Claims

1. A cysteine engineered anti-CD79b antibody comprising one or more free cysteine amino acids wherein said antibody comprises the sequence VTVSSCSTKGP including a free cysteine residue at position 114 of the heavy chain or the sequence GLSSPCTKSFN including a free cysteine residue at position 205 of the light chain, wherein numbering is according to Kabat numbering convention and wherein said anti-CD79b antibody comprises:

a. a heavy chain variable domain comprising the HVR-H1, HVR-H2, and HVR-H3 of SEQ ID NO: 202-204 and a light chain variable domain comprising the HVR-L1, HVR-L2 and HVR-L3 of SEQ ID NO: 194-196; or

b. a heavy chain variable domain comprising the HVR-H1, HVR-H2 and HVR-H3 of SEQ ID NO: 164-166 and a light chain variable domain comprising the HVR-L1, HVR-l2 and HVR-L3 of SEQ ID NO: 156-158; or

c. a heavy chain variable domain comprising the HVR-H1, HVR-H2 and HVR-H3 of SEQ ID NO: 183-185 and a light chain variable domain comprising the HVR-L1, HVR-L2 and HVR-L3 of SEQ ID NO: 175-177; or

d. a heavy chain variable domain comprising the HVR-H1, HVR-H2 and HVR-H3 of SEQ ID NO: 221-223 and a light chain variable domain comprising the HVR-L1, HVR-L2 and HVR-L3 of SEQ ID NO: 213-215.

2. The cysteine-engineered anti-CD79b antibody of claim 1 wherein said antibody comprises the sequence VTVSSCSTKGP including a free cysteine at position 114 of the heavy chain.

3. The cysteine-engineered anti-CD79b antibody of claim 1 wherein said antibody comprises the sequence GLSSPCTKSFN including a free cysteine at position 205 of the light chain.

4. The cysteine-engineered anti-CD79b antibody of claim 1 wherein the light chain variable domain comprises the sequence of SEQ ID NO: 207 and the heavy chain variable domain comprises the sequence of SEQ ID NO: 208.

5. The cysteine-engineered anti-CD79b antibody of claim 1 wherein said antibody comprises a light chain comprising the sequence of SEQ ID NO: 233 and a heavy chain comprising the sequence of SEQ ID NO: 232.

6. The cysteine-engineered anti-CD79b antibody of claim 1 wherein the antibody further comprises a free cysteine amino acid residue located at one or more positions selected from 5, 23, 84, and 112 of

the heavy chain according to Kabat numbering convention or 120, 282, 375, and 400 of the heavy chain according to EU numbering convention.

7. The cysteine-engineered anti-CD79b antibody of claim 1 wherein the antibody is prepared by a process comprising replacing one or more amino acid residue of a parent anti-CD79b antibody by cysteine and wherein the parent antibody comprises a heavy chain sequence selected from: SEQ ID NOS: 308, 304, 306, and 310

and/or

a light chain sequence selected from: SEQ ID NOS: 307, 303, 305, and 309.

8. A polynucleotide encoding the antibody of any one of the preceding claims.

9. A vector comprising the polynucleotide of claim 8.

10. A host cell comprising the vector of claim 9.

11. The host cell of claim 10 wherein the host cell is a prokaryotic host cell.

12. The cysteine engineered anti-CD79b antibody according to any one of claims 1 to 7 wherein the antibody is covalently attached through one or more free cysteine amino acids to a label moiety or a solid support.

13. The cysteine engineered anti-CD79b antibody according to any one of claims 1 to 7 wherein the antibody is covalently attached through the one or more free cysteine amino acids to a growth inhibitory agent or a cytotoxic agent, whereby an antibody drug conjugate is formed.

14. The cysteine engineered anti-CD79b antibody according to any one of claims 1 to 7 wherein the antibody is covalently attached to a drug moiety whereby an antibody-drug conjugate is formed.

15. The cysteine engineered anti-CD79b antibody according to claim 14 comprising a cysteine engineered anti-CD79b antibody (Ab), and a drug moiety (D) wherein the cysteine engineered anti CD79b antibody is attached through one or more free cysteine amino acids by a linker moiety (L) to D; the compound having Formula I:

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$Ab-(L-D)_P$ I

where p is 1, 3, 4, or preferably 2.

16. The cysteine engineered anti-CD79b antibody according to claim 15 wherein L is

$-A_a-W_w-Y_y-$

where:

A is Stretcher unit covalently attached to a cysteine thiol of the cysteine engineered antibody (ab):

a is 0 or 1;

each W is independently an Amino Acid unit;

w is an integer ranging from 0 to 12;

Y is a Spacer unit covalently attached to the drug moiety; and

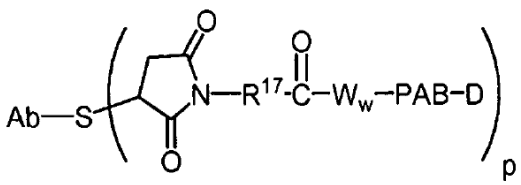
Y is 0, 1 or 2.

17. The cysteine engineered anti-CD79b antibody according to any one of claims 14 to 16 wherein the drug moiety (D) is selected from the group consisting of a maytansinoid, an auristatin, and a dolostatin.

18. The cysteine engineered anti-CD79b antibody according to claim 15 wherein the drug moiety (D) is selected from the group consisting of a maytansinoid and an auristatin.

19. The cysteine engineered anti-CD79b antibody according to claim 18 wherein the antibody-drug conjugate has the formula:

(a)

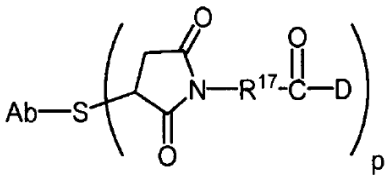


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where PAB is para-aminobenzylcarbamoyl, and R^{17} is a divalent radical selected from $(CH_2)_r$, C_3-C_8 carbocyclyl, O-$(CH_2)_r$ arylene, $(CH_2)_r$-arylene, -arylene-$(CH_2)_r$-, $(CH_2)_r$-(C_3-C_8 carbocyclyl), (C_3-C_8 carbocyclyl)-$(CH_2)_r$, C_3-C_8 heterocyclyl, $(CH_2)_r$-(C_3-C_8 heterocyclyl), -(C_3-C_8 heterocyclyl)-$(CH_2)_r$--$(CH_2)_rC(O)NR^b(CH_2)_r$-, -$(CH_2CH_2O)_r$-, -$(CH_2CH_2O)_r$-CH_2-, -$(CH_2)_rC(O)NR^b(CH_2CH_2^b)_r$-, -$(CH_2)_rC(O)NR^b(CH_2CH_2O)_r$-$CH_2$-, -$(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r$-, -$(CH_2CH_2O)_rC(O)NR^b(CH_2CH_2O)_r$-$CH_2$-, and –$(CH_2CH_2O)_rC(O)NR^b(CH_2)_r$; where R^b is H, C_1-C_6 alkyl, phenyl, or benzyl; and r is independently an integer ranging from 1 to 10.

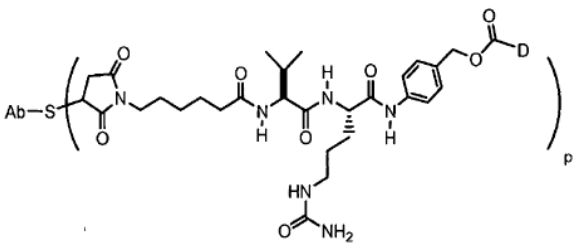
20. The cysteine engineered anti-CD79b antibody of claim 19, wherein W_w is valine-citrulline.

21. The cysteine engineered anti-CD79b antibody of claim 18 having the formula:



22. The cysteine engineered anti-CD79b antibody of claim 21, wherein R^{17} is $(CH_2)_5$ or $(CH_2)_2$.

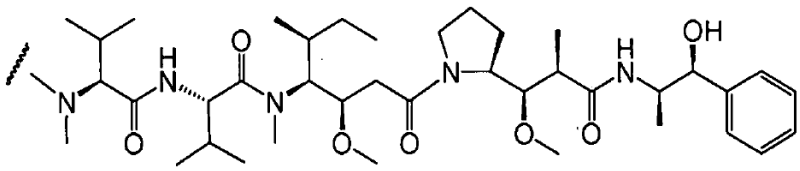
23. The cysteine engineered anti-CD79b antibody of claim 21 having the formula:



24. The cysteine engineered anti-CD79b antibody of claim 21, wherein L is SMCC, SPP, SPDB or BMPEO.

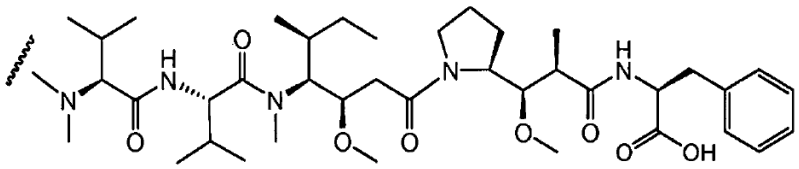
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25. The cysteine engineered anti-CD79b antibody of claim 15, wherein D is either MMAE, preferably having the structure:



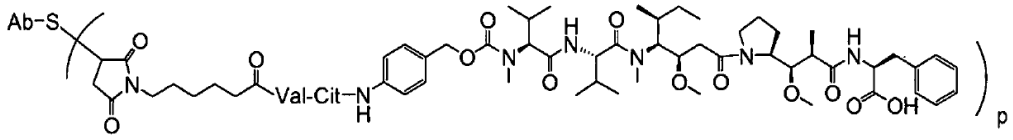
where the wavy line indicated that attachment site to the linker L;

or MMAF, preferably having the structure:



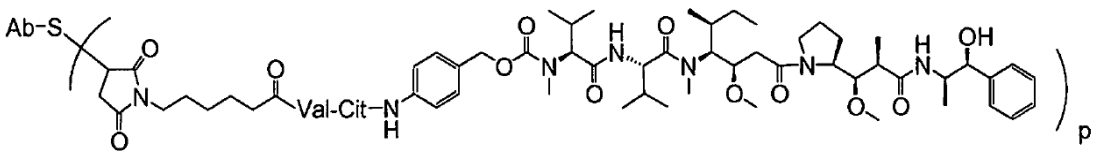
where the wavy line indicated the attachment site to the linker L.

26. An antibody-drug conjugate compound selected from the structures:



Ab-MC-vc-PAB-MMAF

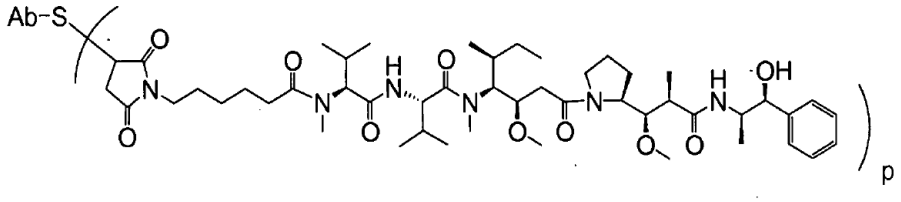
wherein Val is valine; Cit is citrulline; p is 1, 2, 3, or 4;



Ab-MC-vc-PAB-MMAE

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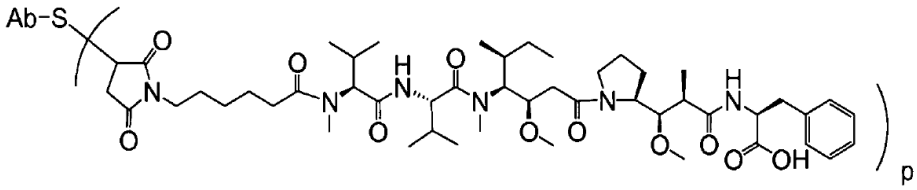
wherein Val is valine; Cit is citrulline; p is 1, 2, 3, or4;



Ab-MC-MMAE

wherein Val is valine; Cit is citrulline; p is 1, 2, 3, or 4;

or



Ab-MC-MMAF

wherein Val is valine; Cit is citrulline; p is 1, 2, 3, or 4 and Ab is an antibody according to any one of claims 1 to 7 and wherein the antibody is conjugated through one or more free cysteine amino acids.

27. The cysteine engineered anti-CD79b antibody of any one of claims 1 to 7 or the antibody-drug conjugate compound of claim 13 to 26 wherein the parent anti-CD79b antibody is selected from a monoclonal antibody, a bispecific antibody, a chimeric antibody, a human antibody, a humanized antibody and an antibody fragment, preferably a Fab fragment.

28. A pharmaceutical formulation comprising the cysteine engineered anti-CD79b antibody of any one of claims 1 to 7 or the antibody drug conjugate of any one of claims 13 to 27, and a pharmaceutically acceptable diluent, carrier, or excipient, and optionally also a therapeutically effective amount of a chemotherapeutic agent.

29. A method of determining the presence of CD79b protein in a sample suspected of containing said protein, said method comprising exposing said sample to a cysteine engineered anti-CD79b antibody of any one of claims 1 to 7 or 12 to 27 and determining binding of said antibody to said CD79b protein in said sample, wherein binding of the antibody to said protein is indicative of the presence of said protein in said sample.

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30. The method of claim 29, wherein said antibody is covalently attached to a label selected from a fluorescent dye, a radioisotope, biotin, or a metal-complexing ligand; or wherein said sample comprises a cell suspected of expressing said CD79b protein, or wherein said cell is a hematopoietic cancer cell.

31. A method of inhibiting cellular proliferation comprising treating mammalian tumor cells ex vivo with an antibody-drug conjugate compound of any one of claims 13 to 27, whereby proliferation of the tumor cells is inhibited, wherein the mammalian tumor cells are preferably hematopoietic tumor cells.

32. The antibody of any one of claims 1 to 7, the antibody-drug conjugate of any one of claims 13 to 27 or the pharmaceutical formulation of claim 28 for use in a method of treating cancer, wherein preferably the cancer is selected from the group consisting of lymphoma, myeloma, non-Hodgkins lymphoma (NHL), diffuse large B-cell lymphoma, aggressive NHL, indolent NHL, follicular lymphoma, relapsed aggressive NHL, relapsed indolent NHL, refractory NHL, refractory indolent NHL, chronic lymphocytic leukemia (CLL), small lymphocytic lymphoma, leukemia, hairy cell leukemia (HCL), acute lymphocytic leukemia (ALL), and mantle cell lymphoma.

33. The antibody, antibody-drug conjugate or pharmaceutical formulation for use according to claim 32, wherein the method comprises administering a therapeutic agent to the patient in combination with the antibody-drug conjugate compound.

34. A method for making an antibody drug conjugate compound comprising a cysteine engineered anti-CD79b antibody (Ab) of any one of claims 1 to 7 and an auristatin drug moiety (D) wherein the cysteine engineered antibody is attached through one or more engineered cysteine amino acids by a linker moiety (L) to D; the compound having Formula I:

$$Ab\text{-}(L\text{-}D)_p \qquad I$$

Where p is 1, 2, 3, or 4; the method comprising the steps of:

(a) reacting an engineered cysteine group of the cysteine engineered antibody with a linker reagent to form antibody-linker intermediate Ab-L; and

(b) reacting AB-L with an activating drug moiety D; whereby the antibody-drug conjugate is formed;

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or comprising the steps of:

(c)reacting a nucleophilic group of a drug moiety with a linker reagent to form drug-linker intermediate D-L; and

(d) reacting D-L with an engineered cysteine group of the cysteine engineered antibody; whereby the antibody-drug conjugate is formed;

wherein the method optionally comprises the step of expressing the cysteine engineered antibody in Chinese hamster ovary (CHO) cells.

35. The method of claim 34, further comprising the step of treating the expressed cysteine engineered antibody with a reducing agent, wherein the reducing agent is preferably selected from TCEP and DTT.

36. The method of claim 35, further comprising the step of treating the expressed cysteine engineered antibody with an oxidizing agent, after treating with the reducing agent, wherein the oxidizing agent is preferably selected from copper sulfate, dehydroascorbic acid, and air.

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