IMMUNOCONJUGATES COMPRISING ANTI-CD79B ANTIBODIES

The invention provides immunoconjugates comprising anti-CD79b antibodies covalently attached to a pyrrolobenzodiazepine and methods of using the same.

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IMMUNOCONJUGATES COMPRISING ANTI-CD79B ANTIBODIES

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 (Iga, mb-1) and CD79b (½β, 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 slg 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 provides anti-CD79b antibodies and immunoconjugates and methods of using the same.

[005] In some embodiments, 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, 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. 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: 1, 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 IgGl, IgG2a or IgG2b antibody.

[007] In some embodiments, 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:

\[ \text{Formula A} \]

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

R^2 is independently selected from H, OH, =0, =CH_2, CN, R, OR, =CH-R^D, =C(R^D)_2,
O-SO_2-R, CO_2-R and COR, and optionally further selected from halo or dihalo, wherein R^D is independently selected from R, C0_2-R, COR, CHO, C0_2-H, and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2, NHR, NRR', N0_2, Me_3Sn and halo;
R⁷ is independently selected from H, R, OH, OR, SH, SR, NH₂, NHR, NRR', N⁰₂, MesSn and halo;
Q is independently selected from O, S and NH;
R¹¹ is either H, or R or, where Q is O, SO₃M, where M is a metal cation;
R and R' are each independently selected from optionally substituted C₁⁻₈ alkyl,
c₃⁻₈ heterocyclyl and c₅⁻₂₀ aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;
R¹², R¹⁶, R¹⁹ and R¹⁷ are as defined for R², R⁶, R⁹ and R⁷ respectively;
R'' is a C₃⁻₁₂ alkyene group, which chain may be interrupted by one or more heteroatoms and/or aromatic rings that are optionally substituted; and
X and X' are independently selected from O, S and N(H).

In some embodiments, D has the structure:

![Chemical Structure](image)

wherein n is 0 or 1.

In some embodiments, D has a structure selected from:

![Chemical Structure](image)

A(I);

![Chemical Structure](image)

A(III);

![Chemical Structure](image)

A(IV); and
wherein \( R^E \) and \( R^E' \) are each independently selected from \( H \) or \( R^D \), wherein \( R^D \) is independently selected from \( R \), \( C_0 \), \( R \), \( COR \), \( CHO \), \( C_0 \), \( H \), and halo; wherein \( Ar^1 \) and \( Ar^2 \) are each independently optionally substituted \( C_5 \) 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 attachment site to the linker; \( R^{v1} \) and \( R^{v2} \) are independently selected from \( H \), methyl, ethyl, phenyl, fluoro-substituted phenyl, and \( C_5 \) 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 immunoconjugate has the formula:

In some embodiments, \( p \) ranges from 1-3.

[014] In some embodiments, an immunoconjugate is provided, wherein the immunoconjugate has 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 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 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-Hodgkins 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.

[018] In some embodiments, a method of inhibiting proliferation of a CD79b-positive cell is provided. In some such embodiments, the method comprises 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 IgGl. 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-Al 18C heavy chain, in which the alanine at EU position 118 (sequential position alanine 118) is altered to a cysteine; Thio-huMA79bv28-
LC-V205C light chain in which a valine at Kabat position 205 (sequential position valine 209) is altered to a cysteine; and Thio-huMA79b.v28-HC-S400C heavy chain in which a serine at EU position 400 (sequential position serine 400) is altered to a cysteine. In each figure, the altered amino acid is shown in bold text with double underlining. Single underlining indicates constant regions. Variable regions are not underlined.

[023] Figure 5 shows the linker and drug structure of huMA79b.v28-PBD, which is described in Example A.

[024] Figure 6 shows efficacy of various antibody-drug conjugates in a WSU-DLCL2 mouse xenograft model, as described in Example B.

[025] Figure 7 shows efficacy of various antibody-drug conjugates in a Granta-519 mouse xenograft model, as described in Example C.

[026] Figure 8 shows efficacy of various antibody-drug conjugates in a SuDHL4-luc mouse xenograft model, as described in Example D.

[027] Figure 9 shows dose-dependent inhibition of tumor growth by huMA79b.v28-PBD in a SuDHL4-luc mouse xenograft model, as described in Example E.

[028] Figure 10 shows dose-dependent inhibition of tumor growth by huMA79b.v28-PBD in a BJAB-luc mouse xenograft model, as described in Example F.

[029] Figure 11 shows inhibition of tumor growth by huMA79b.v28-MMAE in a BJAB-luc mouse xenograft model, as described in Example G.

**DETAILED DESCRIPTION**

I. DEFINITIONS

[030] An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

[031] "Affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic
binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[032] An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

[033] The terms "anti-CD79b antibody" and "an antibody that binds to CD79b" refer to an antibody that is capable of binding CD79b with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting CD79b. In one embodiment, the extent of binding of an anti-CD79b antibody to an unrelated, non-CD79b protein is less than about 10% of the binding of the antibody to CD79b as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to CD79b has a dissociation constant (Kd) of \( \leq 1\mu M \), \( \leq 100\ nM \), \( \leq 10\ nM \), \( \leq 5\ nM \), \( \leq 2\ nM \), \( \leq 1\ nM \), \( \leq 0.1\ nM \), \( \leq 0.01\ nM \), or \( \leq 0.001\ nM \) (e.g., \( 10^{-5}\ M \) or less, e.g. from \( 10^{-5}\ M \) to \( 10^{-13}\ M \), e.g., from \( 10^{-9}\ M \) to \( 10^{-13}\ M \)). In certain embodiments, an anti-CD79b antibody binds to an epitope of CD79b that is conserved among CD79b from different species.

[034] The term "antibody" is used herein in the broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), and antibody fragments so long as they exhibit the desired antigen-binding activity.

[035] An "antibody fragment" refers to a molecule other than an intact antibody that comprises a portion of an intact antibody and that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')_2; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

[036] An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

[037] The terms "cancer" and "cancerous" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation.
Examples of cancer include, but are not limited to, melanoma, carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of cancer include B-cell associated cancers, including for example, high, intermediate and low grade lymphomas (including B cell lymphomas such as, for example, mucosa-associated-lymphoid tissue B cell lymphoma and non-Hodgkin's lymphoma (NHL), mantle cell lymphoma, Burkitt's lymphoma, small lymphocytic lymphoma, marginal zone lymphoma, diffuse large cell lymphoma, follicular lymphoma, and Hodgkin's lymphoma and T cell lymphomas) and leukemias (including secondary leukemia, chronic lymphocytic leukemia (CLL), such as B cell leukemia (CD5+ B lymphocytes), myeloid leukemia, such as acute myeloid leukemia, chronic myeloid leukemia, lymphoid leukemia, such as acute lymphoblastic leukemia (ALL) and myelodysplasia), and other hematological and/or B cell- or T-cell-associated cancers. Also included are cancers of additional hematopoietic cells, including polymorphonuclear leukocytes, such as basophils, eosinophils, neutrophils and monocytes, dendritic cells, platelets, erythrocytes and natural killer cells. Also included are cancerous B cell proliferative disorders selected from the following: lymphoma, non-Hodgkins 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), and mantle cell lymphoma. The origins of B-cell cancers include as follows: marginal zone B-cell lymphoma origins in memory B-cells in marginal zone, follicular lymphoma and diffuse large B-cell lymphoma originates in centrocytes in the light zone of germinal centers, chronic lymphocytic leukemia and small lymphocytic leukemia originates in B1 cells (CD5+), mantle cell lymphoma originates in naive B-cells in the mantle zone and Burkitt's lymphoma originates in centroblasts in the dark zone of germinal centers. Tissues which include hematopoietic cells referred herein to as "hematopoietic cell tissues" include thymus and bone marrow and peripheral lymphoid tissues, such as spleen, lymph nodes, lymphoid tissues associated with mucosa, such as the gut-associated lymphoid tissues, tonsils, Peyer's patches and appendix and lymphoid tissues associated with other mucosa, for example, the bronchial linings. Further particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic
carcinoma, leukemia and other lymphoproliferative disorders, and various types of head and neck cancer.

[038] A "B-cell malignancy" herein includes non-Hodgkin's lymphoma (NHL), including low grade/follicular NHL, small lymphocytic (SL) NHL, intermediate grade/follicular NHL, intermediate grade diffuse NHL, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, mantle cell lymphoma, AIDS-related lymphoma, and Waldenstrom's Macroglobulinemia, non-Hodgkin's lymphoma (NHL), lymphocyte predominant Hodgkin's disease (LPHD), small lymphocytic lymphoma (SLL), chronic lymphocytic leukemia (CLL), indolent NHL including relapsed indolent NHL and rituximab-refractory indolent NHL; leukemia, including acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), Hairy cell leukemia, chronic myeloblasts leukemia; Burkitt's lymphoma; mantle cell lymphoma; and other hematologic malignancies. Such malignancies may be treated with antibodies directed against B-cell surface markers, such as CD79b. Such diseases are contemplated herein to be treated by the administration of an antibody directed against a B cell surface marker, such as CD79b, and includes the administration of an unconjugated ("naked") antibody or an antibody conjugated to a cytotoxic agent as disclosed herein. Such diseases are also contemplated herein to be treated by combination therapy including an anti-CD79b antibody or anti-CD79b antibody drug conjugate of the invention in combination with another antibody or antibody drug conjugate, another cytotoxic agent, radiation or other treatment administered simultaneously or in series. In an exemplary treatment method, an anti-CD79b immunoconjugate is administered in combination with an anti-CD22 antibody, immunoglobulin, or CD22 binding fragment thereof, either together or sequentially. The anti-CD22 antibody may be a naked antibody or an antibody drug conjugate. In another exemplary treatment method, an anti-CD79b immunoconjugate is administered in combination with an anti-CD20 antibody, immunoglobulin, or CD20 binding fragment thereof, either together or sequentially. The anti-CD20 antibody may be a naked antibody or an antibody drug conjugate. In some embodiments of the combination therapy, the anti-CD79b immunoconjugate is administered with Rituxan® (rituximab).

[039] The term "non-Hodgkin's lymphoma" or "NHL", as used herein, refers to a cancer of the lymphatic system other than Hodgkin's lymphomas. Hodgkin's lymphomas can generally be distinguished from non-Hodgkin's lymphomas by the presence of Reed-Sternberg cells in Hodgkin’s lymphomas and the absence of said cells in non-Hodgkin's lymphomas. Examples of non-Hodgkin's lymphomas encompassed by the term as used herein include any that would be identified as such by one skilled in the art (e.g., an oncologist or pathologist) in
accordance with classification schemes known in the art, such as the Revised European-American Lymphoma (REAL) scheme as described in Color Atlas of Clinical Hematology (3rd edition), A. Victor Hoffbrand and John E. Pettit (eds.) (Harcourt Publishers Ltd., 2000). See, in particular, the lists in Fig. 11.57, 11.58 and 11.59. More specific examples include, but are not limited to, relapsed or refractory NHL, front line low grade NHL, Stage II/IV NHL, chemotherapy resistant NHL, precursor B lymphoblastic leukemia and/or lymphoma, small lymphocytic lymphoma, B cell chronic lymphocytic leukemia and/or prolymphocytic leukemia and/or small lymphocytic lymphoma, B-cell prolymphocytic lymphoma, immunocytoma and/or lymphoplasmacytic lymphoma, lymphoplasmacytic lymphoma, marginal zone B cell lymphoma, splenic marginal zone lymphoma, extranodal marginal zone - MALT lymphoma, nodal marginal zone lymphoma, hairy cell leukemia, plasmacytoma and/or plasma cell myeloma, low grade/follicular lymphoma, intermediate grade/follicular NHL, mantle cell lymphoma, follicle center lymphoma (follicular), intermediate grade diffuse NHL, diffuse large B-cell lymphoma, aggressive NHL (including aggressive front-line NHL and aggressive relapsed NHL), NHL relapsing after or refractory to autologous stem cell transplantation, primary mediastinal large B-cell lymphoma, primary effusion lymphoma, high grade immunoblastic NHL, high grade lymphoblastic NHL, high grade small non-cleaved cell NHL, bulky disease NHL, Burkitt's lymphoma, precursor (peripheral) large granular lymphocytic leukemia, mycosis fungoides and/or Sezary syndrome, skin (cutaneous) lymphomas, anaplastic large cell lymphoma, angiocentric lymphoma.

[040] The term "chimeric" antibody refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[041] The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

[042] The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to, radioactive isotopes (e.g., At\(^{211}\), T\(^{131}\), Y\(^{90}\), Re\(^{186}\), Re\(^{188}\), Sm\(^{153}\), Bi\(^{212}\), P\(^{32}\), Pb\(^{212}\) and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth
inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

[043] A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, imposulfan and piposulfan; aziridines such as benzodopa, carboquone, metredopa, and uredop; ethylenimines and methylamlamelamines including altretamine, triethylenemelamine, triethylenephosphoramid, triethylenthiophosphoramid and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARTNOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®, acetylcamptothecin, scopoletin, and 9-aminoacampothecin); bryostatin; callystatin; CC-1065 (including its adzelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CBI-TMI); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammall and calicheamicin omegall (see, e.g., Agnew, Chem Intl. Ed. Engl., 33: 183-186 (1994)); dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, caetinomycin, carabinc, carcinomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peptomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thioguanine; pyrimidine analogs
such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, 
doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone 
propionate, epitiostanol, mepihitostane, testolactone; anti-adrenals such as aminoglutethimide, 
mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide 
glycoside; aminolevulinic acid; eniluracil; ansacrine; bestrabucil; bisantrene; edatraxate; 
defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; 
etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as 
maytansine and ansamitocins; mitouguazone; mitoxantrone; mopedanol; nitraerine; 
pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® 
polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; 
spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes 
-especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, 
FILDESIN®); dacarbazine; mammomustine; mitobronitol; mitolactol; pipobroman; gacytosine; 
arabinoside ("Ara-C"); thiotepa; taxoids, e.g., paclitaxel (TAXOL®; Bristol-Myers Squibb 
Oncology, Princeton, NJ.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle 
formulation of paclitaxel (American Pharmaceutical Partners, Schaumberg, Illinois), and 
docetaxel (TAXOTERE®; Rhone-Poulenc Rorer, Antony, France); chloranbucil; gemcitabine 
(GEMZAR®); 6-thioguanine; mercaptopurine; methotrexate; platinum analogs such as 
cisplatin and carboplatin; vinblastine (VELBAN®); platinum; etoposide (VP-16); ifosfamide; 
mitoxantrone; vincristine (ONCOVIN®); oxaliplatin; leucovorin; vinorelbine 
(NAVELBINE®); novantrone; edatraxate; daunomycin; aminopterin; ibandronate; 
topoisomerase inhibitor RFS 200; difluoromethylornithine (DMFO); retinoids such as retinoic 
acid; capecitabine (XELODA®); pharmaceutically acceptable salts, acids or derivatives of any 
of the above; as well as combinations of two or more of the above such as CHOP, an 
abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and 
prednisolone; CVP, an abbreviation for a combined therapy of cyclophosphamide, vincristine, 
and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin 
(ELOXATIN™) combined with 5-FU and leucovorin.

[044] "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector 
functions include: Clq binding and complement dependent cytotoxicity (CDC); Fc receptor 
binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down 
regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.
An "effective amount" of an agent, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

The term "epitope" refers to the particular site on an antigen molecule to which an antibody binds.

The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

"Framework" or "FR" refers to variable domain residues other than hypervariable region (HVR) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

The term "glycosylated forms of CD79b" refers to naturally occurring forms of CD79b that are post-translationally modified by the addition of carbohydrate residues.

The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

A "human antibody" is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding
sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[053] A "human consensus framework" is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al, *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. In one embodiment, for the VL, the subgroup is subgroup kappa I as in Kabat et al, supra. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al, supra.

[054] A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

[055] The term "hypervariable region" or "HVR," as used herein, refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops ("hypervariable loops"). Generally, native four-chain antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the "complementarity determining regions" (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (HI), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987).) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3. (Kabat et al, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991).) With the exception of CDR1 in VH, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise "specificity determining residues," or "SDRs," which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-
CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008).) Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., supra.

[056] An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[057] An "individual" or "subject" is a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

[058] An "isolated antibody" is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., J. Chromatogr. B 848:79-87 (2007).

[059] An "isolated nucleic acid" refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[060] "Isolated nucleic acid encoding an anti-CD79b antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[061] The term "CD79b," as used herein, refers to any native CD79b from any vertebrate source, including mammals such as primates (e.g. humans, cynomolgus monkey (cyno)) and rodents (e.g., mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed CD79b as well as any form of CD79b that results from processing in the cell. The term also encompasses naturally occurring variants of CD79b, e.g., splice variants, allelic variants, and isoforms. The amino acid sequence of an exemplary human CD79b precursor (with signal sequence) is shown in SEQ ID NO: 40. The amino acid sequence of an exemplary human mature CD79b (without signal sequence) is shown in SEQ ID NO: 41.
The term "CD79b-positive cancer" refers to a cancer comprising cells that express CD79b on their surface.

The term "CD79b-positive cell" refers to a cell that expresses CD79b on its surface.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radionuclide. The naked antibody may be present in a pharmaceutical formulation.

"Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (k) and lambda (λ), based on the amino acid sequence of its constant domain.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications,
usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

"Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU5 10087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \times \frac{X}{Y}$$

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are
obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

[070] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[071] A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[072] As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, immunono conjugates of the invention are used to delay development of a disease or to slow the progression of a disease.

[073] The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. *Kuby Immunology*, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. See, e.g., Portolano et al. *J. Immunol.* 150:880-887 (1993); Clarkson et al, *Nature* 352:624-628 (1991).

[074] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."
The phrase "optionally substituted" as used herein, pertains to a parent group that may be unsubstituted or that may be substituted.

Unless otherwise specified, the term "substituted" as used herein, pertains to a parent group that bears one or more substituents. The term "substituent" is used herein in the conventional sense and refers to a chemical moiety that is covalently attached to, or if appropriate, fused to, a parent group. A wide variety of substituents are known, and methods for their formation and introduction into a variety of parent groups are also known.

In some embodiments, the substituents described herein (which include optional substituents) are limited to those groups that are not reactive to the antibody. In some embodiments, the linker to the antibody is formed from the N10 position of the PBD compound through the linker (L). In some instances, reactive functional groups located at other parts of the PBD structure may be capable of forming additional bonds to the antibody (this may be referred to as crosslinking). Such additional bonds, in some instances, may alter transport and biological activity of the conjugate. Therefore, in some embodiments, the additional substituents are limited to those lacking reactive functionality.

In some embodiments, the substituents are selected from R, OR, SR, NRR', N02, halo, C02R, COR, CONH2, CONHR, and CONRR'. In some embodiments, the substituents are selected from R, OR, SR, NRR', N02, COR, CONH2, CONHR, and CONRR'. In some embodiments, the substituents are selected from R, OR, SR, NRR', N02, and halo. In some embodiments, the substituents are selected from the group consisting of R, OR, SR, NRR', and N02.

Any of the embodiments discussed above may be applied to any of the substituents described herein. Alternatively, the substituents may be selected from one or more of the groups discussed below.

The term "C1-12 alkyl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a carbon atom of a hydrocarbon compound having from 1 to 12 carbon atoms, which is aliphatic, and which may be cyclic or acyclic, and which may be saturated or unsaturated (e.g. partially unsaturated, fully unsaturated). Thus, the term "alkyl" includes the sub-classes alkenyl, alkynyl, cycloalkyl, etc., discussed below.

Examples of saturated alkyl groups include, but are not limited to, methyl (C1), ethyl (C2), propyl (C3), butyl (C4), pentyl (C5), hexyl (C6) and heptyl (C7).

Examples of saturated linear alkyl groups include, but are not limited to, methyl (C1), ethyl (C2), n-propyl (C3), n-butyl (C4), n-pentyl (amyl) (C5), n-hexyl (C6) and n-heptyl (C7).
Examples of saturated branched alkyl groups include, but are not limited to, iso-propyl (C₃), iso-butyl (C₄), sec-butyl (C₄), tert-butyl (C₄), iso-pentyl (C₅), and neo-pentyl (C₅).

An alkyl group may optionally be interrupted by one or more heteroatoms selected from O, N(H) and S. Such groups may be referred to as "heteroalkyl".

The term "C₂₋₁₂ heteroalkyl" as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from a carbon atom of a hydrocarbon compound having from 2 to 12 carbon atoms, and one or more heteroatoms selected from O, N(H) and S, preferably O and S.

Examples of heteroalkyl groups include, but are not limited to, those comprising one or more ethylene glycol units of the type -(OCH₂CH₂)-. The terminus of a heteroalkyl group may be the primary form of a heteroatom, e.g. -OH, -SH or -NH₂. In a preferred embodiment, the terminus is -CH₃.

The term "C₂₋₁₂ alkenyl" as used herein, pertains to an alkyl group having one or more carbon-carbon double bonds.

Examples of unsaturated alkenyl groups include, but are not limited to, ethenyl (vinyl, -CH=CH₂), 1-propenyl (-CH=CH-CH₃), 2-propenyl (allyl, -CH-CH=CH₂), isopropenyl (1-methylvinyl, -C(CH₃)=CH₂), butenyl (C₇), pentenyl (C₈), and hexenyl (C₉).

The term "C₂₋₁₂ alkynyl" as used herein, pertains to an alkyl group having one or more carbon-carbon triple bonds.

Examples of unsaturated alkynyl groups include, but are not limited to, ethynyl (-C≡CH) and 2-propynyl (propargyl, -CH₂-C≡CH).

The term "C₃₋₁₂ cycloalkyl" as used herein, pertains to an alkyl group which is also a cycyl group; that is, a monovalent moiety obtained by removing a hydrogen atom from an alicyclic ring atom of a cyclic hydrocarbon (carbocyclic) compound, which moiety has from 3 to 7 carbon atoms, including from 3 to 7 ring atoms.

Examples of cycloalkyl groups include, but are not limited to, those derived from:

(i) saturated monocyclic hydrocarbon compounds:
cyclopropane (C₃), cyclobutane (C₄), cyclopentane (C₅), cyclohexane (C₆),
cycloheptane (C₇), methylcyclopropane (C₄), dimethylecyclopropane (C₅),
methylcyclobutane (C₅), dimethylcyclobutane (C₆), methylcyclopentane (C₆),
dimethylcyclopentane (C₇) and methylcyclohexane (C₈);

(ii) unsaturated monocyclic hydrocarbon compounds:
cyclopropene \((C_3)\), cyclobutene \((C_4)\), cyclopentene \((C_5)\), cyclohexene \((C_6)\),
methylcyclopropene \((C_4)\), dimethylcyclopropene \((C_5)\), methylcyclobutene \((C_5)\),
dimethylcyclobutene \((C_6)\), methylcyclopentene \((C_6)\), dimethylcyclopentene \((C_7)\) and
methylcyclohexene \((C_7)\); and
(iii) saturated poly cyclic hydrocarbon compounds:
norcarane \((C_7)\), norpinane \((C_7)\), norbornane \((C_7)\).

[093] The term "C\(_{3-20}\) heterocyclyl" as used herein, pertains to a monovalent moiety
obtained by removing a hydrogen atom from a ring atom of a heterocyclic compound, which
moiety has from 3 to 20 ring atoms, of which from 1 to 10 are ring heteroatoms. In some
embodiments, each ring has from 3 to 7 ring atoms, of which from 1 to 4 are ring heteroatoms.

[094] As used herein, the prefixes (e.g. \(C\(_{3-20}\), \(C\(_{5-6}\), etc.) denote the number of
ring atoms, or range of number of ring atoms, whether carbon atoms or heteroatoms. For
example, the term "\(C_5\) \(_{6}\)heterocyclyl", as used herein, pertains to a heterocyclyl group having 5
or 6 ring atoms.

[095] Examples of monocyclic heterocyclyl groups include, but are not limited to,
those derived from:

(i) \(N\)i: aziridine \((C_3)\), azetidine \((C_4)\), pyrrolidine (tetrahydropyrrole) \((C_5)\), pyrrole
(e.g., 3-pyrroline, 2,5-dihydropyrrole) \((C_5)\), 2H-pyrrole or 3H-pyrrole (isopyrrole,
isoazole) \((C_5)\), piperidine \((C_6)\), dihydropyridine \((C_6)\), tetrahydropyridine \((C_6)\),
azepine \((C_7)\);
(ii) \(O\)i: oxirane \((C_3)\), oxetane \((C_4)\), oxolane (tetrahydrofuran) \((C_5)\), oxole
(dihydrofuran) \((C_5)\), oxane (tetrahydropropyn) \((C_6)\), dihydropropyn \((C_6)\), pyran \((C_6)\),
oxepin \((C_7)\);
(iii) \(S\)i: thiirane \((C_3)\), thietane \((C_4)\), thiolane (tetrahydrothiophene) \((C_5)\), thiane
(tetrahydrothiopyran) \((C_6)\), thiepane \((C_7)\);
(iv) \(O\)2: dioxolane \((C_5)\), dioxane \((C_6)\), and dioxypane \((C_7)\);
(v) \(0\)3: trioxane \((C_6)\);
(vi) \(N\)2: imidazolidine \((C_5)\), pyrazolidine (diazolidine) \((C_5)\), imidazoline \((C_5)\),
pyrazoline (dihydroazazole) \((C_5)\), piperezine \((C_6)\);
(vii) \(N\)1O\(1\): tetrahydrooxazole \((C_5)\), dihydrooxazole \((C_5)\), tetrahydroisoxazole \((C_5)\),
dihydroisoxazole \((C_5)\), morpholine \((C_6)\), tetrahydrooxazine \((C_6)\), dihydroxazine \((C_6)\),
oxazine \((C_6)\);
(viii) \(N\)1S\(1\): thiazoline \((C_5)\), thiazolidine \((C_5)\), thiomorpholine \((C_6)\);
(ix) \(N\)2O\(1\): oxadiazine \((C_6)\);
(x) \(O\)1S\(1\): oxathiole \((C_5)\) and oxathiane (thioxane) \((C_6)\); and,
(xi) N1O1S1: oxathiazine (Ce).

[096] Examples of substituted monocyclic heterocycl groups include, but are not limited to, those derived from saccharides, in cyclic form, for example, furanos (C5), such as arabinofuranose, lyxofuranose, ribofuranose, and xylofuranose, and pyranoses (Ce), such as allopypyranose, altropyranose, glucopyranose, mannopyranose, gulopyranose, idopyranose, galactopyranose, and talopyranose.

[097] The term “C5-20 aryl”, as used herein, pertains to a monovalent moiety obtained by removing a hydrogen atom from an aromatic ring atom of an aromatic compound, which moiety has from 3 to 20 ring atoms. In some embodiments, each ring has from 5 to 7 ring atoms.

[098] In some embodiments, the ring atoms are all carbon atoms, as in "carboaryl groups". Examples of carboaryl groups include, but are not limited to, those derived from benzene (i.e. phenyl) (Ce), naphthalene (C10), azulene (C10), anthracene (C14), phenanthrene (C14), naphthacene (C14b), and pyrene (C2).

[099] Examples of aryl groups which comprise fused rings, at least one of which is an aromatic ring, include, but are not limited to, groups derived from indane (e.g. 2,3-dihydro-1H-indene) (C9g), indene (C9g), isoindene (C9g), tetralin (1,2,3,4-tetrahydronaphthalene (C10)),acenaphthene (C12), fluorene (C13), phenalene (C13), acephenanthrene (C15), and acceanthrene (C16).

[0100] In some embodiments, the ring atoms may include one or more heteroatoms, as in "heteroaryl groups". Examples of monocyclic heteroaryl groups include, but are not limited to, those derived from:

(i) Ni: pyrrole (azole) (C5), pyridine (azine) (Ce);
(ii) Oi: furan (oxole) (C5);
(iii) Si: thiophene (thiole) (C5);
(iv) N1O1: oxazole (C5), isoxazole (C5), isoxazine (Ce);
(v) N2O1: oxadiazole (furazan) (C5);
(vi) N3O1: oxatriazole (C5);
(vii) N1S1: thiazole (C5), isothiazole (C5);
(viii) N2: imidazole (1,3-diazole) (C5), pyrazole (1,2-diazole) (C5), pyridazine (1,2-diazine) (Ce), pyrimidine (1,3-diazine) (Ce) (e.g., cytosine, thymine, uracil), pyrazine (1,4-diazine) (Ce);
(ix) N3: triazole (C5), triazine (Ce); and,
(x) N4: tetrazole (C5).
Examples of heteroaryl which comprise fused rings, include, but are not limited to:

(i) C₉ (with 2 fused rings) derived from benzofuran (Oi), isobenzofuran (Oi), indole (Ni), isoindole (Ni), indolizine (Ni), indoline (Ni), isoindoline (Ni), purine (N₄) (e.g., adenine, guanine), benzimidazole (N₂), indazole (N₂), benzoazole (N1O1), benzisoxazole (N1O1), benzodioxole (O2), benzofurazan (N2O1), benzotriazole (N3), benzothiophen (Si), benzothiazole (N1S1), benzothiadiazole (N₂S);

(ii) C₁₁ (with 2 fused rings) derived from chromene (Oi), isochromene (Oi), chroman (Oi), isochroman (Oi), benzodioxan (O2), quinoline (Ni), isoquinoline (Ni), quinolizine (Ni), benzoazine (N1O1), benzodiazine (N₂), pyridopyridine (N₂), quinoxaline (N₃), quinazoline (N₂), cinnoline (N₂), phthalazine (N₂), naphthyridine (N₃), pteridine (N₄);

(iii) C₁₅ (with 2 fused rings) derived from benzodiazepine (N₂);

(iv) C₁₃ (with 3 fused rings) derived from carbazole (Ni), dibenzofuran (Oi), dibenzothiophene (Si), carboline (N₂), perimidine (N₂), pyridoindole (N₂); and,

(v) C₁₄ (with 3 fused rings) derived from acridine (Ni), xanthene (Oi), thioxanthene (Si), oxanthrene (O₂), phenoxathiin (O1S1), phenazine (N₂), phenozone (N1O1), phenothiazine (N1S1), thianthrene (S₂), phenanthridine (Ni), phenanthroline (N₂), phenazine (N₂).

The above groups, whether alone or part of another substituent, may themselves optionally be substituted with one or more groups selected from themselves and the additional substituents listed below.

Halo: -F, -Cl, -Br, and -I.

Hydroxy: -OH.

Ether: -OR, wherein R is an ether substituent, for example, a C₁₋₇ alkyl group (also referred to as a C₁₋₇ alkoxy group, discussed below), a C₃₋₂₀ heterocyclyl group (also referred to as a C₃₋₂₀ heterocyclyloxy group), or a Cs⁶oaryl group (also referred to as a Cs₅₋₂₀ aryloxy group). In some embodiments, R is a C₁₋₇ alkyl group.

Alkoxy: -OR, wherein R is an alkyl group, for example, a C₁₋₇ alkyl group. Examples of C₁₋₇ alkoxy groups include, but are not limited to, -OMe (methoxy), -OEt (ethoxy), -O(nPr) (n-propoxy), -O(iPr) (isopropoxy), -O(nBu) (n-butoxy), -O(sBu) (sec-butoxy), -O(iBu) (isobutoxy), and -O(tBu) (tert-butoxy).

Acetal: -CH(OR₁)(OR₂), wherein R₁ and R₂ are independently acetal substituents, for example, a C₁₋₇ alkyl group, a C₃₋₂₀ heterocyclyl group, or a Cs₅₋₂₀ aryloxy group. In some embodiments, R₁ and/or R₂ are independently C₁₋₇ alkyl group. In some
embodiments, in the case of a "cyclic" acetal group, R\textsuperscript{1} and R\textsuperscript{2}, taken together with the two oxygen atoms to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Examples of acetal groups include, but are not limited to, -CH(OMe)\textsubscript{2}, -CH(OEt)\textsubscript{2}, and -CH(OMe)(OEt).

[0108] Hemiacetal: -CH(OH)(OR\textsuperscript{1}), wherein R\textsuperscript{1} is a hemiacetal substituent, for example, a C\textsubscript{1}-7 alkyl group, a C\textsubscript{5}-20 heterocyclyl group, or a C\textsubscript{5}-20 aryl group. In some embodiments, R\textsuperscript{1} is a C\textsubscript{1}-7 alkyl group. Examples of hemiacetal groups include, but are not limited to, -CH(OH)(OMe) and -CH(OH)(OEt).

[0109] Ketal: -CRCOR\textsuperscript{1}OR\textsuperscript{2}, where R\textsuperscript{1} and R\textsuperscript{2} are as defined for acetics, and R is a ketal substituent other than hydrogen, for example, a C\textsubscript{1}-7 alkyl group, a C\textsubscript{5}-20 heterocyclyl group, or a C\textsubscript{5}-20 aryl group. In some embodiments, R is a C\textsubscript{1}-7 alkyl group. Examples ketal groups include, but are not limited to, -C(Me)(OMe)\textsubscript{2}, -C(Me)(OEt)\textsubscript{2}, -C(Me)(OMe)(OEt), -C(Et)(OMe)\textsubscript{2}, -C(Et)(OEt)\textsubscript{2}, and -C(Et)(OMe)(OEt).

[0110] Hemiketal: -CRiOHXOR\textsuperscript{1}, where R\textsuperscript{1} is as defined for hemiacetals, and R is a hemiketel substituent other than hydrogen, for example, a C\textsubscript{1}-7 alkyl group, a C\textsubscript{5}-20 heterocyclyl group, or a C\textsubscript{5}-20 aryl group. In some embodiments, R is a C\textsubscript{1}-7 alkyl group. Examples of hemiketal groups include, but are not limited to, -C(Me)(OH)(OMe), -C(Et)(OH)(OMe), -C(Me)(OH)(OEt), and -C(Et)(OH)(OEt).

[0111] Oxo (keto, -one): =O.
[0112] Thione (thioketone): =S.
[0113] Imino (imine): =NR, wherein R is an imino substituent, for example, hydrogen, C\textsubscript{1}-7 alkyl group, a C\textsubscript{5}-20 heterocyclyl group, or a C\textsubscript{5}-20 aryl group. In some embodiments, R is hydrogen or a C\textsubscript{1}-7 alkyl group. Examples of imino groups include, but are not limited to, =NH, =NMe, =NEt, and =NPh.

[0114] Formyl (carbaldehyde, carboxaldehyde): -C(=0)H.
[0115] Acyl (keto): -C(=0)R, wherein R is an acyl substituent, for example, a C\textsubscript{1}-7 alkyl group (also referred to as C\textsubscript{1}-7 alkylacyl or C\textsubscript{1}-7 alkanoyl), a C\textsubscript{5}-20 heterocyclyl group (also referred to as C\textsubscript{5}-20 heterocyclylacyl), or a C\textsubscript{5}-20 aryl group (also referred to as C\textsubscript{5}-20 arylacyl). In some embodiments, R is a C\textsubscript{1}-7 alkyl group. Examples of acyl groups include, but are not limited to, -C(=0)CH\textsubscript{3} (acetyl), -C(=0)CH\textsubscript{2}CH\textsubscript{3} (propionyl), -C(=0)C(CH\textsubscript{3})\textsubscript{3} (t-butyryl), and -C(=0)Ph (benzoyl, phenone).

[0116] Carboxy (carboxylic acid): -C(=0)OH.
[0117] Thiocarboxy (thiocarboxylic acid): -C(=S)SH.
[0118] Thiolocarboxy (thiolocarboxylic acid): -C(=S)SH.
[0119] Thionocarboxy (thionocarboxylic acid): -C(=S)OH.
[0120] Imidic acid: -C(=NH)OH.
[0121] Hydroxamic acid: -C(=NOH)OH.
[0122] Ester (carboxylate, carboxylic acid ester, oxycarbonyl): -C(=0)OR, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group. In some embodiments, R is a C_{1-7} alkyl group. Examples of ester groups include, but are not limited to, -C(=0)OCH_{3}, -C(=0)OCH_{2}CH_{3}, -C(=0)OC(CH_{3})_{3}, and -C(=0)OPh.
[0123] Acyloxy (reverse ester): -OC(=0)R, wherein R is an acyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group. In some embodiments, R is a C_{1-7} alkyl group. Examples of acyloxy groups include, but are not limited to, -OC(=0)CH_{3} (acetoxy), -OC(=0)CH_{2}CH_{3}, -OC(=0)OC(CH_{3})_{3}, -OC(=0)Ph, and -OC(=0)CH_{2}Ph.
[0124] Oxy carbonyloxy: -OC(=0)OR, wherein R is an ester substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group. In some embodiments, R is a C_{1-7} alkyl group. Examples of oxy carbonyloxy groups include, but are not limited to, -OC(=0)OCH_{3}, -OC(=0)OCH_{2}CH_{3}, -OC(=0)OC(CH_{3})_{3}, and -OC(=0)OPh.
[0125] Amino: -NR^{1}R^{2}, wherein R^{1} and R^{2} are independently amino substituents, for example, hydrogen, a C_{1-7} alkyl group (also referred to as C_{1-7} alkylamino or di-C_{1-7} alkylamino), a C_{3-20} heterocyclyl group, or a C_{5-20} aryl group. In some embodiments, R^{1} and R^{2} are independently H or a C_{1-7} alkyl group. In some embodiments, in the case of a "cyclic" amino group, R^{1} and R^{2}, taken together with the nitrogen atom to which they are attached, form a heterocyclic ring having from 4 to 8 ring atoms. Amino groups may be primary (-NH_{2}), secondary (-NH{R^{1}}), or tertiary (-NHR^{1}R^{2}), and in cationic form, may be quaternary (-NR^{1}R^{2}R^{3}). Examples of amino groups include, but are not limited to, -NH_{2}, -NHCH_{3}, -NHC(CH_{3})_{2}, -N(CH_{3})_{2}, -N(CH_{2}CH_{3})_{2}, and -NHPh. Examples of cyclic amino groups include, but are not limited to, aziridino, azetidino, pyrrolidino, piperidino, piperazino, morpholino, and thiomorpholino.
[0126] Amido (carbamoyl, carbamyl, aminocarbonyl, carboxamide): -C(=0)NR^{1}R^{2}, wherein R^{1} and R^{2} are independently amino substituents, as defined for amino groups. Examples of amido groups include, but are not limited to, -C(=0)NH_{2}, -C(=0)NHCH_{3}, -C(=0)N(CH_{3})_{2}, -C(=0)NHCH_{2}CH_{3}, and -C(=0)N(CH_{2}CH_{3})_{2}, as well as amido groups in which R^{1} and R^{2}, together with the nitrogen atom to which they are attached, form a heterocyclic structure as in, for example, piperidinocarbonyl, morpholinocarbonyl, thiomorpholinocarbonyl, and piperazinocarbonyl.
[0127] Thioamido (thiocarbamyl): \(-\text{C}(=\text{S})\text{NR}_1\text{R}_2\), wherein \(\text{R}_1\) and \(\text{R}_2\) are independently amino substituents, as defined for amino groups. Examples of thioamido groups include, but are not limited to, \(-\text{C}(=\text{S})\text{NH}_2\), \(-\text{C}(=\text{S})\text{NHCH}_3\), \(-\text{C}(=\text{S})\text{N(CH}_3)_2\), and \(-\text{C}(=\text{S})\text{NHCH}_2\text{CH}_3\).

[0128] Acylamido (acylamino): \(-\text{NR}^1\text{C}(=0)\text{R}_2\), wherein \(\text{R}^1\) is an amide substituent, for example, hydrogen, a \(\text{C}_1-\text{C}_7\) alkyl group, a \(\text{C}_3-\text{C}_20\) heterocyclyl group, or a \(\text{C}_5-\text{C}_20\) aryl group, and \(\text{R}_2\) is an acyl substituent, for example, a \(\text{C}_1-\text{C}_7\) alkyl group, a \(\text{C}_3-\text{C}_20\) heterocyclyl group, or a \(\text{C}_8-\text{C}_20\text{aryl}\) group. In some embodiments, \(\text{R}^1\) and/or \(\text{R}_2\) is hydrogen or a \(\text{C}_1-\text{C}_7\) alkyl group. Examples of acylamide groups include, but are not limited to, \(-\text{NHC}(=0)\text{CH}_3\), \(-\text{NHC}(=0)\text{CH}_2\text{CH}_3\), and \(-\text{NHC}(=0)\text{Ph}\). \(\text{R}^1\) and \(\text{R}_2\) may together form a cyclic structure, as in, for example, succinimidyl, maleimidyl, and phthalimidyl:

\[
\begin{align*}
\text{succinimidyl} & \quad \text{maleimidyl} & \quad \text{phthalimidyl}
\end{align*}
\]

[0129] Aminocarbonyloxy: \(-\text{OC}(=0)\text{NR}_1\text{R}_2\), wherein \(\text{R}_1\) and \(\text{R}_2\) are independently amino substituents, as defined for amino groups. Examples of aminocarbonyloxy groups include, but are not limited to, \(-\text{OC}(=0)\text{NH}_2\), \(-\text{OC}(=0)\text{NHMe}\), \(-\text{OC}(=0)\text{NMe}_2\), and \(-\text{OC}(=0)\text{NEt}_2\).

[0130] Ureido: \(-\text{N}(\text{R}^1)\text{CONR}_2\text{R}_3\) wherein \(\text{R}_2\) and \(\text{R}_3\) are independently amino substituents, as defined for amino groups, and \(\text{R}^1\) is a ureido substituent, for example, hydrogen, a \(\text{C}_1-\text{C}_7\) alkyl group, a \(\text{C}_3-\text{C}_20\) heterocyclyl group, or a \(\text{C}_5-\text{C}_20\) aryl group. In some embodiments, \(\text{R}^1\) is hydrogen or a \(\text{C}_1-\text{C}_7\) alkyl group. Examples of ureido groups include, but are not limited to, \(-\text{NCONH}_2\), \(-\text{NCONHMe}\), \(-\text{NCONHET}\), \(-\text{NCONMe}_2\), \(-\text{NCONET}_2\), \(-\text{NMeCONH}_2\), \(-\text{NMeCONHMe}\), \(-\text{NMeCONHET}\), \(-\text{NMeCONMe}_2\), and \(-\text{NMeCONET}_2\).

[0131] Guanidino: \(-\text{NH-C}(=\text{NH})\text{NH}_2\).

[0132] Tetrazolyl: a five membered aromatic ring having four nitrogen atoms and one carbon atom.

\[
\begin{align*}
\text{Tetrazolyl}
\end{align*}
\]

[0133] Amidine (amidino): \(-\text{C}(=\text{NR})\text{NR}_2\), wherein each \(\text{R}\) is an amidine substituent, for example, hydrogen, a \(\text{C}_1-\text{C}_7\) alkyl group, a \(\text{C}_3-\text{C}_20\) heterocyclyl group, or a \(\text{C}_5-\text{C}_20\) aryl group. In some embodiments, each \(\text{R}\) is \(\text{H}\) or a \(\text{C}_1-\text{C}_7\) alkyl group. Examples of amidine groups include, but are not limited to, \(-\text{C}(=\text{NH})\text{NH}_2\), \(-\text{C}(=\text{NH})\text{NMe}_2\), and \(-\text{C}(=\text{NMe})\text{NMe}_2\).
[0135] Nitroso: -NO.
[0137] Cyano (nitrile, carbonitrile): -CN.
[0138] Isocyno: -NC.
[0139] Cyanato: -OCN.
[0140] Isocyanato: -NCO.
[0141] Thiocynano (thiocyanato): -SCN.
[0142] Isothiocyanato: -NCS.
[0143] Sulhydryl (thiol, mercapto): -SH.
[0144] Thioether (sulfide): -SR, wherein R is a thioether substituent, for example, a
Ci-7 alkyl group (also referred to as a Ci7alkylthio group), a C₃-2₀ heterocycl group, or a C₅-2₀
aryl group. In some embodiments, R is a C₁-7 alkyl group. Examples of thioether groups
include, but are not limited to, -SCH₃ and -SCH₂CH₃.
[0145] Disulfide: -SS-R, wherein R is a disulfide substituent, for example, a C₁-7 alkyl
group, a C₃-2₀ heterocycl group, or a C₅-2₀ heterocycl group. In some embodiments, R is a C₁-7 alkyl
group (also referred to herein as C₁-7 alkyl disulfide). Examples of disulfide groups include,
but are not limited to, -SSCH₃ and -SSCH₂CH₃.
[0146] Sulfine (sulfinyl, sulfoxide): -SO(=0)R, wherein R is a sulfine substituent, for
example, a C₁,7 alkyl group, a C₃-2₀ heterocycl group, or a C₅-2₀ aryl group. In some
embodiments, R is a C₁-7 alkyl group. Examples of sulfine groups include, but are not limited
to, -SO(=0)CH₃ and -SO(=0)CH₂CH₃.
[0147] Sulfone (sulfonyl): -SO(=0)₂R, wherein R is a sulfone substituent, for example, a
C₁-7 alkyl group, a C₃-2₀ heterocycl group, or a C₅-2₀ aryl group. In some embodiments, R is a
C₁-7 alkyl group, including, for example, a fluorinated or perfluorinated C₁-7 alkyl group.
Examples of sulfone groups include, but are not limited to, -SO(=0)₂CH₃ (methanesulfonyl,
mesyl), -SO(=0)₂CF₃ (triflyl), -SO(=0)₂CH₂CH₃ (esyl), -SO(=0)₂C₆F₅ (nonaflyl), -SO(=0)₂CH₂CF₃
(tresyl), -SO(=0)₂CH₂CH₂NH₂ (tauryl), -SO(=0)₂Ph (phenylsulfonyl, besyl), 4-
methylphenylsulfonyl (tosyl), 4-chlorophenylsulfonyl (closyl), 4-bromophenylsulfonyl
(brosyl), 4-nitrophenyl (nosyl), 2-naphthalenesulfonate (napsyl), and 5-dimethylamino-
naphthalen-1-ylsulfonate (dansyl).
[0148] Sulfinic acid (sulfino): -SO(=0)OH, -SO₂H.
[0149] Sulfonic acid (sulfo): -SO(=0)₂OH, -SO₃H.
[0150] Sulfinate (sulfinic acid ester): -SO(=0)OR; wherein R is a sulfinate substituent,
for example, a C₁-7 alkyl group, a C₃-2₀ heterocycl group, or a C₅-2₀ aryl group. In some
embodiments, R is a C_{1-7} alkyl group. Examples of sulfinate groups include, but are not limited to, -S(=0)OCH \_3 (methoxysulfonyl; methyl sulfinate) and -S(=0)OCH \_2CH \_3 (ethoxysulfonyl; ethyl sulfinate).

[0151] Sulfonate (sulfonic acid ester): -S(=0) \_2OR, wherein R is a sulfonate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a Cs\^oaryl group. In some embodiments, R is a C_{1-7} alkyl group. Examples of sulfonate groups include, but are not limited to, -S(=0) \_2OCH \_3 (methoxysulfonate; methyl sulfonate) and -S(=0) \_2OCH \_2CH \_3 (ethoxysulfonate; ethyl sulfonate).

[0152] Sulfinyloxy: -OS(=0)\_2R, wherein R is a sulfinyloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a Cs\^oaryl group. In some embodiments, R is a C_{1-7} alkyl group. Examples of sulfinyloxy groups include, but are not limited to, -OS(=0)\_2CH \_3 (mesylate) and -OS(=0)\_2CH \_2CH \_3 (esylate).

[0153] Sulfonyloxy: -OS(=0)\_2R, wherein R is a sulfonloxy substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a Cs\^oaryl group. In some embodiments, R is a C_{1-7} alkyl group. Examples of sulfonyloxy groups include, but are not limited to, -OS(=0)\_2OCH \_3 and -SO(=0)\_2OCH \_2CH \_3.

[0154] Sulfate: -OS(=0)\_2OR; wherein R is a sulfate substituent, for example, a C_{1-7} alkyl group, a C_{3-20} heterocyclyl group, or a Cs\^oaryl group. In some embodiments, R is a C_{1-7} alkyl group. Examples of sulfate groups include, but are not limited to, -OS(=0)\_2OCH \_3 and -SO(=0)\_2OCH \_2CH \_3.

[0155] Sulffamyl (sulffamoyl; sulfinic acid amide; sulfinamide): -S(=0)NR \_1R \_2, wherein R \_1 and R \_2 are independently amino substituents, as defined for amino groups. Examples of sulfamyl groups include, but are not limited to, -S(=0)NH \_2, -S(=0)NH(CH \_3), -S(=0)N(CH \_3)\_2, -S(=0)NH(CH \_2CH \_3), -S(=0)N(CH \_2CH \_3)\_2, and -S(=0)NHPh.

[0156] Sulffamidno (sulffnamoyl; sulfonic acid amide; sulfonamide): -S(=0)\_2NR\_1R\_2, wherein R \_1 and R \_2 are independently amino substituents, as defined for amino groups. Examples of sulffnamidno groups include, but are not limited to, -S(=0)\_2NH\_2, -S(=0)\_2NH(CH\_3), -S(=0)\_2N(CH\_3)\_2, -S(=0)\_2NH(CH\_2CH\_3), -S(=0)\_2N(CH\_2CH\_3)\_2, and -S(=0)\_2NHPh.

[0157] Sulffamino: -NR\_1S(=0)\_2OH, wherein R \_1 is an amino substituent, as defined for amino groups. Examples of sulffamino groups include, but are not limited to, -NHS(=0)\_2OH and -N(CH\_3)S(=0)\_2OH.

[0158] Sulffnamido: -NR\_1S(=0)\_2R, wherein R \_1 is an amino substituent, as defined for amino groups, and R is a sulfnamido substituent, for example, a C_{1-7} alkyl group, a C_{3-20}
heterocyclyl group, or a C5-20 aryl group. In some embodiments, R is a C1-7 alkyl group. Examples of sulfonamino groups include, but are not limited to, -NHS(=O)2CH3 and -N(CH3)S(=O)2C6H5.

[0159] Sulfamino: -NR1S(=O)R, wherein R1 is an amino substituent, as defined for amino groups, and R is a sulfamino substituent, for example, a C1-7 alkyl group, a C3-20 heterocyclyl group, or a C5-20 aryl group. In some embodiments, R is a C1-7 alkyl group. Examples of sulfamino groups include, but are not limited to, -NHS(=O)CH3 and -N(CH3)S(=O)C6H5.

[0160] Phosphino (phosphine): -PR2, wherein R is an a phosphino substituent, for example, -H, a C1-7 alkyl group, a C3-20 heterocyclyl group, or a C5-20 aryl group. In some embodiments, R is -H, a C1-7 alkyl group, or a Cs^oaryl group. Examples of phosphino groups include, but are not limited to, -PH2, -P(CH3)2, -P(CH2CH3)2, -P(t-Bu)2, and -P(Ph)2.

[0161] Phospho: -P(=O)2.

[0162] Phoshinyl (phosphino oxide): -P(=O)OR, wherein R is an a phosphinyl substituent, for example, a C1-7 alkyl group, a C3-20 heterocyclyl group, or a Cs^oaryl group. In some embodiments, R is a C1-7 alkyl group or a C5-20 ary1 group. Examples of phosphinyl groups include, but are not limited to, -P(=O)(CH3)2, -P(=O)(CH2CH3)2, -P(=O)(t-Bu)2, and -P(=O)(Ph)2.


[0164] Phosphonate (phosphono ester): -P(=O)(OR)2, where R is an a phosphonate substituent, for example, -H, a C1-7 alkyl group, a C3-20 heterocyclyl group, or a C5-20 ary1 group. In some embodiments, R is -H, a C1-7 alkyl group, or a C5-20 ary1 group. Examples of phosphonate groups include, but are not limited to, -P(=O)(OCH3)2, -P(=O)(OCH2CH3)2, -P(=O)(0-t-Bu)2, and -P(=O)(OPh)2.


[0166] Phosphate (phosphonoxy ester): -OP(=O)(OR)2, where R is a phosphate substituent, for example, -H, a C1-7 alkyl group, a C3-20 heterocyclyl group, or a C5-20 ary1 group. In some embodiments, R is -H, a C1-7 alkyl group, or a C5-20 ary1 group. Examples of phosphate groups include, but are not limited to, -OP(=O)(OCH3)2, -OP(=O)(OCH2CH3)2, -OP(=O)(0-t-Bu)2, and -OP(=O)(OPh)2.

[0167] Phosphorous acid: -OP(OH)2.

[0168] Phosphate: -OP(OH)2, where R is a phosphate substituent, for example, -H, a C1-7 alkyl group, a C3-20 heterocyclyl group, or a Cs^oaryl group. In some embodiments, R is -H, a C1-7 alkyl group, or a C5-20 ary1 group. Examples of phosphate groups include, but are not limited to, -OP(OCH3)2, -OP(OCH2CH3)2, -OP(0-t-Bu)2, and -OP(OPh)2.
Phosphoramidite: \(-\text{OP(OR}_1^-\text{NR}_2^2\), where R}_1^1 and \text{R}_2^2 are phosphoramidite substituents, for example, \(-\text{H}, a (optionally substituted) \text{C}_3^3 \text{alkyl group, a C}_3^n \text{heterocyclenot group, or a C}_5^n \text{aryl group. In some embodiments, R is -H, a C}_1^1 \text{alkyl group, or a C}_5^n \text{aryl group. Examples of phosphoramidite groups include, but are not limited to, -OP(OCH}_2^2\text{CH}_2^2\)-N(CH}_3_-CH(=CH-CH(CH}_3^-)_2^2, -OP(OCH}_2^2\text{CH}_\text{CN})\text{-N(i-Pr)}_2^2\) and -OP(=0)(OCH}_2^2\text{CH}_2^2\)-N(i-Pr)_2^2.

Phosphoramidate: -OP(=0)(OR}_1^-\text{NR}_2^2\), where \text{R}_1^1 and \text{R}_2^2 are phosphoramidate substituents, for example, \(-\text{H}, a (optionally substituted) \text{C}_3^3 \text{alkyl group, a C}_3^n \text{heterocyclenot group, or a C}_5^n \text{aryl group. In some embodiments, R}_1^1 and \text{R}_2^2 are \(-\text{H}, a \text{C}_1^1 \text{alkyl group, or a C}_5^n \text{aryl group. Examples of phosphoramidate groups include, but are not limited to, -OP(=0)(OCH}_2^2\text{CH}_2^2\)-N(CH}_3_-CH(=CH-CH(CH}_3^-)_2^2, -OP(=0)(OCH}_2^2\text{CH}_\text{CN})\text{-N(i-Pr)}_2^2\) and -OP(=0)(OCH}_2^2\text{CH}_2^2\)-N(i-Pr)_2^2.

The term "C}_3^n \text{alkylene"}, as used herein, pertains to a bidentate moiety obtained by removing two hydrogen atoms, either both from the same carbon atom, or one from each of two different carbon atoms, of a hydrocarbon compound having from 3 to 12 carbon atoms (unless otherwise specified), which is aliphatic, and which may be cyclic or acyclic, and which may be saturated, partially unsaturated, or fully unsaturated. Thus, the term "alkylene" includes the sub-classes alkenylene, alkynylene, cycloalkylene, etc., discussed below.

Examples of linear saturated C}_3^n \text{alkylene groups include, but are not limited to, -(CH}_2^2)_n^-\) where \text{n is an integer from 3 to 12, for example, -(CH}_2^2\text{CH}_2^2\)- (propylene), -(CH}_2^2\text{CH}_2^2\text{CH}_2^2\)- (butylene), -(CH}_2^2\text{CH}_2^2\text{CH}_2^2\text{CH}_2^2\)- (pentylene) and -(CH}_2^2\text{CH}_2^2\text{CH}_2^2\text{CH}_2^2\)- (heptylene).

Examples of branched saturated C}_3^n \text{alkylene groups include, but are not limited to, -(CH}_2^2\text{CH}_2^2\)-, -(CH}_2^2\text{CH}_2^2\text{CH}_2^2\)-, -(CH}_2^2\text{CH}_2^2\text{CH}_2^2\text{CH}_2^2\)-, -(CH}_2^2\text{CH}_2^2\text{CH}_2^2\text{CH}_2^2\)-, -(CH}_2^2\text{CH}_2^2\text{CH}_2^2\text{CH}_2^2\)- and -(CH}_2^2\text{CH}_2^2\text{CH}_2^2\text{CH}_2^2\)-.

Examples of linear partially unsaturated C}_3^n \text{alkylene groups (C}_3^n \text{alkylene, and alkynylene groups) include, but are not limited to, -(CH}=CH\text{-CH}_2^2\)-, -(CH}=CH\text{-CH}_2^2\text{-}, -(CH}=CH\text{-CH}_2^2\text{-CH}_2^2\text{-}, -(CH}=CH\text{-CH}=CH\text{-CH}_2^2\text{-}, -(CH}=CH\text{-CH}=CH\text{-CH}_2^2\text{-CH}_2^2\text{-}, -(CH}=CH\text{-CH)=CH\text{-CH}_2^2\text{-CH}_2^2\text{-CH}_2^2\text{-}, and -(CH}_2^2\text{C}=-\text{C}-(CH}_2^2\text{-}.

Examples of branched partially unsaturated C}_3^n \text{alkylene groups (C}_3^n \text{alkylene and alkynylene groups) include, but are not limited to, -(CH}_3^-\text{CH}=CH\text{-}, -(CH}_3^-\text{CH}=CH\text{-CH}_2^2\text{-}, -(CH}_3^-\text{CH}=CH\text{-CH}_2^2\text{-CH}_2^2\text{-}, -(CH}_3^-\text{CH}=CH\text{-CH}_2^2\text{-CH}_2^2\text{-CH}_2^2\text{-}, -(CH}_3^-\text{CH}=CH\text{-CH}_2^2\text{-CH}_2^2\text{-CH}_2^2\text{-CH}_2^2\text{-} and -(CH}_2^2\text{C}=-\text{C}-(CH}_3^-\text{-}.
Examples of alicyclic saturated C$_{3-12}$ alkylene groups (C$_{3-12}$ cycloalkylenes) include, but are not limited to, cyclopentylene (e.g. cyclopent-1,3-ylene), and cyclohexylene (e.g. cyclohex-1,4-ylene).

Examples of alicyclic partially unsaturated C$_{3-12}$ alkylene groups (C$_{3-12}$ cycloalkylenes) include, but are not limited to, cyclopentenylene (e.g. 4-cyclopenten-1,3-ylene), cyclohexenylene (e.g. 2-cyclohexen-1,4-ylene; 3-cyclohexen-1,2-ylene; 2,5-cyclohexadien-1,4-ylene).

"Linker" refers to a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety. Nonlimiting exemplary linkers are described herein.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliei, E. and Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or R and S, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur
where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

[0184] "Leaving group" refers to a functional group that can be substituted by another functional group. Certain leaving groups are well known in the art, and examples include, but are not limited to, a halide (e.g., chloride, bromide, iodide), methanesulfonyl (mesyl), p-toluene sulfonate (tosyl), trifluormethylsulfonate (triflate), and trifluormethylsulfonate.

[0185] The term "protecting group" refers to a substituent that is commonly employed to block or protect a particular functionality while reacting other functional groups on the compound. For example, an "amino-protecting group" is a substituent attached to an amino group that blocks or protects the amino functionality in the compound. Suitable amino-protecting groups include, but are not limited to, acetyl, trifluoroacetyl, t-butoxycarbonyl (BOC), benzylxycarbonyl (CBZ) and 9-fluorenymethylenoxycarbonyl (Fmoc). For a general description of protecting groups and their use, see T. W. Greene, Protective Groups in Organic Synthesis, John Wiley & Sons, New York, 1991, or a later edition.

II. COMPOSITIONS AND METHODS

[0186] In one aspect, the invention is based, in part, on antibodies that bind to CD79b and immunoconjugates comprising such antibodies. Antibodies and immunoconjugates of the invention are useful, e.g., for the diagnosis or treatment of CD79b-positive cancers.

A. Exemplary Anti-CD79b Antibodies

[0187] In some embodiments, isolated antibodies that bind to CD79b are provided. CD79b heterodimerizes with CD79a to form CD79, a B-cell-restricted receptor. CD79b is expressed in various B-cell related disorders and cancers, including various lymphomas, such as Non-Hodgkin’s lymphoma.

[0188] An exemplary naturally occurring human CD79b precursor sequence, with signal sequence (amino acids 1-28) is provided in SEQ ID NO: 40, and the corresponding mature CD79b sequence is shown in SEQ ID NO: 41 (corresponding to amino acids 29 to 229 of SEQ ID NO: 40).

[0189] In some embodiments, an anti-CD79b antibody binds human CD79b. In some embodiments, an anti-CD79b antibody binds human CD79b with an affinity of ≤ 10 nM, or ≤ 5 nM, or ≤ 4 nM, or ≤ 3 nM, or ≤ 2 nM and optionally ≥ 0.0001 nM, or ≥ 0.001 nM, or ≥ 0.01 nM. Exemplary such antibodies include huMA79bv28 and huMA79bv32, which bind to
human CD79b with an affinity of 0.44 nM and 0.24 nM, respectively. See, e.g., US 8,088,378 B2.

Assays

[0190] Whether an anti-CD79b antibody "binds with an affinity of" ≤ 10 nM, or ≤ 5 nM, or ≤ 4 nM, or ≤ 3 nM, or ≤ 2 nM, is determined using Scatchard analysis, as described, e.g., US 8,088,378 B2. Briefly, 125I labeled antibody is competed against serial dilutions of unlabeled antibody in the presence of BJAB cells expressing human CD79b. Following the incubation, cells are washed and cell pellet counts read by a gamma counter. See, e.g., US 8,088,378 B2. Binding affinity, K_D, of the antibodies may be determined in accordance with standard Scatchard analysis performed utilizing a non-linear curve fitting program (see, for example, Munson et al, Anal Biochem, 107: 220-239, 1980).

Antibody MA79b and other embodiments

[0191] In some embodiments, the invention provides an anti-CD79b antibody or immunoconjugate comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 23; (d) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 18, 24, and 35; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some such embodiments, the antibody or immunoconjugate comprises at least one of: (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23, and/or (ii) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 24 and 35.

[0192] In some embodiments, the invention provides an anti-CD79b antibody or immunoconjugate comprising at least one, two, three, four, five, or six HVRs selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; (d) HVR-L1 comprising an amino acid sequence of SEQ ID NO: 24; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some such embodiments, the antibody or immunoconjugate comprises at least one of: (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23, and/or (ii) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24.

[0193] In one aspect, the invention provides an antibody or immunoconjugate comprising at least one, at least two, or all three VH HVR sequences selected from (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21; (b) HVR-H2 comprising the
amino acid sequence of SEQ ID NO: 22; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23. In some embodiments, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23. In another embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23 and HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In a further embodiment, the antibody comprises HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23, HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26, and HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22. In a further embodiment, the antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23.

[0194] In another aspect, the invention provides an antibody or immunoconjugate comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 18, 24, and 35; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In another aspect, the invention provides an antibody or immunoconjugate comprising at least one, at least two, or all three VL HVR sequences selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In one embodiment, the antibody comprises (a) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 24 and 35; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24 or SEQ ID NO: 35. In some embodiments, the antibody comprises an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24. In some embodiments, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the antibody comprises (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 35; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0195] In another aspect, an antibody or immunoconjugate comprises (a) a VH domain comprising at least one, at least two, or all three VH HVR sequences selected from (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 an amino acid sequence selected from SEQ ID NOs: 17 and 23; and (b) a VL domain comprising at least one, at least
two, or all three VL HVR sequences selected from (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 such embodiments, the antibody or immunoconjugate comprises at least one of: (i) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23, and/or (ii) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24 or SEQ ID NO: 35.

[0196] In another aspect, the invention provides an antibody or immunoconjugate comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 23; (d) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 18, 24, and 35; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some such embodiments, the antibody or immunoconjugate comprises at least one of: HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23 and/or HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 24 and 35. In another aspect, the invention provides an antibody or immunoconjugate comprising (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; (d) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0197] In any of the above embodiments, an anti-CD79b antibody is humanized. In one embodiment, an anti-CD79b antibody comprises HVRs as in any of the above embodiments, and further comprises a human acceptor framework, e.g. a human immunoglobulin framework or a human consensus framework. In certain embodiments, the human acceptor framework is the human VL kappa 1 (VLKI) framework and/or the VH framework V\¾i. In some embodiments, a humanized anti-CD79b antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 23; (d) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 18, 24, and 35; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, a humanized anti-CD79b antibody comprises (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21; (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22; (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 23; (d)
HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24; (e) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (f) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0198] In another aspect, an anti-CD79b antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 11. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 11 contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-CD79b antibody comprising that sequence retains the ability to bind to CD79b. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 11. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 11. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs).

[0199] Optionally, the anti-CD79b antibody comprises the VH sequence of any one of SEQ ID NOs: 5, 7, 9, 11, and 13, including post-translational modifications of that sequence. In some embodiments, the anti-CD79b antibody comprises the VH sequence of SEQ ID NO: 11, including post-translational modifications of that sequence. In a particular embodiment, the VH comprises one, two or three HVRs selected from: (a) HVR-H1 comprising the amino acid sequence of SEQ ID NO: 21, (b) HVR-H2 comprising the amino acid sequence of SEQ ID NO: 22, and (c) HVR-H3 comprising the amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 23.

[0200] In some embodiments, an anti-CD79b antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of SEQ ID NO: 12. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to the amino acid sequence of SEQ ID NO: 12 contains substitutions (e.g., conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-CD79b antibody comprising that sequence retains the ability to bind to CD79b. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 12. In certain embodiments, a total of 1 to 5 amino acids have been substituted, inserted and/or deleted in SEQ ID NO: 12. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (i.e., in the FRs). Optionally, the anti-CD79b antibody comprises the VL sequence of any one of SEQ ID NOs: 6, 8, 10, 12, and 14, including post-translational modifications of that sequence.
In some embodiments, the anti-CD79b antibody comprises the VL sequence of SEQ ID NO: 12, including post-translational modifications of that sequence. In a particular embodiment, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising an amino acid sequence selected from SEQ ID NOs: 18, 24, and 35; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26. In some embodiments, the VL comprises one, two or three HVRs selected from (a) HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24 or SEQ ID NO: 35; (b) HVR-L2 comprising the amino acid sequence of SEQ ID NO: 25; and (c) HVR-L3 comprising the amino acid sequence of SEQ ID NO: 26.

[0201] In another aspect, an anti-CD79b antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 11 and SEQ ID NO: 12, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 13 and SEQ ID NO: 14, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 9 and SEQ ID NO: 10, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the VH and VL sequences in SEQ ID NO: 7 and SEQ ID NO: 8, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the heavy chain and light chain sequences in SEQ ID NO: 38 and SEQ ID NO: 37, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the heavy chain and light chain sequences in SEQ ID NO: 39 and SEQ ID NO: 37, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the heavy chain and light chain sequences in SEQ ID NO: 38 and SEQ ID NO: 54, respectively, including post-translational modifications of those sequences. In some embodiments, the antibody comprises the heavy chain and light chain sequences in SEQ ID NO: 55 and SEQ ID NO: 37, respectively, including post-translational modifications of those sequences.

[0202] In a further aspect, the invention provides an antibody or immunoconjugate that binds to the same epitope as an anti-CD79b antibody provided herein. For example, in certain embodiments, an antibody or immunoconjugate is provided that binds to the same epitope as an anti-CD79b antibody comprising a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 12.
[0203] In a further aspect of the invention, an anti-CD79b antibody according to any of
the above embodiments is a monoclonal antibody, including a chimeric, humanized or human
antibody. In one embodiment, an anti-CD79b antibody is an antibody fragment, e.g., a Fv,
Fab, Fab', scFv, diabody, or F(ab')2 fragment. In another embodiment, the antibody is a
substantially full length antibody, e.g., an IgGl antibody or other antibody class or isotype as
defined herein.

[0204] In any of the immunoconjugates described above, the antibody may be
conjugated to a drug moiety. In some embodiments, the antibody is conjugated to a cytotoxic
agent. In some such embodiments, the cytotoxic agent is a pyrrolobenzodiazepine (PBD), such
as a PBD dimer. Various nonlimiting exemplary PBD dimers are discussed herein.

[0205] In a further aspect, an anti-CD79b antibody or immunoconjugate according to
any of the above embodiments may incorporate any of the features, singly or in combination,
as described in Sections 1-7 below.

1. Antibody Affinity

[0206] In certain embodiments, an antibody provided herein has a dissociation constant
(Kd) of ≤ 1µM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤ 0.001 nM, and
optionally is ≥ 10⁻¹³ M. (e.g. 10⁻⁸ M or less, e.g. from 10⁻⁸ M to 10⁻¹³ M, e.g., from 10⁻⁹ M to
10⁻¹³ M).

[0207] In one embodiment, Kd is measured by a radiolabeled antigen binding assay
(RIA) performed with the Fab version of an antibody of interest and its antigen as described by
the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating
Fab with a minimal concentration of (¹²⁵I)-labeled antigen in the presence of a titration series
of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see,
e.g., Chen et al, J. Mol. Biol. 293:865-881(1999)). To establish conditions for the assay,
MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 µg/ml of a
capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and
subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at
room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or
26 pM [¹²⁵I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with
assessment of the anti-VEGF antibody, Fab-12, in Presta et al, Cancer Res. 57:4593-4599
(1997)). The Fab of interest is then incubated overnight; however, the incubation may
continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached.
Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature
(e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1%
polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 µl/well of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0208] According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIACore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with N-ethyl-N’-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier’s instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (-0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25°C at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio k_{off}/k_{on}. See, e.g., Chen et al., J. Mol. Biol. 293:865-881 (1999). If the on-rate exceeds 10^4 M⁻¹ s⁻¹ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoScientific) with a stirred cuvette.

2. Antibody Fragments

[0209] In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab’, Fab’-SH, F(ab’)2, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson et al. Nat. Med. 9:129-134 (2003). For a review of scFv fragments, see, e.g., Pluckthun, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S.
Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(\(ab')2\) fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.


[0211] Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, e.g., U.S. Patent No. 6,248,516 B1).

[0212] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. \(E.\ coli\) or phage), as described herein.

3. **Chimeric and Humanized Antibodies**

[0213] In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, e.g., in U.S. Patent No. 4,816,567; and Morrison et al, Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0214] In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, e.g., CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the HVR residues are derived), e.g., to restore or improve antibody specificity or affinity.


4. Human Antibodies


[0218] Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal’s chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, *Nat. Biotech.* 23:11 17-1125 (2005). See also, e.g., U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No.
Human variable regions from intact antibodies generated by such animals may be further modified, e.g., by combining with a different human constant region.


Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

5. Library-Derived Antibodies


In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage

[0223] Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

6. *Multispecific Antibodies*

[0224] In certain embodiments, an antibody provided herein is a multispecific antibody, e.g. bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for CD79b and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of CD79b. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express CD79b. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, e.g. US 2006/0025576A1).

The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to CD79b as well as another, different antigen (see, US 2008/0069820, for example).

**7. Antibody Variants**

In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

- **a) Substitution, Insertion, and Deletion Variants**

In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "preferred substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

<table>
<thead>
<tr>
<th>Original Residue</th>
<th>Exemplary Substitutions</th>
<th>Preferred Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala (A)</td>
<td>Val; Leu; Ile</td>
<td>Val</td>
</tr>
<tr>
<td>Arg (R)</td>
<td>Lys; Gln; Asn</td>
<td>Lys</td>
</tr>
<tr>
<td>Asn (N)</td>
<td>Gln; His; Asp, Lys; Arg</td>
<td>Gln</td>
</tr>
<tr>
<td>Asp (D)</td>
<td>Glu; Asn</td>
<td>Glu</td>
</tr>
<tr>
<td>Original Residue</td>
<td>Exemplary Substitutions</td>
<td>Preferred Substitutions</td>
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</tr>
<tr>
<td>Cys (C)</td>
<td>Ser; Ala</td>
<td>Ser</td>
</tr>
<tr>
<td>Gin (Q)</td>
<td>Asn; Glu</td>
<td>Asn</td>
</tr>
<tr>
<td>Glu (E)</td>
<td>Asp; Gin</td>
<td>Asp</td>
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<tr>
<td>Gly (G)</td>
<td>Ala</td>
<td>Ala</td>
</tr>
<tr>
<td>His (H)</td>
<td>Asn; Gin; Lys; Arg</td>
<td>Arg</td>
</tr>
<tr>
<td>Ile (I)</td>
<td>Leu; Val; Met; Ala; Phe; Norleucine</td>
<td>Leu</td>
</tr>
<tr>
<td>Leu (L)</td>
<td>Norleucine; He; Val; Met; Ala; Phe</td>
<td>He</td>
</tr>
<tr>
<td>Lys (K)</td>
<td>Arg; Gin; Asn</td>
<td>Arg</td>
</tr>
<tr>
<td>Met (M)</td>
<td>Leu; Phe; Ile</td>
<td>Leu</td>
</tr>
<tr>
<td>Phe (F)</td>
<td>Trp; Leu; Val; He; Ala; Tyr</td>
<td>Tyr</td>
</tr>
<tr>
<td>Pro (P)</td>
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<td>Trp; Phe; Thr; Ser</td>
<td>Phe</td>
</tr>
<tr>
<td>Val (V)</td>
<td>He; Leu; Met; Phe; Ala; Norleucine</td>
<td>Leu</td>
</tr>
</tbody>
</table>

Amino acids may be grouped according to common side-chain properties:

1. hydrophobic: Norleucine, Met, Ala, Val, Leu, He;
2. neutral hydrophilic: Cys, Ser, Thr, Asn, Gin;
3. acidic: Asp, Glu;
4. basic: His, Lys, Arg;
5. residues that influence chain orientation: Gly, Pro;
6. aromatic: Trp, Tyr, Phe.

[0230] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0231] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based
affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0232] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots," i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, Methods Mol. Biol. 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in Methods in Molecular Biology 178:1-37 (O'Brien et al, ed., Human Press, Totowa, NJ, (2001)). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0233] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0234] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244:1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex is used to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for
substitution. Variants may be screened to determine whether they contain the desired properties.

[0235] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

[0236] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0237] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. TIBTECH 15:26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody may be made in order to create antibody variants with certain improved properties.

[0238] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See,

[0239] Antibodies variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/01 1878 (Jean-Mairet et al); US Patent No. 6,602,684 (Umana et al); and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

c) Fc region variants

[0240] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgGl, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[0241] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. In vitro and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure
that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, e.g. Hellstrom, I. et al. *Proc. Nat'l Acad. Sci. USA* 83:7059-7063 (1986)) and Hellstrom, I et al, *Proc. Nat'l Acad. Sci. USA* 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. et al, *J. Exp. Med.* 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA* 95:652-656 (1998). Clq binding assays may also be carried out to confirm that the antibody is unable to bind Clq and hence lacks CDC activity. See, e.g., Clq and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., *J. Immunol. Methods* 202:163 (1996); Cragg, M.S. et al, *Blood* 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, *Blood* 103:2738-2743 (2004)). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S.B. et al, *Int'l. Immunol.* 18(12): 1759-1769 (2006)).

[0242] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

[0243] Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Patent No. 6,737,056; WO 2004/056312, and Shields et al, *J. Biol. Chem.* 9(2): 6591-6604 (2001).)

[0244] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).
In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) Clq binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie et al. J. Immunol. 164: 4178-4184 (2000).

Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., J. Immunol. 117:587 (1976) and Kim et al, J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton et al.). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (US Patent No. 7,371,826).


d) Cysteine engineered antibody variants

In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunonoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Nonlimiting exemplary cysteine engineered heavy chains and light chains of a huMA79bv28 antibody are shown in Figure 4 (SEQ ID NOs: 39, 54, and 55). Cysteine engineered antibodies may be generated as described, e.g., in U.S. Patent No. 7,521,541.

e) Antibody Derivatives

In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol,
carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1, 3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

[0250] In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al, Proc. Natl. Acad. Sci. USA 102: 11600-1 1605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

B. Recombinant Methods and Compositions

[0251] Antibodies may be produced using recombinant methods and compositions, e.g., as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-CD79b antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (e.g., the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (e.g., expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (e.g., has been transformed with): (1) a vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, e.g. a Chinese Hamster
Ovary (CHO) cell or lymphoid cell (e.g., Y0, NSO, Sp20 cell). In one embodiment, a method of making an anti-CD79b antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

[0252] For recombinant production of an anti-CD79b antibody, nucleic acid encoding an antibody, e.g., as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0253] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, e.g., U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in E. coli.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0254] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, Nat. Biotech. 22:1409-1414 (2004), and Li et al, Nat. Biotech. 24:210-215 (2006).

[0255] Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of Spodoptera frugiperda cells.

[0256] Plant cell cultures can also be utilized as hosts. See, e.g., US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

[0257] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of usable mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, e.g., in Graham et al, J. Gen Virol. 36:59 (1977));
baby hamster kidney cells (BHK); mouse Sertoli cells (TM4 cells as described, e.g., in Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, e.g., in Mather et al, Annals N.Y. Acad. Sci. 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR- CHO cells (Urlaub et al, Proc. Natl. Acad. Sci. USA 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

C. Assays

[0258] Anti-CD79b antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

[0259] In one aspect, an antibody is tested for its antigen binding activity, e.g., by known methods such as ELISA, BIACore®, FACS, or Western blot.

[0260] In another aspect, competition assays may be used to identify an antibody that competes with any of the antibodies described herein for binding to CD79b. In certain embodiments, such a competing antibody binds to the same epitope (e.g., a linear or a conformational epitope) that is bound by an antibody described herein. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ).

[0261] In an exemplary competition assay, immobilized CD79b is incubated in a solution comprising a first labeled antibody that binds to CD79b (e.g., murine MA79b antibody, humanized MA79b.v17 antibody and/or humanized MA79b.v18 antibody and/or humanized MA79b.v28 and/or humanized MA79b.v32) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to CD79b. The second antibody may be present in a hybridoma supernatant. As a control, immobilized CD79b is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to CD79b, excess unbound antibody is removed, and the amount of label associated with immobilized CD79b is measured. If the amount of label associated with immobilized
CD79b is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to CD79b. In certain embodiments, immobilized CD79b is present on the surface of a cell or in a membrane preparation obtained from a cell expressing CD79b on its surface. See Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

D. Immunoconjugates

[0262] The invention also provides immunoconjugates comprising an anti-CD79b antibody herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes (i.e., a radioconjugate).

[0263] Immunoconjugates allow for the targeted delivery of a drug moiety to a tumor, and, in some embodiments intracellular accumulation wherein, where systemic administration of unconjugated drugs may result in unacceptable levels of toxicity to normal cells (Polakis P. (2005) Current Opinion in Pharmacology 5:382-387).


[0265] The ADC compounds of the invention include those with anticancer activity. In some embodiments, the ADC compounds include an antibody conjugated, i.e. covalently attached, to the drug moiety. In some embodiments, the antibody is covalently attached to the drug moiety through a linker. The antibody-drug conjugates (ADC) of the invention selectively deliver an effective dose of a drug to tumor tissue whereby greater selectivity, i.e. a lower efficacious dose, may be achieved while increasing the therapeutic index ("therapeutic window").

[0266] The drug moiety (D) of the antibody-drug conjugates (ADC) may include any compound, moiety or group that has a cytotoxic or cytostatic effect. Exemplary drug moieties include, but are not limited to, pyrrolobenzodiazepine (PBD) and derivatives thereof that have cytotoxic activity. Nonlimiting examples of such immunoconjugates are discussed in further detail below.
1. **Exemplary Antibody-drug Conjugates**

[0267] An exemplary embodiment of an antibody-drug conjugate (ADC) compound comprises an antibody (Ab) which targets a tumor cell, a drug moiety (D), and a linker moiety (L) that attaches Ab to D. In some embodiments, the antibody is attached to the linker moiety (L) through one or more amino acid residues, such as lysine and/or cysteine.

[0268] An exemplary ADC has Formula 1:

\[ \text{Ab-}(L-D)_p \]

where \( p \) is 1 to about 20. In some embodiments, the number of drug moieties that can be conjugated to an antibody is limited by the number of free cysteine residues. In some embodiments, free cysteine residues are introduced into the antibody amino acid sequence by the methods described herein. Exemplary ADC of Formula 1 include, but are not limited to, antibodies that have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon, R. et al (2012) *Methods in Enzym. 502*:123-138). In some embodiments, one or more free cysteine residues are already present in an antibody, without the use of engineering, in which case the existing free cysteine residues may be used to conjugate the antibody to a drug. In some embodiments, an antibody is exposed to reducing conditions prior to conjugation of the antibody in order to generate one or more free cysteine residues.

a) **Exemplary Linkers**

[0269] 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 1. 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.

[0270] 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, a-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.

[0271] 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.

[0272] 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 ("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.

[0273] 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).

[0274] In certain embodiments, a linker has the following Formula II:

$$\begin{align*}
- & \quad \text{A}_a \quad \text{W}_w \quad \text{Y}_y- \\
\text{II}
\end{align*}$$

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. An ADC comprising the linker of Formula II has the Formula 1(A): Ab-(A\_a-W\_w-Y\_y-D)p, wherein 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.

[0275] In some embodiments, a linker component comprises a "stretcher unit" (A) that links an antibody to another linker component or to a drug moiety. Nonlimiting exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody, drug, or additional linker components):
In some embodiments, a linker component comprises an "amino acid unit" (W). In some such embodiments, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug from the immunoconjugate upon exposure to intracellular proteases, such as lysosomal enzymes (Doronina et al. (2003) Nat. Biotechnol. 21:778-784). Exemplary amino acid units include, but are not limited to, dipeptides, tripeptides, tetrapeptides, and pentapeptides. Exemplary dipeptides include, but are not limited to, valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe); phenylalanine-lysine (fk or phe-lys); phenylalanine-homolysine (phe-homolys); and N-methyl-valine-citrulline (Me-val-cit). Exemplary tripeptides include, but are not limited to, glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). An amino acid unit may comprise amino acid residues that occur naturally and/or minor amino acids and/or non-naturally occurring amino acid analogs, such as citrulline. Amino acid units can be designed and optimized for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

Typically, peptide-type linkers can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to a liquid phase synthesis method (e.g., E. Schroder and K. Lubke (1965) "The Peptides", volume 1, pp 76-136, Academic Press).

In some embodiments, a linker component comprises a "spacer" unit that links the antibody to a drug moiety, either directly or through a stretcher unit and/or an amino acid unit. A spacer unit may be "self-immolative" or a "non-self-immolative." A "non-self-
immolative" spacer unit is one in which part or all of the spacer unit remains bound to the drug moiety upon cleavage of the ADC. Examples of non-self-immolative spacer units include, but are not limited to, a glycine spacer unit and a glycine-glycine spacer unit. In some embodiments, enzymatic cleavage of an ADC containing a glycine-glycine spacer unit by a tumor-cell associated protease results in release of a glycine-glycine-drug moiety from the remainder of the ADC. In some such embodiments, the glycine-glycine-drug moiety is subjected to a hydrolysis step in the tumor cell, thus cleaving the glycine-glycine spacer unit from the drug moiety.

[0279] A "self-immolative" spacer unit allows for release of the drug moiety. In certain embodiments, a spacer unit of a linker comprises a p-aminobenzyl unit. In some such embodiments, a p-aminobenzyl alcohol is attached to an amino acid unit via an amide bond, and a carbamate, methylcarbamate, or carbonate is made between the benzyl alcohol and the drug (Hamann et al. (2005) Expert Opin. Ther. Patents (2005) 15:1087-1103). In some embodiments, the spacer unit comprises p-aminobenzyl oxycarbonyl (PAB). In some embodiments, an ADC comprising a self-immolative linker has the structure:

\[
\text{Ab} \rightarrow \text{A}_a \text{W}_w \text{NH} \rightarrow \text{C}_x \text{D}
\]

wherein Q is -Ci-Cs alkyl, -0-(Ci-Cs alkyl), -halogen, -nitro, or -cyano; m is an integer ranging from 0 to 4; X may be one or more additional spacer units or may be absent; and p ranges from 1 to about 20. In some embodiments, p ranges from 1 to 10, 1 to 7, 1 to 5, or 1 to 4. Nonlimiting exemplary X spacer units include:

\[
\text{and } \text{R}_1 \text{R}_2 \quad \text{; wherein } \text{R}_1 \text{ and } \text{R}_2 \text{ are independently selected from } \text{H and C1-C6 alkyl.}
\]

In some embodiments, R1 and R2 are each -CH3.

[0280] Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group, such as 2-aminoimidazol-5-methanol derivatives (U.S. Patent No. 7,375,078; Hay et al. (1999) Bioorg. Med. Chem. Lett. 9:2237) and ortho- or para-aminobenzylacetals. In some embodiments, spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted...

[0281] In some embodiments, linker L may be a dendritic type linker for covalent attachment of more than one drug moiety to an antibody through a branching, multifunctional linker moiety (Sun et al (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215; Sun et al (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768). Dendritic linkers can increase the molar ratio of drug to antibody, i.e. loading, which is related to the potency of the ADC. Thus, where an antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

[0282] Nonlimiting exemplary linkers are shown below in the context of an ADC of Formula I:

![Diagram of an ADC of Formula I](image-url)
wherein \( R_1 \) and \( R_2 \) are independently selected from H and Ci-Ce alkyl. In some embodiments, \( R_1 \) and \( R_2 \) are each -CH\(_3\).

Further nonlimiting exemplary ADCs include the structures:

- [Structure 1]
- [Structure 2]
- [Structure 3]
- [Structure 4]
where X is:

\[
\begin{align*}
&\text{CH}_3 - \text{CH}_2 - \text{O} - \text{N} - \text{C} - \text{CH} - \text{C} - \text{H} - \\
&\hspace{1cm} \text{(CH}_2)_n - \text{CH} - \text{CH}_2 - \text{CH}_3
\end{align*}
\]

Y is:

\[
\begin{align*}
&\text{CH}_3 - \text{O} - \text{N} - \text{C} - \text{H}_2 - \text{CH}_2 - \text{CH}_3 - \\
&\hspace{1cm} \text{(CH}_2)_n - \text{CH} - \text{N} - \text{C} - \text{H}_2 - \text{CH}_2 - \text{CH}_3
\end{align*}
\]

each R is independently H or \( \text{Ci-Ce} \) alkyl; and n is 1 to 12.

[0285] In some embodiments, a linker is substituted with groups that modulate solubility and/or reactivity. As a nonlimiting example, a charged substituent such as sulfonate (-SO_3) or ammonium may increase water solubility of the linker reagent and facilitate the coupling reaction of the linker reagent with the antibody and/or the drug moiety, or facilitate the coupling reaction of Ab-L (antibody-linker intermediate) with D, or D-L (drug-linker intermediate) with Ab, depending on the synthetic route employed to prepare the ADC. In some embodiments, a portion of the linker is coupled to the antibody and a portion of the linker is coupled to the drug, and then the Ab-(linker portion)\(^a\) is coupled to drug-(linker portion)\(^b\) to form the ADC of Formula I.

[0286] The compounds of the invention expressly contemplate, but are not limited to, ADC prepared with the following linker reagents: bis-maleimido-trioxy ethylene glycol (BMPEO), N-(\( \beta \)-maleimidopropoxy)-N-hydroxy succinimide ester (BMPS), N-(\( \varepsilon \)-maleimidocaproyloxy) succinimide ester (EMCS), N-[\( \gamma \)-maleimidobutyroloxy]succinimide ester (GMBS), 1,6-hexane-bis-vinylsulfone (HBVS), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), 4-(4-N-Maleimidophenyl)butyric acid hydrazide (MPBH), succinimidyl 3-(bromoacetamido)propionate (SBAP), succinimidyl
iodoacetate (SIA), succinimidyl (4-iodoacetyl)aminobenzoate (SIAB), N-succinimidyl-3-(2-pyridyl)propionate (SPDP), N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), succinimidyl 6-[(beta-maleimidopropionamido)hexanoate] (SMPH), iminothiolane (IT), sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and succinimidyl-(4-vinylsulfone)benzoate (SVSB), and including bis-maleimide reagents: dithiobismaleimidoethane (DTME), 1,4-Bismaleimidobutane (BMB), 1,4-Bismaleimido-2,3-dihydroxybutane (BMDB), bismaleimidohexane (BMH), bismaleimidoethane (BMOE), BM(PEG)2 (shown below), and BM(PEG)3 (shown below); bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). In some embodiments, bis-maleimide reagents allow the attachment of the thiol group of a cysteine in the antibody to a thiol-containing drug moiety, linker, or linker-drug intermediate. Other functional groups that are reactive with thiol groups include, but are not limited to, iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.

![Image of BM(PEG)2 and BM(PEG)3]


[0288] Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, e.g., W094/1 1026.
b) **Exemplary Drug Moieties**


[0290] PBD dimers have been conjugated to antibodies and the resulting ADC shown to have anti-cancer properties. Nonlimiting exemplary linkage sites on the PBD dimer include the five-membered pyrrolo ring, the tether between the PBD units, and the N10-C1 imine group (WO 2009/016516; US 2009/034710; US 2010/047257; US 2009/036431; US 2011/0256157; WO 2011/130598).

[0291] Nonlimiting exemplary PBD dimer components of ADCs are of Formula A:

![Diagram](image)

and salts and solvates thereof, wherein:

- the wavy line indicates the covalent attachment site to the linker;
- 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, =0, =CH\(_2\), CN, R, OR, =CH-\( R^D \), =C(\( R^D \))\(_2\), O-SO\(_2\)-R, CO\(_2\)R and COR, and optionally further selected from halo or dihalo, wherein \( R^D \) is independently selected from R, C\(_2\)R, COR, CHO, C\(_2\)H, and halo;
R\(^6\) and R\(^9\) are independently selected from H, R, OH, OR, SH, SR, NH\(_2\), NHR, NRR', NO\(_2\), Me\(_3\)Sn and halo;

R\(^2\) is independently selected from H, R, OH, OR, SH, SR, NH\(_2\), NHR, NRR', NO\(_2\), MesSn and halo;

Q is independently selected from O, S and NH;

R\(^{11}\) is either H, or R or, where Q is O, SO\(_3\)M, where M is a metal cation;

R and R' are each independently selected from optionally substituted C\(_{1-12}\) alkyl, C\(_{3-20}\) heterocyclyl and C\(_{5-20}\) aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

R\(^{12}\), R\(^{16}\), R\(^{19}\) and R\(^{17}\) are as defined for R\(^2\), R\(^6\), R\(^9\) and R\(^7\) respectively;

R\(^\circ\) is a C\(_{3-12}\) alkylene group, which chain may be interrupted by one or more heteroatoms, e.g. O, S, N(H), NMe and/or aromatic rings, e.g. benzene or pyridine, which rings are optionally substituted; and

X and X' are independently selected from O, S and N(H).

[0292] In some embodiments, R\(^9\) and R\(^{19}\) are H.

[0293] In some embodiments, R\(^6\) and R\(^{16}\) are H.

[0294] In some embodiments, R\(^7\) are R\(^{17}\) are both OR\(^{7\text{A}}\), where R\(^{7\text{A}}\) is optionally substituted C\(_{1-4}\) alkyl. In some embodiments, R\(^{7\text{A}}\) is Me. In some embodiments, R\(^{7\text{A}}\) is is CH\(_2\)Ph, where Ph is a phenyl group.

[0295] In some embodiments, X is O.

[0296] In some embodiments, R\(^{11}\) is H.

[0297] In some embodiments, there is a double bond between C2 and C3 in each monomer unit.

[0298] In some embodiments, R\(^2\) and R\(^{12}\) are independently selected from H and R. In some embodiments, R\(^2\) and R\(^{12}\) are independently selected R. In some embodiments, R\(^2\) and R\(^{12}\) are independently optionally substituted C\(_{5-20}\) aryl or C\(_{5-7}\) aryl or C\(_{5-10}\) aryl. In some embodiments, R\(^2\) and R\(^{12}\) are independently optionally substituted phenyl, thienyl, naphthyl, pyridyl, quinoliny1, or isoquinoliny1. In some embodiments, R\(^2\) and R\(^{12}\) are independently selected from =0, =CH\(_2\), =CH-R\(^{D}\), and =C(R\(^D\))\(_2\). In some embodiments, R\(^2\) and R\(^{12}\) are each =CH\(_2\). In some embodiments, R\(^2\) and R\(^{12}\) are each H. In some embodiments, R\(^2\) and R\(^{12}\) are each =0. In some embodiments, R\(^2\) and R\(^{12}\) are each =CF\(_2\). In some embodiments, R\(^2\) and R\(^{12}\) are each C\(^2\) and/or R\(^{12}\) are independently =C(R\(^D\))\(_2\). In some embodiments, R\(^2\) and/or R\(^{12}\) are independently =CH-R\(^D\).

[0299] In some embodiments, when R\(^2\) and/or R\(^{12}\) is =CH-R\(^D\), each group may independently have either configuration shown below:
In some embodiments, a =CH-R \text{D} is in configuration (I).

[0300] In some embodiments, R'' is a C\text{3} alkylene group or a C\text{5} alkylene group.

[0301] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(I):

\begin{center}
\includegraphics[width=0.8\textwidth]{formula_a1.png}
\end{center}

wherein n is 0 or 1.

[0302] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(II):

\begin{center}
\includegraphics[width=0.8\textwidth]{formula_a2.png}
\end{center}

wherein n is 0 or 1.

[0303] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(III):

\begin{center}
\includegraphics[width=0.8\textwidth]{formula_a3.png}
\end{center}

wherein R^E and R^{E''} are each independently selected from H or R^D, wherein R^D is defined as above; and

wherein n is 0 or 1.

[0304] In some embodiments, n is 0. In some embodiments, n is 1. In some embodiments, R^E and/or R^{E''} is H. In some embodiments, R^E and R^{E''} are H. In some
embodiments, \(R^E\) and/or \(R^E\) is \(RD\), wherein \(RD\) is optionally substituted \(C_{1-12}\) alkyl. In some embodiments, \(R^E\) and/or \(R^E\) is \(RD\), wherein \(RD\) is methyl.

[0305] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(IV):

![Formula A(IV)](image)

wherein \(Ar^1\) and \(Ar^2\) are each independently optionally substituted \(C_{5-20}\) aryl; wherein \(Ar^1\) and \(Ar^2\) may be the same or different; and wherein \(n\) is 0 or 1.

[0306] In some embodiments, an exemplary PBD dimer component of an ADC has the structure of Formula A(V):

![Formula A(V)](image)

wherein \(Ar^1\) and \(Ar^2\) are each independently optionally substituted \(C_{5-20}\) aryl; wherein \(Ar^1\) and \(Ar^2\) may be the same or different; and wherein \(n\) is 0 or 1.

[0307] In some embodiments, \(Ar^1\) and \(Ar^2\) are each independently selected from optionally substituted phenyl, furanyl, thiophenyl and pyridyl. In some embodiments, \(Ar^1\) and \(Ar^2\) are each independently optionally substituted phenyl. In some embodiments, \(Ar^1\) and \(Ar^2\) are each independently optionally substituted thien-2-yl or thien-3-yl. In some embodiments, \(Ar^1\) and \(Ar^2\) are each independently optionally substituted quinolinyl or isoquinolinyl. The quinolinyl or isoquinolinyl group may be bound to the PBD core through any available ring position. For example, the quinolinyl may be quinolin-2-yl, quinolin-3-yl, quinolin-4-yl, quinolin-5-yl, quinolin-6-yl, quinolin-7-yl and quinolin-8-yl. In some embodiments, the quinolinyl is selected from quinolin-3-yl and quinolin-6-yl. The isoquinolinyl may be isoquinolin-1-yl, isoquinolin-3-yl, isoquinolin-4yl, isoquinolin-5-yl, isoquinolin-6-yl, isoquinolin-7-yl and isoquinolin-8-yl. In some embodiments, the isoquinolinyl is selected from isoquinolin-3-yl and isoquinolin-6-yl.

[0308] Further nonlimiting exemplary PBD dimer components of ADCs are of Formula B.
and salts and solvates thereof, wherein:

the wavy line indicates the covalent attachment site to the linker;
the wavy line connected to the OH indicates the S or R configuration;

R<sup>V1</sup> and R<sup>V2</sup> are independently selected from H, methyl, ethyl and phenyl (which phenyl may be optionally substituted with fluoro, particularly in the 4 position) and C<sub>3-6</sub> heterocycl; wherein R<sup>V1</sup> and R<sup>V2</sup> may be the same or different; and

n is 0 or 1.

[0309] In some embodiments, R<sup>V1</sup> and R<sup>V2</sup> are independently selected from H, phenyl, and 4-fluorophenyl.

[0310] In some embodiments, a linker may be attached at one of various sites of the PBD dimer drug moiety, including the N10 imine of the B ring, the C-2 endo/exo position of the C ring, or the tether unit linking the A rings (see structures C(I) and C(II) below).

[0311] Nonlimiting exemplary PBD dimer components of ADCs include Formulas C(I) and C(II):

[0312] Formulas C(I) and C(II) are shown in their N10-C1 1 imine form. Exemplary PBD drug moieties also include the carbinolamine and protected carbinolamine forms as well, as shown in the table below:

<table>
<thead>
<tr>
<th></th>
<th>Imine</th>
<th>Carbinolamine</th>
<th>Protected Carbinolamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

wherein:
X is CH₂ (n = 1 to 5), N, or O;

Z and Z’ are independently selected from OR and NR₂, where R is a primary, secondary or tertiary alkyl chain containing 1 to 5 carbon atoms;

R₁, R’₁, R₂ and R’₂ are each independently selected from H, Ci-Cs alkyl, C₂-C₅ alkenyl, C₂-C₅ alkynyl, C₅-2₀ aryl (including substituted aryls), C₅-2₀ heteroaryl groups, -NH₂, -NHMe, -OH, and -SH, where, in some embodiments, alkyl, alkenyl and alkynyl chains comprise up to 5 carbon atoms;

R₃ and R’₃ are independently selected from H, OR, NHR, and NR₂, where R is a primary, secondary or tertiary alkyl chain containing 1 to 5 carbon atoms;

R₄ and R’₄ are independently selected from H, Me, and OMe;

R₅ is selected from Ci-Cs alkyl, C₂-C₅ alkenyl, C₂-C₅ alkynyl, C₅-2₀ aryl (including aryls substituted by halo, nitro, cyano, alkoxy, alkyl, heterocyclyl) and C₅-2₀ heteroaryl groups, where, in some embodiments, alkyl, alkenyl and alkynyl chains comprise up to 5 carbon atoms;

R₁₁ is H, Ci-Cs alkyl, or a protecting group (such as acetyl, trifluoroacetyl, t-butoxycarbonyl (BOC), benzyloxycarbonyl (CBZ), 9-fluorenylmethyloxycarbonyl (Fmoc), or a moiety comprising a self-immolating unit such as valine-citrulline-PAB);

R₁₂ is H, Ci-Cs alkyl, or a protecting group;

wherein a hydrogen of one of R₁, R’₁, R₂, R’₂, or R₁₂ or a hydrogen of the -OCH₂CH₂(X)nCH₂CH₂₀ - spacer between the A rings is replaced with a bond connected to the linker of the ADC.

[0313] Exemplary PBD dimer portions of ADC include, but are not limited to (the wavy line indicates the site of covalent attachment to the linker):

[0314] Nonlimiting exemplary embodiments of ADCs comprising PBD dimers have the following structures:
PBD dimer-val-cit-PAB-Ab; where:

- **n** is 0 to 12. In some embodiments, **n** is 2 to 10. In some embodiments, **n** is 4 to 8. In some embodiments, **n** is selected from 4, 5, 6, 7, and 8.

- [0315] The linkers of PBD dimer-val-cit-PAB-Ab and the PBD dimer-Phe-homoLys-PAB-Ab are protease cleavable.


c) **Drug Loading**

- [0317] Drug loading is represented by **p**, the average number of drug moieties per antibody in a molecule of Formula I. Drug loading may range from 1 to 20 drug moieties (D) per antibody. ADCs of Formula I include collections of antibodies conjugated with a range of
drug moieties, from 1 to 20. The average number of drug moieties per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of ADC in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

[0318] For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in certain exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In certain embodiments, higher drug loading, e.g. p >5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the average drug loading for an ADC ranges from 1 to about 8; from about 2 to about 6; or from about 3 to about 5. Indeed, it has been shown that for certain ADCs, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5 (US 7498298).

[0319] In certain embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, lysine residues that do not react with the drug-linker intermediate or linker reagent, as discussed below. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to a drug moiety; indeed most cysteine thiol residues in antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonylthylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

[0320] The loading (drug/antibody ratio) of an ADC may be controlled in different ways, and for example, by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

[0321] 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

**d) Certain Methods of Preparing Immunoconjugates**

[0322] 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.

[0323] 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 tricarbonylhexylphosphine (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 modification of lysine residues, e.g. by reacting lysine residues with 2-iminothiolane (Traut's reagent), resulting in conversion of an amine into a thiol. Reactive thiol groups may also be introduced
into an antibody by introducing one, two, three, four, or more cysteine residues (e.g., by preparing variant antibodies comprising one or more non-native cysteine amino acid residues).

[0324] Antibody-drug conjugates of the invention may also be produced by reaction between an electrophilic group on an antibody, such as an aldehyde or ketone carbonyl group, with a nucleophilic group on a linker reagent or drug. Useful nucleophilic groups on a linker reagent include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. In one embodiment, an antibody is modified to introduce electrophilic moieties that are capable of reacting with nucleophilic substituents on the linker reagent or drug. In another embodiment, the sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either galactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the antibody that can react with appropriate groups on the drug (Hermanson, Bioconjugate Techniques). In another embodiment, antibodies containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, (1992) Bioconjugate Chem. 3:138-146; US 5362852). Such an aldehyde can be reacted with a drug moiety or linker nucleophile.

[0325] Exemplary nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups 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; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

[0326] Nonlimiting exemplary cross-linker reagents that may be used to prepare ADC are described herein in the section titled "Exemplary Linkers." Methods of using such cross-linker reagents to link two moieties, including a proteinaceous moiety and a chemical moiety, are known in the art. In some embodiments, a fusion protein comprising an antibody and a cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. A recombinant DNA molecule may comprise regions encoding the antibody and cytotoxic portions of the conjugate either adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.
In yet another embodiment, an antibody may be conjugated to a "receptor" (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a drug or radionucleotide).

E. Methods and Compositions for Diagnostics and Detection

In certain embodiments, any of the anti-CD79b antibodies provided herein is useful for detecting the presence of CD79b in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. A "biological sample" comprises, e.g., a cell or tissue (e.g., biopsy material, including cancerous or potentially cancerous lymph tissue, including tissue from subjects having or suspected of having a B cell disorder and/or a B cell proliferative disorder, including, but not limited to, 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 one embodiment, an anti-CD79b antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of CD79b in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-CD79b antibody as described herein under conditions permissive for binding of the anti-CD79b antibody to CD79b, and detecting whether a complex is formed between the anti-CD79b antibody and CD79b in the biological sample. Such method may be an in vitro or in vivo method. In one embodiment, an anti-CD79b antibody is used to select subjects eligible for therapy with an anti-CD79b antibody, e.g. where CD79b is a biomarker for selection of patients. In a further embodiment, the biological sample is a cell or tissue (e.g., cancerous or potentially cancerous lymph tissue, including tissue of subjects having or suspected of having a B cell disorder and/or a B cell proliferative disorder, including, but not limited to, 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 a further embodiment, an anti-CD79b antibody is used in vivo to detect, e.g., by in vivo imaging, a CD79b-positive cancer in a subject, e.g., for the purposes of diagnosing,
prognosing, or staging cancer, determining the appropriate course of therapy, or monitoring response of a cancer to therapy. One method known in the art for in vivo detection is immuno-
positron emission tomography (immuno-PET), as described, e.g., in van Dongen et al, *The
Oncologist* 12:1379-1389 (2007) and Verel et al, *J. Nucl. Med.* 44:1271-1281 (2003). In such embodiments, a method is provided for detecting a CD79b-positive cancer in a subject, the method comprising administering a labeled anti-CD79b antibody to a subject having or suspected of having a CD79b-positive cancer, and detecting the labeled anti-CD79b antibody in the subject, wherein detection of the labeled anti-CD79b antibody indicates a CD79b-positive cancer in the subject. In certain of such embodiments, the labeled anti-CD79b antibody comprises an anti-CD79b antibody conjugated to a positron emitter, such as $^{68}$Ga, $^{18}$F, $^{64}$Cu, $^{86}$Y, $^{76}$Br, $^{89}$Zr, and $^{124}$I. In a particular embodiment, the positron emitter is $^{89}$Zr.

[0331] In further embodiments, a method of diagnosis or detection comprises contacting a first anti-CD79b antibody immobilized to a substrate with a biological sample to be tested for the presence of CD79b, exposing the substrate to a second anti-CD79b antibody, and detecting whether the second anti-CD79b is bound to a complex between the first anti-CD79b antibody and CD79b in the biological sample. A substrate may be any supportive medium, e.g., glass, metal, ceramic, polymeric beads, slides, chips, and other substrates. In certain embodiments, a biological sample comprises a cell or tissue (e.g., biopsy material, including cancerous or potentially cancerous lymph tissue, including tissue from subjects having or suspected of having a B cell disorder and/or a B cell proliferative disorder, including, but not limited to, 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 certain embodiments, the first or second anti-CD79b antibody is any of the antibodies described herein.

[0332] Exemplary disorders that may be diagnosed or detected according to any of the above embodiments include CD79b-positive cancers, such as CD79b-positive lymphoma, CD79b-positive non-Hogkins lymphoma (NHL; including, but not limited to CD79b-positive aggressive NHL, CD79b-positive relapsed aggressive NHL, CD79b-positive relapsed indolent NHL, CD79b-positive refractory NHL, and CD79b-positive refractory indolent NHL), CD79b-positive chronic lymphocytic leukemia (CLL), CD79b-positive small lymphocytic lymphoma, CD79b-positive leukemia, CD79b-positive hairy cell leukemia (HCL), CD79b-positive acute lymphocytic leukemia (ALL), CD79b-positive Burkitt's lymphoma, and CD79b-positive mantle cell lymphoma. In some embodiments, a CD79b-positive cancer is a cancer that
receives an anti-CD79b immunohistochemistry (IHC) score greater than "0," which corresponds to very weak or no staining in >90% of tumor cells. In some embodiments, 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 an in situ hybridization (ISH) assay. In some such embodiments, a scoring system similar to that used for IHC is used. 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.

[0333] In certain embodiments, labeled anti-CD79b antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes $^{35}$P, $^{14}$C, $^{125}$I, $^{3}$H, and $^{131}$I, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like. In another embodiment, a label is a positron emitter. Positron emitters include but are not limited to $^{68}$Ga, $^{18}$F, $^{64}$Cu, $^{86}$Y, $^{76}$Br, $^{89}$Zr, and $^{124}$I. In a particular embodiment, a positron emitter is $^{89}$Zr.

F. Pharmaceutical Formulations

[0334] Pharmaceutical formulations of an anti-CD79b antibody or immunoconjugate as described herein are prepared by mixing such antibody or immunoconjugate having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid
and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycansases such as chondroitinases.

[0335] Exemplary lyophilized antibody or immunoconjugate formulations are described in US Patent No. 6,267,958. Aqueous antibody or immunoconjugate formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0336] The formulation herein may also contain more than one active ingredient as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other.

[0337] Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0338] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody or immunoconjugate, which matrices are in the form of shaped articles, e.g. films, or microcapsules.
[0339] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

**G. Therapeutic Methods and Compositions**

[0340] Any of the anti-CD79b antibodies or immunoconjugates provided herein may be used in methods, e.g., therapeutic methods.

[0341] In one aspect, an anti-CD79b antibody or immunoconjugate provided herein is used in a method of inhibiting proliferation of a CD79b-positive cell, the method comprising exposing the cell to the anti-CD79b antibody or immunoconjugate under conditions permissive for binding of the anti-CD79b antibody or immunoconjugate to CD79b on the surface of the cell, thereby inhibiting the proliferation of the cell. In certain embodiments, the method is an *in vitro* or an *in vivo* method. In some embodiments, the cell is a B cell. In some embodiments, the cell is a neoplastic B cell, such as a lymphoma cell or a leukemia cell.

[0342] Inhibition of cell proliferation in vitro may be assayed using the CellTiter-Glo™ Luminescent Cell Viability Assay, which is commercially available from Promega (Madison, WI). That assay determines the number of viable cells in culture based on quantitation of ATP present, which is an indication of metabolically active cells. *See* Crouch et al. (1993) *J. Immunol. Meth.* 160:81-88, US Pat. No. 6602677. The assay may be conducted in 96- or 384-well format, making it amenable to automated high-throughput screening (HTS). *See* Cree et al. (1995) *Anticancer Drugs* 6:398-404. The assay procedure involves adding a single reagent (CellTiter-Glo® Reagent) directly to cultured cells. This results in cell lysis and generation of a luminescent signal produced by a luciferase reaction. The luminescent signal is proportional to the amount of ATP present, which is directly proportional to the number of viable cells present in culture. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is expressed as relative light units (RLU).

[0343] In another aspect, an anti-CD79b antibody or immunoconjugate for use as a medicament is provided. In further aspects, an anti-CD79b antibody or immunoconjugate for use in a method of treatment is provided. In certain embodiments, an anti-CD79b antibody or immunoconjugate for use in treating CD79b-positive cancer is provided. In certain embodiments, the invention provides an anti-CD79b antibody or immunoconjugate for use in a method of treating an individual having a CD79b-positive cancer, the method comprising administering to the individual an effective amount of the 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, e.g., as described below.
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.

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.

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.

In some embodiments, immunoconjugates comprising a pyrrolobenzodiazepine cytotoxic moiety 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 F. The immunoconjugate for use in treating diffuse large B-cell lymphomas, mantle cell lymphomas, and/or Burkitt's lymphoma, may, in some embodiments, comprise a PBD dimer having the structure:
wherein \( n \) is 0 or 1. In some embodiments, the PBD dimer is covalently attached to the antibody through a protease cleavable linker, such as, for example, the immunoconjugate shown in Figure 5, which has a val-cit linker.

[0348] An "individual" according to any of the above embodiments may be a human.

[0349] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-CD79b antibodies or immunoconjugate provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-CD79b antibodies or immunoconjugates provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-CD79b antibodies or immunoconjugates provided herein and at least one additional therapeutic agent, e.g., as described below.

[0350] Antibodies or immunoconjugates of the invention can be used either alone or in combination with other agents in a therapy. For instance, an antibody or immunoconjugate of the invention may be co-administered with at least one additional therapeutic agent.

[0351] In some embodiments, an anti-CD79b immunoconjugate is administered in combination with an anti-CD22 antibody or immunoconjugate. A nonlimiting exemplary anti-CD22 antibody or immunoconjugate comprises the hypervariable regions of 10F4v3, such that the anti-CD22 antibody or immunoconjugate comprises (i) HVR H1 having the sequence of SEQ ID NO: 42, (ii) HVR H2 having the sequence of SEQ ID NO: 43, (iii) HVR H3 having the sequence of SEQ ID NO: 44, (iv) HVR L1 having the sequence of SEQ ID NO: 45, (v) HVR L2 having the sequence of SEQ ID NO: 46, and (vi) HVR L3 having the sequence of SEQ ID NO: 47. In some embodiments, an anti-CD22 antibody or immunoconjugate comprises the heavy chain variable region and light chain variable region of 10F4v3. In some such embodiments, the anti-CD22 antibody or immunoconjugate comprises a heavy chain variable region having the sequence of SEQ ID NO: 48 and a light chain variable region having the sequence of SEQ ID NO: 49. An anti-CD22 immunoconjugate comprises, in some embodiments, a cytotoxic agent selected from an auristatin, a nemorubicin derivative, and a pyrrolobenzodiazepine. In some embodiments, an anti-CD22 immunoconjugate comprises a cytotoxic agent selected from MMAE, PNU-159682, and a PBD dimer having the structure:
wherein \( n \) is 0 or 1. In some embodiments, an anti-CD22 immunoconjugate is selected from a Thio Hu anti-CD22 10F4v3 HC A1 18C-MC-val-cit-PAB-MMAE, a Thio Hu anti-CD22 10F4v3 HC S400C-MC-val-cit-PAB-MMAE, and a Thio Hu anti-CD22 10F4v3 LC V205C-MC-val-cit-PAB-MMAE immunoconjugate, which are described, e.g., in US 2008/0050310; a Thio Hu anti-CD22 10F4v3 HC A1 18C-MC-val-cit-PAB-PNU-159682, a Thio Hu anti-CD22 10F4v3 HC A1 18C-MC-acetal-PNU-159682, a Thio Hu anti-CD22 10F4v3 HC A1 18C-MC-val-cit-PAB-PBD, a Thio Hu anti-CD22 10F4v3 HC S400C-MC-val-cit-PAB-PNU-159682, a Thio Hu anti-CD22 10F4v3 HC S400C-MC-acetal-PNU-159682, a Thio Hu anti-CD22 10F4v3 HC S400C-MC-val-cit-PAB-PBD, a Thio Hu anti-CD22 10F4v3 HC S400C-MC-val-cit-PAB-PNU-159682, a Thio Hu anti-CD22 10F4v3 HC S400C-MC-acetal-PNU-159682, a Thio Hu anti-CD22 10F4v3 HC S400C-MC-val-cit-PAB-PBD, a Thio Hu anti-CD22 10F4v3 HC S400C-MC-val-cit-PAB-PNU-159682, a Thio Hu anti-CD22 10F4v3 HC S400C-MC-acetal-PNU-159682, a Thio Hu anti-CD22 10F4v3 HC S400C-MC-val-cit-PAB-PBD. The heavy chain and light chain sequences for Thio Hu anti-CD22 10F4v3 HC A1 18C are shown in SEQ ID NOs: 50 and 51, respectively. The heavy chain and light chain sequences for Thio Hu anti-CD22 10F4v3 HC S400C are shown in SEQ ID NOs: 52 and 51, respectively. The heavy chain and light chain sequences for Thio Hu anti-CD22 10F4v3 LC V205C are shown in SEQ ID NOs: 56 and 53, respectively. Apart from the specific antibody sequence, the structures of the anti-CD22 immunoconjugates are analogous to the structures of the anti-CD79b immunoconjugates described herein, and the anti-CD22 immunoconjugates described in US 2008/0050310.

Nonlimiting exemplary immunoconjugates comprising PNU-159682 have the structures:

\[
\text{Ab-MC-acetal-PNU-159682}
\]

\[
\text{Ab-MC-val-cit-PAB-PNU-159682}
\]
In some embodiments, an anti-CD22 immunoconjugate is administered in combination with an anti-CD20 antibody (either a naked antibody or an ADC). In some embodiments, the anti-CD20 antibody is rituximab (Rituxan®) or 2H7 (Genentech, Inc., South San Francisco, CA). In some embodiments, an anti-CD22 immunoconjugate is administered in combination with an anti-VEGF antibody (e.g., bevacizumab, trade name Avastin®).

Other therapeutic regimens may be combined with the administration of an anti-CD22 immunoconjugate, including, without limitation, radiation therapy and/or bone marrow and peripheral blood transplants, and/or a cytotoxic agent. In some embodiments, a cytotoxic agent is an agent or a combination of agents such as, for example, cyclophosphamide, hydroxydaunorubicin, adriamycin, doxorubicin, vincristine (Oncovin™), prednisolone, CHOP (combination of cyclophosphamide, doxorubicin, vincristine, and prednisolone), CVP (combination of cyclophosphamide, vincristine, and prednisolone), or immunotherapeutics such as anti-CD20 (e.g., rituximab, trade name Rituxan®), anti-VEGF (e.g., bevacizumab, trade name Avastin®), taxanes (such as paclitaxel and docetaxel) and anthracycline antibiotics.

Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody or immunoconjugate of the invention can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies or immunoconjugates of the invention can also be used in combination with radiation therapy.

An antibody or immunoconjugate of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intraleisonal administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, e.g. by injections, such as
intravenous or subcutaneous injections, depending in part on whether the administration is
brief or chronic. Various dosing schedules including but not limited to single or multiple
administrations over various time-points, bolus administration, and pulse infusion are
contemplated herein.

[0356] Antibodies or immunoconjugates of the invention would be formulated, dosed,
and administered in a fashion consistent with good medical practice. Factors for consideration
in this context include the particular disorder being treated, the particular mammal being
treated, the clinical condition of the individual patient, the cause of the disorder, the site of
delivery of the agent, the method of administration, the scheduling of administration, and other
factors known to medical practitioners. The antibody or immunoconjugate need not be, but is
optionally formulated with one or more agents currently used to prevent or treat the disorder in
question. The effective amount of such other agents depends on the amount of antibody or
immunoconjugate present in the formulation, the type of disorder or treatment, and other
factors discussed above. These are generally used in the same dosages and with administration
routes as described herein, or about from 1 to 99% of the dosages described herein, or in any
dosage and by any route that is empirically/clinically determined to be appropriate.

[0357] For the prevention or treatment of disease, the appropriate dosage of an
antibody or immunoconjugate of the invention (when used alone or in combination with one or
more other additional therapeutic agents) will depend on the type of disease to be treated, the
type of antibody or immunoconjugate, the severity and course of the disease, whether the
antibody or immunoconjugate is administered for preventive or therapeutic purposes, previous
therapy, the patient's clinical history and response to the antibody or immunoconjugate, and the
discretion of the attending physician. The antibody or immunoconjugate is suitably
administered to the patient at one time or over a series of treatments. Depending on the type
and severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1mg/kg-10mg/kg) of antibody or
immunoconjugate can be an initial candidate dosage for administration to the patient, whether,
for example, by one or more separate administrations, or by continuous infusion. One typical
daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors
mentioned above. For repeated administrations over several days or longer, depending on the
condition, the treatment would generally be sustained until a desired suppression of disease
symptoms occurs. One exemplary dosage of the antibody or immunoconjugate would be in the
range from about 0.05 mg/kg to about 10 mg/kg. Thus, one or more doses of about 0.5 mg/kg,
2.0 mg/kg, 4.0 mg/kg or 10 mg/kg (or any combination thereof) may be administered to the
patient. Such doses may be administered intermittently, e.g. every week or every three weeks
(e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of
the antibody). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0358] In some embodiments, a lower dose of ahuMA79b (such as huMA79bv28) ADC comprising a pyrrolobenzodiazepine (PBD) dimer may be used to achieve the same efficacy as a higher dose of a huMA79b ADC comprising an MMAE moiety.

[0359] It is understood that any of the above formulations or therapeutic methods may be carried out using both an immunoconjugate of the invention and an anti-CD79b antibody.

H. Articles of Manufacture

[0360] In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the disorder and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody or immunoconjugate of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody or immunoconjugate of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution or dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.
III. EXAMPLES

[0361] The following are examples of methods and compositions of the invention. It is understood that various other embodiments may be practiced, given the general description provided above.

A. Production of Anti-CD79b Antibody Drug Conjugates

[0362] Anti-CD79b antibody MA79b and certain variants, including humanized huMA79b graft and humanized variants huMA79bv17, huMA79bv18, huMA79bv28, and huMA79bv32, are described, e.g., in US 8,088,378 B2. Table 2 shows the SEQ ID NOs corresponding to the heavy chain, light chain, and hypervariable regions (HVRs) for each antibody.

Table 2: Sequences corresponding to MA79b and humanized variants

<table>
<thead>
<tr>
<th>Antibody</th>
<th>HC variable region SEQ ID NO:</th>
<th>LC variable region SEQ ID NO:</th>
<th>HVR H1 SEQ ID NO:</th>
<th>HVR H2 SEQ ID NO:</th>
<th>HVR H3 SEQ ID NO:</th>
<th>HVR L1 SEQ ID NO:</th>
<th>HVR L2 SEQ ID NO:</th>
<th>HVR L3 SEQ ID NO:</th>
</tr>
</thead>
<tbody>
<tr>
<td>MA79b</td>
<td>3</td>
<td>4</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>huMA79b graft</td>
<td>5</td>
<td>6</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>huMA79bv17</td>
<td>7</td>
<td>8</td>
<td>15</td>
<td>16</td>
<td>17</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>huMA79bv18</td>
<td>9</td>
<td>10</td>
<td>15</td>
<td>16</td>
<td>23</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>huMA79bv28</td>
<td>11</td>
<td>12</td>
<td>21 (identical to 15)</td>
<td>22 (identical to 16)</td>
<td>23</td>
<td>24</td>
<td>25 (identical to 19)</td>
<td>26 (identical to 20)</td>
</tr>
<tr>
<td>huMA79bv32</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>16</td>
<td>23</td>
<td>35</td>
<td>19</td>
<td>20</td>
</tr>
</tbody>
</table>

[0363] The heavy chain framework regions for antibodies huMA79bv17, huMA79bv18, huMA79bv28, and huMA79bv32, HC FR1 to FR4, are shown in SEQ ID NOs: 27 to 30, respectively. The light chain framework regions for antibodies huMA79bv17, huMA79bv18, and huMA79bv28, LC FR1 to FR4, are shown in SEQ ID NOs: 31 to 34, respectively. The light chain framework regions for antibody huMA79bv32, LC FR1 to FR4, are shown in SEQ ID NOs: 31, 36, 33, and 34, respectively. The binding affinity of huMA79b was found to be about 0.4 nM using Scatchard analysis. See, e.g., US 8,088,378 B2.

[0364] For larger scale antibody production, antibodies were produced in CHO cells. Vectors coding for VL and VH were transfected into CHO cells and IgG was purified from cell culture media by protein A affinity chromatography.

[0365] Anti-CD79b antibody-drug conjugates (ADCs) were produced by conjugating Thio huMA79bv28 HC A18C antibodies to certain drug moieties. Thio huMA79bv28 HC A18C is a huMA79bv28 antibody with an A18C mutation in the heavy chain that adds a
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-val-cit-PAB-PBD** ("huMA79bv28-PBD")

[0366] 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-PBD ("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 1.6 to about 1.8, as indicated in the Examples below. HuMA79bv28-PBD has the structure shown in Figure 5 (p = drug load).

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

[0367] 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 huMA79bv28 HC A118C-MC-val-cit-PAB-MMAE is described, e.g., in US 8,088,378 B2.
B. *In vivo* anti-tumor activity of humanized anti-CD79b antibody drug conjugates in a WSU-DLCL2 xenograft model

[0368] To test the efficacy of Thio huMA79bv28 HC A118C conjugate with PBD ("huMA79bv28-PBD"), the effects of the conjugated antibodies in a mouse xenograft model of WSU-DLCL2 tumors (diffuse large B-cell lymphoma cell line) was examined.

[0369] Female CB17 ICR SCID mice (11-12 weeks of age from Charles Rivers Laboratories; Hollister, CA) were each inoculated subcutaneously in the flank with $2 \times 10^7$ WSU-DLCL2 cells (DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). When the xenograft tumors reached an average tumor volume of 150-250 mm$^3$ (referred to as Day 0), the first and only dose of treatment was administered. Tumor volume was calculated based on two dimensions, measured using calipers, and was expressed in mm$^3$ according to the formula: $V = 0.5a \times b^2$, wherein $a$ and $b$ are the long and the short diameters of the tumor, respectively. To analyze the repeated measurement of tumor volumes from the same animals over time, a mixed modeling approach was used (*see, e.g.*, Pinheiro J, et al. nlme: linear and nonlinear mixed effects models. 2009; R package, version 3.1-96). This approach can address both repeated measurements and modest dropout rates due to non-treatment related removal of animals before the study end. Cubic regression splines were used to fit a non-linear profile to the time courses of log2 tumor volume at each dose level. These non-linear profiles were then related to dose within the mixed model.

[0370] Groups of 8 mice were treated with a single intravenous (i.v.) dose of 0.5 or 2 mg ADC/kg of Thio huMA79bv28 HC A118C immunoconjugate or control antibody-drug conjugates (control ADCs). One group of mice received 12.86 µg/kg free PBD dimer, SG2057. *See Hartley et al., Invest. New Drugs, 30: 950-958 (2012); Epub March 8, 2011.* The control ADCs bind to a protein that is not expressed on the surface of WSU-DLCL2 cells. Tumors and body weights of mice were measured 1-2 times a week throughout the experiment. Mice were euthanized before tumor volumes reached 3000 mm$^3$ or when tumors showed signs of impending ulceration. All animal protocols were approved by an Institutional Animal Care and Use Committee (IACUC).

[0371] The results of that experiment are shown in Table 3 and Figure 6. Table 3 shows each treatment group, the number of mice with observable tumors at the end of the study ("TI"), the number of mice showing a partial response ("PR"; where the tumor volume at any time after administration dropped below 50% of the tumor volume measured at day 0), the number of mice showing a complete response ("CR"; where the tumor volume at any time after administration dropped to 0 mm$^3$), the drug dose for each group, the antibody dose for each group, and the drug load for each ADC administered.
Table 3: Anti-CD79b ADC administration to mice with WSU-DLCL2 xenografts

<table>
<thead>
<tr>
<th>Antibody administered (Treatment)</th>
<th>TI</th>
<th>PR</th>
<th>CR</th>
<th>Drug Dose (µg/kg)</th>
<th>Ab Dose (mg/kg)</th>
<th>Drug Load (Drug/Ab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle*</td>
<td>8/8</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>huMA79bv28-PBD</td>
<td>8/8</td>
<td>0</td>
<td>0</td>
<td>3.22</td>
<td>0.5</td>
<td>1.65</td>
</tr>
<tr>
<td>huMA79bv28-PBD</td>
<td>3/8</td>
<td>2</td>
<td>6</td>
<td>12.86</td>
<td>2</td>
<td>1.65</td>
</tr>
<tr>
<td>Control ADC-MC-val-cit-PAB-PBD</td>
<td>8/8</td>
<td>0</td>
<td>0</td>
<td>14.03</td>
<td>2</td>
<td>1.8</td>
</tr>
<tr>
<td>Thio huMA79bv28 HC A118C-MC-vc-PAB-MMAE (“huMA79bv28-MMAE”)</td>
<td>8/8</td>
<td>0</td>
<td>0</td>
<td>19.24</td>
<td>2</td>
<td>2.01</td>
</tr>
<tr>
<td>SG2057</td>
<td>8/8</td>
<td>0</td>
<td>0</td>
<td>12.86</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

* Vehicle = 20 mM histidine acetate, pH 5.5, 240 mM sucrose, 0.02% PS20; n/a = not applicable.

[0372] In a 35 day time course with drug conjugates and doses as shown in Table 3, thio huMA79bv28 ADCs conjugated through a protease cleavable linker with PBD (“huMA79bv28-PBD”) showed inhibition of tumor growth in SCID mice with WSU-DLCL2 tumors compared to the vehicle and the control ADC (“Control-PBD”). See Figure 6.

[0373] Furthermore, when administered at 2 mg/kg, huMA79bv28-PBD better inhibited tumor growth than huMA79bv28 conjugated with the auristatin drug MMAE (“huMA79bv28-MMAE”). See Figure 6. The PBD free drug SG2057 did not show inhibition of tumor growth when given intravenously at 12.86 µg/kg, which is approximately equivalent to the drug dose of 2 mg/kg of huMA79bv28-PBD. As shown in Table 3, mice receiving 2 mg/kg huMA79bv28-PBD had six complete responses.

[0374] In this study, the percent body weight change was determined in each dosage group. The results indicated that administration of the huMA79bv28 ADCs did not cause a significant decrease in body weight during the study.

C. In vivo anti-tumor activity of humanized anti-CD79b antibody drug conjugates in a Granta-519 xenograft model

[0375] To test the efficacy of Thio huMA79bv28 HC A118C conjugates with PBD (“huMA79bv28-PBD”), the effects of the conjugated antibodies in a mouse xenograft model of Granta-519 tumors (human mantle cell lymphoma cell line) was examined.

[0376] Female CB17 ICR SCID mice (10-11 weeks of age from Charles Rivers Laboratories; Hollister, CA) were each inoculated subcutaneously in the flank with 2 x 10^7 Granta-519 cells (DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). When the xenograft tumors reached an average tumor volume of 150-250 mm^3 (referred to as Day 0), the first and only dose of treatment was administered. Tumor volume was calculated based on two dimensions, measured using calipers, and was
expressed in mm³ according to the formula: \( V = 0.5a \times b^2 \), wherein \( a \) and \( b \) are the long and the short diameters of the tumor, respectively. To analyze the repeated measurement of tumor volumes from the same animals over time, a mixed modeling approach was used (see, e.g., Pinheiro et al. 2009). This approach can address both repeated measurements and modest dropout rates due to non-treatment related removal of animals before the study end. Cubic regression splines were used to fit a non-linear profile to the time courses of log2 tumor volume at each dose level. These non-linear profiles were then related to dose within the mixed model.

[0377] Groups of 8 mice were treated with a single intravenous (i.v.) dose of 0.25, 0.5, or 1 mg ADC/kg of huMA79bv28 immunoconjugate or control antibody-drug conjugates (control ADCs). The control ADCs bind to a protein that is not expressed on the surface of Grant-519 cells. One group of mice received 3.22 µg/kg free PBD dimer, SG2057. Tumors and body weights of mice were measured 1-2 times a week throughout the experiment. Mice were euthanized before tumor volumes reached 3000 mm³ or when tumors showed signs of impending ulceration. All animal protocols were approved by an Institutional Animal Care and Use Committee (IACUC).

[0378] The results of that experiment are shown in Table 4 and Figure 7. Table 4 shows each treatment group, the number of mice with observable tumors at the end of the study ("TI"), the number of mice showing a partial response ("PR"; where the tumor volume at any time after administration dropped below 50% of the tumor volume measured at day 0), the number of mice showing a complete response ("CR"; where the tumor volume at any time after administration dropped to 0 mm³), the drug dose for each group, the antibody dose for each group, and the drug load for each ADC administered.

<table>
<thead>
<tr>
<th>Antibody administered (Treatment)</th>
<th>TI</th>
<th>PR</th>
<th>CR</th>
<th>Drug Dose - (µg/kg)</th>
<th>Ab Dose (mg/kg)</th>
<th>Drug Load (Drug/Ab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle*</td>
<td>8/8</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>huMA79bv28-PBD</td>
<td>0/8</td>
<td>0</td>
<td>8</td>
<td>3.22</td>
<td>0.5</td>
<td>1.65</td>
</tr>
<tr>
<td>huMA79bv28-PBD</td>
<td>0/8</td>
<td>0</td>
<td>8</td>
<td>1.61</td>
<td>0.25</td>
<td>1.65</td>
</tr>
<tr>
<td>Control-PBD</td>
<td>3/8</td>
<td>3</td>
<td>5</td>
<td>3.51</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>huMA79bv28-MMAE</td>
<td>6/8</td>
<td>1</td>
<td>2</td>
<td>9.62</td>
<td>1</td>
<td>2.01</td>
</tr>
<tr>
<td>SG2057</td>
<td>8/8</td>
<td>0</td>
<td>0</td>
<td>3.22</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

*Vehicle = 20 mM histidine acetate, pH 5.5, 240 mM sucrose, 0.02% PS20; n/a = not applicable.
In a 31 day time course with the ADCs and doses shown in Table 4, Thio huMA79bv28 conjugated through a protease cleavable linker with PBD ("huMA79bv28-PBD") showed inhibition of tumor growth in SCID mice with Granta-519 tumors compared to the vehicle. However, the control ADC conjugated to PBD ("Control-PBD") also showed anti-tumor activity, indicating that this tumor model is very sensitive to PBD. HuMA79bv28-PBD at a lower dose of 0.25 or 0.5 mg/kg was more effective at inhibiting tumor growth than huMA79bv28-MMAE at 1 mg/kg. The PBD free drug SG2057 did not show inhibition of tumor growth when given intravenously at 3.22 µg/kg, which is approximately equivalent to the drug dose of 0.5 mg/kg of huMA79bv28-PBD.

All 16 mice receiving huMA79bv28-PBD at 0.25 mg/kg or 0.5 mg/kg showed complete response, while only two mice that received 1 mg/kg huMA79bv28-MMAE showed complete response. See Table 4.

In this study, the percent body weight change was determined in each dosage group. The results indicated that administration of the huMA79bv28 ADCs did not cause a significant decrease in body weight during the study.

D. In vivo anti-tumor activity of humanized anti-CD79b antibody drug conjugates in a SuDHL4-luc xenograft model

To test the efficacy of Thio huMA79bv28 HC A118C conjugates with PBD ("huMA79bv28-PBD"), the effects of the conjugated antibodies in a mouse xenograft model of SuDHL4-luc tumors (diffuse large B-cell lymphoma cell line) was examined.

Female CB17 ICR SCID mice (10-11 weeks of age from Charles Rivers Laboratories; Hollister, CA) were each inoculated subcutaneously in the flank with 2 x 10^7 SuDHL4-luc cells (obtained from DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, and engineered at Genentech to stably express a luciferase gene). When the xenograft tumors reached an average tumor volume of 150-250 mm^3 (referred to as Day 0), the first and only dose of treatment was administered. Tumor volume was calculated based on two dimensions, measured using calipers, and was expressed in mm^3 according to the formula: V = 0.5a x b^2, wherein a and b are the long and the short diameters of the tumor, respectively. To analyze the repeated measurement of tumor volumes from the same animals over time, a mixed modeling approach was used (see, e.g., Pinheiro et al. 2008). This approach can address both repeated measurements and modest dropout rates due to non-treatment related removal of animals before the study end. Cubic regression splines were used to fit a non-linear profile to the time courses of log2 tumor volume at each dose level. These non-linear profiles were then related to dose within the mixed model.
Groups of 7 mice were treated with a single intravenous (i.v.) dose of 1 mg ADC/kg of huMA79bv28 immunoconjugate or control antibody-drug conjugates (control ADCs). The control ADCs bind to a protein that is not expressed on the surface of SuDHL4-luc cells. Tumors and body weights of mice were measured 1-2 times a week throughout the experiment. Mice were euthanized before tumor volumes reached 3000 mm$^3$ or when tumors showed signs of impending ulceration. All animal protocols were approved by an Institutional Animal Care and Use Committee (IACUC).

The results of that experiment are shown in Table 5 and Figure 8. Table 5 shows each treatment group, the number of mice with observable tumors at the end of the study ("TI"), the number of mice showing a partial response ("PR"; where the tumor volume at any time after administration dropped below 50% of the tumor volume measured at day 0), the number of mice showing a complete response ("CR"; where the tumor volume at any time after administration dropped to 0 mm$^3$), the drug dose for each group, the antibody dose for each group, and the drug load for each ADC administered.

Table 5: Anti-CD79b ADC administration to mice with SuDHL4-luc xenografts.

<table>
<thead>
<tr>
<th>Antibody administered (Treatment)</th>
<th>TI</th>
<th>PR</th>
<th>CR</th>
<th>Drug Dose (µg/kg)</th>
<th>Ab Dose (mg/kg)</th>
<th>Drug Load (Drug/Ab)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle*</td>
<td>7/7</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>huMA79bv28-PBD</td>
<td>6/7</td>
<td>4</td>
<td>3</td>
<td>6.43</td>
<td>1</td>
<td>1.65</td>
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<td>Control-PBD</td>
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<td>0</td>
<td>7.02</td>
<td>1</td>
<td>1.8</td>
</tr>
<tr>
<td>huMA79bv28-MMAE</td>
<td>5/7</td>
<td>1</td>
<td>3</td>
<td>9.62</td>
<td>1</td>
<td>2.01</td>
</tr>
<tr>
<td>Control-ADC-A118C-MC-vc-PAB-MMAE</td>
<td>7/7</td>
<td>0</td>
<td>0</td>
<td>10.05</td>
<td>1</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*Vehicle = 20 mM histidine acetate, pH 5.5, 240 mM sucrose, 0.02% PS20; n/a = not applicable.

In a 21 day time course with drug conjugates and doses as shown in Table 5, Thio Hu anti-CD79b ADC conjugated through a protease cleavable linker with PBD ("huMA79bv28-PBD") showed inhibition of tumor growth in SCID mice with SuDHL4-luc tumors compared to the vehicle and the control ADC ("Control-PBD"). See Figure 8.

Furthermore, 1 mg/kg of huMA79bv28-PBD showed comparable anti-tumor activity to the humanized anti-CD79b thiomab conjugated with auristatin drug MMAE ("huMA79bv28-MMAE"). However, three mice from the group administered huMA79bv28-PBD showed a complete response, and another four mice showed a partial response, in contrast to huMA79bv28-MMAE, which produced three complete responses and one partial response. See Table 5.
In this study, the percent body weight change was determined in each dosage group. The results indicated that administration of the huMA79bv28 ADCs did not cause a significant decrease in body weight during the study.

E. Dose escalation study of huMA79bv28-PBD in a SuDHL4-luc xenograft model

The efficacy of huMA79bv28-PBD at various dose levels in a mouse xenograft model of SuDHL4-luc tumors (diffuse large B-cell lymphoma cell line) was examined.

Female CB17 ICR SCID mice (12-13 weeks of age from Charles Rivers Laboratories; Hollister, CA) were each inoculated subcutaneously in the flank with $2 \times 10^7$ SuDHL4-luc cells (obtained from DSMZ, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, and engineered at Genentech to stably express a luciferase gene). When the xenograft tumors reached an average tumor volume of 150-300 mm³ (referred to as Day 0), the first and only dose of treatment was administered. Tumor volume was calculated based on two dimensions, measured using calipers, and was expressed in mm³ according to the formula: $V = 0.5a \times b^2$, wherein $a$ and $b$ are the long and the short diameters of the tumor, respectively. To analyze the repeated measurement of tumor volumes from the same animals over time, a mixed modeling approach was used (see, e.g., Pinheiro et al. 2008). This approach can address both repeated measurements and modest dropout rates due to non-treatment related removal of animals before the study end. Cubic regression splines were used to fit a non-linear profile to the time courses of log₂ tumor volume at each dose level. These non-linear profiles were then related to dose within the mixed model.

Groups of 8 mice were treated with a single intravenous (i.v.) dose of 0.2, 0.5, 1, or 2 mg ADC/kg of huMA79bv28-PBD or Control-PBD, which binds to a protein that is not expressed on the surface of SuDHL4-luc cells. Tumors and body weights of mice were measured 1-2 times a week throughout the experiment. Mice were euthanized before tumor volumes reached 3000 mm³ or when tumors showed signs of impending ulceration. All animal protocols were approved by an Institutional Animal Care and Use Committee (IACUC).

The results of that experiment are shown in Table 6 and Figure 9. Table 7 shows each treatment group, the number of mice with observable tumors at the end of the study ("TI"), the number of mice showing a partial response ("PR"; where the tumor volume at any time after administration dropped below 50% of the tumor volume measured at day 0), the number of mice showing a complete response ("CR"; where the tumor volume at any time after administration dropped to 0 mm³), the drug dose for each group, the antibody dose for each group, and the drug load for each ADC administered.
Table 6: Anti-CD79a ADC administration to mice with SuDHL4-luc xenografts

<table>
<thead>
<tr>
<th>Antibody administered (Treatment)</th>
<th>TI</th>
<th>PR</th>
<th>CR</th>
<th>Drug Dose (μg/kg)</th>
<th>Ab Dose (mg/kg)</th>
<th>Drug Load (Drug/Ab)</th>
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</thead>
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<tr>
<td>Vehicle*</td>
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<td>0</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
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<td>huMA79bv28-PBD</td>
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<td>3.39</td>
<td>0.5</td>
<td>1.74</td>
</tr>
<tr>
<td>huMA79bv28-PBD</td>
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<td>2</td>
<td>5</td>
<td>6.78</td>
<td>1</td>
<td>1.74</td>
</tr>
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<td>2</td>
<td>6</td>
<td>13.56</td>
<td>2</td>
<td>1.74</td>
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<tr>
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<td>0</td>
<td>7.02</td>
<td>1</td>
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<td>0</td>
<td>14.03</td>
<td>2</td>
<td>1.8</td>
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</table>

*Vehicle = 20 mM histidine acetate, pH 5.5, 240 mM sucrose, 0.02% PS20; n/a = not applicable.

[0393] In a 31 day time course with drug conjugates and doses as shown in Table 6, huMA79bv28-PBD showed dose-dependent inhibition of tumor growth in SCID mice with SuDHL4-luc tumors. When administered at 0.2 mg/kg or higher dose, huMA79bv28-PBD showed clear inhibitory activity compared to vehicle or the control ADC. See Figure 9. In addition, a single dose of 1 or 2 mg/kg huMA79bv28-PBD resulted in complete response in 5/8 and 6/8 treated animals, respectively. See Table 6.

[0394] In this study, the percent body weight change was determined in each dosage group. The results indicated that administration of huMA79bv28-PBD did not cause a significant decrease in body weight during the study.

F. Dose escalation study of huMA79bv28-PBD in a BJAB-luc xenograft model

[0395] The efficacy of huMA79bv28-PBD at various dose levels in a mouse xenograft model of BJAB-luc tumors (Burkitt’s lymphoma cell line) was examined.

[0396] Female CB17 ICR SCID mice (8-9 weeks of age from Charles Rivers Laboratories; Hollister, CA) were each inoculated subcutaneously in the flank with 2 x 10^7 BJAB-luc cells (available, e.g., from Lonza, Basel, Switzerland, and engineered at Genentech to stably express a luciferase gene). When the xenograft tumors reached an average tumor volume of 150-300 mm^3 (referred to as Day 0), the first and only dose of treatment was administered. Tumor volume was calculated based on two dimensions, measured using calipers, and was expressed in mm^3 according to the formula: \( V = 0.5a \times b^2 \), wherein \( a \) and \( b \) are the long and the short diameters of the tumor, respectively. To analyze the repeated measurement of tumor volumes from the same animals over time, a mixed modeling approach was used (see, e.g., Pinheiro et al. 2008). This approach can address both repeated measurements and modest dropout rates due to non-treatment related removal of animals.
before the study end. Cubic regression splines were used to fit a non-linear profile to the time courses of log2 tumor volume at each dose level. These non-linear profiles were then related to dose within the mixed model.

[0397] Groups of 9 mice were treated with a single intravenous (i.v.) dose of 0.05, 0.2, 0.5, or 1 mg ADC/kg of huMA79bv28-PBD or Control-PBD, which binds to a protein that is not expressed on the surface of BJAB-luc cells. Tumors and body weights of mice were measured 1-2 times a week throughout the experiment. Mice were euthanized before tumor volumes reached 3000 mm³ or when tumors showed signs of impending ulceration. All animal protocols were approved by an Institutional Animal Care and Use Committee (IACUC).

[0398] The results of that experiment are shown in Table 7 and Figure 10. Table 7 shows each treatment group, the number of mice with observable tumors at the end of the study ("TI"), the number of mice showing a partial response ("PR"; where the tumor volume at any time after administration dropped below 50% of the tumor volume measured at day 0), the number of mice showing a complete response ("CR"; where the tumor volume at any time after administration dropped to 0 mm³), the drug dose for each group, the antibody dose for each group, and the drug load for each ADC administered.

Table 7: Anti-CD79a ADC administration to mice with BJAB-luc xenografts

<table>
<thead>
<tr>
<th>Antibody administered (Treatment)</th>
<th>TI</th>
<th>PR</th>
<th>CR</th>
<th>Drug Dose (µg/kg)</th>
<th>Ab Dose (mg/kg)</th>
<th>Drug Load (Drug/Ab)</th>
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<td>n/a</td>
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<td>5</td>
<td>1.36</td>
<td>0.2</td>
<td>1.74</td>
</tr>
<tr>
<td>huMA79bv28-PBD</td>
<td>0/9</td>
<td>0</td>
<td>9</td>
<td>3.39</td>
<td>0.5</td>
<td>1.74</td>
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<tr>
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<td>9</td>
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*Vehicle = 20 mM histidine acetate, pH 5.5, 240 mM sucrose, 0.02% PS20; n/a = not applicable.

[0399] In a 35 day time course with drug conjugates and doses as shown in Table 7, huMA79bv28-PBD showed dose-dependent inhibition of tumor growth in SCID mice with BJAB-luc tumors. When administered at 0.2 mg/kg or higher dose, huMA79bv28-PBD showed clear inhibitory activity compared to vehicle or the control ADC administered at 0.5 mg/kg. *See Figure 10. In addition, a single dose of 0.5 or 1 mg/kg huMA79bv28-PBD resulted in complete tumor remission in all treated animals. Control-PBD at 1 mg/kg also showed substantial anti-tumor activity, indicating that this model is very sensitive to PBD.
In this study, the percent body weight change was determined in each dosage group. The results indicated that administration of huMA79bv28-PBD did not cause a significant decrease in body weight during the study.

G. Dose escalation study of huMA79bv28-MMAE in a BJAB-luc xenograft model

The efficacy of huMA79bv28-MMAE at various dose levels in a mouse xenograft model of BJAB-luc tumors (Burkitt’s lymphoma cell line) was examined.

Female CB 17 ICR SCID mice (13-14 weeks of age from Charles Rivers Laboratories; Hollister, CA) were each inoculated subcutaneously in the flank with $2 \times 10^7$ BJAB-luc cells (available, e.g., from Lonza, Basel, Switzerland, and engineered at Genentech to stably express a luciferase gene). When the xenograft tumors reached an average tumor volume of 150-300 mm$^3$ (referred to as Day 0), the first and only dose of treatment was administered. Tumor volume was calculated based on two dimensions, measured using calipers, and was expressed in mm$^3$ according to the formula: $V = 0.5a \times b^2$, wherein $a$ and $b$ are the long and the short diameters of the tumor, respectively. To analyze the repeated measurements of tumor volumes from the same animals over time, a mixed modeling approach was used (see, e.g., Pinheiro et al. 2008). This approach can address both repeated measurements and modest dropout rates due to non-treatment related removal of animals before the study end. Cubic regression splines were used to fit a non-linear profile to the time courses of log2 tumor volume at each dose level. These non-linear profiles were then related to dose within the mixed model.

Groups of 8 mice were treated with a single intravenous (i.v.) dose of 0.1, 0.5, 1, 2, or 4 mg ADC/kg of huMA79bv28-MMAE, unconjugated huMA79bv28, or Control-MMAE, which binds to a protein that is not expressed on the surface of BJAB-luc cells. Tumors and body weights of mice were measured 1-2 times a week throughout the experiment. Mice were euthanized before tumor volumes reached 3000 mm$^3$ or when tumors showed signs of impending ulceration. All animal protocols were approved by an Institutional Animal Care and Use Committee (IACUC).

The results of that experiment are shown in Table 8 and Figure 11. Table 8 shows each treatment group, the number of mice with observable tumors at the end of the study ("TI"), the number of mice showing a partial response ("PR"; where the tumor volume at any time after administration dropped below 50% of the tumor volume measured at day 0), the number of mice showing a complete response ("CR"; where the tumor volume at any time
after administration dropped to 0 mm³), the drug dose for each group, the antibody dose for each group, and the drug load for each ADC administered.

Table 8: Anti-CD79a ADC administration to mice with BJAB-luc xenografts

<table>
<thead>
<tr>
<th>Antibody administered (Treatment)</th>
<th>TI</th>
<th>PR</th>
<th>CR</th>
<th>Drug Dose (µg/kg)</th>
<th>Ab Dose (mg/kg)</th>
<th>Drug Load (Drug/Ab)</th>
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</thead>
<tbody>
<tr>
<td>Vehicle*</td>
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<td>0</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
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*Vehicle = 20 mM histidine acetate, pH 5.5, 240 mM sucrose, 0.02% PS20; n/a = not applicable.

[0405] In a 42 day time course with drug conjugates and doses as shown in Table 8, huMA79bv28-MMAE showed dose-dependent inhibition of tumor growth in SCID mice with BJAB-luc tumors. In contrast to huMA79bv28-PBD, which showed complete inhibition at 0.2 mg ADC/kg, huMA79bv28-MMAE did not show complete inhibition until a dose of 2 mg ADC/kg.

[0406] In this study, the percent body weight change was determined in each dosage group. The results indicated that administration of huMA79bv28-MMAE did not cause a significant decrease in body weight during the study.

[0407] 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.
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<td>A118C cysteine engineered heavy chain (IgGl)</td>
<td>ILPGGGDTNY NEIFKGRATF SADTSKNTAY LQMNSLRAED TAVYYCTRRV PIRLDWYGQG TLTVSTSCST KGPSVFPLAP SSKSTSGGTA ALGCLVKDVF PEPLVSVWNS GALTSGVHTF PAVLQSSGLY SLSSWTVPS SSLGQTQTYIC NVNHHPSNTK VDKKVEKSC DKTTHCPPCP APELLGGSV FLFPKKPKDT LMIQRTPETY CWVDVSHED PEVKFNWYVD GVENVNATKXK PREEQNYSTQ RVVSVTLTVH QDWLNGKEKY CKVSNKALPA PIEKTISKAK GQPREPQYVT LPPSRREEMTK NQVSITLCLV GFYPISDIAVE WESNGQPENN YKTTTPVLDS DGSFRFLYSKL TVDSRKSWQOG NVFSCSVMHE ALHNHYTRKQ LSLPGL</td>
<td></td>
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<tr>
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<td>MARALALSPVF SHMVAVALLLL LSAPDFVPAAK SEDRYRNFK SACSRIWQSP RFIAHRGKF VTKHCMYNSA SGNVSWLWKO EMDENPQQLK LEKGRMEESQ NESLATTLTIQ GIRFEDNGIY FCQQKCNNTS EVYQCGTLEG RVMGFSLILAQ LKQRNTLKDQ IIMIQTLLI LIFFIIVFILL IDQATYEDY VTLRTGEVKW SVGEHPQGE</td>
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<tr>
<td>Human mature CD79b, without signal sequence; amino acids 29 to 229</td>
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<td>EVQLVESGGG LVPQPSLRLLR SCAASGYESF RSWMNNVR QA PKGKLEWVGR IYPGDGDNTY SGKFKGRFTI SADTSKNTAY LQMNSLRAED TAVYYCARDG SSWDWFYDVW GQGLTVTRESS</td>
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<td>52 Anti-CD22</td>
<td><strong>hulOF4v3</strong></td>
<td>EVQLVESGGG LVQPGGSLRL SCAASGYEFS RSWMNVRQQA PGKLEGWVGR</td>
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<tr>
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<td></td>
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<tr>
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<td>V205C cysteine engineered light chain (Igs)</td>
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<td>DIQMTQSPSS LSASVGDRTV ITCKRASQSIV HSVGNTFLFW YQQPKGKAPK</td>
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</table>
WHAT IS CLAIMED IS:

1. An immunoconjugate comprising an antibody that binds CD79b covalently attached to a cytotoxic agent, 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.

2. The immunoconjugate of claim 1, wherein 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.

3. The immunoconjugate of claim 2, wherein the antibody comprises HVR-L1 comprising the amino acid sequence of SEQ ID NO: 24.

4. The immunoconjugate of any one of claims 1 to 3, wherein 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).

5. The immunoconjugate of any one of claims 1 to 4, comprising a VH sequence having an amino acid sequence selected from SEQ ID NOs: 7, 9, 11, and 13.

6. The immunoconjugate of claim 5, comprising a VH sequence having the amino acid sequence of SEQ ID NO: 11.

7. The immunoconjugate of any one of claims 1 to 6, comprising a VL sequence having an amino acid sequence selected from SEQ ID NOs: 8, 10, 12, and 14.

8. The immunoconjugate of claim 7, comprising a VL sequence having the amino acid sequence of SEQ ID NO: 12.

9. An immunoconjugate comprising an antibody that binds CD79b covalently attached to a cytotoxic agent, 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.

10. The immunoconjugate of any one of claims 1 to 9, wherein the antibody is an IgGl, IgG2a or IgG2b antibody.

11. An immunoconjugate of any one of claims 1 to 10, wherein 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.

12. The immunoconjugate of claim 11, wherein D is a pyrrolobenzodiazepine of Formula A:

![Chemical Structure]

wherein the wavy line indicates the covalent attachment site to the linker;
the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;
R² is independently selected from H, OH, =O, =CH₂, CN, R, OR, =CH-R¹-D, =C(R¹>D)₂,
O-SO₂-R, CO₂R and COR, and optionally further selected from halo or dihalo, wherein
R¹D is independently selected from R, C0₂R, COR, CHO, C0₂H and halo;
R⁶ and R⁹ are independently selected from H, R, OH, OR, SH, SR, NH₂, NHR, NRR', N0₂,
N0₂, Me₃Sn and halo;
R⁷ is independently selected from H, R, OH, OR, SH, SR, NH₂, NHR, NRR', N0₂,
N0₂, MesSn and halo;
Q is independently selected from O, S and NH;
R¹¹ is either H, or R or, where Q is O, SO₃M, where M is a metal cation;
R and R' are each independently selected from optionally substituted Ci-i₂ alkyl,
c₃-i₄ heterocyclyl and c₅-i₄ ary1 groups, and optionally in relation to the group NRR', R
and R' together with the nitrogen atom to which they are attached form an optionally
substituted 4-, 5-, 6- or 7-membered heterocyclic ring;
R¹₂, R¹⁶, R¹⁹ and R¹⁷ are as defined for R², R⁶, R⁹ and R⁷ respectively;
R² is a c₃-i₂ alkylene group, which chain may be interrupted by one or more
heteroatoms and/or aromatic rings that are optionally substituted; and
X and X' are independently selected from O, S and N(H).
13. The immunoconjugate of claim 12, wherein D has the structure:

![Chemical structure](image)

wherein n is 0 or 1.

14. The immunoconjugate of claim 12, wherein D has a structure selected from:

![Chemical structure](image)

wherein 

wherein R^E and R^E' are each independently selected from H or R^D, wherein R^D is independently selected from R, C0_2R, COR, CHO, C0_2H, and halo;

wherein Ar^1 and Ar^2 are each independently optionally substituted C5-20 aryl; and

wherein n is 0 or 1.
15. The immunoconjugate of claim 11, wherein D is a pyrrolobenzodiazepine of Formula B:

![Chemical Structure](image)

wherein the horizontal wavy line indicates the covalent attachment 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.

16. The immunoconjugate of any one of claims 11 to 15, wherein the linker is cleavable by a protease.

17. The immunoconjugate of claim 16, wherein the linker comprises a val-cit dipeptide or a Phe-homoLys dipeptide.

18. The immunoconjugate of claim 13 having the formula:

![Chemical Structure](image)

19. The immunoconjugate of any one of claims 11 to 18, wherein p ranges from 1-3.
20. An immunoconjugate 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.

21. The immunoconjugate of claim 20, wherein the antibody comprises a VH sequence of SEQ ID NO: 11 and a VL sequence of SEQ ID NO: 12.

22. The immunoconjugate of claim 21, wherein the antibody comprises a heavy chain of SEQ ID NO: 39 and a light chain of SEQ ID NO: 37.

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 CD79b.

26. The immunoconjugate of any one of the preceding claims, wherein the antibody binds human CD79b.

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 of the immunoconjugate of any one of claims 1 to 27.

31. The 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 method of claim 31, further comprising administering an additional therapeutic agent to the individual.

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

34. The 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 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.

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

37. The method of claim 36, wherein the cell is a lymphoma cell.
### VH Sequences

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<th>huMA79b.v28</th>
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#### Kabat# 42 43 44 45 46 47 48 49 50 51 52 52a 52b 52c 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80

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**FIG. 1A**
VL Sequences

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Kabat - CDR L1
Chothisa - CDR L1
Contact - CDR L1

huKL  D I Q H T Q S P S L S A S V G D R V T I T C  R A S Q  S I N Y L A W Y Q
MA79b  D I Q H T Q S P S L S A S V G D R V T I T C  R A S Q  S I N Y L A W Y Q
huMA79b v17  D I Q L T Q S P S L S A S V G D R V T I T C  R A S Q  S I N Y L A W Y Q
huMA79b v18  D I Q L T Q S P S L S A S V G D R V T I T C  R A S Q  S I N Y L A W Y Q
huMA79b v28  D I Q L T Q S P S L S A S V G D R V T I T C  R A S Q  S I N Y L A W Y Q
huMA79b v32  D I Q L T Q S P S L S A S V G D R V T I T C  R A S Q  S I N Y L A W Y Q

Kabat#  38  39  40  41  42  43  44  45  46  47  48  49  50  51  52  53  54  55  56  57  58  59  60  61  62  63  64  65  66  67  68  69  70  71  72  73  74  75  76  77  78  79  80

Kabat - CDR L2
Chothisa - CDR L2
Contact - CDR L2


FIG. 2A
**VL Sequences**

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* | 3 6 7 | | 3 9 |

**FIG. 2B**
huMA79bv28 Light Chain

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SGSGTDFTLTISLSQPEDFATYCCQSQSNEPQFPTFQGQGKVEIKRTVAAPSVFIFPSDEQLKSGTASV
VCLMNFYPREAVQWKNALQSGNSQESTVQDEKDKSTYSTLSTLSLKLAYEKYYACEVTHGC
LSSPVTKSFNREGECSQID NO: 37

huMA79bv28 Heavy Chain

EVQLVESGGGLVQPGGLSRLSCAASGYTFSYYIWVRQAPGKGLEWIGIEILPQGDTNYIEIFKGRA
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FIG. 3

5/13
Thio-huMA79b.v28-HC-A118C (HC)

EVQLVESGGGLVQPGSPGSLSLCAASGYTFSSYICEWVRQAPKGLEWIGELPGGDTNYEIKGRATFSADTSKNTAYLMNLSLRAEDTAVYCTRRVPILDYWGQTTLVTVSSQGSKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEVPVTVSNNSGALTSGVHFFTPAVLQLSSGLSSVVTSLQSSSLGTQTYICNVNHKFSNTKVKVEPKSDCTKTCPQQPAPELLEGGPSVFLEFPKPDLSMTLRSPVEVTCDVVIDCADEPEVKFNKTVDGVEVHNAKTPREEQYNGSTNRVSVVLTLHQDLNNGKEYCKVSNKALPAEKTISKAQGQPREFQVTLPPPSEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLSYSKLTVDKSRWQQGNFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO: 39

Thio-huMA79b.v28-LC-V205C (LC)

DIQLTQSSPLSLASVGRVTITCKASQSVDYEGDSFLLNYQQFPGKAPKLLYIASHLESGLVESRSFSGSSTGDFLTISSLQEDFATYCQOSNEDPLTFQGGTKVEIKPTVAAPSIFIPDNSDEQLKSSSTASVVCNLNFYPREKAVYQKVHNALSGHEGSVEQDSKDTYSLSLSTLSDKADYEHKHYACEVTQHQLSSRCPKSFNREGEC

SEQ ID NO: 54

Thio-huMA79b.v28-HC-S400C (HC)

EVQLVESGGGLVQPGSPGSLSLCAASGYTFSSYICEWVRQAPKGLEWIGELPGGDTNYEIKGRATFSADTSKNTAYLMNLSLRAEDTAVYCTRRVPILDYWGQTTLVTVSSQGSKGPSVFLAPSSKSTSGGTAALGCLVKDYFPEVPVTVSNNSGALTSGVHFFTPAVLQLSSGLSSVVTSLQSSSLGTQTYICNVNHKFSNTKVKVEPKSDCTKTCPQQPAPELLEGGPSVFLEFPKPDLSMTLRSPVEVTCDVVIDCADEPEVKFNKTVDGVEVHNAKTPREEQYNGSTNRVSVVLTLHQDLNNGKEYCKVSNKALPAEKTISKAQGQPREFQVTLPPPSEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLSYSKLTVDKSRWQQGNFSCSVMHEALHNHYTQKSLSLSPG

SEQ ID NO: 55

FIG. 4
Thio huMA79bv28 HC A118C-MC-val-cit-PAB-PBD ("huMA79bv28-PBD")

FIG. 5
FIG. 6
FIG. 7
FIG. 9
FIG. 10
FIG. 11
A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/48 A61P35/00

ADD.

According to International Patent Classification (IPC) and/or both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO/Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Date of the actual completion of the international search

14 October 2013

Date of mailing of the international search report

21/10/2013

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk

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Biri kaki, Lemonia
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