ANTI-CD79B ANTIBODIES AND IMMUNOCONJUGATES

FIELD OF THE INVENTION

[001] The present invention relates to immunoconjugates comprising anti-CD79b antibodies and methods of using the same.

BACKGROUND

[002] CD79 is the signaling component of the B-cell receptor consisting of a covalent heterodimer containing CD79a (Igα, mb-1) and CD79b (Igβ, B29). CD79a and CD79b each contain an extracellular immunoglobulin (Ig) domain, a transmembrane domain, and an intracellular signaling domain, an immunoreceptor tyrosine-based activation motif (ITAM) domain. CD79 is expressed on B cells and, for example, in Non-Hodgkin's Lymphoma cells (NHLs) (Cabezudo et al., *Haematologica,* 84:413-418 (1999); D'Arena et al., *Am. J. Hematol.,* 64: 275-281 (2000); Olejniczak et al., *Immunol. Invest.,* 35: 93-114 (2006)). CD79a and CD79b and sIg are all required for surface expression of the CD79 (Matsuuchi et al., *Curr. Opin. Immunol.,* 13(3): 270-7)). The average surface expression of CD79b on NHLs is similar to that on normal B-cells, but with a greater range (Matsuuchi et al., *Curr. Opin. Immunol.,* 13(3): 270-7 (2001)).

[003] There is a need in the art for agents that target CD79b for the diagnosis and treatment of CD79b-associated conditions, such as cancer. The invention fulfills that need and provides other benefits.

SUMMARY

[004] The ~~invention~~disclosure provides anti-CD79b antibodies and immunoconjugates and methods of using the same.

[005] In ~~some embodiments~~one aspect, there is provided an immunoconjugate comprising an antibody that binds CD79b covalently attached to a cytotoxic agent~~, is provided. In some embodiments, the cytotoxic agent is a pyrrolobenzodiazepine. In some embodiments,~~ wherein the antibody that binds CD79b comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; and wherein the cytotoxic agent is a pyrrolobenzodiazepine. In some embodiments, the antibody further comprises (i) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 18, 24, and 35, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25, and (iii)

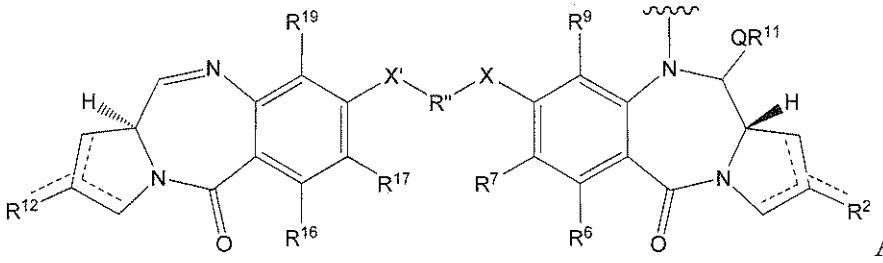
HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody comprises HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24.

[006] In some embodiments, the antibody comprises: a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 11; or b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 12; or c) a VH sequence as in (a) and a VL sequence as in (b). in some embodiments, the antibody comprises a VH sequence having an amino acid sequence selected from SEQ ID NOs: 7, 9, 11, and 13. In some embodiments, the antibody comprises a VH sequence having the amino acid sequence of SEQ ID NO: 11. In some embodiments, the antibody comprises a VL sequence having an amino acid sequence selected from SEQ ID NOs: 8, 10, 12, and 14. In some embodiments, the antibody comprises a VL sequence having the amino acid sequence of SEQ ID NO: 12. In some embodiments, the antibody is an IgG1, IgG2a or IgG2b antibody.

[007] In ~~some embodiments~~one aspect, there is provided an immunoconjugate comprising an antibody that binds CD79b covalently attached to a cytotoxic agent ~~is provided~~, wherein the antibody comprises (a) a VH sequence having the amino acid sequence of SEQ ID NO: 11 and a VL sequence having the amino acid sequence of SEQ ID NO: 12, and wherein the cytotoxic agent is a pyrrolobenzodiazepine.

[008] In some embodiments, the immunoconjugate has the formula Ab-(L-D)p, wherein: (a) Ab is the antibody; (b) L is a linker; (c) D is the cytotoxic agent; and (d) p ranges from 1-8.

[009] In some such embodiments, D is a pyrrolobenzodiazepine of Formula A:



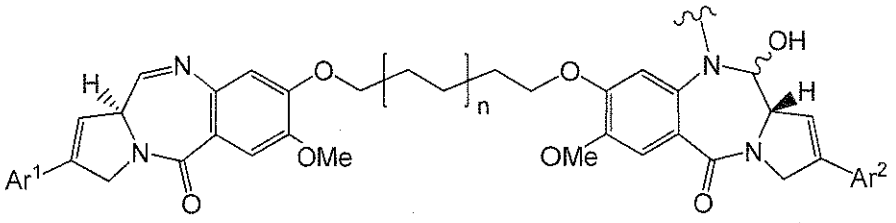
A;

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

R^2 is independently selected from H, OH, =O, $=CH_2$, CN, R, OR, $=CH\text{-}R^D$, $=C(R^D)_2$, $O\text{-}SO_2\text{-}R$, 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 independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;



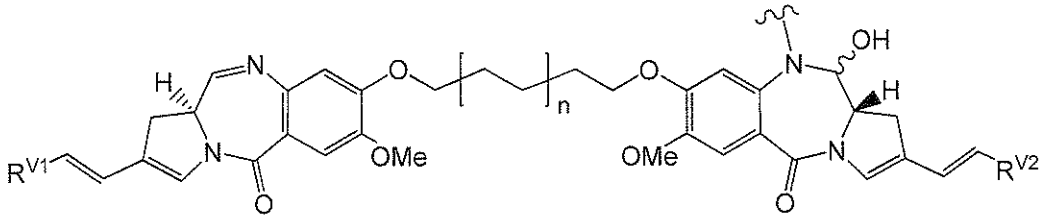
A(V);

wherein R^E and $R^{E"}$ are each independently selected from H or R^D, wherein R^D is independently selected from R, CO_2R, COR, CHO, CO_2H, and halo;

wherein Ar^1 and Ar^2 are each independently optionally substituted C_{5-20} aryl; and

wherein n is 0 or 1.

[012] In some embodiments, D is a pyrrolobenzodiazepine of Formula B:

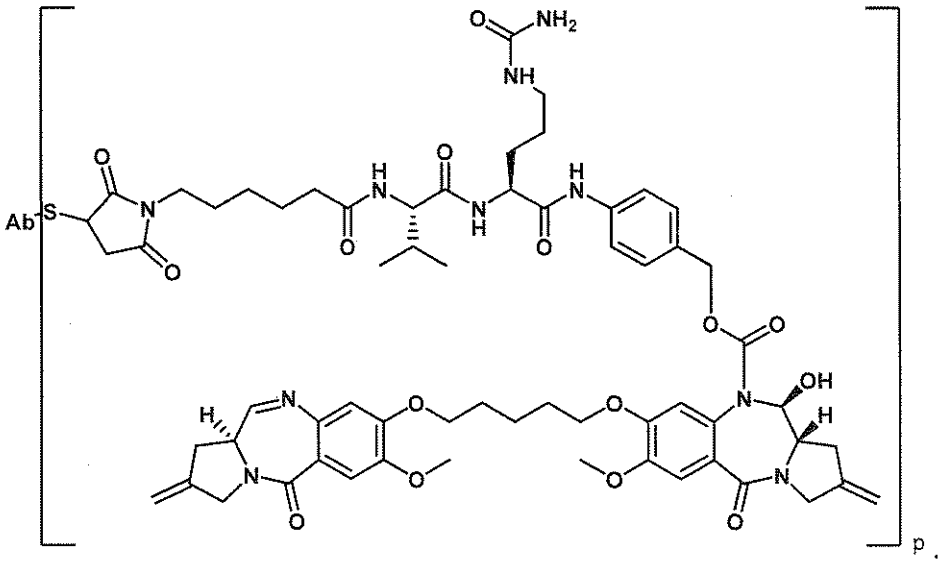


wherein the horizontal wavy line indicates the covalent attachement site to the linker;

R^{V1} and R^{V2} are independently selected from H, methyl, ethyl, phenyl, fluoro-substituted phenyl, and C_{5-6} heterocyclyl; and

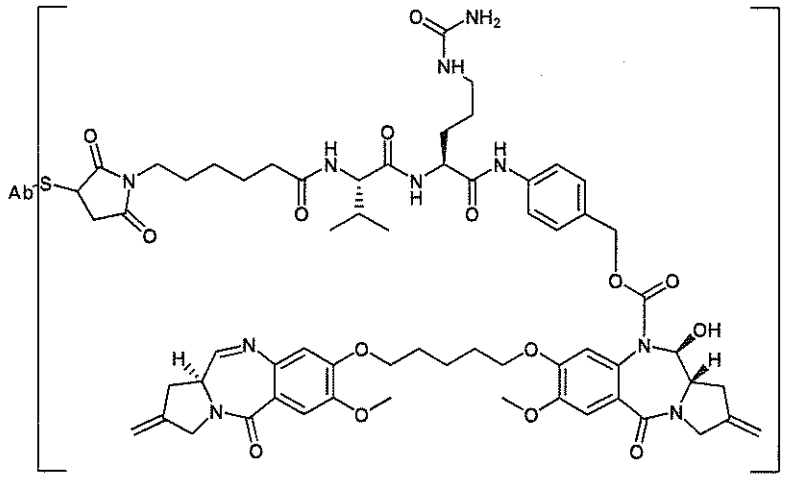
n is 0 or 1.

[013] In some embodiments, the immunoconjugate comprises a linker that is cleavable by a protease. In some such embodiments, the linker comprises a val-cit dipeptide or a Phe-homoLys dipeptide. In some embodiments, the immunoconjuge has the formula:



In some embodiments, p ranges from 1-3.

[014] In ~~some embodiments~~one aspect, there is provided an immunoconjugate ~~is provided, wherein the immunoconjugate has~~having the formula:



wherein Ab is an antibody comprising (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26; and wherein p ranges from 1 to 3. In some embodiments, the antibody comprises a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 12. In some embodiments, the antibody comprises a heavy chain of SEQ ID NO: 39 and a light chain of SEQ ID NO: 37.

[015] In any of the embodiments discussed herein, the antibody may be a monoclonal antibody. In some embodiments, the antibody may be a human, humanized, or chimeric antibody. In some embodiments, the antibody is an antibody fragment that binds CD79b. in some embodiments, the antibody binds human CD79b. In some such embodiments, human CD79b has the sequence of SEQ ID NO: 40 or SEQ ID NO: 41.

[016] ~~In some embodiments, pharmaceutical formulations are provided, wherein the~~ In one aspect, there is provided a pharmaceutical formulation comprisinges ~~an~~ the immunoconjugate described herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical formulation comprises an additional therapeutic agent.

[016a] In one aspect, there is provided the use of the immunoconjugate as described herein in the manufacture of a medicament for the treatment of an individual having a CD79b-positive cancer.

[017] In some embodiments, methods of treating an individual with a CD79b-positive cancer are provided. In some embodiments, a method comprises administering to the individual an effective amount of the immunoconjugate described herein. In some embodiments, the CD79b-positive cancer is selected from lymphoma, non-Hogkins lymphoma (NHL), aggressive NHL, 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), Burkitt's lymphoma, and mantle cell lymphoma. In some embodiments, the method further comprises

administering an additional therapeutic agent to the individual. In some such embodiments, the additional therapeutic agent comprises an antibody that binds CD22. In some embodiments, the additional therapeutic agent is an immunoconjugate comprising an antibody that binds CD22 covalently attached to a cytotoxic agent.

[0017a] In one aspect, there is provided the use of the immunoconjugate as described herein in the manufacture of a medicament for the inhibition of proliferation of a CD79b-positive cell, wherein the cell is to be exposed to the immunoconjugate under conditions permissive for binding of the immunoconjugate to CD79b on the surface of the cell, thereby inhibiting proliferation of the cell.

[018] In ~~some embodiments~~one aspect, there is provided an *in vitro*~~a~~ method of inhibiting proliferation of a CD79b-positive cell, ~~is provided. In some such embodiments,~~ the method comprising~~es~~ exposing the cell to the immunoconjugate described herein under conditions permissive for binding of the immunoconjugate to CD79b on the surface of the cell, thereby inhibiting proliferation of the cell. In some embodiments, the cell is a neoplastic B cell. In some embodiments, the cell is a lymphoma cell.

BRIEF DESCRIPTION OF THE FIGURES

[019] **Figures 1A-1B** show the amino acid sequence of the heavy chain variable region of murine anti-CD79b antibody MA79b aligned with the humanized MA79b graft and humanized versions 17, 18, 28, and 32 (huMA79b graft, huMA79bv17, huMA79bv18, huMA79bv28, and huMA79bv32, respectively), and aligned with the human subgroup III sequence. The HVRs are boxed (HVR-H1, HVR-H2, HVR-H3). The sequences bracketing the HVRs are the framework sequences (FR-H1 to FR-H4). The sequences are numbered according to Kabat numbering. The Kabat, Chothia, and contact CDRs are indicated about the boxed HVRs.

[020] **Figures 2A-2B** show the amino acid sequence of the light chain variable region of murine anti-CD79b antibody MA79b aligned with the humanized MA79b graft and humanized versions 17, 18, 28, and 32 (huMA79b graft, huMA79bv17, huMA79bv18, huMA79bv28, and huMA79bv32, respectively), and aligned with the human subgroup kappa I sequence. The HVRs are boxed. The FR-L1, FR-L2, FR-L3, and FR-L4 sequences bracket the HVRs (HVR-L1, HVR-L2, HVR-L3). The sequences are numbered according to Kabat numbering. The Kabat, Chothia, and contact CDRs are indicated about the boxed HVRs.

[021] **Figure 3** shows the full length amino acid sequences (variable and constant regions) of the light and heavy chains of humanized anti-CD79b antibody huMA79bv28, isotype IgG1. The underlined portions are the constant domains.

[022] **Figure 4** shows the amino acid sequences of the anti-CD79b cysteine engineered antibodies in which the light chain or heavy chain or Fc region is altered to replace an amino acid with a cysteine at selected amino acid positions. The cysteine engineered antibodies shown include Thio-huMA79bv28-HC-A118C heavy chain, in which the alanine at EU position 118 (sequential position alanine 118) is altered to a cysteine; Thio-huMA79b.v28-

30. ~~A method of treating an individual having a CD79b-positive cancer, the method comprising administering to the individual an effective amount~~Use of the immunoconjugate of any one of claims 1 to 27 in the manufacture of a medicament for the treatment of an individual having a CD79b-positive cancer.

31. The use~~method~~ of claim 30, wherein the CD79b-positive cancer is selected from lymphoma, non-Hogkins lymphoma (NHL), aggressive NHL, 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), Burkitt's lymphoma, and mantle cell lymphoma.

32. The use~~method~~ of claim 31, ~~further comprising administering~~wherein the immunoconjugate is to be administered with an additional therapeutic agent to the individual.

33. The use~~method~~ of claim 32, wherein the additional therapeutic agent comprises an antibody that binds CD22.

34. The use~~method~~ of claim 33, wherein the additional therapeutic agent is an immunoconjugate comprising an antibody that binds CD22 covalently attached to a cytotoxic agent.

35. ~~A method of inhibiting proliferation of a CD79b-positive cell, the method comprising exposing the cell to~~Use of the immunoconjugate of any one of claims 1 to 27 in the manufacture of a medicament for the inhibition of proliferation of a CD79b-positive cell, wherein the cell is to be exposed to the immunoconjugate under conditions permissive for binding of the immunoconjugate to CD79b on the surface of the cell, thereby inhibiting proliferation of the cell.

36. The use~~method~~ of claim 35, wherein the cell is a neoplastic B cell.

37. The use~~method~~ of claim 36, wherein the cell is a lymphoma cell.

38. An *in vitro* method of inhibiting proliferation of a CD79b-positive cell, the method comprising exposing the cell to the immunoconjugate of any one of claims 1 to 27 under conditions permissive for binding of the immunoconjugate to CD79b on the surface of the cell, thereby inhibiting proliferation of the cell.

39. The method of claim 38, wherein the cell is a neoplastic B cell.

40. The method of claim 39, wherein the cell is a lymphoma cell.