enhanced apoptosis, which suggests a role of this antigen in B-cell survival (Otipoby, K.L. et al., Nature (Lond) 384:634-637 (1996)). After binding with its natural ligand(s) or antibodies, CD22 is rapidly internalized, providing a costimulatory signal in primary B cells and proapoptotic signals in neoplastic B cells (Sato, S. et al., Immunity 5:551-562 (1996)).

[005] There is a need in the art for agents that target CD22 for the diagnosis and treatment of CD22-associated conditions, such as cancer. The invention fulfills that need and provides other benefits.

SUMMARY

[006] The ~~invention~~ disclosure provides anti-CD22 antibodies and immunoconjugates and methods of using the same.

[006a] In one aspect, there is provided an immunoconjugate comprising an antibody that binds CD22 covalently attached to a cytotoxic agent, wherein the antibody binds an epitope within amino acids 20 to 240 of SEQ ID NO: 28, and wherein the cytotoxic agent is a nemorubicin derivative.

[007] In some embodiments, an immunoconjugate comprising an antibody that binds CD22 covalently attached to a cytotoxic agent is provided, wherein the antibody binds an epitope within amino acids 20 to 240 of SEQ ID NO: 28. In some embodiments, the cytotoxic agent is a nemorubicin derivative.

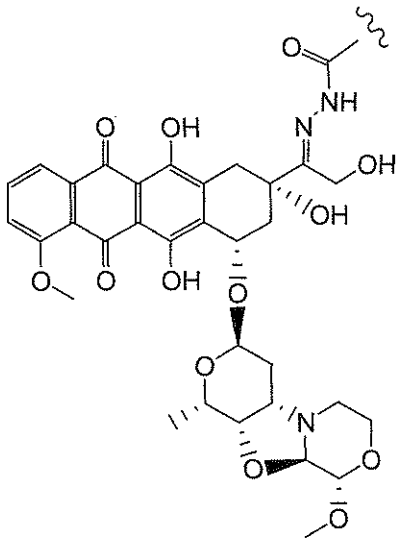
[008] In some embodiments, the antibody comprises (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 11, (ii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14, and (iii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 10. In some embodiments, the antibody comprises (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 10, and (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 11. In some embodiments, the antibody comprises: a) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 10, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 11, (iv) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 12 and 15 to 22, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 13, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14; or b) (i) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 9, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 10, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 11, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 13, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14. In some embodiments, the antibody comprises: a) (i) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 12 and 15 to 22, (ii) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 13, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14; or b) (i) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) HVR-L2 comprising the amino

acid sequence of SEQ ID NO: 13, and (iii) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14. 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: 7; or b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 8; 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 the amino acid sequence of SEQ ID NO: 7. In some embodiments, the antibody comprises a VL sequence having the amino acid sequence of SEQ ID NO: 6 or a VL sequence having the amino acid sequence of SEQ ID NO: 8. In some embodiments, the antibody is an IgG1, IgG2a or IgG2b antibody.

[009] In ~~some embodiments,~~one aspect, there is provided an immunoconjugate comprising an antibody that binds CD22 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: 7 and a VL sequence having the amino acid sequence of SEQ ID NO: 8, and wherein the cytotoxic agent is a nemorubicin derivative.

[010] 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.

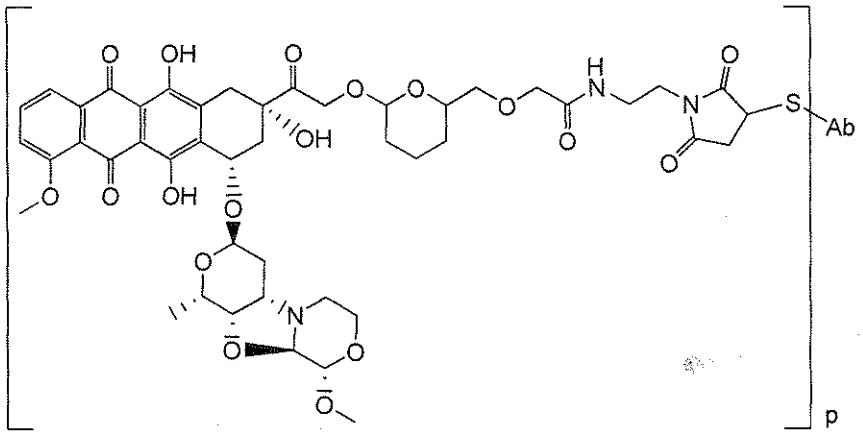
[011] In some embodiments, D is a nemorubicin derivative. In some such embodiments, D has a structure selected from:



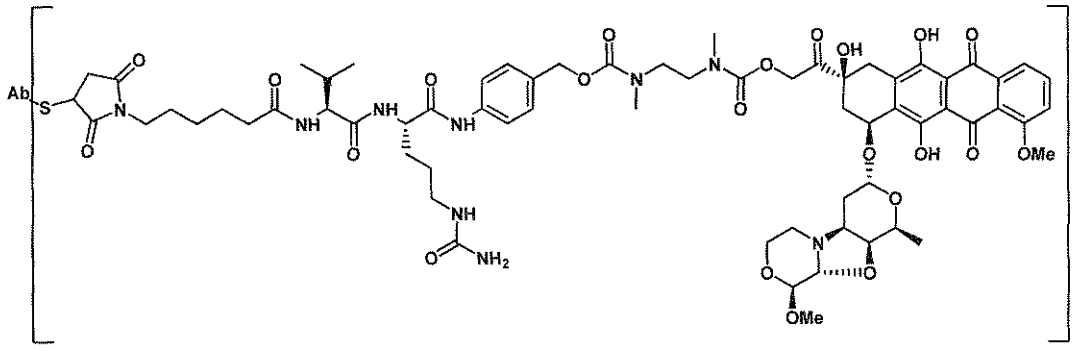
; and

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.

[014] ~~In some embodiments, an immunoconjugate is provided, wherein the~~In one aspect, there is provided an 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: 9, (ii) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 10, (iii) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 11, (iv) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 15, (v) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 13, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14; and wherein p ranges from 1 to 3. In some such embodiments, the antibody comprises a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 8. In some embodiments, the antibody comprises a heavy chain of SEQ ID NO: 26 and a light chain of SEQ ID NO: 23.

[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 CD22. In some embodiments, the antibody binds human CD22. In some such embodiments, human CD22 has the sequence of SEQ ID NO: 28 or SEQ ID NO: 29.

[015a] In one aspect, there is provided a pharmaceutical formulation comprising the immunoconjugate as described herein and a pharmaceutically acceptable carrier.

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

[017] In some embodiments, methods of treating an individual with a CD22-positive cancer are provided.

[017a] In one aspect, there is provided use of the immunoconjugate as described herein in the manufacture of a medicament for the treatment of an individual having a CD22-positive cancer.

In some embodiments, a method comprises administering to the individual an effective amount of an immunoconjugate described herein. In some embodiments, the CD22-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 CD79b. In some embodiments, the additional therapeutic agent is an immunoconjugate comprising an antibody that binds CD79b covalently attached to a cytotoxic agent.

[018] In some embodiments, a method of treating an individual having a CD22-positive cancer, wherein the CD22-positive cancer is resistant to a first therapeutic is provided.

[018a] In one aspect, there is provided use of the immunoconjugate as described herein in the manufacture of a medicament for the treatment of an individual having a CD22-positive cancer, wherein the CD22-positive cancer is resistant to a first therapeutic.

In some embodiments, the method comprises administering to the individual an effective amount of an immunoconjugate described herein. In some embodiments, the CD22-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 CD22. In some embodiments, the first therapeutic is a first immunoconjugate

comprising a first antibody that binds an antigen other than CD22 and a first cytotoxic agent. In some embodiments, the first antibody binds CD79b. In some embodiments, the first therapeutic comprises a first antibody that binds CD22. In some embodiments, the first therapeutic is a first immunoconjugate comprising a first antibody that binds CD22 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 CD22-positive cancer that is resistant to a first therapeutic expresses P-glycoprotein (P-gp).

[019] In some embodiments, a method of treating an individual having a CD22-positive cancer is provided, wherein the CD22-positive cancer expresses P-gp.

[019a] In one aspect, there is provided use of the immunoconjugate as described herein in the manufacture of a medicament for the treatment of an individual having a CD22-positive cancer, wherein the CD22-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 CD22-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 CD22 positive / P-gp positive cancer expresses higher levels of P-gp mRNA and/or protein than control cells or tissue.

[020] In some embodiments, a method of inhibiting proliferation of a CD22-positive cell is provided.

[020a] In one aspect, there is provided use of the immunoconjugate as described herein in the manufacture of a medicament for the inhibition of proliferation of a CD22-positive cell, wherein the cell is to be exposed to the immunoconjugate under conditions permissive for binding of the immunoconjugate to CD22 on the surface of the cell, thereby inhibiting proliferation of the cell.

[020b] In one aspect, there is provided an *in vitro* method of inhibiting proliferation of a CD22-positive cell, the method comprising exposing the cell to the immunoconjugate as described herein under conditions permissive for binding of the immunoconjugate to CD22 on the surface of the cell, thereby inhibiting proliferation of the cell.

In some such embodiments, the method comprises exposing the cell to the immunoconjugate described herein under conditions permissive for binding of the immunoconjugate to CD22 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

[021] **Figures 1A-1B**: Figure 1A shows the amino acid sequence of the heavy chain variable region of murine 10F4 anti-CD22 antibody (m10F4) aligned with the humanized 10F4 version 1 (hu10F4v1) heavy chain variable region and the humanized 10F4 version 3

(hu10F4v3) heavy chain variable region, 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. Figure 1B shows the amino acid sequence of the light chain variable region of murine 10F4 anti-CD22 antibody (m10F4) aligned with the humanized 10F4 version 1 (hu10F4v1) light chain variable region and the humanized 10F4 version 3 (hu10F4v3) light chain variable region, and aligned with the human kappa I sequence. The antibody hu10F4v3 differ from hu10F4v1 at amino acid 28 of the HVR-L1 (N28V). 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.

the medicament is for treatment of CD22-positive cancer. In a further embodiment, the medicament is for use in a method of treating CD22-positive cancer, the method comprising administering to an individual having CD22-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.

[0257] In a further aspect, the invention provides a method for treating CD22-positive cancer. In one embodiment, the method comprises administering to an individual having such CD22-positive cancer an effective amount of an anti-CD22 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.

[0258] A CD22-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 CD22-positive cancer is a cancer that receives an anti-CD22 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 CD22-positive cancer expresses CD22 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 CD22-positive cancer is a cancer that expresses CD22 according to a reverse-transcriptase PCR (RT-PCR) assay that detects CD22 mRNA. In some embodiments, the RT-PCR is quantitative RT-PCR.

[0259] In some embodiments, immunoconjugates comprising a nemorubicin derivative covalently attached to the anti-CD22 antibody through a protease-cleavable linker are useful for treating diffuse large B-cell lymphomas and mantle cell lymphomas as evidenced, for example, by the xenograft models shown in Examples B, C, and D. The immunoconjugate for use in treating diffuse large B-cell lymphomas and mantle cell lymphomas may, in some embodiments, comprise PNU-159682 and a linker comprising val-cit, such as the immunoconjugate shown in Figure ~~4C~~4B. In some embodiments, immunoconjugates comprising a nemorubicin derivative covalently attached to the anti-CD22 antibody through a protease-cleavable linker are useful for treating Burkitt's lymphoma.

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 1.8, as indicated in the Examples below. 10F4v3-PNU-2 has the structure shown in Figure ~~4B~~ 4A (p = drug load).

Thio Hu anti-CD22 10F4v3 HC A118C-MC-val-cit-PAB-PNU-159682 ("10F4v3-PNU-1")

[0282] 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 1.9, as indicated in the Example below. 10F4v3-PNU-1 has the structure shown in Figure ~~4C~~ 4B (p = drug load).

Thio Hu anti-CD22 10F4v3 HC A118C-MC-val-cit-PAB-MMAE ("10F4v3-MMAE")

[0283] 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-MMAE ("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 determined to be about 2, as indicated in the examples below. Thio Hu anti-CD22 10F4v3 HC A118C-MC-val-cit-PAB-MMAE is described, e.g., in US 2008/0050310.

SEQ ID NO: 13, and (vi) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 14; and wherein p ranges from 1 to 3.

21. The immunoconjugate of claim 20, wherein the antibody comprises a VH sequence of SEQ ID NO: 7 and a VL sequence of SEQ ID NO: 8.

22. The immunoconjugate of claim 21, wherein the antibody comprises a heavy chain of SEQ ID NO: 26 and a light chain of SEQ ID NO: 23.

23. The immunoconjugate of any one of the preceding claims, wherein the antibody is a monoclonal antibody.

24. The immunoconjugate of any one of the preceding claims, wherein the antibody is a human, humanized, or chimeric antibody.

25. The immunoconjugate of any one of the preceding claims, wherein the antibody is an antibody fragment that binds CD22.

26. The immunoconjugate of claim any one of the preceding claims, wherein the antibody binds human CD22.

27. The immunoconjugate of claim 26, wherein human CD22 has the sequence of SEQ ID NO: 28 or SEQ ID NO: 29.

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 CD22-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 CD22-positive cancer.

31. The use~~method~~ of claim 30, wherein the CD22-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 CD79b.

34. The use~~method~~ of claim 33, wherein the additional therapeutic agent is an immunoconjugate comprising an antibody that binds CD79b covalently attached to a cytotoxic agent.

35. ~~A method of inhibiting proliferation of a CD22-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 CD22-positive cell, wherein the cell is to be exposed to the immunoconjugate under conditions permissive for binding of the immunoconjugate to CD22 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 CD22-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 CD22-positive cancer, wherein the CD22-positive cancer is resistant to a first therapeutic.

39. The use~~method~~ of claim 38, wherein the CD22-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 CD22.

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 CD22 and a first cytotoxic agent.

42. The use~~method~~ of claim 40 or claim 41, wherein the first antibody binds CD79b.

43. The use~~method~~ of claim 38 or claim 39, wherein the first therapeutic comprises a first antibody that binds CD22.

44. The use~~method~~ of claim 43, wherein the first therapeutic is a first immunoconjugate comprising a first antibody that binds CD22 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 CD22-positive cancer expresses P-glycoprotein (P-gp).

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

49. The use~~method~~ of claim 48, wherein the CD22-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 CD22-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 CD22 on the surface of the cell, thereby inhibiting proliferation of the cell.

51. The method of claim 50, wherein the cell is a neoplastic B cell.

52. The method of claim 51, wherein the cell is a lymphoma cell.