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.

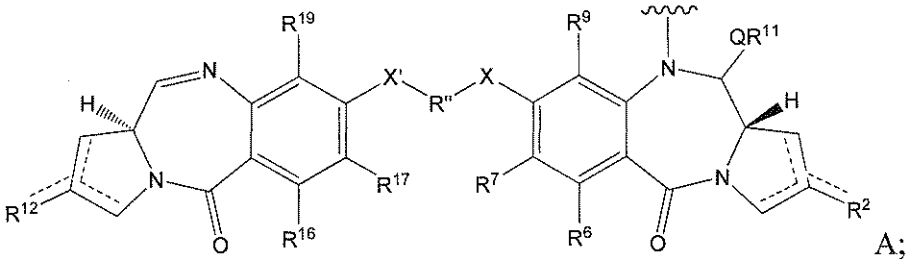
[007] In ~~some embodiments~~one aspect, there is provided an immunoconjugate comprising an antibody that binds CD22 covalently attached to a cytotoxic agent ~~is provided~~, wherein the antibody binds an epitope within amino acids 20 to 240 of SEQ ID NO: 28, and wherein ~~. In some embodiments,~~ the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

[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 pyrrolobenzodiazepine.

[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. 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;

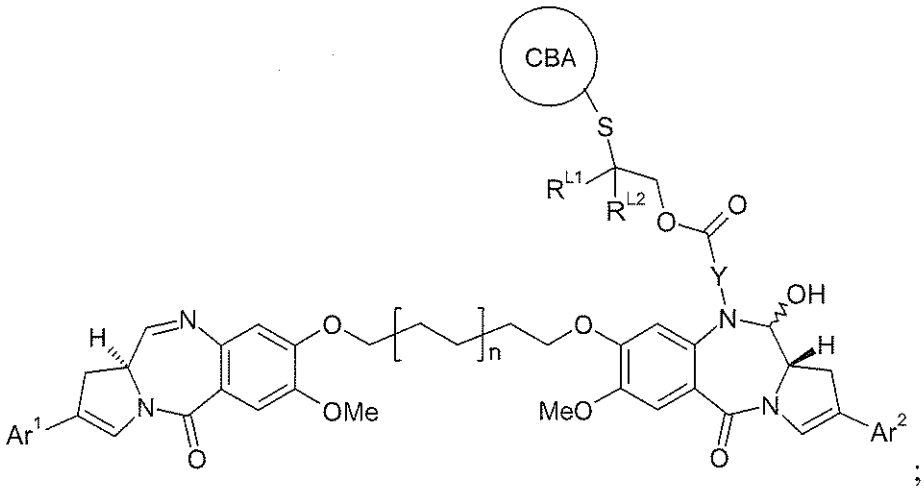
R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', NO_2, Me_3Sn and halo;

Q is independently selected from O, S and NH;

R^{11} is either H, or R or, where Q is O, SO_3M, where M is a metal cation;

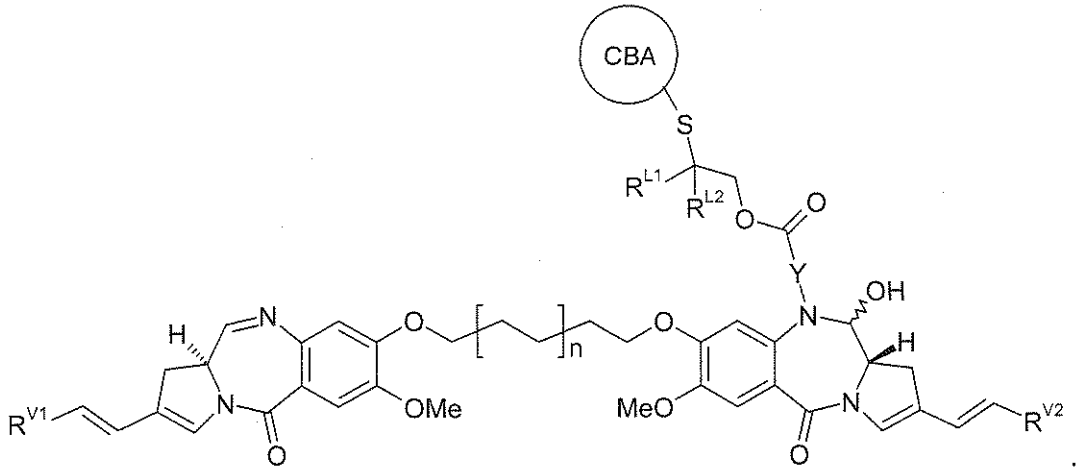
[018] In some embodiments, the immunoconjugate comprises the structure:



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wherein Ar^1 and Ar^2 are each independently optionally substituted C_{5-20} aryl. In some embodiments, Ar^1 and Ar^2 are each independently selected from optionally substituted phenyl, furanyl, thiophenyl and pyridyl.

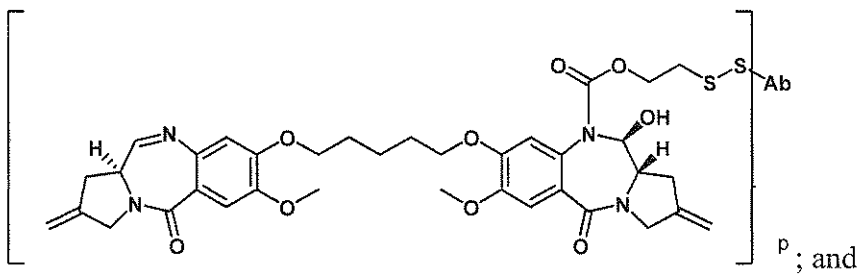
[019] In some embodiments, the immunoconjugate comprises the structure:



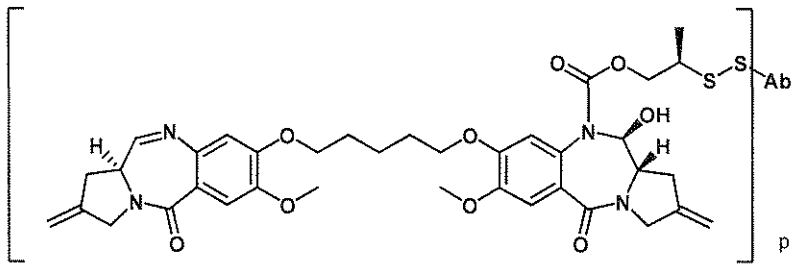
;

wherein R^{V1} and R^{V2} are each independently selected from H, methyl, ethyl, optionally substituted phenyl, and C_{5-6} heterocyclyl. In some embodiments, R^{V1} and R^{V2} are each independently selected from H, phenyl, and 4-fluorophenyl.

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

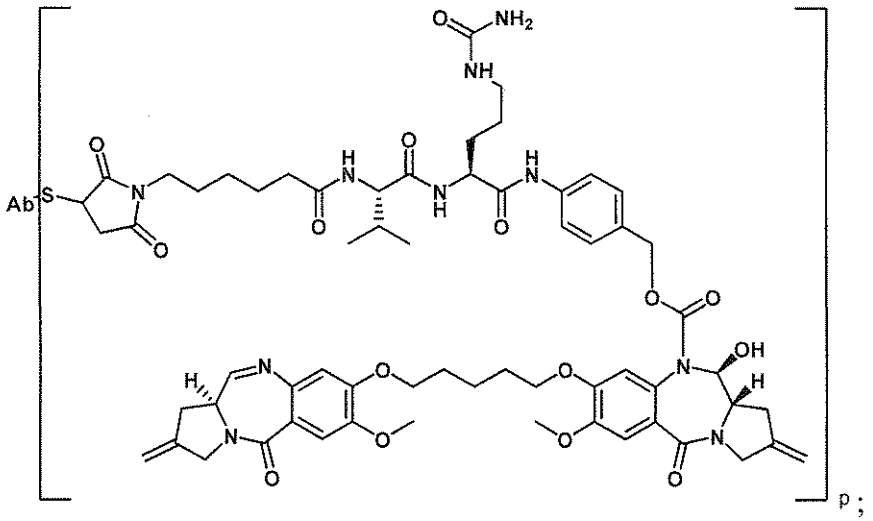


; and



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.

[021] In one aspect~~some embodiments~~, 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: 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.

[022] 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.

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

[0023a] 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 CD22-positive cancer.

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

[0024a] 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 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.

[025] In one aspect~~some embodiments~~, there is provided an *in vitro* method of inhibiting proliferation of a CD22-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 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

[026] **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

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

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

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

35. The immunoconjugate of claim 34, wherein human CD22 has the sequence of SEQ ID NO: 28 or SEQ ID NO: 29.

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

37. The pharmaceutical formulation of claim 36, further comprising an additional therapeutic agent.

38. ~~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 35 in the manufacture of a medicament for the treatment of an individual having a CD22-positive cancer.

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 39, ~~further comprising administering~~wherein the immunoconjugate is to be administered with an additional therapeutic agent to the individual.

41. The use~~method~~ of claim 40, wherein the additional therapeutic agent comprises an antibody that binds CD79b.

42. The use~~method~~ of claim 41, wherein the additional therapeutic agent is an immunoconjugate comprising an antibody that binds CD79b covalently attached to a cytotoxic agent.

43. ~~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 35 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.

44. The use~~method~~ of claim 43, wherein the cell is a neoplastic B cell.

45. The use~~method~~ of claim 44, wherein the cell is a lymphoma cell.

46. 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 35 under conditions permissive for binding of the immunoconjugate to CD22 on the surface of the cell, thereby inhibiting proliferation of the cell.

47. The method of claim 46, wherein the cell is a neoplastic B cell.

48. The method of claim 47, wherein the cell is a lymphoma cell.