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 nemorubicin derivative. 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 nemorubicin derivative. 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)

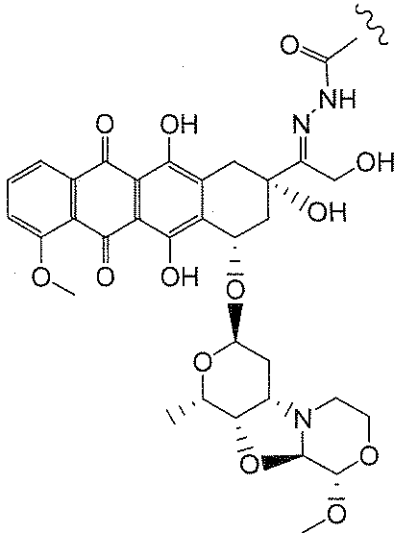
HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody comprises HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24.

[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 nemorubicin derivative.

[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.

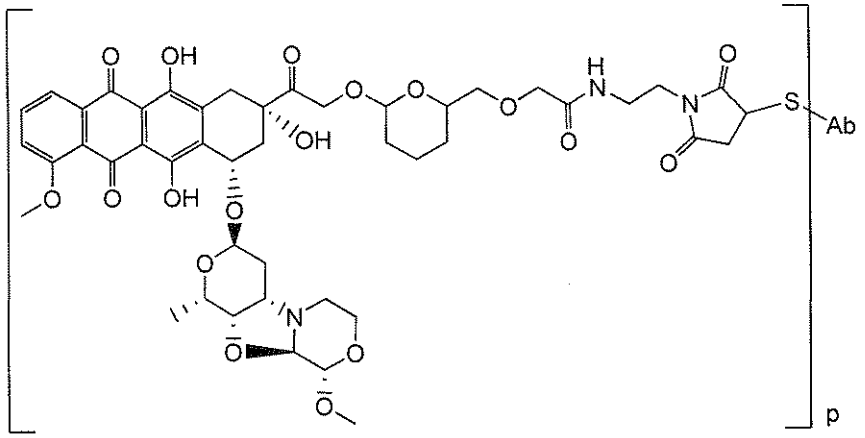
[009] In some embodiments, D is a nemorubicin derivative. In some such embodiments, D has a structure selected from:



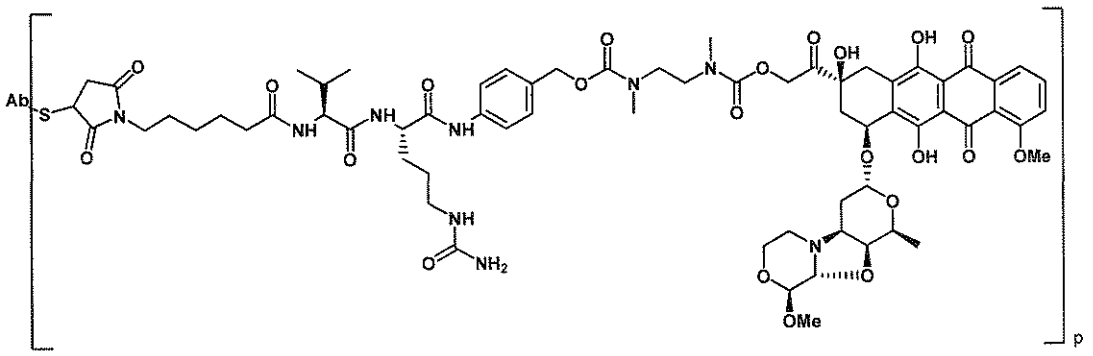
; and

wherein R_1 and R_2 are independently selected from H and C_1-C_6 alkyl. In some embodiments, p ranges from 1-3.

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



; and



;

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.

[013] 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.

[014] ~~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.

[0014a] In one aspect 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.

[015] 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.

[0015a] 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, wherein the CD79b-positive cancer is resistant to a first therapeutic.

[016] In some embodiments, a method of treating an individual having a CD79b-positive cancer, wherein the CD79b-positive cancer is resistant to a first therapeutic is provided. In some embodiments, the method comprises administering to the individual an effective amount of an 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 first therapeutic comprises a first antibody that binds an antigen other than CD79b. In some embodiments, the first therapeutic is a first immunoconjugate comprising a first antibody that binds an antigen other than CD79b and a first cytotoxic agent. In some embodiments, the first antibody binds CD22. In some embodiments, the first therapeutic comprises a first antibody that binds CD79b. In some embodiments, the first therapeutic is a first immunoconjugate comprising a first antibody that binds CD79b and a first cytotoxic agent. In some embodiments, the first cytotoxic agent and the cytotoxic agent of the immunoconjugate described herein are different. In some embodiments, the first cytotoxic agent is MMAE. In some embodiments, the first cytotoxic agent is a pyrrolobenzodiazepine. In some embodiments, the first cytotoxic agent is a

maytansinoid. In some embodiments, the CD79b-positive cancer that is resistant to a first therapeutic expresses P-glycoprotein (P-gp).

[0016a] In one aspect, there is provided the use of an immunoconjugate as described herein in the manufacture of a medicament for the treatment of an individual having a CD79b-positive cancer, wherein the CD79-positive cancer expresses P-gp.

[017] In some embodiments, a method of treating an individual having a CD79b-positive cancer is provided, wherein the CD79b-positive cancer expresses P-gp. In some embodiments, the method comprises administering to the individual an effective amount of an immunoconjugate described herein. In some embodiments, the CD79b-positive / P-gp 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 CD79b positive / P-gp positive cancer expresses higher levels of P-gp mRNA and/or protein than control cells or tissue.

[0017a] In one aspect, there is provided the use of an 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 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* method of inhibiting proliferation of a CD79b-positive cell ~~is provided. In some such embodiments~~, the method comprisinges 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.

[0252] In a further aspect, the invention provides for the use of an anti-CD79b antibody or immunoconjugate in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of CD79b-positive cancer. In a further embodiment, the medicament is for use in a method of treating CD79b-positive cancer, the method comprising administering to an individual having CD79b-positive cancer an effective amount of the medicament. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below.

[0253] In a further aspect, the invention provides a method for treating CD79b-positive cancer. In one embodiment, the method comprises administering to an individual having such CD79b-positive cancer an effective amount of an anti-CD79b antibody or immunoconjugate. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below.

[0254] A CD79b-positive cancer according to any of the above embodiments may be, e.g., 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, a CD79b-positive cancer is a cancer that receives an anti-CD79b immunohistochemistry (IHC) or in situ hybridization (ISH) score greater than "0," which corresponds to very weak or no staining in >90% of tumor cells. In another embodiment, a CD79b-positive cancer expresses CD79b at a 1+, 2+ or 3+ level, wherein 1+ corresponds to weak staining in >50% of neoplastic cells, 2+ corresponds to moderate staining in >50% neoplastic cells, and 3+ corresponds to strong staining in >50% of neoplastic cells. In some embodiments, a CD79b-positive cancer is a cancer that expresses CD79b according to a reverse-transcriptase PCR (RT-PCR) assay that detects CD79b mRNA. In some embodiments, the RT-PCR is quantitative RT-PCR.

[0255] In some embodiments, immunoconjugates comprising a nemorubicin derivative covalently attached to the anti-CD79b antibody through a protease-cleavable linker are particularly useful for treating diffuse large B-cell lymphomas, mantle cell lymphomas, and Burkitt's lymphoma as evidenced, for example, by the xenograft models shown in Examples B, C, D, E, and G. The immunoconjugate for use in treating diffuse large B-cell lymphomas, mantle cell lymphomas, and/or Burkitt's lymphoma, may, in some embodiments, comprise PNU-159682. In some such embodiments, the immunoconjugate comprises PNU-159682 and a linker comprising val-cit, such as the immunoconjugate shown in Figure ~~5C~~5B.

conjugatable thiol group. *See, e.g.*, US 8,088,378 B2. The amino acid sequence of the heavy chain of Thio huMA79bv28 HC A118C is shown in SEQ ID NO: 39 (*see* Figure 4), and the amino acid sequence of the light chain of Thio huMA79bv28 HC A118C is shown in SEQ ID NO: 37 (*see* Figure 3). The immunoconjugates were prepared as follows.

Thio huMA79bv28 HC A118C-MC-acetal-PNU-159682 ("huMA79bv28-PNU-2")

[0278] Prior to conjugation, the antibody was reduced with dithiothreitol (DTT) to remove blocking groups (e.g. cysteine) from the engineered cysteines of the thio-antibody. This process also reduces the interchain disulfide bonds of the antibody. The reduced antibody was purified to remove the released blocking groups and the interchain disulfides were reoxidized using dehydro-ascorbic acid (dhAA). The intact antibody was then combined with the drug-linker moiety MC-acetal-PNU-159682 to allow conjugation of the drug-linker moiety to the engineered cysteine residues of the antibody. The conjugation reaction was quenched by adding excess N-acetyl-cysteine to react with any free linker-drug moiety, and the ADC was purified. The drug load (average number of drug moieties per antibody) for the ADC was about 1.86, as indicated in the Examples below. huMA79bv28-PNU-2 has the structure shown in Figure ~~5B~~ 5A (p = drug load).

Thio huMA79bv28 HC A118C-MC-val-cit-PAB-PNU-159682 ("huMA79bv28-PNU-1")

[0279] Prior to conjugation, the antibody was reduced with dithiothreitol (DTT) to remove blocking groups (e.g. cysteine) from the engineered cysteines of the thio-antibody. This process also reduces the interchain disulfide bonds of the antibody. The reduced antibody was purified to remove the released blocking groups and the interchain disulfides were reoxidized using dehydro-ascorbic acid (dhAA). The intact antibody was then combined with the drug-linker moiety MC-val-cit-PAB-PNU-159682 ("val-cit" may also be referred to herein as "vc") to allow conjugation of the drug-linker moiety to the engineered cysteine residues of the antibody. The conjugation reaction was quenched by adding excess N-acetyl-cysteine to react with any free linker-drug moiety, and the ADC was purified. The drug load (average number of drug moieties per antibody) for the ADC was in the range of about 1.8 to about 1.9, as indicated in the Example below. huMA79bv28-PNU-1 has the structure shown in Figure ~~5C~~ 5B (p = drug load).

Thio huMA79bv28 HC A118C-MC-val-cit-PAB-MMAE ("huMA79bv28-MMAE")

[0280] Prior to conjugation, the antibody was reduced with dithiothreitol (DTT) to remove blocking groups (e.g. cysteine) from the engineered cysteines of the thio-antibody. This process also reduces the interchain disulfide bonds of the antibody. The reduced antibody was purified to remove the released blocking groups and the interchain disulfides were

27. The immunoconjugate of claim 26, wherein human CD79b has the sequence of SEQ ID NO: 40 or SEQ ID NO: 41.

28. A pharmaceutical formulation comprising the immunoconjugate of any one of the preceding claims and a pharmaceutically acceptable carrier.

29. The pharmaceutical formulation of claim 28, further comprising an additional therapeutic agent.

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. ~~A method of treating an individual having a CD79b-positive cancer, wherein the CD22-positive cancer is resistant to a first therapeutic, 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, wherein the CD79b-positive cancer is resistant to a first therapeutic.

39. The use~~method~~ of claim 38, 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.

40. The use~~method~~ of claim 38 or claim 39, wherein the first therapeutic comprises a first antibody that binds an antigen other than CD79b.

41. The use~~method~~ of claim 40, wherein the first therapeutic is a first immunoconjugate comprising a first antibody that binds an antigen other than CD79b and a first cytotoxic agent.

42. The use~~method~~ of claim 40 or claim 41, wherein the first antibody binds CD22.

43. The use~~method~~ of claim 38 or claim 39, wherein the first therapeutic comprises a first antibody that binds CD79b.

44. The use~~method~~ of claim 43, wherein the first therapeutic is a first immunoconjugate comprising a first antibody that binds CD79b and a first cytotoxic agent.

45. The use~~method~~ of any one of claims 41 to 44, wherein the first cytotoxic agent and the cytotoxic agent of the immunoconjugate of any one of claims 1 to 27 are different.

46. The use~~method~~ of claim 45, wherein the first cytotoxic agent is MMAE.

47. The use~~method~~ of claim 46, wherein the CD79b-positive cancer expresses P-glycoprotein (P-gp).

48. ~~A method of treating an individual having a CD79b-positive cancer, wherein the CD22-positive cancer expresses P-gp, 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, wherein the CD79b-positive cancer expresses P-gp.

49. The use~~method~~ of claim 48, 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.

50. 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.

51. The method of claim 70, wherein the cell is a neoplastic B cell.

52. The method of claim 71, wherein the cell is a lymphoma cell.