[0029] In one embodiment, an immunoconjugate of the disclosure as described herein comprises an antibody comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO:10, (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:12, (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO:7; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO:8; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO:9. In another embodiment, an immunoconjugate of the diclsoure as described herein comprises a VH sequence of SEQ ID NO:5 and a VL sequence of SEQ ID NO:3.

[0029a] In one aspect, there is provided a pharmaceutical formulation comprising the immunoconjugate as defined herein and a pharmaceutical acceptable carrier.

[0030] In one embodiment, a pharmaceutical formulation comprising the immunoconjugate of the disclosure as described herein and a pharmaceutically acceptable carrier is provided. In some embodiments, a pharmaceutical formulation further comprises an additional therapeutic agent. In some embodiments, the additional therapeutic agent is a chemotherapeutic agent, such as, for example, a platinum complex.

[0030a] In one aspect, there is provided the use of an effective amount of the immunoconjugate as defined herein in the manufacture of a medicament for treating an individual having an Ly6E-positive cancer.

[0031] In one embodiment, methods of treating an individual having a Ly6E-positive cancer are provided. In some embodiments, such methods comprise administering a pharmaceutical formulation comprising an immunoconjugate of the disclosure as described herein comprising an antibody that binds Ly6E, e.g., as described herein. In other embodiments, the Ly6E-positive cancer is selected from breast cancer, pancreatic cancer, colon cancer, colorectal cancer, melanoma, ovarian cancer, non-small cell lung cancer, or gastric cancer. In some embodiments, a method of the disclosure comprises administering an additional therapeutic agent to the individual. In some embodiments, the additional therapeutic agent is a chemotherapeutic agent, such as, for example, a platinum complex.

[0031a] ~~In one aspect, there is provided~~ Disclosed is the use of an immunoconjugate as defined herein in the manufacture of a medicament for inhibiting proliferation of an Ly6E-positive cell, wherein the cell is to be exposed to the medicament under conditions permissive for binding of the immunoconjugate to Ly6E on the surface of the cell.

[0032] In one aspect, there is provided an *in vitro* method of inhibiting proliferation of an Ly6E-positive cell, the method comprising exposing the cell to the immunoconjugate described herein under conditions permissive for binding of the immunoconjugate to Ly6E on the surface of the cell, thereby inhibiting proliferation of the cell. In some embodiments, methods of inhibiting proliferation of a Ly6E-positive cell are provided. In one embodiment, such methods comprise exposing a Ly6E-positive cell to an immunoconjugate of the disclosure as described herein comprising an antibody that binds Ly6E under conditions permissive for binding of the immunoconjugate to Ly6E on the surface of the cell. In some embodiments, an antibody that binds Ly6E is an antibody as described herein. In some embodiments, proliferation of the Ly6E-positive cell is thereby inhibited. In some embodiments, the cell is a breast

a) Exemplary Linkers

[0219] A "Linker" (L) is a bifunctional or multifunctional moiety that can be used to link one or more drug moieties (D) to an antibody (Ab) to form an antibody-drug conjugate (ADC) of Formula I. In some embodiments, antibody-drug conjugates (ADC) can be prepared using a Linker having reactive functionalities for covalently attaching to the drug and to the antibody. For example, in some embodiments, a cysteine thiol of an antibody (Ab) can form a bond with a reactive functional group of a linker or a drug-linker intermediate to make an ADC.

[0220] In one aspect, a linker has a functionality that is capable of reacting with a free cysteine present on an antibody to form a covalent bond. Nonlimiting exemplary such reactive functionalities include maleimide, haloacetamides, α-haloacetyl, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates, and isothiocyanates. *See, e.g.*, the conjugation method at page 766 of Klussman, et al (2004), *Bioconjugate Chemistry* 15(4):765-773, and the Examples herein.

[0221] In some embodiments, a linker has a functionality that is capable of reacting with an electrophilic group present on an antibody. Exemplary such electrophilic groups include, but are not limited to, aldehyde and ketone carbonyl groups. In some embodiments, a heteroatom of the reactive functionality of the linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Nonlimiting exemplary such reactive functionalities include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

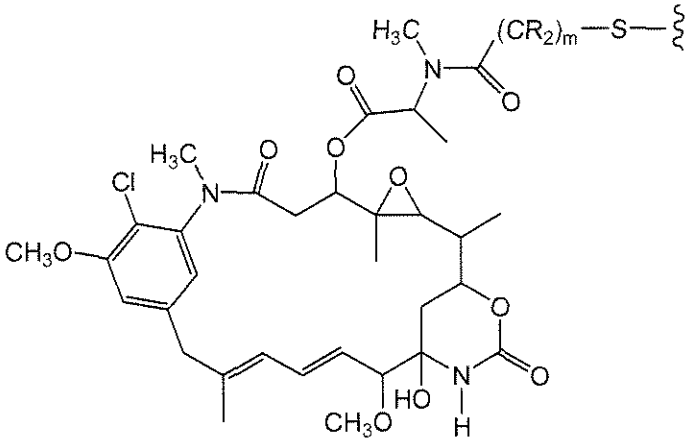
[0222] A linker may comprise one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl (a "PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), and 4-(N-maleimidomethyl) cyclohexane-1 carboxylate ("MCC"). Various linker components are known in the art, some of which are described below.

[0223] A linker may be a "cleavable linker," facilitating release of a drug. Nonlimiting exemplary cleavable linkers include acid-labile linkers (e.g., comprising hydrazone), protease-sensitive (e.g., peptidase-sensitive) linkers, photolabile linkers, or disulfide-containing linkers (Chari et al., Cancer Research 52:127-131 (1992); US 5208020).

[0224] In certain embodiments, a linker has the following Formula II:

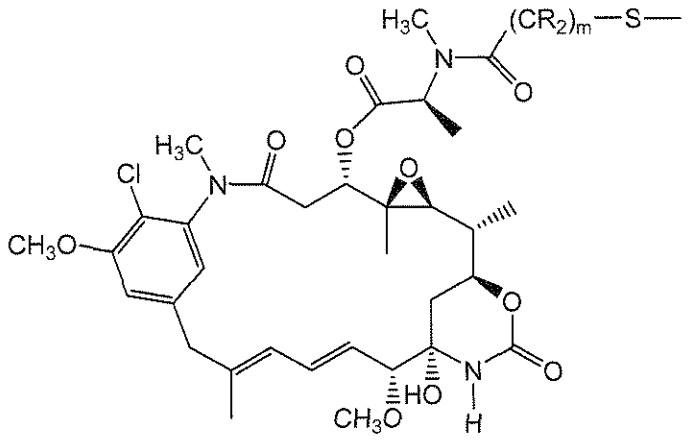
$$-A_a-W_w-Y_y- \qquad \text{Formula II}$$

[0225] wherein A is a "stretcher unit", and a is an integer from 0 to 1; W is an "amino acid unit", and w is an integer from 0 to 12; Y is a "spacer unit", and y is 0, 1, or 2; and Ab, D, and p are defined as above for Formula I. Exemplary embodiments of such linkers are described in U.S. Patent No. 7,498,298~~, which is expressly incorporated herein by reference~~.



[0246] where the wavy line indicates the covalent attachment of the sulfur atom of the maytansinoid drug moiety to a linker of an ADC. Each R may independently be H or a C_1–C_6 alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propyl, i.e., m is 1, 2, or 3 (US 633410; US 5208020; Chari et al (1992) *Cancer Res.* 52:127-131; Liu et al (1996) *Proc. Natl. Acad. Sci USA* 93:8618-8623).

[0247] All stereoisomers of the maytansinoid drug moiety are contemplated for the ADC of the invention, i.e. any combination of *R* and *S* configurations at the chiral carbons (US 7276497; US 6913748; US 6441163; US 633410 (RE39151); US 5208020; Widdison et al (2006) J. Med. Chem. 49:4392-4408~~, which are incorporated by reference in their entirety~~). In some embodiments, the maytansinoid drug moiety has the following stereochemistry:



[0248] Exemplary embodiments of maytansinoid drug moieties include, but are not limited to, DM1; DM3; and DM4, having the structures:

[0252] where Ab is antibody; n is 0, 1, or 2; and p is 1 to about 20. In some embodiments, p is 1 to 10, p is 1 to 7, p is 1 to 5, or p is 1 to 4.

[0253] Immunoconjugates containing maytansinoids, methods of making the same, and their therapeutic use are disclosed, for example, in U.S. Patent Nos. 5,208,020 and 5,416,064; US 2005/0276812 A1; and European Patent EP 0 425 235 B1~~, the disclosures of which are hereby expressly incorporated by reference~~. *See also* Liu et al. *Proc. Natl. Acad. Sci. USA* 93:8618-8623 (1996); and Chari et al. *Cancer Research* 52:127-131 (1992).

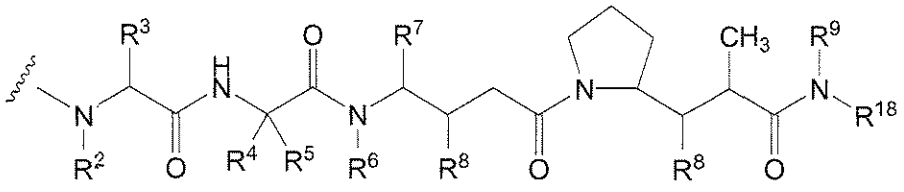
[0254] In some embodiments, antibody-maytansinoid conjugates may be prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. *See, e.g.*, U.S. Patent No. 5,208,020 ~~(the disclosure of which is hereby expressly incorporated by reference)~~. In some embodiments, ADC with an average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody. In some instances, even one molecule of toxin/antibody is expected to enhance cytotoxicity over the use of naked antibody.

[0255] Exemplary linking groups for making antibody-maytansinoid conjugates include, for example, those described herein and those disclosed in U.S. Patent No. 5208020; EP Patent 0 425 235 B1; Chari et al. *Cancer Research* 52:127-131 (1992); US 2005/0276812 A1; and US 2005/016993 A1~~, the disclosures of which are hereby expressly incorporated by reference~~.

(2) Auristatins and dolastatins

[0256] Drug moieties include dolastatins, auristatins, and analogs and derivatives thereof (US 5635483; US 5780588; US 5767237; US 6124431). Auristatins are derivatives of the marine mollusk compound dolastatin-10. While not intending to be bound by any particular theory, dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) *Antimicrob. Agents and Chemother.* 45(12):3580-3584) and have anticancer (US 5663149) and antifungal activity (Pettit et al (1998) *Antimicrob. Agents Chemother.* 42:2961-2965). The dolastatin/auristatin drug moiety may be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172; Doronina et al (2003) *Nature Biotechnology* 21(7):778-784; Francisco et al (2003) *Blood* 102(4):1458-1465).

[0257] Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties D_E and D_F, disclosed in US 7498298 and US 7659241~~, the disclosures of which are expressly incorporated by reference in their entirety~~:



D_E

antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

[0327] It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate or linker reagent, then the resulting product is a mixture of ADC compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, e.g. hydrophobic interaction chromatography (*see, e.g.*, McDonagh et al (2006) Prot. Engr. Design & Selection 19(7):299-307; Hamblett et al (2004) Clin. Cancer Res. 10:7063-7070; Hamblett, K.J., et al. "Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate," Abstract No. 624, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; Alley, S.C., et al. "Controlling the location of drug attachment in antibody-drug conjugates," Abstract No. 627, American Association for Cancer Research, 2004 Annual Meeting, March 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). In certain embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

d) Certain Methods of Preparing Immunoconjugates

[0328] An ADC of Formula I may be prepared by several routes employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent to form Ab-L via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with a nucleophilic group of an antibody. Exemplary methods for preparing an ADC of Formula I via the latter route are described in US 7498298~~, which is expressly incorporated herein by reference~~.

[0329] Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; and (iii) aldehydes, ketones, carboxyl, and maleimide groups. Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol) or tricarbonylethylphosphine (TCEP), such that the antibody is fully or partially reduced. Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through

with a currently valid license for experiments on vertebrate animals, issued by the French Ministry for Agriculture and Fisheries to Dr. Truong-An TRAN (No. A 91-541 dated 21 December 2010; validity: 5 years). 6- to 9-week old female immunodeficient mice were inoculated subcutaneously in the dorsal right flank and average tumor volumes with SDs were determined from 9-10 mice per group.

[0401] For efficacy studies with xenografts derived from cell lines, NCR nude mice from Taconic were inoculated with 5 million cells in HBSS with Matrigel or C.B-17 SCID (inbred) mice From Charles River were inoculated with 2 million cells in HBSS with Matrigel. 0.36mg estrogen implants were used for the HCC1569 X2 xenograft model. For efficacy studies with tumor explants at XenTech and Oncotest, athymic nude or NMRI nu/nu mice from Harlan or Charles River were implanted with primary breast or pancreatic cancer patient derived materials from models HBCx-8, HBCx-9, MAXF-1162 and PAXF-1657. When tumor volumes reached approximately 80-200 mm3 (day 0), animals were randomized into groups of 9-10 each and administered a single intravenous (IV) injection of either vehicle control or the ADC at the appropriate dose. Tumor volumes were measured twice per week until study end.

[0402] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. ~~The disclosures of all patent and scientific literature cited herein are expressly incorporated in their entirety by reference.~~

21. The immunoconjugate of any one of claims 9 to 20, comprising the antibody of claim 1.

22. A pharmaceutical formulation comprising the immunoconjugate of any one of claims 9 to 21 and a pharmaceutically acceptable carrier.

23. The pharmaceutical formulation of claim 22, further comprising an additional therapeutic agent.

24. The pharmaceutical formulation of claim 23, wherein the additional therapeutic agent is a platinum complex.

25. Use of an effective amount of the immunoconjugate of any one of claims 9 to 21 in the manufacture of a medicament for treating an individual having an Ly6E-positive cancer.

26. The use of claim 25, wherein the Ly6E-positive cancer is selected from a breast cancer, pancreatic cancer, colon cancer, colorectal cancer, melanoma, ovarian cancer, non-small cell lung cancer, or gastric cancer.

27. The use of claim 26, wherein the medicament is to be further administered with an additional therapeutic agent to the individual.

28. The use of claim 27, wherein the additional therapeutic agent is a platinum complex.

29. An *in vitro* method of ~~Use of the immunoconjugate of any one of claims 9 to 21 in the manufacture of a medicament for~~ inhibiting proliferation of an Ly6E-positive cell, the method comprising exposing ~~wherein~~ the cell to the immunoconjugate of any one of claims 9 to 21 ~~is to be exposed to the medicament~~ under conditions permissive for binding of the immunoconjugate to Ly6E on the surface of the cell, thereby inhibiting proliferation of the cell.

30. The method ~~use~~ of claim 29, wherein the cell is a breast, pancreatic, colon, colorectal, melanoma, ovarian non-small cell lung or gastric cancer cell.