[00006] There is a need in the art for safe and effective agents that target AML including CLL-1 for the diagnosis and treatment of CLL-1-associated conditions, such as cancer. The invention fulfills that need and provides other benefits.

SUMMARY

[00007] The disclosure provides anti-CLL-1 antibodies and immunoconjugates and methods of using the same.

[00008] Provided herein are isolated monoclonal anti-CLL-1 antibodies, wherein the antibody binds an epitope and/or binds an overlapping epitope comprising amino acids of SEQ ID NO:49 and does not bind an epitope comprising SEQ ID NO:50 and/or SEQ ID NO:51. In some embodiments, the anti-CLL-1 antibody binds an epitope comprising amino acids of SEQ ID NO:49. In some embodiments, the anti-CLL-1 antibody binds an epitope consisting or consisting essentially of the amino acids of SEQ ID NO:49. In some embodiments, the epitope is determined by hydroxyl radical footprinting.

[00009] Further provided herein isolated antibody that binds to CLL-1, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:45; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7. In some embodiments, the antibody comprises HVR-H2 comprising the amino acid sequence of SEQ ID NO:9. In some embodiments, the antibody comprises HVR-H2 comprising the amino acid sequence of SEQ ID NO:47. In some embodiments, the antibody comprises HVR-H2 comprising the amino acid sequence of SEQ ID NO:11. In some embodiments, the antibody comprises HVR-H2 comprising the amino acid sequence of SEQ ID NO:43. In some embodiments, the antibody comprises HVR-H2 comprising the amino acid sequence of SEQ ID NO:44.

[00009a] In one aspect, there is provided an isolated monoclonal anti-CLL1 antibody, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:11; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7.

[00010] In some embodiments, the antibody comprises: (a) a heavy chain variable region comprising the sequence of SEQ ID NO: 33 and a light chain variable region comprising the sequence of SEQ ID NO: 32; (b) a heavy chain variable region comprising the sequence of SEQ ID NO: 34 and a light chain variable region comprising the sequence of SEQ ID NO: 32; (c) a heavy chain variable region comprising the sequence of SEQ ID NO: 46 and a light chain variable region comprising the sequence of SEQ ID NO: 32; or (d) a heavy chain variable region comprising the sequence of SEQ ID NO: 48 and a light chain variable region comprising the sequence of SEQ ID NO: 32. In some embodiments, the antibody comprises a heavy chain variable region comprising the sequence of SEQ ID NO: 48 and a light chain variable region comprising the sequence of SEQ ID NO: 32. In some embodiments, the antibody comprises a heavy chain variable region comprising the

free cysteine amino acid residues is located in the light chain. In some embodiments, the one or more engineered free cysteine amino residues in the light chain comprises V205C according to Kabat numbering. In some embodiments, the one or more engineered free cysteine amino residues in the light chain comprises K149C according to Kabat numbering. In some embodiments, the one or more engineered free cysteine amino acid residues is located in the heavy chain. In some embodiments, the one or more engineered free cysteine amino residues in the heavy chain comprises A118C according to EU numbering. In some embodiments, the one or more engineered free cysteine amino residues in the heavy chain comprises S400C according to EU numbering.

[00014] In some embodiments of any of the antibodies, the antibody is a monoclonal antibody. In some embodiments of any of the antibodies, the antibody is a human or chimeric antibody. In some embodiments of any of the antibodies, the antibody is an antibody fragment that binds CLL-1. In some embodiments of any of the antibodies, the antibody is an IgG1, IgG2a or IgG2b antibody.

[00015] In another aspect, provided herein are isolated nucleic acids encoding the antibodies described herein. In a further aspect, provided herein are host cells comprising the nucleic acid encoding the antibodies described herein. In yet a further aspect, provided herein are methods of producing an antibody comprising culturing the host cell comprising the nucleic acid encoding the antibodies described herein so that the antibody is produced, and isolating the antibody.

[00016] In one aspect, provided herein are immunoconjugates comprising the antibodies described herein and a cytotoxic drug. In particular, in one aspect, provided herein are immunoconjugates having the formula Ab-(L-D)p, wherein:

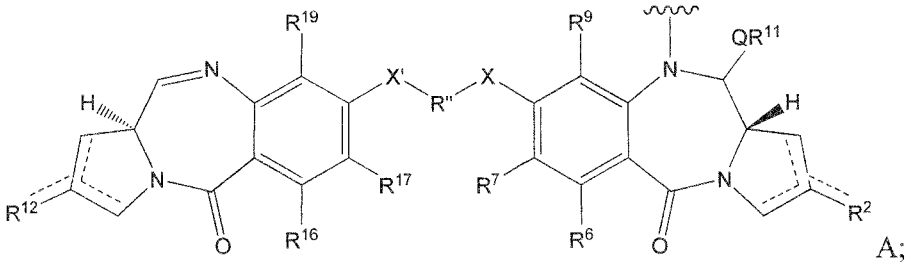
(a) Ab is the antibody described herein;

(b) L is a linker that links the Ab to D;

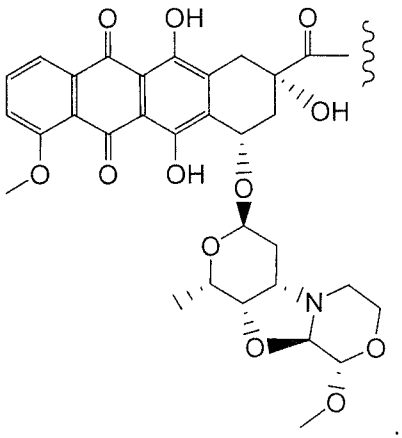
(c) D is a cytotoxic drug; and

(d) p ranges from 1-8.

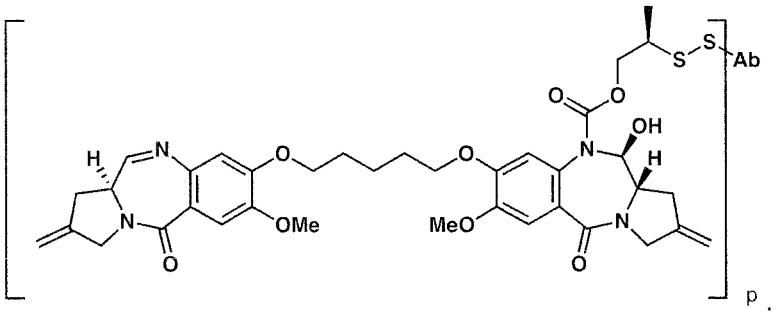
[00017] In some embodiments of any of the immunoconjugates, the cytotoxic agent is selected from a maytansinoid, a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative. In some embodiments of any of the immunoconjugates, D is a pyrrolobenzodiazepine of Formula A:



A;

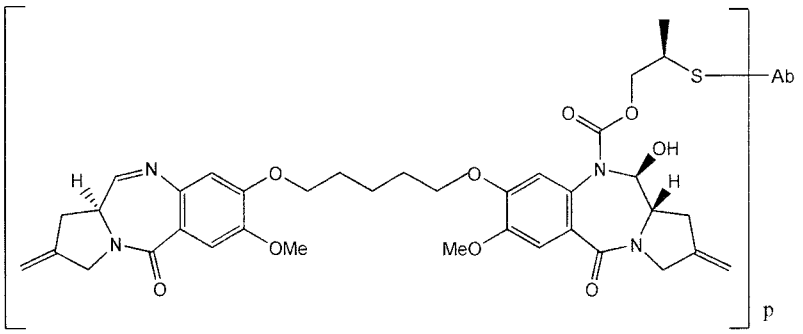


[00021] In some embodiments of any of the immunoconjugates, L is cleavable by a protease. In some embodiments of any of the immunoconjugates, L is acid-labile. In some embodiments of any of the immunoconjugates, L comprises hydrazone.

[00022] In some embodiments of any of the immunoconjugates, the immunoconjugate has a structure: 

[00023] In some embodiments of any of the immunoconjugates, p ranges from 2-5.

[00023a] In one aspect, there is provided an immunoconjugate comprising a structure:

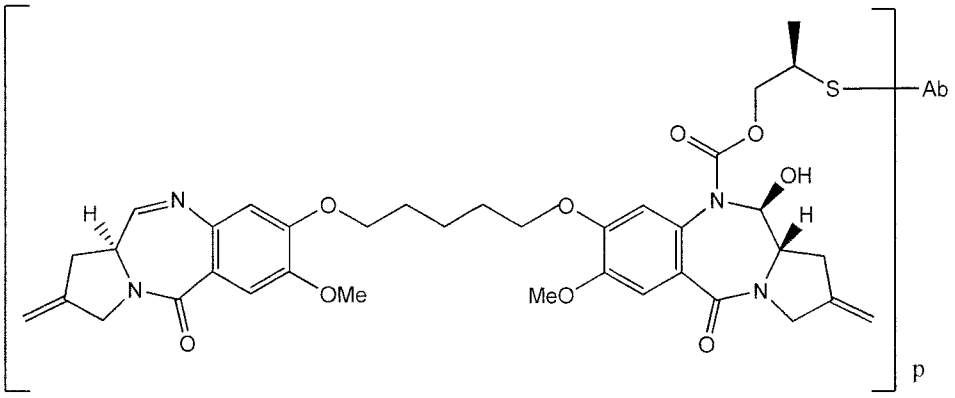


wherein Ab is an isolated monoclonal anti-CLL-1 antibody, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:11; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7;

wherein the antibody comprises an engineered free cysteine residue located in the light chain at K149C according to Kabat numbering; and

wherein p is 2.

[00023b] In one aspect, there is provided an immunoconjugate comprising a structure:



wherein Ab is an isolated monoclonal anti-CLL-1 antibody, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:11; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7;

wherein the antibody comprises an engineered free cysteine residue located in the light chain at K149C according to Kabat numbering; and

wherein p is 1.

[00024] In some embodiments, pharmaceutical formulations are provided. In one aspect, there is provided a pharmaceutical formulation comprising an immunoconjugate described herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical formulation comprises an additional therapeutic agent. In some embodiments, the additional therapeutic agent is an anthracycline. In some embodiments, the anthracycline is daunorubicin or idarubicin. In some embodiments, the additional therapeutic agent is cytarabine. In some embodiments, the additional therapeutic agent is cladribine. In some embodiments, the additional therapeutic agent is fludarabine or topotecan. In some embodiments, the additional therapeutic agent is 5-azacytidine or decitabine.

[00025] In some embodiments, methods of treatment are provided. In some embodiments, methods of treating CLL-1-positive cancers are provided. In one aspect, there is provided use of the immunoconjugate described herein or the pharmaceutical formulation described herein in the manufacture of a medicament for treating an individual having a CLL-1-positive cancer. In some embodiments, a method of treatment comprises administering to an individual an effective amount of an immunoconjugate described herein or a pharmaceutical formulation described herein. In some embodiments, the cancer is a cancer. In some embodiments, the cancer is acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and/or myelodysplastic syndrome (MDS). In some embodiments, the cancer is CLL-1 positive. In some embodiments, the CLL-1-positive cancer is AML. In some embodiments, the method comprises administering an additional therapeutic agent to the individual.

In some embodiments, the additional therapeutic agent is an anthracycline. In some embodiments, the anthracycline is daunorubicin or idarubicin. In some embodiments, the additional therapeutic agent is cytarabine. In some embodiments, the additional therapeutic agent is cladribine. In some embodiments, the additional therapeutic agent is fludarabine or topotecan. In some embodiments, the additional therapeutic agent is 5-azacytidine or decitabine.

[00026] In some embodiments of any of the methods, the method further comprises administering to the subject a PD-1 axis binding antagonist or an additional therapeutic agent. In some embodiments, the PD-1 axis binding antagonist is a PD-1 binding antagonist. In some embodiments, the PD-1 axis binding antagonist is a PD-L1 binding antagonist. In some embodiments, the PD-1 axis binding antagonist is a PD-L2 binding antagonist.

[00027] In some embodiments, methods of inhibiting proliferation of a CLL-1-positive cell are provided. In some embodiments, the method comprises exposing the cell to an immunoconjugate described herein under conditions permissive for binding of the immunoconjugate to CLL-1 on the surface of the cell, thereby inhibiting proliferation of the cell. In one aspect, there is provided an *in vitro* method of inhibiting proliferation of a CLL-1-positive cell, the method comprising exposing the cell to the immunoconjugate described herein under conditions permissive for binding of the immunoconjugate to CLL-1 on the surface of the cell, thereby inhibiting proliferation of the cell. In some embodiments, the cell is an AML cancer cell.

[00028] In some embodiments, a method of detecting human CLL-1 in a biological sample is provided. In some embodiments, a method comprises contacting the biological sample with an anti-CLL-1 antibody under conditions permissive for binding of the anti-CLL-1 antibody to a naturally occurring human CLL-1, and detecting whether a complex is formed between the anti-CLL-1 antibody and a naturally occurring human CLL-1 in the biological sample. In one aspect, there is provided an *in vitro* method of detecting human CLL-1 in a biological sample comprising contacting the biological sample with the anti-CLL-1 antibody described herein under conditions permissive for binding of the anti-CLL-1 antibody to a naturally occurring human CLL-1, and detecting whether a complex is formed between the anti-CLL-1 antibody and a naturally occurring human CLL-1 in the biological sample. In some embodiments, an anti-CLL-1 antibody is an antibody described herein. In some embodiments, the biological sample is an AML cancer sample.

[00029] In one aspect, there is provided an antibody described herein for use in detecting a CLL-1-positive cancer in a subject. In some embodiments, a method for detecting a CLL-1-positive cancer is provided. In some such embodiments, a method comprises (i) administering a labeled anti-CLL-1 antibody to a subject having or suspected of having a CLL-1-positive cancer, and (ii) detecting the labeled anti-CLL-1 antibody in the subject, wherein detection of the labeled anti- CLL-1 antibody indicates a CLL-1-positive cancer in the subject. In some embodiments, an anti-CLL-1 antibody is an antibody described herein. In some such embodiments, the labeled anti-CLL-1 antibody comprises an anti-CLL-1 antibody conjugated to a positron emitter. In some embodiments, the positron emitter is ^{89}Zr.

BRIEF DESCRIPTION OF THE FIGURES

[00030] **Figures 1A-B** show alignment of the light chain variable region sequences (A) and heavy chain variable region sequences (B) of murine (m) 6E7, m21C9, m20B1, and m28H12.

[00031] **Figures 2A-B** show alignment of the light chain variable region sequences (A) and heavy chain variable region sequences (B) of K1H1, m6E7, humanized (h) 6E7.L4H1e, and h6E7.L4H1e.A54.

WHAT IS CLAIMED IS:

1. An isolated monoclonal anti-CLL1 antibody, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:11; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7.

2. The antibody of claim 1, wherein the antibody comprises:

a heavy chain variable region comprising the sequence of SEQ ID NO: 34 and a light chain variable region comprising the sequence of SEQ ID NO: 32.

3. The antibody of claim 1 or claim 2, wherein the antibody comprises one or more engineered free cysteine amino acids residues.

4. The antibody of claim 3, wherein the one or more engineered free cysteine amino acid residues is located in the light chain.

5. The antibody of claim 3, wherein the one or more engineered free cysteine amino acid residues in the light chain comprises K149C according to Kabat numbering.

6. The antibody of any one of the preceding claims, wherein the antibody is a humanized antibody.

7. The antibody of any one of the preceding claims, wherein the antibody is an antibody fragment that binds CLL-1.

8. The antibody of any one of the preceding claims, wherein the antibody is an IgG1, IgG2a or IgG2b antibody.

9. The antibody of any one of the preceding claims, wherein the antibody is an IgG1 antibody.

10. An isolated nucleic acid encoding the antibody of any one of the preceding claims.

11. A host cell comprising the nucleic acid of claim 10.

12. A method of producing an antibody comprising culturing the host cell of claim 11 so that the antibody is produced, and isolating the antibody.

13. An immunoconjugate comprising the antibody of any one of claims 1 to 9 and a cytotoxic drug.

14. An immunoconjugate having the formula Ab-(L-D)p, wherein:

(a) Ab is the antibody of any one of claim 1 to 9;

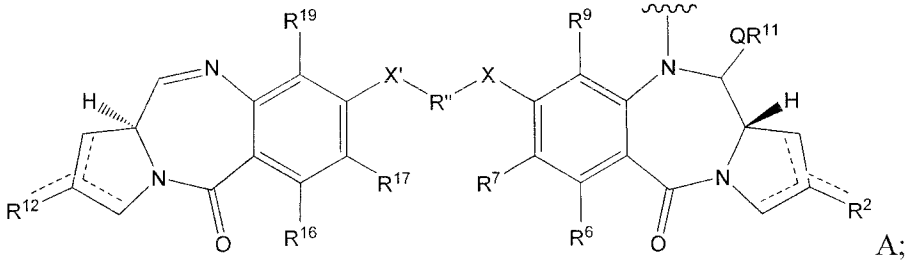
(b) L is a linker that links the Ab to D;

(c) D is a cytotoxic drug; and

(d) p ranges from 1-8.

15. The immunoconjugate of claim 13 or claim 14, wherein the cytotoxic drug is selected from a maytansinoid, a calicheamicin, a pyrrolobenzodiazepine, and a nemorubicin derivative.

16. The immunoconjugate of claim 14, wherein D is a pyrrolobenzodiazepine of Formula A:



A;

wherein the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

R^2 is selected from H, OH, =O, $=CH_2$, CN, R, OR, $=CH\text{-}R^D$, $=C(R^D)_2$, O-SO_2-R, CO_2R and COR, and optionally further selected from halo or dihalo, wherein R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

R^7 is selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

Q is selected from O, S and NH, and R^{11} is either H or R; or Q is O and R^{11} is SO_3M, wherein M is a metal cation;

R and R' are each independently selected from optionally substituted C_{1-8} alkyl, C_{3-8} heterocyclyl and C_{5-20} aryl groups, and optionally in relation to the group NRR', R and

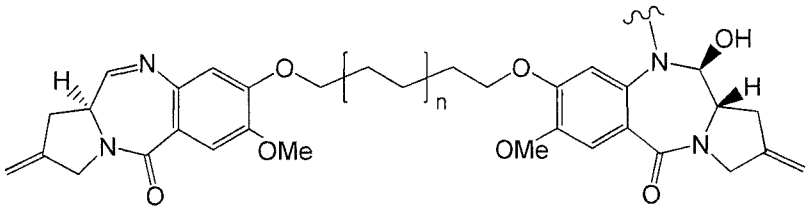
R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

R^{12}, R^{16}, R^{19} and R^{17} are as defined for R^2, R^6, R^9 and R^7 respectively;

R'' is a C_{3-12} alkylene group, which chain may be interrupted by one or more heteroatoms and/or aromatic rings that are optionally substituted; and

X and X' are independently selected from O, S and NH.

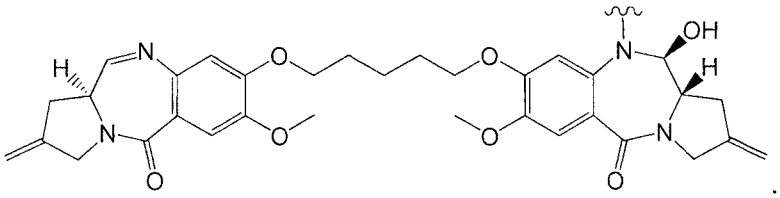
17. The immunoconjugate of claim 14, wherein D has a structure:



;

wherein n is 0 or 1.

18. The immunoconjugate of claim 17, wherein D has a structure:



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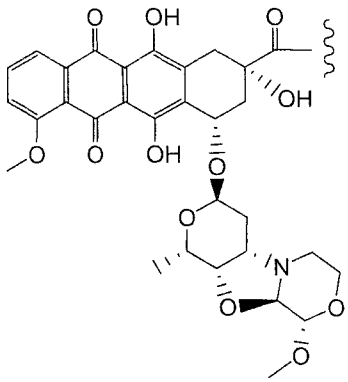
19. The immunoconjugate of claim 14, wherein D is a nemorubicin derivative.

20. The immunoconjugate of claim 19, wherein D has a structure:



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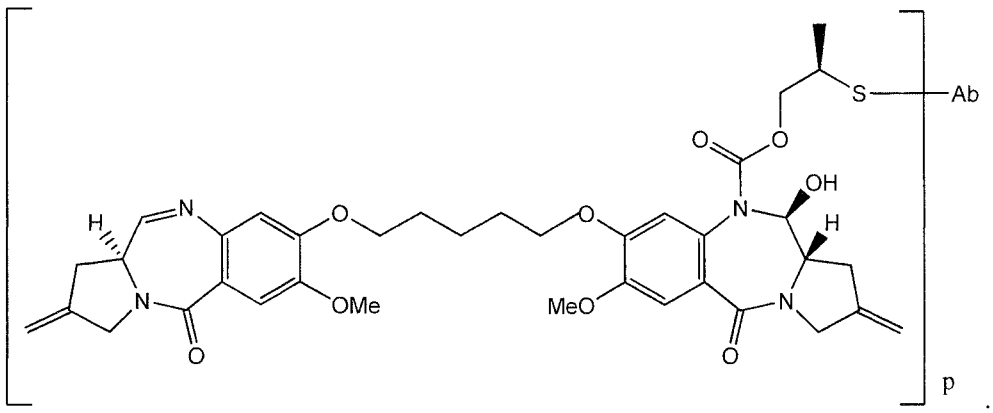
21. The immunoconjugate of claim 14, wherein the immunoconjugate has the structure:



.

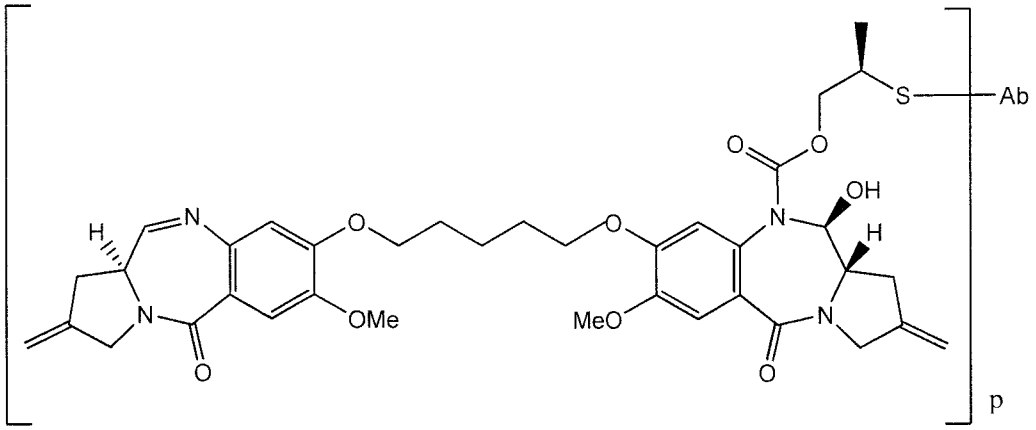
22. The immunoconjugate of any one of claims 14 to 21, wherein p ranges from 2-5.

23. The immunoconjugate of any one of claims 14 to 21, wherein p is 1.

24. The immunoconjugate of any one of claims 14 to 21, wherein p is 2.

25. The immunoconjugate of any one of claims 14 to 24, wherein Ab comprises an engineered free cysteine amino acid residue located in the light chain at K149C according to Kabat numbering and wherein Ab is an IgG1 antibody.

26. An immunoconjugate comprising a structure:

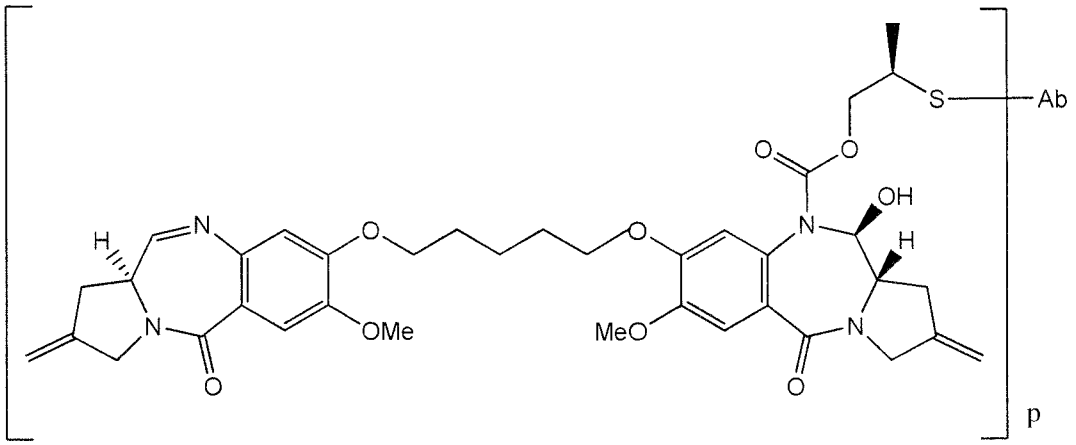


wherein Ab is an isolated monoclonal anti-CLL-1 antibody, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:11; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7;

wherein the antibody comprises an engineered free cysteine residue located in the light chain at K149C according to Kabat numbering; and

wherein p is 2.

27. An immunoconjugate comprising a structure:



wherein Ab is an isolated monoclonal anti-CLL-1 antibody, wherein the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:8; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO:11; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO:10; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:5; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:6; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:7;

wherein the antibody comprises an engineered free cysteine residue located in the light chain at K149C according to Kabat numbering; and

wherein p is 1.

28. The immunoconjugate of claim 26 or claim 27, wherein the antibody is a humanized antibody.

29. The immunoconjugate of any one of claims 26 to 28, wherein the antibody is an IgG1, IgG2a, or IgG2b antibody.

30. The immunoconjugate of claim 29, wherein the antibody is an IgG1 antibody.

31. A pharmaceutical formulation comprising the immunoconjugate of any one of claims 14 to 30 and a pharmaceutically acceptable carrier.

32. The pharmaceutical formulation of claim 31, further comprising an additional therapeutic agent.

33. The pharmaceutical formulation of claim 32, wherein the additional therapeutic agent is selected from 5-azacytidine and decitabine.

34. The pharmaceutical formulation of claim 33, wherein the additional therapeutic agent is 5-azacytidine.

35. The pharmaceutical formulation of claim 33, wherein the additional therapeutic agent is decitabine.

36. Use of the immunoconjugate of any one of claims 14 to 30 or the pharmaceutical formulation of any one of claims 31 to 35 in the manufacture of a medicament for treating an individual having a CLL-1-positive cancer.

37. The use of claim 36, wherein the CLL-1-positive cancer is AML.

38. The use of claim 36 or claim 37, wherein the medicament is to be administered with an additional therapeutic agent to the individual.

39. An *in vitro* method of inhibiting proliferation of a CLL-1-positive cell, the method comprising exposing the cell to the immunoconjugate of any one of claims 14 to 30 under conditions permissive for binding of the immunoconjugate to CLL-1 on the surface of the cell, thereby inhibiting proliferation of the cell.

40. The method of claim 39, wherein the cell is an AML cancer cell.

41. The antibody of any one of claims 1 to 9 conjugated to a label.

42. The antibody of claim 41, wherein the label is a positron emitter.

43. The antibody of claim 42, wherein the positron emitter is ^{89}Zr.

44. An *in vitro* method of detecting human CLL-1 in a biological sample comprising contacting the biological sample with the anti-CLL-1 antibody of any one of claims 1 to 9 under conditions permissive for binding of the anti-CLL-1 antibody to a naturally occurring human CLL-1, and detecting whether a complex is formed between the anti-CLL-1 antibody and a naturally occurring human CLL-1 in the biological sample.

45. The method of claim 44, wherein the biological sample is an AML cancer sample.

46. An antibody of any one of claims 1 to 9 for use in detecting a CLL-1-positive cancer in a subject.

47. The antibody of claim 46, wherein the labeled anti-CLL-1 antibody comprises an anti-CLL-1 antibody conjugated to a positron emitter.

48. The antibody of claim 47, wherein the positron emitter is ^{89}Zr.