conjugates have been engineered with drug attachment at different parts of the antibody (light chain-Fab, heavy chain-Fab and heavy chain-Fc). The *in vitro & in vivo* stability, efficacy and PK properties of TDC provide a unique advantage over conventional ADC due to their homogeneity and site-specific conjugation to cytotoxic drugs.

[0010] SUMMARY

[0011] The ~~invention~~disclosure includes an isolated cysteine engineered antibody comprising a free cysteine amino acid in the heavy chain or light chain.

[0011a] In one aspect, there is provided an isolated cysteine engineered antibody comprising a free cysteine amino acid and a sequence in the heavy chain selected from

HC-F27C	CAASGCNIKDT	SEQ ID NO: 56
HC-K76C	SADTSCNTAYL	SEQ ID NO: 66
HC-T78C	DTSKNCAYLQM	SEQ ID NO: 67
HC-L81C	KNTAYCQMNSL	SEQ ID NO: 69

or a sequence in the light chain selected from

LC-I75C	DFTLTCSSLQP	SEQ ID NO: 106
LC-Y92C	YCQQHCTTPPT	SEQ ID NO: 110
LC-N137C	VVCLLCNFYPR	SEQ ID NO: 127
LC-N138C	VCLLNCFYPRE	SEQ ID NO: 128
LC-K149C	AKVQWCVDNAL	SEQ ID NO: 133

wherein a cysteine in the sequence is the free cysteine amino acid.

[0012] Also disclosed~~An aspect of the invention~~ is a process to prepare the isolated cysteine engineered antibody by mutagenizing a nucleic acid sequence of a parent antibody by replacing one or more amino acid residues by cysteine to encode the cysteine engineered antibody; expressing the cysteine engineered antibody; and isolating the cysteine engineered antibody.

[0013] Further disclosed~~Another aspect of the invention~~ is a conjugate of the isolated cysteine engineered antibody wherein the antibody is covalently attached to a capture label, a detection label, a drug moiety, or a solid support.

[0014] BRIEF DESCRIPTION OF THE DRAWINGS

[0015] Figure 1A shows a three-dimensional representation of the hu4D5Fabv7 antibody fragment derived by X-ray crystal coordinates. The structure positions of the exemplary engineered Cys residues of the heavy and light chains are numbered (according to a sequential numbering system).

[0016] Figure 1B shows a sequential numbering scheme (top row), starting at the N-terminus in comparison with the Kabat numbering scheme (bottom row) for 4D5v7fabH. Kabat numbering insertions are noted by a,b,c.

[0017] Figures 2A and 2B show binding measurements with detection of absorbance at 450nm of hu4D5Fabv8 and hu4D5Fabv8 Cys mutant (ThioFab) phage variants: (A) non-biotinylated phage-hu4D5Fabv8 and (B) biotinylated phage-hu4D5Fabv8 (B) by the PHESELECTOR assay for interactions with BSA (open bar), HER2 (striped bar) or streptavidin (solid bar).

[0018] Figures 3A and 3B show binding measurements with detection of absorbance at 450nm of hu4D5Fabv8 (left) and hu4D5Fabv8 Cys mutant (ThioFab) variants: (A) non-biotinylated phage-hu4D5Fabv8 and (B) biotinylated phage-hu4D5Fabv8 by the PHESELECTOR assay for interactions with: BSA (open bar), HER2 (striped bar) and

We claim:

1. An isolated cysteine engineered antibody comprising a free cysteine amino acid and a sequence in the heavy chain selected from SEQ ID NOS 50-98 or a sequence in the light chain selected from SEQ ID NOS 99-147 wherein a cysteine in the sequence is the free cysteine amino acid.

2. The isolated cysteine engineered antibody of claim 1 prepared by a process comprising:

(i) mutagenizing a nucleic acid sequence of a parent antibody by replacing one or more amino acid residues by cysteine to encode the cysteine engineered antibody;

(ii) expressing the cysteine engineered antibody; and

(iii) isolating the cysteine engineered antibody.

3. The cysteine engineered antibody of claim 2 wherein mutagenizing comprises site-directed mutagenesis.

4. The cysteine engineered antibody of claim 2 wherein the cysteine engineered antibody is expressed on a viral particle selected from a phage or a phagemid particle.

5. The cysteine engineered antibody of claim 2 further comprising:

(i) reacting the cysteine engineered antibody with a thiol-reactive affinity reagent to generate an affinity labelled, cysteine engineered antibody; and

(ii) measuring the binding of the affinity labelled, cysteine engineered antibody to a capture media.

6. The cysteine engineered antibody of claim 5 wherein the thiol-reactive affinity reagent comprises a biotin moiety.

7. The cysteine engineered antibody of claim 5 wherein the thiol-reactive reagent comprises a maleimide moiety.

8. The cysteine engineered antibody of claim 5 wherein the capture media comprises streptavidin.

9. The cysteine engineered antibody of claim 1 wherein the cysteine engineered

antibody is a fusion protein comprising the albumin-binding peptide (ABP).

10. The cysteine engineered antibody of claim 9 wherein the ABP comprises a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5.

11. The cysteine engineered antibody of claim 1 wherein the cysteine engineered antibody is selected from a monoclonal antibody, an antibody fragment, a bispecific antibody, a chimeric antibody, a human antibody, and a humanized antibody.

12. The cysteine engineered antibody of claim 11 wherein the antibody fragment is a Fab fragment.

13. The cysteine engineered antibody of claim 11 wherein the cysteine engineered antibody is an anti-HER2 antibody.

14. The cysteine engineered antibody of claim 1 wherein the cysteine engineered antibody binds to one or more of receptors (1)-(36):

(1) BMPR1B (bone morphogenetic protein receptor-type IB);

(2) E16 (LAT1, SLC7A5);

(3) STEAP1 (six transmembrane epithelial antigen of prostate);

(4) 0772P (CA125, MUC16);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene);

(9) ETBR (Endothelin type B receptor);

(10) MSG783 (RNF124, hypothetical protein FLJ20315);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor);

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792);

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C);

(17) HER2;

(18) NCA;

(19) MDP;

(20) IL20Rα;

(21) Brevican;

(22) EphB2R;

(23) ASLG659;

(24) PSCA;

(25) GEDA;

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3;

(27) CD22 (B-cell receptor CD22-B isoform);

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family);

(34) FcRH1 (Fc receptor-like protein 1);

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2); and

(36) TENB2 (putative transmembrane proteoglycan).

15. The cysteine engineered antibody of claim 1 wherein the antibody is covalently attached to a capture label, a detection label, a drug moiety, or a solid support.

16. The cysteine engineered antibody of claim 15 wherein the antibody is covalently attached to a biotin capture label.

17. The cysteine engineered antibody of claim 15 wherein the antibody is covalently attached to a fluorescent dye detection label.

18. The cysteine engineered antibody of claim 17 wherein the fluorescent dye is selected from a fluorescein type, a rhodamine type, dansyl, Lissamine, a cyanine, a phycoerythrin, Texas Red, and an analog thereof.

19. The cysteine engineered antibody of claim 15 wherein the antibody is covalently attached to a radionuclide detection label selected from ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, and ^{213}Bi.

20. The cysteine engineered antibody of claim 15 wherein the antibody is covalently attached to a detection label by a chelating ligand.

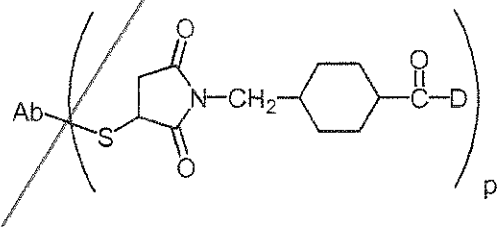
21. The cysteine engineered antibody of claim 20 wherein the chelating ligand is selected from DOTA, DOTP, DOTMA, DTPA and TETA.

22. The cysteine engineered antibody of claim 15 wherein the antibody is covalently attached to a drug moiety selected from a maytansinoid, an auristatin, a dolastatin, a trichothecene, CC1065, a calicheamicin, enediyne antibiotics, a taxane, and an anthracycline to form an antibody-drug conjugate having Formula I:

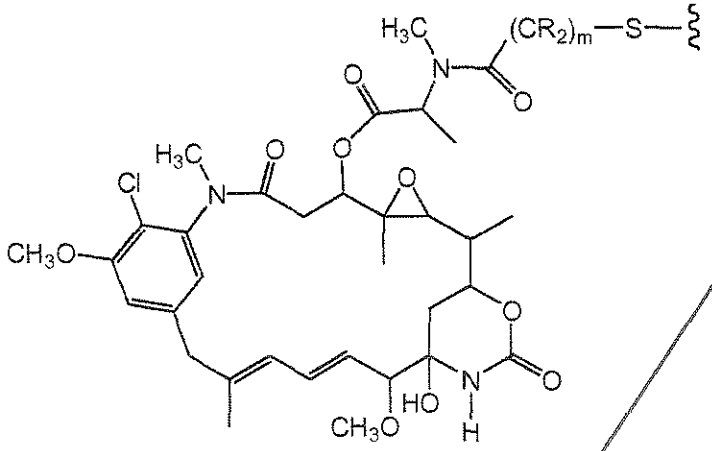
$$Ab-(L-D)_p \qquad I$$

where Ab is the antibody, L is a linker, D is the drug moiety, and p is 1, 2, 3, or 4.

23. The cysteine engineered antibody of claim 22 wherein the antibody-drug conjugate has the structure:



24. The cysteine engineered antibody of claim 22 wherein D is a maytansinoid having the structure:

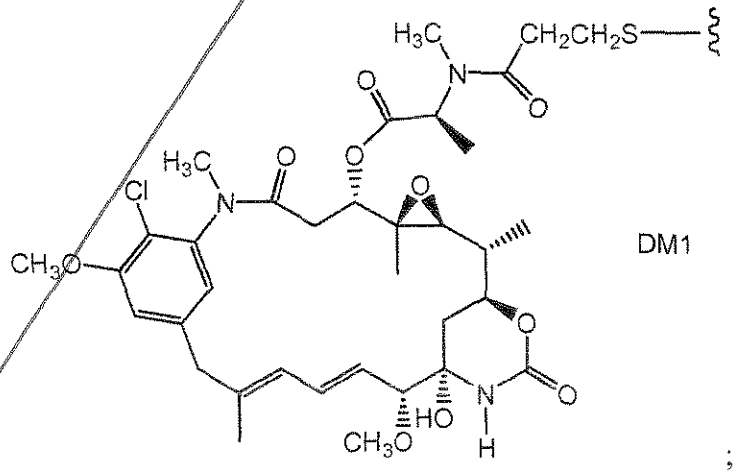


wherein the wavy line indicates the covalent attachment of the sulfur atom of D to the linker;

R is independently selected from H, methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-methyl-1-propyl, 2-butyl, 2-methyl-2-propyl, 1-pentyl, 2-pentyl, 3-pentyl, 2-methyl-2-butyl, 3-methyl-2-butyl, 3-methyl-1-butyl, 2-methyl-1-butyl, 1-hexyl, 2-hexyl, 3-hexyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 3-methyl-3-pentyl, 2-methyl-3-pentyl, 2,3-dimethyl-2-butyl, and 3,3-dimethyl-2-butyl; and

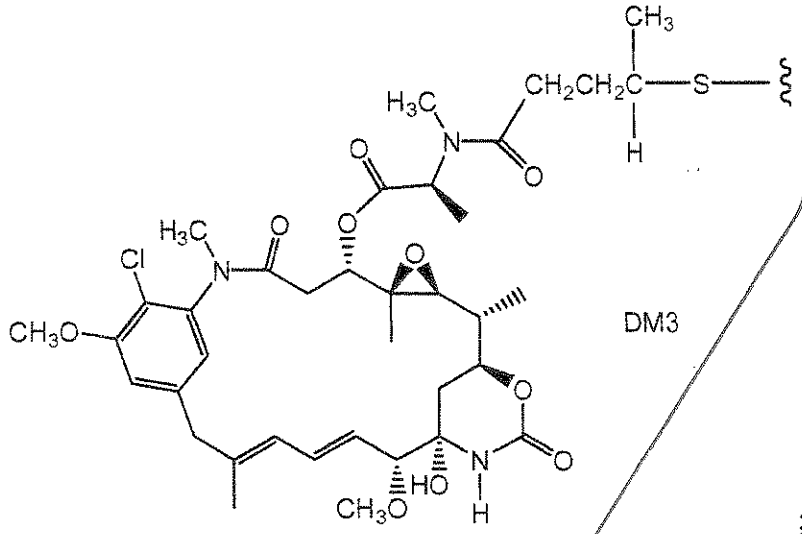
m is 1, 2, or 3.

25. The cysteine engineered antibody of claim 24 wherein D is selected from the structures:



DM1

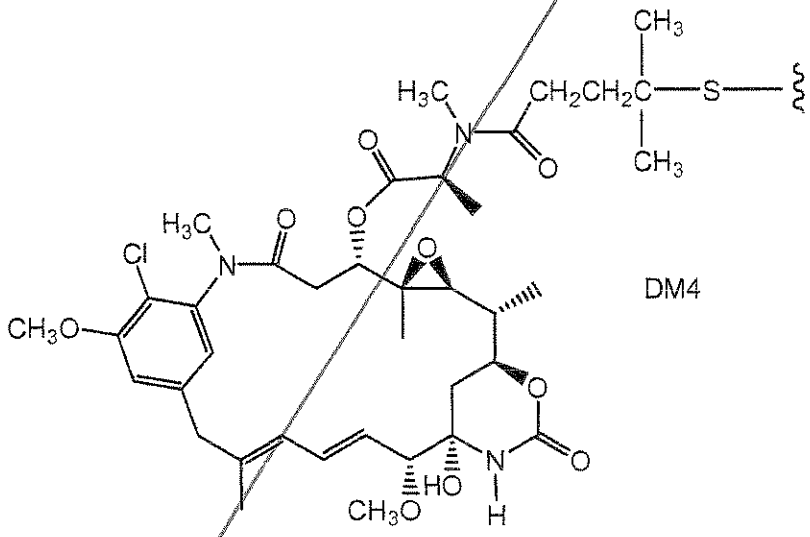
;



DM3

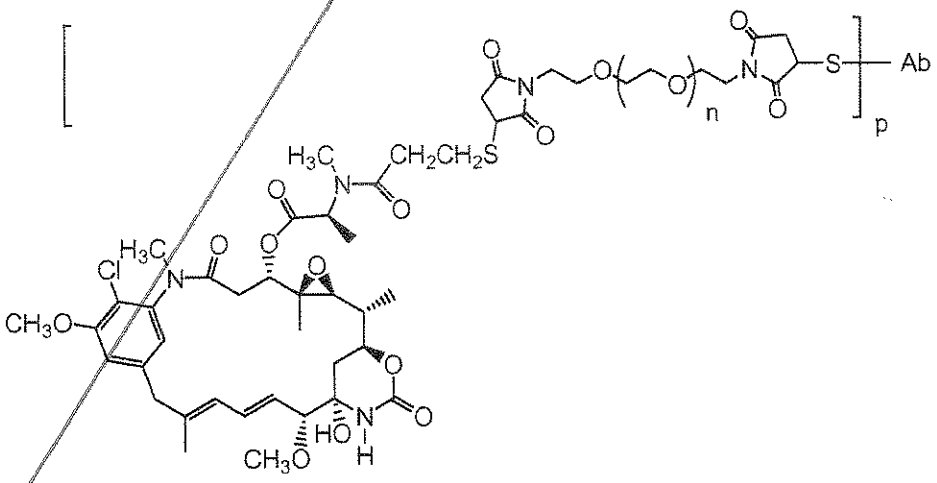
;

and



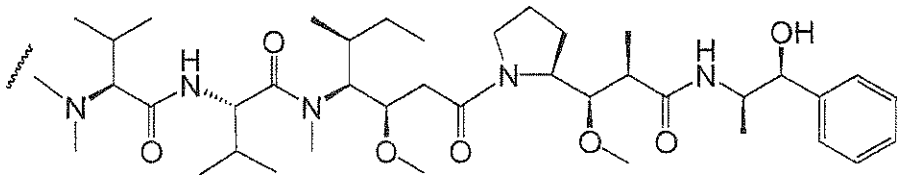
DM4

26. The cysteine engineered antibody of claim 25 having the structure:

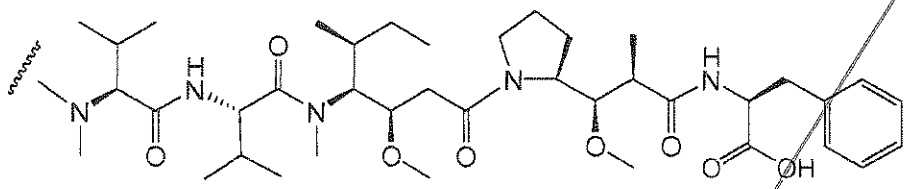


wherein n is 0, 1, or 2.

27. The cysteine engineered antibody of claim 22 wherein D is a monomethylauristatin drug moiety MMAE or MMAF having the structures:

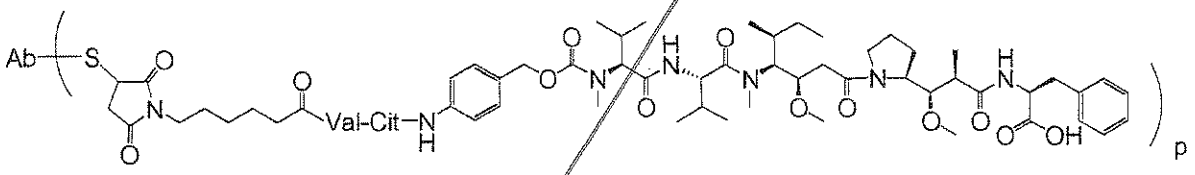


MMAE

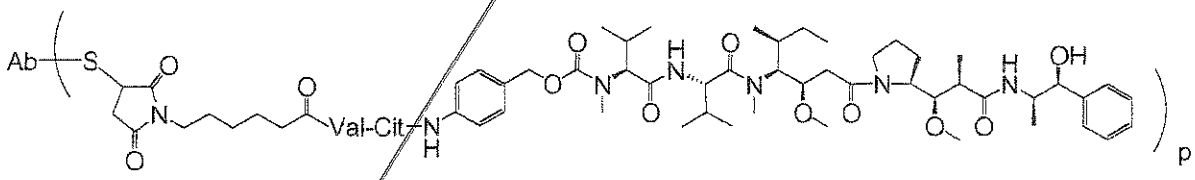


MMAF .

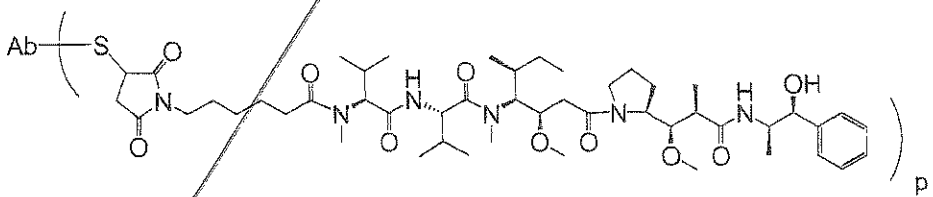
28. The cysteine engineered antibody of claim 27 wherein the antibody-drug conjugate is selected from the structures:



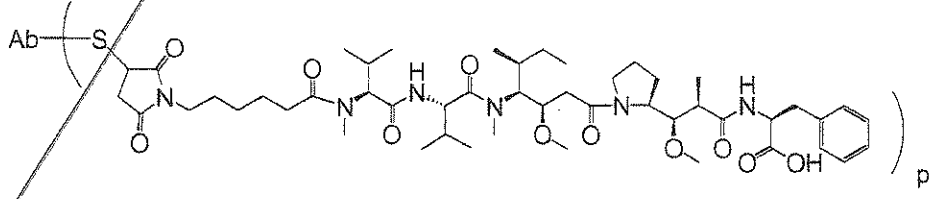
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



where Val is valine; Cit is citrulline; and p is 1, 2, 3, or 4.

CLAIMS:

1. An isolated cysteine engineered antibody comprising a free cysteine amino acid and a sequence in the heavy chain selected from

HC-F27C	CAASGCNIKDT	SEQ ID NO: 56
HC-K76C	SADTSCNTAYL	SEQ ID NO: 66
HC-T78C	DTSKNCAYLQM	SEQ ID NO: 67
HC-L81C	KNTAYCQMNSL	SEQ ID NO: 69

or a sequence in the light chain selected from

LC-I75C	DFTLTCSSLQP	SEQ ID NO: 106
LC-Y92C	YCQQHCTTPPT	SEQ ID NO: 110
LC-N137C	VVCLLCNFYPR	SEQ ID NO: 127
LC-N138C	VCLLNCFYPRE	SEQ ID NO: 128
LC-K149C	AKVQWCVDNAL	SEQ ID NO: 133

wherein a cysteine in the sequence is the free cysteine amino acid.

2. The isolated cysteine engineered antibody of claim 1 prepared by a process comprising:

(i) mutagenizing a nucleic acid sequence of a parent antibody by replacing one or more amino acid residues by cysteine to encode the cysteine engineered antibody;

(ii) expressing the cysteine engineered antibody; and

(iii) isolating the cysteine engineered antibody.

3. The cysteine engineered antibody of claim 2 wherein mutagenizing comprises site-directed mutagenesis.

4. The cysteine engineered antibody of claim 2 wherein the cysteine engineered antibody is expressed on a viral particle selected from a phage or a phagemid particle.

5. The cysteine engineered antibody of claim 2 wherein the process further comprises

(i) reacting the free cysteine of the cysteine engineered antibody with a thiol-reactive affinity reagent to generate an affinity labelled, cysteine engineered antibody comprising the affinity reagent covalently attached to the free cysteine; and

(ii) measuring the binding of the affinity labelled, cysteine engineered antibody to a capture media.

6. The cysteine engineered antibody of claim 5 wherein the thiol-reactive affinity reagent comprises a biotin moiety.

7. The cysteine engineered antibody of claim 5 wherein the thiol-reactive reagent comprises a maleimide moiety.

8. The cysteine engineered antibody of claim 5 wherein the capture media comprises streptavidin.

9. The cysteine engineered antibody of claim 1 wherein the cysteine engineered antibody is a fusion protein comprising the albumin-binding peptide (ABP).

10. The cysteine engineered antibody of claim 9 wherein the ABP comprises a sequence selected from SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, and SEQ ID NO: 5.

11. The cysteine engineered antibody of claim 1 wherein the cysteine engineered antibody is selected from a monoclonal antibody, an antibody fragment, a bispecific antibody, a chimeric antibody, a human antibody, and a humanized antibody.

12. The cysteine engineered antibody of claim 11 wherein the antibody fragment is a Fab fragment.

13. The cysteine engineered antibody of claim 11 wherein the cysteine engineered antibody is an anti-HER2 antibody.

14. The cysteine engineered antibody of claim 1 wherein the cysteine engineered antibody binds to one or more of receptors (1)-(36):

(1) BMPR1B (bone morphogenetic protein receptor-type IB);

(2) E16 (LAT1, SLC7A5);

(3) STEAP1 (six transmembrane epithelial antigen of prostate);

(4) 0772P (CA125, MUC16);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin);

(6) Napi3b (NAPI-3B, NPTIIb, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b);

(7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B);

(8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene);

(9) ETBR (Endothelin type B receptor);

(10) MSG783 (RNF124, hypothetical protein FLJ20315);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein);

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4);

(13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor);

(14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792);

(15) CD79b (CD79B, CD79β, IGb (immunoglobulin-associated beta), B29);

(16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C);

(17) HER2;

(18) NCA;

(19) MDP;

(20) IL20Rα;

(21) Brevican;

(22) EphB2R;

(23) ASLG659;

(24) PSCA;

(25) GEDA;

(26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3;

(27) CD22 (B-cell receptor CD22-B isoform);

(28) CD79a (CD79A, CD79α, immunoglobulin-associated alpha, a B cell-specific protein);

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor);

(30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen);

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5);

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2);

(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family);

(34) FcRH1 (Fc receptor-like protein 1);

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2); and

(36) TENB2 (putative transmembrane proteoglycan).

15. The cysteine engineered antibody of claim 1 wherein the free cysteine of the antibody is covalently attached to a capture label, a detection label, a drug moiety, or a solid support.

16. The cysteine engineered antibody of claim 15 wherein the free cysteine of the antibody is covalently attached to a biotin capture label.

17. The cysteine engineered antibody of claim 15 wherein the free cysteine of the antibody is covalently attached to a fluorescent dye detection label.

18. The cysteine engineered antibody of claim 17 wherein the fluorescent dye is selected from a fluorescein type, a rhodamine type, dansyl, Lissamine, a cyanine, a phycoerythrin, Texas Red, and an analog thereof.

19. The cysteine engineered antibody of claim 15 wherein the detection label comprises a radionuclide selected from ^{3}H, ^{11}C, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{64}Cu, ^{68}Ga, ^{86}Y, ^{89}Zr, ^{99}Tc, ^{111}In, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{133}Xe, ^{177}Lu, ^{211}At, and ^{213}Bi.

20. The cysteine engineered antibody of claim 15 wherein the free cysteine of the antibody is covalently attached to a detection label by a chelating ligand.

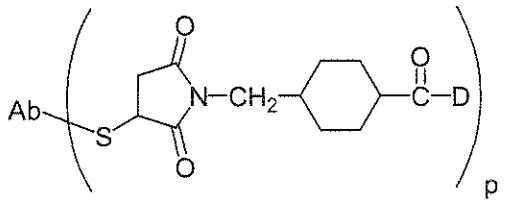
21. The cysteine engineered antibody of claim 20 wherein the chelating ligand is selected from DOTA, DOTP, DOTMA, DTPA and TETA.

22. The cysteine engineered antibody of claim 15 wherein the free cysteine of the antibody is covalently attached to a drug moiety selected from a maytansinoid, an auristatin, a dolastatin, a trichothecene, CC1065, a calicheamicin, enediyne antibiotics, a taxane, and an anthracycline to form an antibody-drug conjugate having Formula I:

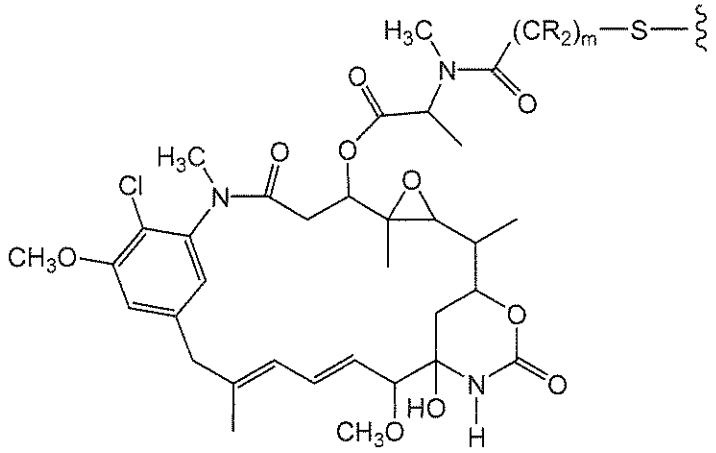
$$Ab–(L–D)_p \qquad I$$

where Ab is the antibody, L is a linker, D is the drug moiety, and p is 1, 2, 3, or 4.

23. The cysteine engineered antibody of claim 22 wherein the antibody-drug conjugate has the structure:



24. The cysteine engineered antibody of claim 22 wherein D is a maytansinoid having the structure:



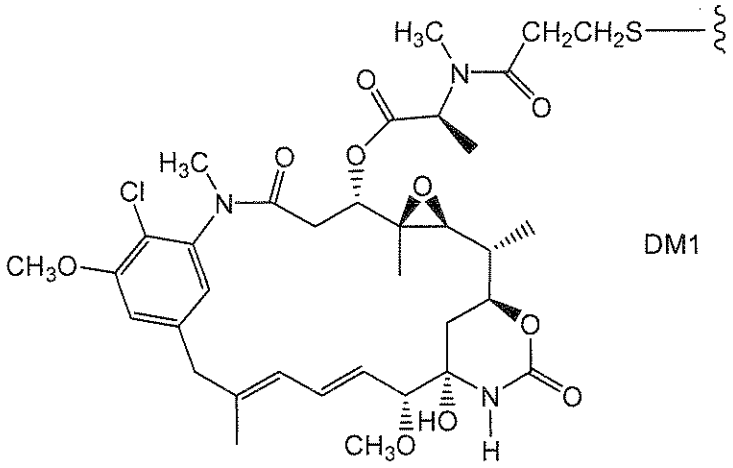
wherein the wavy line indicates the covalent attachment of the sulfur atom of D to the linker;

R is independently selected from H, methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-methyl-1-propyl, 2-butyl, 2-methyl-2-propyl, 1-pentyl, 2-pentyl, 3-pentyl, 2-methyl-2-butyl, 3-methyl-2-

butyl, 3-methyl-1-butyl, 2-methyl-1-butyl, 1-hexyl, 2-hexyl, 3-hexyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 3-methyl-3-pentyl, 2-methyl-3-pentyl, 2,3-dimethyl-2-butyl, and 3,3-dimethyl-2-butyl; and

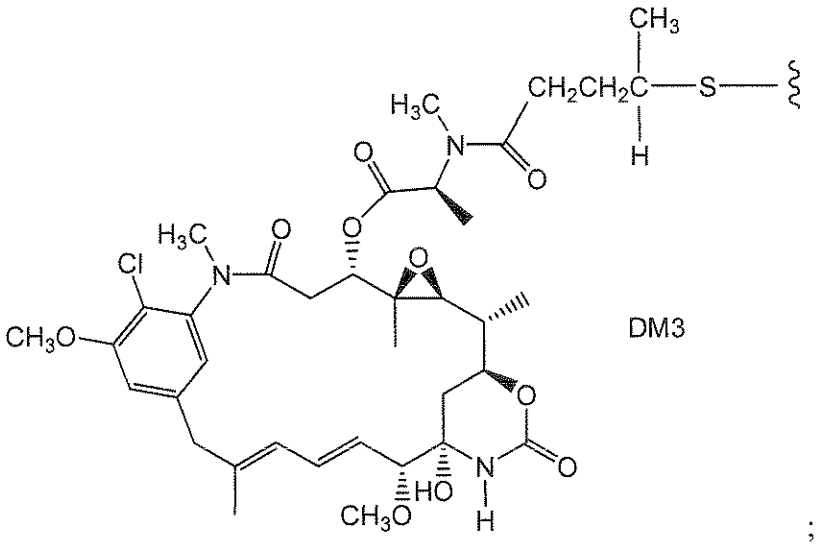
m is 1, 2, or 3.

25. The cysteine engineered antibody of claim 24 wherein D is selected from the structures:



DM1

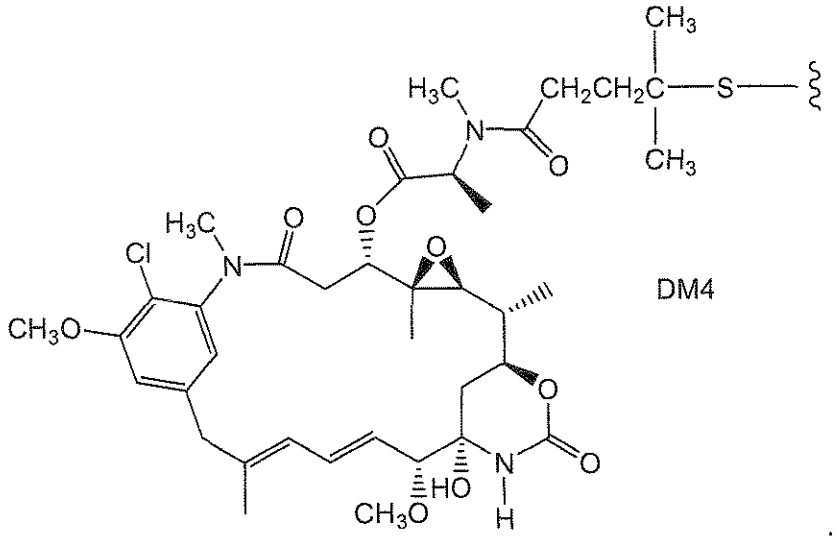
;



DM3

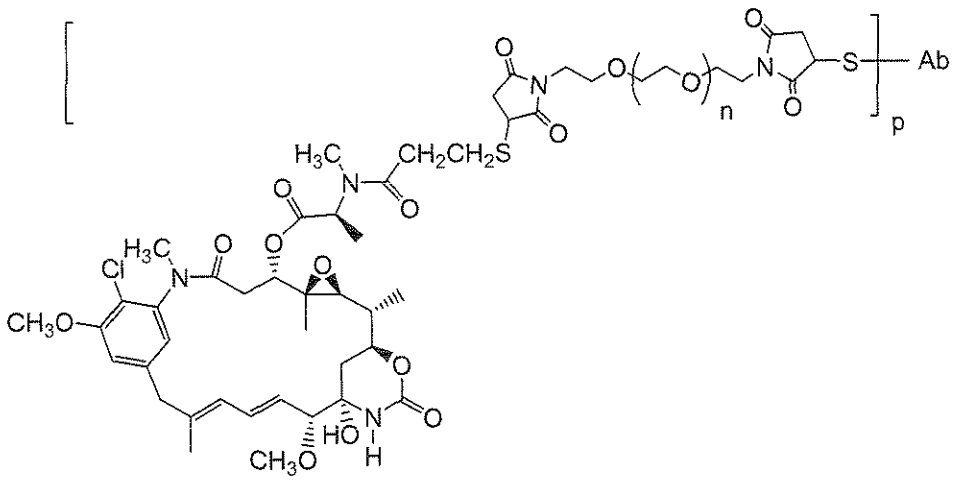
;

and



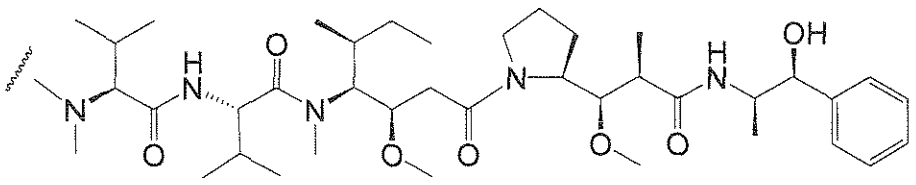
DM4

26. The cysteine engineered antibody of claim 25 having the structure:

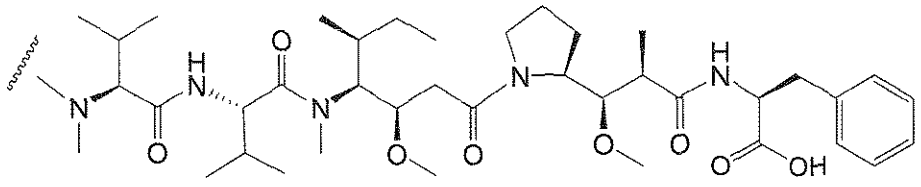


wherein n is 0, 1, or 2.

27. The cysteine engineered antibody of claim 22 wherein D is a monomethylauristatin drug moiety MMAE or MMAF having the structures:

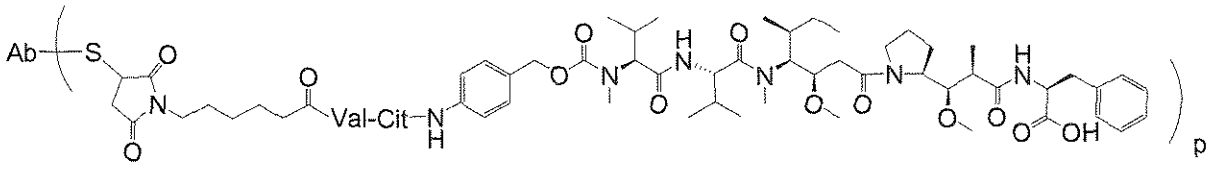


MMAE

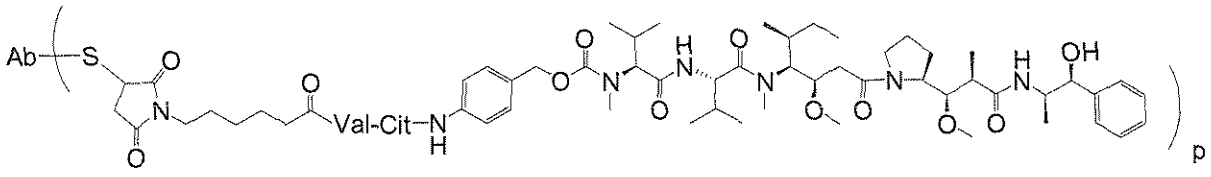


MMAF .

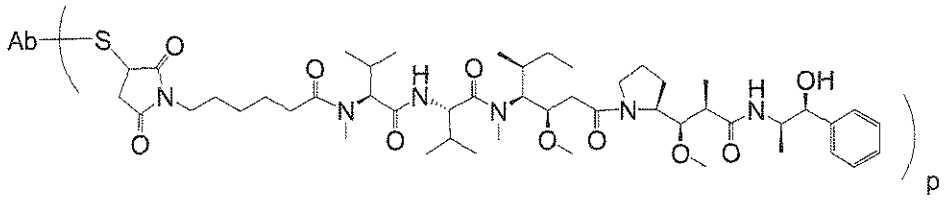
28. The cysteine engineered antibody of claim 27 wherein the antibody-drug conjugate is selected from the structures:



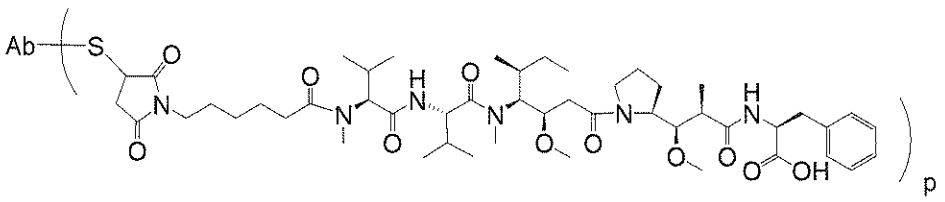
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



Ab-MC-MMAF

where Val is valine; Cit is citrulline; and p is 1, 2, 3, or 4.