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

[0010a] In one aspect, there is provided use of an antibody-drug conjugate of Formula I:

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

in the manufacture of a medicament for treating cancer, where Ab is a cysteine engineered antibody, L is a linker, D is a drug moiety, and p is 1, 2, 3, or 4; wherein the cysteine engineered antibody comprises one or more free cysteine amino acids, wherein at least one free cysteine amino acid is located at a position selected from heavy chain positions 4, 20, 23, 27, 32, 47, 68, 76, 78, 80, 81, 82, 94, 98, 108, 113, 154, 162, and 164 by Kabat numbering and light chain positions 49, 53, 73, 75, 80, 92, 95, 99, 101, 137, 138, 149, and 183 by Kabat numbering.

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

[0012] Disclosed also 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 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. Use of an antibody-drug conjugate of formula I:

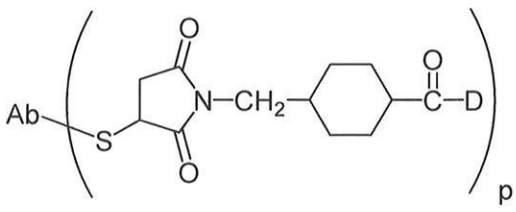
$$Ab–(L–D)_p \qquad I$$

in the manufacture of a medicament for treating cancer,
where Ab is a cysteine engineered antibody, L is a linker, D is a drug moiety, and p is 1, 2, 3, or 4; wherein the cysteine engineered antibody comprises one or more free cysteine amino acids, wherein at least one free cysteine amino acid is located at a position selected from heavy chain positions 4, 20, 23, 27, 32, 47, 68, 76, 78, 80, 81, 82, 94, 98, 108, 113, 154, 162, and 164 by Kabat numbering and light chain positions 49, 53, 73, 75, 80, 92, 95, 99, 101, 137, 138, 149, and 183 by Kabat numbering.

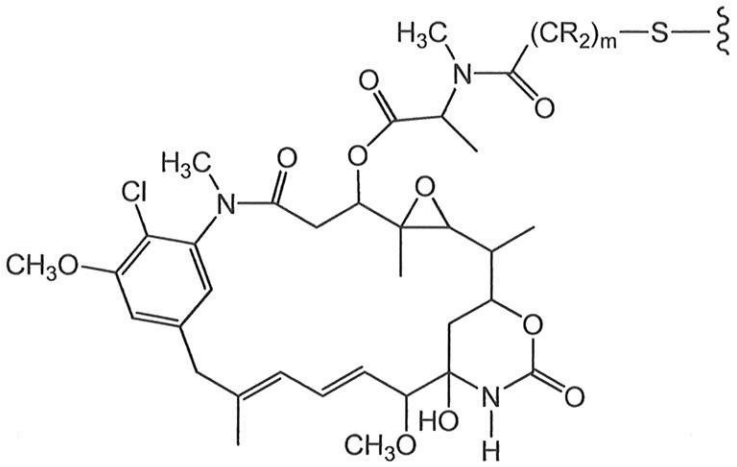
2. The use of claim 1, wherein the drug is selected from a maytansinoid, an auristatin, a dolastatin, a trichothecene, CC1065, a calicheamicin, an enediyne antibiotics, a taxane, and an anthracycline.

3. The use of claim 1, wherein the cancer is selected from lung cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, stomach cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial cancer, uterine carcinoma, salivary gland carcinoma, kidney cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, and head and neck cancer.

4. The use of claim 1, wherein the antibody-drug conjugate has the structure:



5. The use of claim 1, wherein D is a maytansinoid having the structure:

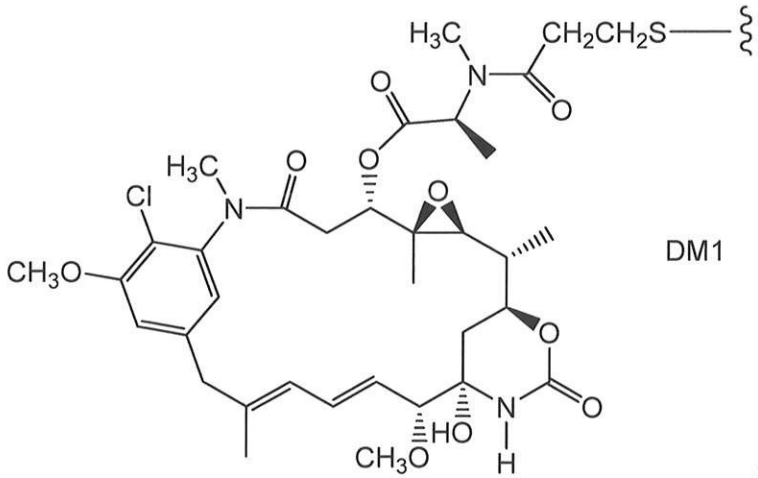


wherein the wavy line indicates the covalent attachment of the sulfur atom of D to the linker;

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

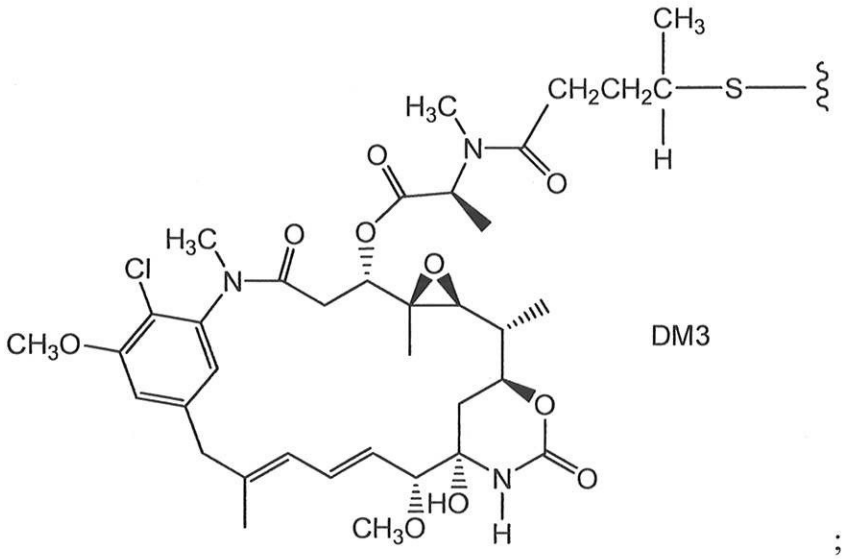
m is 1, 2, or 3.

6. The use of claim 5 wherein D is selected from the structures:



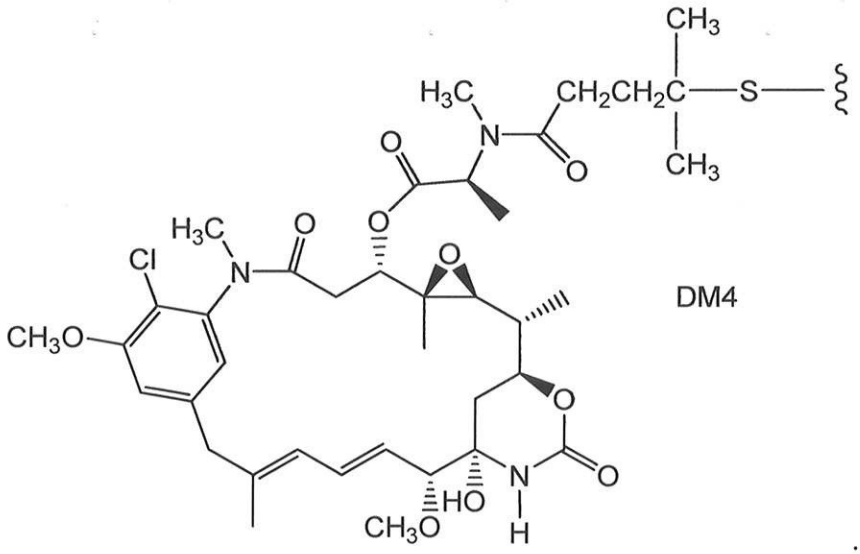
DM1

;



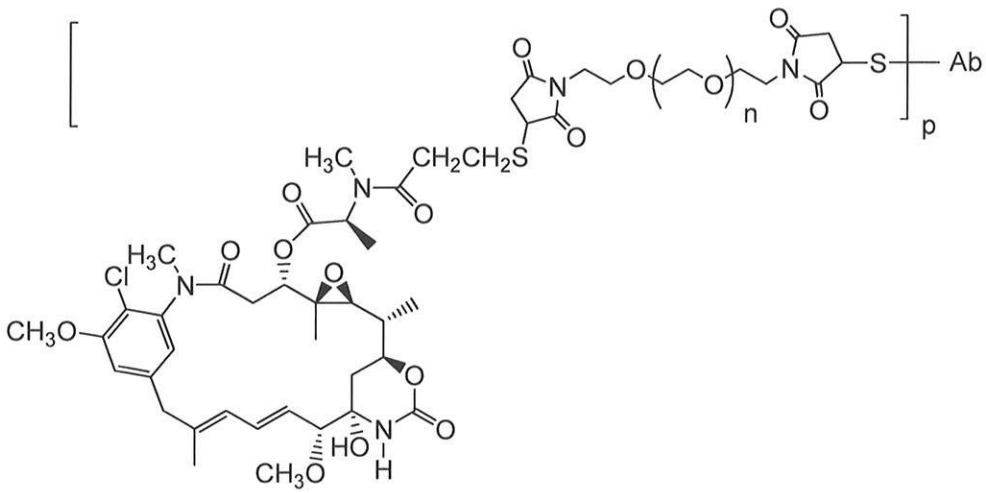
;

and



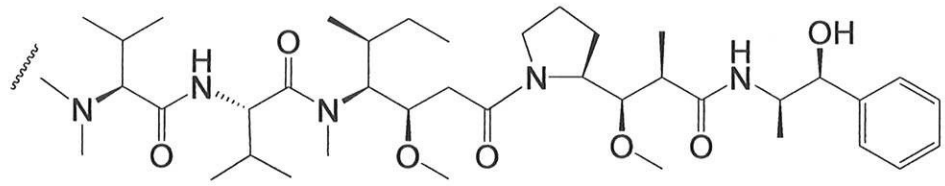
.

7. The use of claim 6 having the structure:

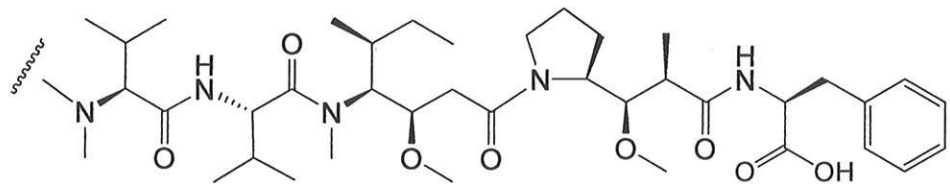


wherein n is 0, 1, or 2.

8. The use of claim 1 wherein D is a monomethylauristatin drug moiety MMAE or MMAF having the structures:

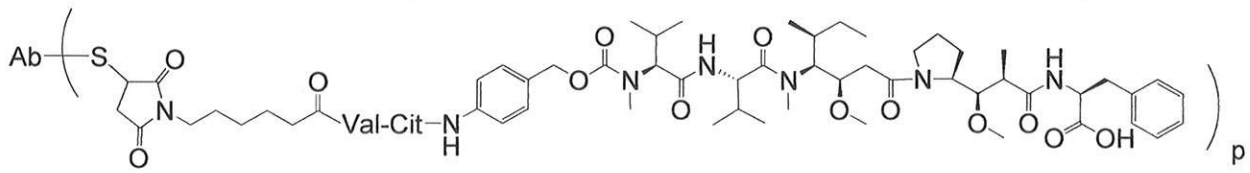


MMAE

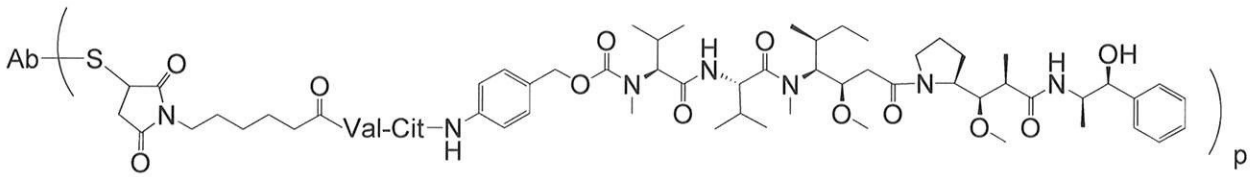


MMAF .

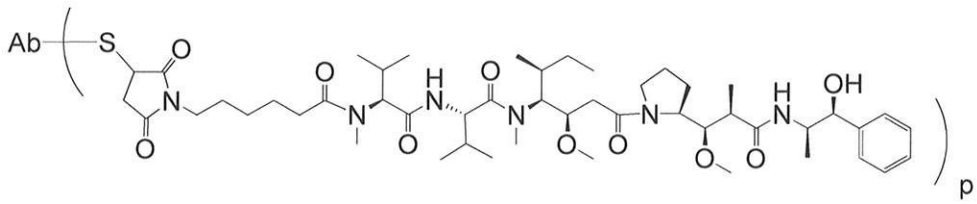
9. The use of claim 8 wherein the antibody-drug conjugate is selected from the structures:



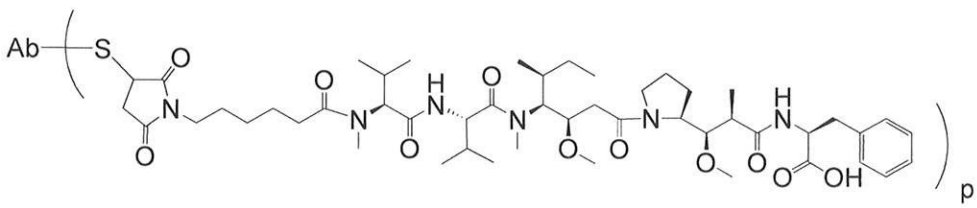
Ab-MC-vc-PAB-MMAF



Ab-MC-vc-PAB-MMAE



Ab-MC-MMAE



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

10. The use 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).