcinoma, and pancreatic cancer,

further optionally,

wherein the cancer is characterized by cancer cells expressing Nectin-4,

yet further optionally

wherein the conjugate or the antibody binds to Nectin-4.