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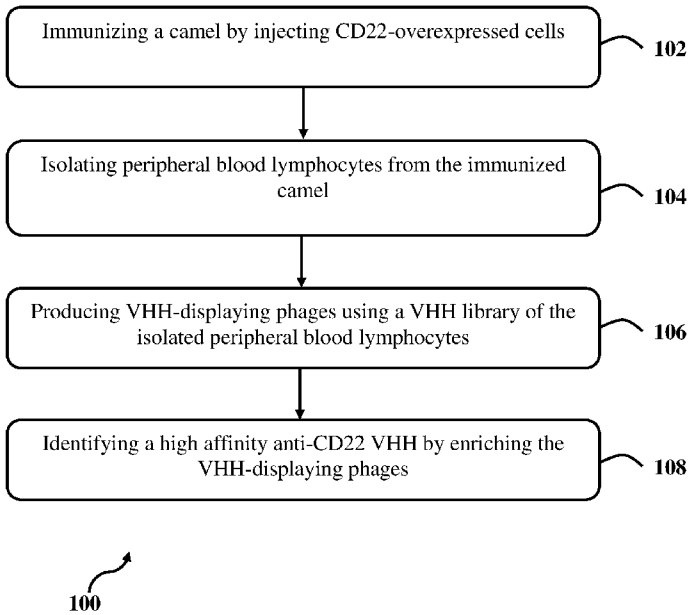

FIG. 1

(57) Abstract: Disclosed herein is an anti-CD22 heavy-chain variable domain (anti-CD22 VHH) antibody including an amino acid sequence as set forth in at least one of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, and combinations thereof.

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ANTI-CD22 HEAVY-CHAIN VARIABLE DOMAIN ANTIBODY

STATEMENT REGARDING SEQUENCE LISTING

[0001] The Sequence Listing associated with this application is provided in text format in lieu of a paper copy and is hereby incorporated by reference into the specification. The name of the text file containing the Sequence Listing is (057-PCT9705) Sequence Listing.txt. The text file is 11 KB, was created on September 10, 2018, and is being submitted electronically via ePCT.

TECHNICAL FIELD

[0002] The present disclosure generally relates to the field of antibody engineering, particularly to single domain antibodies, and more particularly to anti-CD22 heavy-chain variable domain (VHH) antibodies.

BACKGROUND ART

[0003] A cluster of differentiation-22 (CD22) antigen is a B-cell-specific transmembrane glycoprotein which modulates B-cell function, survival, and apoptosis. Generally, the CD22 antigen is overexpressed in B-cell malignancies and rapidly internalized into the B-cells upon binding to its antibody. Therefore, the CD22 antigen may be considered as an attractive therapeutic biomarker for treating B-cell malignancies such as non-Hodgkin's lymphoma (NHL), hairy cell leukemia (HCL), Burkitt's lymphoma, acute lymphoblastic leukemia (ALL), and B-cell autoimmune disorders.

[0004] Conventional therapeutic antibodies for B-cell malignancies such as monoclonal antibodies (mAbs) against CD22 antigen have limited efficacy due to their severe toxicity, large size, thermosensitivity, instability at low or high pH levels, poor penetration into tumors, pH-dependent activity, and complex production. In order to resolve the limitations of the conventional therapeutic antibodies, antibody fragments may be introduced by modifying therapeutic antibodies using antibody engineering. However, these modifications may decrease the stability and affinity of the antibodies and may lead to antibody aggregation in many cases.

[0005] Therefore, there is a need for a stable anti-CD22 antibody fragment with high efficacy, high affinity, high shelf-life, a small size, rapid clearance from blood, and low immunogenicity. Moreover, there is a need for a functional human anti-CD22 single-domain antibody fragment,

anti-CD22 antibody-drug conjugate (ADC), and other anti-CD22 antibody fusion proteins with a simple and cheap manufacturing process.

SUMMARY OF THE DISCLOSURE

[0006] This summary is intended to provide an overview of the subject matter of this patent, and is not intended to identify essential elements or key elements of the subject matter, nor is it intended to be used to determine the scope of the claimed implementations. The proper scope of this patent may be ascertained from the claims set forth below in view of the detailed description below and the drawings.

[0007] In one general aspect, the present disclosure describes an anti-cluster of differentiation-22 (anti-CD22) heavy-chain variable-domain (VHH) antibody (anti-CD22 VHH) including an amino acid sequence as set forth in at least one of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, and combinations thereof.

[0008] The above general aspect may include one or more of the following features. In some exemplary embodiments, the anti-CD22 VHH antibody may have a molecular weight between about 15 kilodaltons (kDa) and about 17 kDa. In some exemplary embodiments, a complex of the anti-CD22 VHH antibody and a CD22 antigen may have a dissociation constant (Kd) between 10−7 M and about 10−8 M. According to some exemplary embodiments, the anti-CD22 VHH antibody may be a Camelidae VHH antibody.

[0009] In another general aspect, the present disclosure describes a formulation for treating a B-cell malignancy. The formulation may include an anti-CD22 VHH antibody conjugated to at least one of a therapeutic agent and a diagnostic agent. In some exemplary embodiments, the anti-CD22 VHH antibody may include an amino acid sequence as set forth in at least one of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, and combinations thereof.

[0010] The above general aspect may include one or more of the following features. In some exemplary embodiments, the B-cell malignancy may include at least one of non-Hodgkin's lymphoma (NHL), hairy cell leukemia (HCL), Burkitt's lymphoma, acute lymphoblastic leukemia (ALL), and combinations thereof.

[0011] According to some exemplary embodiments, the therapeutic agent may include at least one of a radionuclide, an immunomodulator, an anti-angiogenic agent, a pro-apoptotic agent,

a cytokine, a chemokine, a drug, a toxin, a hormone, a siRNA, an enzyme, and combinations thereof. In some exemplary embodiments, the toxin may include at least one of ricin, abrin, alpha toxin, saporin, ribonuclease (RNase), onconase, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, and combinations thereof.

[0012] According to some exemplary embodiments, the drug may include at least one of 5-fluorouracil, aplidin, azaribine, anastrozole, anthracyclines, bendamustine, bleomycin, bortezomib, bryostatin-1, busulfan, calicheamycin, camptothecin, carboplatin, 10-hydroxycamptothecin, carmustine, celecoxib, chlorambucil, cisplatinum, Cox-2 inhibitors, irinotecan, carboplatin, cladribine, camptothecans, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunorubicin, doxorubicin, 2-pyrrolinodoxorubicine (2P-DOX), pro-2-pyrrolinodoxorubicine (pro-2P-DOX), cyano-morpholino doxorubicin, doxorubicin glucuronide, epirubicin glucuronide, estramustine, epipodophyllotoxin, estrogen receptor binding agents, etoposide (VP16), etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3′,5′-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, farnesyl-protein transferase inhibitors, gemcitabine, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, lenolidamide, leucovorin, lomustine, mechlorethamine, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, navelbine, nitrosourea, plicomycin, procarbazine, paclitaxel, pentostatin, raloxifene, semustine, streptozocin, tamoxifen, paclitaxel, temazolomide, transplatinum, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinorelbine, vinblastine, vincristine, a vinca alkaloid, a tyrophostin, canertinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, leflunomide, nilotinib, pazopanib, semaxinib, sorafenib, sunitinib, sutent, vatalanib, ibrutinib, and combinations thereof.

[0013] According to some exemplary embodiments, the immunomodulator may include at least one of a cytokine, a stem cell growth factor, a lymphotoxin, a hematopoietic factor, a colony stimulating factor (CSF), an interleukin (IL), erythropoietin, thrombopoietin, tumor necrosis factor (TNF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon-α, interferon-β, interferon-γ, interferon-λ, transforming growth factor-alpha (TGF-α), transforming growth factor-beta (TGF-β), interleukin-1 (IL-1), interleukin-1α (IL-1α), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7),

interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11), interleukin-12 (IL-12), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-18 (IL-18), interleukin-21 (IL-21), interleukin-23 (IL-23), interleukin-25 (IL-25), Leukemia inhibitory factor (LIF), Fms-like tyrosine kinase 3 (FLT-3), angiostatin, thrombospondin, endostatin, lymphotoxin, and combinations thereof.

[0014] According to some exemplary embodiments, the radionuclide may include at least one of 110In, 111In, 177Lu, 18F, 52Fe, 62Cu, 64Cu, 67Cu, 67Ga, 68Ga, 86Y, 90Y,89Zr, 94mTc, 94Tc, 99Tc, 120I, 123I, 124I, 125I, 131I, 154Gd, 155Gd, 156Gd, 157Gd, 158Gd, 32F, 11C, 13N, 15O,186Re, 188Re, 51Mn, 52Mn, 55Co, 72As, 75Br, 76Br, 82Rb, 83Sr, 99Mo, 105Rh, 149Pm, 169Er, 194Ir, 58Co, 80Br, 99Tc, 103Rh, 109Pt, 119Sb, 125I, 189mOs, 192Ir, 219Rn, 215Po, 221Fr, 255Fm, 11C, 13N, 15O, 75Br, 198Au, 199Au, 224Ac, 77Br, 113mIn, 95Ru, 97Ru, 103Ru, 105Ru, 107Hg, 203Hg, 121Te, 122Te, 227Th, 125Te, 165Tm, 167Tm, 168Tm, 197Pt, 109Pd, 142Pr, 143Pr, 161Tb, 57Co, 58Co, 51Cr, 59Fe, 75Se, 201Tl, 76Br, 169Yb, and combinations thereof.

[0015] According to some exemplary embodiments, the diagnostic agent may include at least one of a radionuclide, a contrast agent, a fluorescent agent, a chemiluminescent agent, a bioluminescent agent, a paramagnetic ion, an enzyme, a photoactive diagnostic agent, and combinations thereof. In some exemplary embodiments, the paramagnetic ion may include at least one of chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III), erbium (III), and combinations thereof.

[0016] According to some exemplary embodiments, the fluorescent agent may include at least one of fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde, fluorescamine, and combinations thereof. In some exemplary embodiments, the chemiluminescent agent may include at least one of luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt, an oxalate ester, and combinations thereof. In some exemplary embodiments, the bioluminescent agent may include at least one of luciferin, luciferase, aequorin, and combinations thereof.

[0017] In another general aspect, the present disclosure describes a method for treating a B-cell malignancy. The method may include administering an exemplary formulation including the exemplary anti-CD22 VHH antibody conjugated to at least one of the therapeutic agent and

the diagnostic agent. The exemplary anti-CD22 VHH antibody may include an amino acid sequence as set forth in at least one of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] The drawing figures depict one or more implementations in accord with the present teachings, by way of example only, not by way of limitation. In the figures, like reference numerals refer to the same or similar elements.

[0019] FIG. 1 illustrates a method for identifying an exemplary anti-CD22 heavy-chain variable domain (VHH) antibody, consistent with one or more exemplary of the present disclosure.

[0020] FIG. 2A illustrates a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profile of an exemplary anti-CD22 VHH antibody under non-reducing condition, consistent with one or more exemplary of the present disclosure.

[0021] FIG. 2B illustrates a western blot profile of an exemplary anti-CD22 VHH antibody under non-reducing condition, consistent with one or more exemplary embodiments of the present disclosure.

[0022] FIG. 3A illustrates an SDS-PAGE profile of Raji cell lysate as CD22-overexpressed cells and Jurkat cell lysate as a negative control treated with an exemplary anti-CD22 VHH antibody, consistent with one or more exemplary embodiments of the present disclosure.

[0023] FIG. 3B illustrates a western blot profile of Raji cell lysate as CD22-overexpressed cells and Jurkat cell lysate as a negative control with an exemplary anti-CD22 VHH antibody, consistent with one or more exemplary embodiments of the present disclosure.

[0024] FIG. 4A illustrates a graph of a binding study of anti-CD22 VHH antibodies by flow cytometry using Jurkat cells as a negative control, consistent with one or more exemplary embodiments of the present disclosure.

[0025] FIG. 4B illustrates a graph of a binding study of anti-CD22 VHH antibodies by flow cytometry using Raji cells as CD22-overexpressed cells, consistent with one or more exemplary embodiments of the present disclosure.

[0026] FIG. 5A illustrates results of an internalization assay of an exemplary anti-CD22 VHH antibody in Raji cells as CD22-overexpressed cells, consistent with one or more exemplary embodiments of the present disclosure.

[0027] FIG. 5B illustrates results of an internalization assay of an exemplary anti-CD22 VHH antibody in Jurkat cells as a negative control, consistent with one or more exemplary embodiments of the present disclosure.

[0028] FIG. 6A illustrates results of a proliferation inhibition study of an exemplary anti-CD22 VHH antibody in Raji cells as CD22-overexpressed cells, consistent with one or more exemplary embodiments of the present disclosure.

[0029] FIG. 6B illustrates results of a proliferation inhibition study of an exemplary anti-CD22 VHH antibody in Jurkat cells as a negative control, consistent with one or more exemplary embodiments of the present disclosure.

DESCRIPTION OF EMBODIMENTS

[0030] In the following detailed description, numerous specific details are set forth by way of examples in order to provide a thorough understanding of the relevant teachings. However, it should be apparent that the present teachings may be practiced without such details. In other instances, well-known methods, procedures, components, and/or circuitry have been described at a relatively high-level, without detail, in order to avoid unnecessarily obscuring aspects of the present teachings.

[0031] Single-domain antibodies (sdAbs) are antibody fragments of a single monomeric variable antibody domain. The sdAbs have a small size and low molecular weight and may selectively bind to a specific antigen as same as a whole antibody. The low molecular weight of sdAbs leads to their better permeability to tissues and tumors and leads to a short plasma half-life since they may be eliminated renally. Unlike whole antibodies, the sdAbs have low immunogenicity because they do not trigger the complement system.

[0032] A heavy-chain variable domain (VHH) antibody is a kind of sdAbs which corresponds to the variable domain of a heavy chain of the antibody. The VHH antibodies may have a very small size which enables their binding to hidden epitopes which are not accessible to whole antibodies. The VHH antibodies may be more stable and robust than a whole antibody and may be easily expressed in bacterial cells without any need for a supramolecular assembly in contrast to a full antibody made of 4 chains.

[0033] In the present disclosure, an exemplary anti-CD22 VHH antibody is produced against human CD22 antigen for detecting the overexpression of CD22 antigen on the cells and for treating B-cells malignancies by internalizing desired therapeutic agents into the malignant B-cells. The CD22 marker is a diagnostic and therapeutic biomarker because CD22 marker modulates function, survival, and apoptosis of B-cells and has an important role in the regulation of humoral immunity and the growth of malignant B-cells.

[0034] Disclosed herein is the exemplary anti-CD22 VHH antibody with an amino acid sequence as set forth in at least one of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, and combinations thereof. In some exemplary embodiments, the exemplary anti-CD22 VHH antibody may have a molecular weight between about 15 kilodaltons (kDa) and about 17 kDa.

[0035] In some exemplary embodiments, a complex of the exemplary anti-CD22 VHH antibody and a CD22 antigen may have a dissociation constant (K_d) between about 10^{-7} M and about 10^{-8} M. In some exemplary embodiments, the exemplary anti-CD22 VHH antibody may be very stable, resistant to extreme pH levels, temperatures, and different proteases. In some exemplary embodiments, the exemplary anti-CD22 VHH antibody may be produced by immunizing the Camelidae species including at least one of a camel, a dromedary, a llama, an alpaca, a guanaco, and combinations thereof. In some exemplary embodiments, the exemplary anti-CD22 VHH antibody may be a Camelidae VHH antibody. In one or more exemplary embodiments, the exemplary anti-CD22 VHH antibody may be a humanized Camelidae VHH antibody.

[0036] The present disclosure further discloses an exemplary formulation for treating a B-cell malignancy. In some exemplary embodiments, the exemplary formulation may include the anti-CD22 VHH antibody conjugated to at least one of a therapeutic agent or diagnostic agent. In some exemplary embodiments, the B-cell malignancy may include at least one of non-Hodgkin's lymphoma (NHL), hairy cell leukemia (HCL), Burkitt's lymphoma, acute lymphoblastic leukemia (ALL), and combinations thereof. In some exemplary embodiments, treating the B-cell malignancy may include reducing the number of CD22 antigens on the surface of B-cells by internalizing the CD22 antigens into the B-cells.

[0037] In some exemplary embodiments, the therapeutic agent may include at least one of a radionuclide, an immunomodulator, an anti-angiogenic agent, a pro-apoptotic agent, a cytokine, a chemokine, a drug, a toxin, a hormone, a siRNA, an enzyme, and combinations

thereof. In some exemplary embodiments, the toxin may include at least one of ricin, abrin, alpha toxin, saporin, ribonuclease (RNase), onconase, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, pseudomonas exotoxin, Pseudomonas endotoxin, and combinations thereof.

[0038] In some exemplary embodiments, the drug may include at least one of 5-fluorouracil, aplidin, azaribine, anastrozole, anthracyclines, bendamustine, bleomycin, bortezomib, bryostatin-1, busulfan, calicheamycin, camptothecin, carboplatin, 10-hydroxycamptothecin, carmustine, celecoxib, chlorambucil, cisplatinum, Cox-2 inhibitors, irinotecan, carboplatin, cladribine, camptothecans, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunorubicin, doxorubicin, 2-pyrrolinodoxorubicine (2P-DOX), pro-2-pyrrolinodoxorubicine (pro-2P-DOX), cyano-morpholino doxorubicin, doxorubicin glucuronide, epirubicin glucuronide, estramustine, epipodophyllotoxin, estrogen receptor binding agents, etoposide (VP16), etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3′,5′-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, farnesyl-protein transferase inhibitors, gemcitabine, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, lenolidamide, leucovorin, lomustine, mechlorethamine, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, navelbine, nitrosourea, plicomycin, procarbazine, paclitaxel, pentostatin, raloxifene, semustine, streptozocin, tamoxifen, paclitaxel, temazolomide, transplatinum, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinorelbine, vinblastine, vincristine, a vinca alkaloid, a tyrophostin, canertinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, leflunomide, nilotinib, pazopanib, semaxinib, sorafenib, sunitinib, sutent, vatalanib, ibrutinib, and combinations thereof.

[0039] In some exemplary embodiments, the immunomodulator may include at least one of a cytokine, a stem cell growth factor, a lymphotoxin, a hematopoietic factor, a colony stimulating factor (CSF), an interleukin (IL), erythropoietin, thrombopoietin, tumor necrosis factor (TNF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon-α, interferon-β, interferon-γ, interferon-λ, transforming growth factor-alpha (TGF-α), transforming growth factor-beta (TGF-β), interleukin-1 (IL-1), interleukin-1α (IL-1α), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11), interleukin-12 (IL-12), interleukin-13

(IL-13), interleukin-14 (IL-14), interleukin-15 (IL-15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-18 (IL-18), interleukin-21 (IL-21), interleukin-23 (IL-23), interleukin-25 (IL-25), Leukemia inhibitory factor (LIF), Fms-like tyrosine kinase 3 (FLT-3), angiostatin, thrombospondin, endostatin, lymphotoxin, and combinations thereof.

[0040] In some exemplary embodiments, the diagnostic agent may include at least one of a radionuclide, a contrast agent, a fluorescent agent, a chemiluminescent agent, a bioluminescent agent, a paramagnetic ion, an enzyme, a photoactive diagnostic agent, and combinations thereof. In some exemplary embodiments, the paramagnetic ion may include at least one of chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III), erbium (III), and combinations thereof.

[0041] In some exemplary embodiments, the radionuclide may include at least one of ^{110}In, ^{111}In, ^{177}Lu, ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y, ^{89}Zr, ^{94}mTc, ^{94}Tc, ^{99}Tc, ^{120}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{154}Gd, ^{155}Gd, ^{156}Gd, ^{157}Gd, ^{158}Gd, ^{32}F, ^{11}C, ^{13}N, ^{15}O, ^{186}Re, ^{188}Re, ^{51}Mn, ^{52}Mn, ^{55}Co, ^{72}As, ^{75}Br, ^{76}Br, ^{82}Rb, ^{83}Sr, ^{99}Mo, ^{105}Rh, ^{149}Pm, ^{169}Er, ^{194}Ir, ^{58}Co, ^{80}Br, ^{99}Tc, ^{103}Rh, ^{109}Pt, ^{119}Sb, ^{125}I, ^{189}mOs, ^{192}Ir, ^{219}Rn, ^{215}Po, ^{221}Fr, ^{255}Fm, ^{11}C, ^{13}N, ^{15}O, ^{75}Br, ^{198}Au, ^{199}Au, ^{224}Ac, ^{77}Br, ^{113}mIn, ^{95}Ru, ^{97}Ru, ^{103}Ru, ^{105}Ru, ^{107}Hg, ^{203}Hg, ^{121}Te, ^{122}Te, ^{227}Th, ^{125}Te, ^{165}Tm, ^{167}Tm, ^{168}Tm, ^{197}Pt, ^{109}Pd, ^{142}Pr, ^{143}Pr, ^{161}Tb, ^{57}Co, ^{58}Co, ^{51}Cr, ^{59}Fe, ^{75}Se, ^{201}Tl, ^{76}Br, ^{169}Yb, and combinations thereof.

[0042] In some exemplary embodiments, the fluorescent agent may include at least one of fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, fluorescamine, and combinations thereof. In some exemplary embodiments, the chemiluminescent agent may include at least one of luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt, an oxalate ester, and combinations thereof. In some exemplary embodiments, the bioluminescent agent comprises at least one of luciferin, luciferase, aequorin, and combinations thereof.

[0043] In some exemplary embodiments, the exemplary anti-CD22 VHH antibody may be identified as described in **FIG. 1**. **FIG. 1** shows method **100** for identifying the exemplary anti-CD22 VHH antibody, consistent with one or more exemplary of the present disclosure. Method **100** may include immunizing a camel by injecting CD22-overexpressed cells (**102**), isolating peripheral blood lymphocytes from the immunized camel (**104**), producing VHH-displaying

phages using a VHH library of the isolated peripheral blood lymphocytes (**106**), identifying a high-affinity anti-CD22 VHH antibody by enriching the VHH-displaying phages (**108**).

[0044] Step **102** may include immunizing the camel by injecting the CD22-overexpressed cells. In some exemplary embodiments, the camel may be immunized by injecting about 5×10^7 number of CD22-overexpressed cells. In some exemplary embodiments, the camel may be immunized by subcutaneous injection of the CD22-overexpressed cells for between about 4 and about 6 times.

[0045] In some exemplary embodiments, immunizing the camel may include between about 4 and about 6 booster immunizations with an interval of about one week. In some exemplary embodiments, the camel may include at least one of a camel, a dromedary, a llama, an alpaca, a guanaco, and combinations thereof.

[0046] Step **104** may include isolating peripheral blood lymphocytes from the immunized camel. In some exemplary embodiments, isolating peripheral blood lymphocytes from the immunized camel may be done one week after that last booster immunization. In some exemplary embodiments, the peripheral blood lymphocytes may be isolated from a blood sample using a ficol solution. In some exemplary embodiments, the peripheral blood lymphocytes may be isolated from a blood sample with an amount of about 100 ml.

[0047] Step **106** may include producing VHH-displaying phages using a VHH library of the isolated peripheral blood lymphocytes. In some exemplary embodiments, constructing the library of VHH genes of the isolated peripheral blood lymphocytes may include synthesizing complementary DNA (cDNA) of the isolated peripheral blood lymphocytes, amplifying cDNA of the VHH and VH genes of the isolated peripheral blood lymphocytes, forming VHH vectors by ligating the amplified VHH cDNAs to a vector, constructing a VHH library by transforming host cells with the library vectors, and producing VHH-displaying phages by infecting the host cells of the VHH library with helper phages.

[0048] In some exemplary embodiments, cDNA of the VHH and VH genes of the isolated peripheral blood lymphocytes may be amplified using a leader primer with a nucleic acid sequence as set forth in SEQ ID NO. 9 and a CH2-specific primer with a nucleic acid sequence as set forth in SEQ ID NO. 10 by polymerase chain reaction (PCR). In some exemplary embodiments, the VHH cDNAs may have a size of about 600 base pair (bp). In some exemplary embodiments, VHH vectors may be formed by ligating the amplified VHH cDNAs with a vector.

[0049] In some exemplary embodiments, constructing the VHH library by transforming host cells with the library vectors may include transforming host cells with the library vectors using an electroporation method, calcium chloride ($CaCl_2$) transformation method, and combinations thereof. In some exemplary embodiments, the VHH library may include about 4×10^7 transformed host cells containing the VHH vector. In some exemplary embodiments, the host cells may include bacterial cells such as Escherichia coli (E.coli) cells or its derivatives. In some exemplary embodiments, after producing the VHH-displaying phages by infecting the host cells of the VHH library with helper phages, the VHH-displaying phages may present different VHHs on their surfaces.

[0050] Step **108** may include identifying the high-affinity anti-CD22 VHH antibody by enriching the VHH-displaying phages. In some exemplary embodiments, enriching the VHH-displaying phages may include adding the VHH-displaying phages to the CD22 antigen and panning the VHH-displaying phages. In some exemplary embodiments, identifying a high-affinity anti-CD22 VHH antibody by enriching the VHH-displaying phages may include at least about 3 rounds of panning.

[0051] In some exemplary embodiments, in each round of panning, enriched phages may be used to infect the host cells for the next round of panning. In some exemplary embodiments, in the last round of panning, phages displaying the high affinity anti-CD22 VHH antibody may be identified using the ELISA technique. In some exemplary embodiments, the identified anti-CD22 VHH antibody may be sequenced, produced in an expression host, and purified for further characterization.

EXAMPLES

[0052] **EXAMPLE 1: IDENTIFYING AND PRODUCING THE EXEMPLARY HUMAN ANTI-CD22 VHH ANTIBODY**

[0053] In this example, the exemplary human anti-CD22 VHH antibody was produced through the steps of immunizing a camel by injecting CD22-overexpressed cells, isolating peripheral blood lymphocytes from the immunized camel, producing VHH-displaying phages using a VHH library of the isolated peripheral blood lymphocytes, identifying a high-affinity anti-CD22 VHH antibody by enriching the VHH-displaying phages.

[0054] In the first step, a young male camel (Camelus dromedarius) was immunized by five subcutaneous injections of Raji cells at weekly intervals. The Raji cells with a number of about

5×10^7 were used for the first and following booster immunizations, and both the first and booster immunizations were done in absence of adjuvant. Prior to each immunization, a blood specimen was collected from the jugular vein of the camel and separated sera were used to evaluate the immunization process by enzyme-linked immunosorbent assay (ELISA) on a plate coated with a recombinant human CD22 antigen. Also, polyclonal rabbit anti-camel IgGs and an anti-Rabbit-IgG horseradish peroxidase (HRP)-conjugated mAb was used to detect the bound camel antibodies.

[0055] One week after the last booster immunization, a blood sample with an amount of about 100 mL of was collected from the immunized camel with anticoagulant, and peripheral blood lymphocytes of the blood sample were isolated using a ficol solution. Afterward, total RNA was extracted by TRIzol reagent and cDNA was synthesized using a reverse transcription kit and an oligo-dT primer. In the next step, cDNA of the variable-havy chains (VHs) and VHHs genes of the isolated peripheral blood lymphocytes were amplified in two PCRs, In the first PCR, a leader primer and a CH2-specific primer were used Parameters of a first PCR is shown in TABLE. 1.

[0056] TABLE. 1: Parameters of a first PCR program for amplifying VHH cDNAs

Cycles: 32 cycles from step 2 to 4			
Number	**Step**	**Time**	**Temperature**
1	Initial denaturation	10 min	95 °C
2	Denaturation	60 sec	95 °C
3	Annealing	60 sec	55 °C
4	Extension	60 sec	72 °C
5	Final extension	10 min	72 °C

[0057] After the first PCR, the 600-bp fragments corresponding to the amplified VHH cDNAs were purified from a gel and used for amplification in a second PCR with specific primers which had the restriction sites for PstI and NotI restriction enzymes. Parameters of the second PCR program were the same as the parameters of the first PCR.

[0058] The PCR products were digested using PstI and NotI enzymes and VHH vectors were produced by ligating amplified DNA strands of VHHs into the vector. After that, the VHH library was produced by transforming TG1 cells as competent E. coli cells with the VHH vectors using electroporation. The VHH library included about 4×10^7 transformed E. coli cells containing different VHHs. In order to produce VHH-displaying phages, the TG1 cells of the

VHH library were infected with helper phages and different VHH antibodies were presented on the surfaces of the phages.

[0059] In the next step, a high-affinity anti-CD22 VHH antibody was identified by enriching the VHH-displaying phages. Enriching the VHH-displaying phages was done through three sequential rounds of panning on ELISA microtiter plate coated with 5 μg of recombinant human CD22 antigen in 100 μl of phosphate-buffered saline (PBS). In each round of panning, in order to elute bound phages, 100 mM of triethylamine solution with a pH level of about 10.0 was used to detach the phages followed by instantly neutralizing by 1 M Tris–HCl solution with a pH level of about 8.0. After each round of panning, eluted phages were utilized to infect E. coli cells in an exponential phase of growth.

[0060] Furthermore, after three rounds of panning and polyclonal phage-ELISA, an effective enrichment was seen. Analysis of the 48 colonies from the third step of panning demonstrated that about 85% of the colonies carried a phagemid which had an insert of the desired 600 bp size for a VHH cDNA. In the last round of panning, about 48 single colonies were randomly selected to express soluble periplasmic VHH antibodies via inducing with 1 mM isopropyld-1-thiogalactopyranoside. Afterward, a solid phase periplasmic extract (PE) ELISA was performed to find a high-affinity anti-CD22 VHH antibody and the identified high-affinity anti-CD22 VHH antibody was sequenced.

[0061] In the last step, the identified high-affinity anti-CD22 VHH antibody was expressed in E. coli WK6 expression strain, and the recombinant high-affinity anti-CD22 VHH antibody that was secreted into the periplasmic space was purified by Ni-NTA affinity purification method. Also, the molecular weight of the purified anti-CD22 VHH antibody was studied by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

[0062] **FIG. 2A** illustrates an SDS-PAGE profile of the exemplary anti-CD22 VHH antibody with an amino acid sequence of SEQ ID NO. 8 under non-reducing condition, consistent with one or more exemplary of the present disclosure. Referring to **FIG 2A**, the exemplary anti-CD22 VHH antibody with an amino acid sequence of SEQ ID NO. 8 has a single protein band with a molecular weight of about 17 kDa.

[0063] **EXAMPLE 2: CHARACTERIZATION OF THE ANTI-CD22 VHH ANTIBODY**

[0064] In this example, the exemplary anti-CD22 VHH antibody was characterized by measuring its binding affinity and specificity toward the CD22 antigen. The binding affinity of the exemplary anti-CD22 VHH antibody to recombinant CD22 antigen was measured by a

competition enzyme-linked immunosorbent assay (ELISA). According to the results, the dissociation constant (K_D) of the exemplary anti-CD22 VHH antibodies were between about 10^{-7} M and about 10^{-8} M.

[0065] In order to evaluate the reactivity of the anti-CD22 VHH antibodies with CD22 antigen in a cell lysate, a western blotting was performed on the Raji cell lysate as CD22-overexpressed cells and Jurkat cell lysate as a negative control. **FIG. 2B** illustrates a western blot profile of the exemplary anti-CD22 VHH antibody under non-reducing condition, consistent with one or more exemplary embodiments of the present disclosure. Referring to **FIG. 2B**, western-blot profile showed that the exemplary anti-CD22 VHH antibodies were recognized by an anti-hemagglutinin (anti-HA) tag monoclonal antibody.

[0066] **FIG. 3A** illustrates an SDS-PAGE profile of Raji cell lysate as $CD22^+$ cells and Jurkat cell lysate as $CD22^-$ cells with the exemplary anti-CD22 VHH antibody, consistent with one or more exemplary embodiments of the present disclosure. Referring to **FIG. 3A**, several protein bands were stained.

[0067] **FIG. 3B** illustrates a western blot profile of Raji cell lysate as CD22-overexpressed cells and Jurkat cell lysate as the negative control with the exemplary anti-CD22 VHH antibody, consistent with one or more exemplary embodiments of the present disclosure. Referring to **FIG. 3B**, a single protein band with a molecular weight of about 135 kDa, which was equal to the human CD22 antigen, appeared after staining. This result indicates that the exemplary anti-CD22 VHH was able to recognize the linear form of the human CD22 antigen.

[0068] Furthermore, binding of the exemplary anti-CD22 VHH antibodies to the native CD22 antigen on the cell surface was studied. Raji cells as CD22-overexpressed cells and Jurkat cells as a negative control were detected by flow cytometry after incubation with the anti-CD22 VHH antibodies. **FIG. 4A** illustrates a graph of the binding study of anti-CD22 monoclonal antibody (Monoclonal Ab) **400**, exemplary anti-CD22 VHH **404,** and isotype control **406** by flow cytometry using Jurkat cells as the negative control, consistent with one or more exemplary embodiments of the present disclosure. In this study, an anti-CD22 monoclonal antibody (Monoclonal Ab) group **400** was used as a positive control and an isotype control **406** and non-stained Jurkat cells **402** were used as negative controls.

[0069] **FIG. 4B** illustrates a graph of the binding study of anti-CD22 monoclonal antibody (Monoclonal Ab) **408**, exemplary anti-CD22 VHH antibody **412**, and isotype control **414** by flow cytometry using Raji cells as the CD22-overexpressed cells, consistent with one or more

exemplary embodiments of the present disclosure. In this study, an anti-CD22 monoclonal antibody (Monoclonal Ab) group **408** was used as a positive control and an isotype control **414** and non-stained Raji cells **410** were used as negative controls.

[0070] Referring to **FIGs. 4A** and **4B,** it was revealed that in comparison with Jurkat cells as the negative control in **FIG. 4A**, binding of the exemplary anti-CD22 VHH antibody **412** to CD22 on Raji cell surface in **FIG. 4B** produced a strong positive signal of about 93% in flow cytometry, whereas no positive signal was detected in the negative controls. Therefore, the exemplary anti-CD22 VHH antibody **412** is bound to the CD22 antigens on the surface of Raji cells with a high affinity.

[0071] **EXAMPLE 3: INTERNALIZATION ASSAY OF THE ANTI-CD22 VHH ANTIBODY**

[0072] In this example, internalization assay of the exemplary anti-CD22 VHH antibody was done to show that anti-CD22 VHH antibodies were able to be internalized after binding to a CD22 antigen on the cell surface. At first, Raji cells with a number of about 10^6 were incubated with the exemplary anti-CD22 VHH antibody at a concentration between about 10^{-6} µg/cell and about 20^{-6} µg/cell for a time period of about 30 minutes at a temperature of about 37 °C. After that, the treated Raji cells were stained with an anti-mouse FITC conjugated antibody and internalization was evaluated by flow cytometry.

[0073] **FIG. 5A** shows the results of internalization assay of the exemplary anti-CD22 VHH antibody in Raji cells as CD22 positive cells, consistent with one or more exemplary embodiments of the present disclosure. **FIG. 5B** shows the results of internalization assay of the exemplary anti-CD22 VHH antibody in Jurkat cells as a negative control, consistent with one or more exemplary embodiments of the present disclosure.

[0074] Referring to **FIG. 5A**, signal intensity and percentage of FITC positive cells (fluorescent percentage) reduced after incubation of the Raji cells with the exemplary anti-CD22 VHH antibody for a time period of about 30 minutes at a temperature of about 37 °C in comparison with time 0. Therefore, it indicates that about 22% of internalization of the anti-CD22 VHH antibodies occurred after incubation of the Raji cells and Jurkat cells with the exemplary anti-CD22 VHH antibody for a time period of about 30 minutes at a temperature of about 37 °C. Referring to **FIG. 5B**, there was no binding signal neither in time 0 nor after 30 minutes of incubation of the Jurkat cells as the negative control with the exemplary anti-CD22 VHH antibody at a temperature of about 37 °C.

[0075] EXAMPLE 4: PROLIFERATION INHIBITION STUDY OF THE HUMAN ANTI-CD22 VHH ANTIBODIES

[0076] In this example, the effect of the exemplary anti-CD22 VHH antibody on the proliferation of Raji cells as CD22 positive cells was studied using carboxyfluorescein diacetate succinimidyl ester (CFSE) by flow cytometry. Also, Jurkat cells were used as a negative control. The CFSE is an intracellular fluorescent dye that is able to covalently bind to intracellular molecules and be retained within the cells.

[0077] During cellular division, the intracellular CFSE concentration is halved. Also, the cells were incubated with the anti-CD22 VHH antibody with a concentration of between about 5 µg/ml and about 10 µg/Ml for a time period of about 1 hour at a temperature of 4 °C and then stimulated using sheep anti-human B cell receptor (BCR) IgM antibody for a time period of about 24 hours at a temperature of about 37 °C with 5% CO_2.

[0078] **FIG. 6A** shows results of proliferation inhibition study of the exemplary anti-CD22 VHH antibody and anti-BCR IgM in Raji cells as the CD22 positive cells, consistent with one or more exemplary embodiments of the present disclosure. **FIG. 6B** shows results of proliferation inhibition study of the exemplary anti-CD22 VHH antibody in Jurkat cells as a negative control, consistent with one or more exemplary embodiments of the present disclosure.

[0079] Referring to **FIG. 6A**, there is a comparable shift in the proliferation histogram of Raji cells treated with the exemplary anti-CD22 VHH antibody **604** in comparison with a histogram of day 0 **606**. Therefore, it indicates that the exemplary anti-CD22 VHH antibody had an inhibitory effect on the proliferation of Raji cells.

[0080] Moreover, the median fluorescent intensities (MFI) of Raji cells treated with the exemplary anti-CD22 VHH antibody followed by stimulation with anti-human BCR IgM antibody **608** and Raji cells treated only with anti-CD22 VHH antibody **604** were 219 in day 0 and did not decrease significantly after 24 hours in comparison with a graph **600** of the Raji cells treated only with anti-human IgM antibody as the control group.

[0081] Furthermore, the anti-CD22 VHH antibodies inhibited between about 60% and 70% of the cell proliferation of the Raji cells treated with the exemplary anti-CD22 VHH antibody and the anti-human BCR IgM antibody **608** in comparison with the Raji cells treated only with the anti-human BCR IgM antibody **600**. The percent of divided cells had a remarkable decrease ($P<0.05$) in the group which was treated with only the exemplary anti-CD22 VHH antibody

604 in comparison with the group treated with only Anti-BCR IgM antibody **600**. Referring to **FIG. 6B**, no change was observed in the proliferation of Jurkat cells as the negative control.

INDUSTRIAL APPLICABILITY

[0082] Applicants have found that the exemplary anti-CD22 VHH antibody of the present disclosure is particularly suited for industrial applications. By way of example, industrial applications may include pharmaceutical industry. The exemplary anti-CD22 VHH may be conjugated to a therapeutic agent for treating a B-cell malignancy by targeting over-expressed CD22 antigens on a surface of B-cells, such as malignant cells in non-Hodgkin's lymphoma (NHL), hairy cell leukemia (HCL), Burkitt's lymphoma, acute lymphoblastic leukemia (ALL), and B-cell autoimmune disorders. The exemplary anti-CD22 VHH may reduce the cost and provide an efficient and simple method for treating a B-cell malignancy. Moreover, the exemplary anti-CD22 VHH may be conjugated to a diagnostic agent for diagnosis purpose of B-cell malignancies.

[0083] While the foregoing has described what are considered to be the best mode and/or other examples, it is understood that various modifications may be made therein and that the subject matter disclosed herein may be implemented in various forms and examples, and that the teachings may be applied in numerous applications, only some of which have been described herein. It is intended by the following claims to claim any and all applications, modifications and variations that fall within the true scope of the present teachings.

[0084] Unless otherwise stated, all measurements, values, ratings, positions, magnitudes, sizes, and other specifications that are set forth in this specification, including in the claims that follow, are approximate, not exact. They are intended to have a reasonable range that is consistent with the functions to which they relate and with what is customary in the art to which they pertain.

[0085] The scope of protection is limited solely by the claims that now follow. That scope is intended and should be interpreted to be as broad as is consistent with the ordinary meaning of the language that is used in the claims when interpreted in light of this specification and the prosecution history that follows and to encompass all structural and functional equivalents. Notwithstanding, none of the claims are intended to embrace subject matter that fails to satisfy the requirement of Sections 101, 102, or 103 of the Patent Act, nor should they be interpreted in such a way. Any unintended embracement of such subject matter is hereby disclaimed.

[0086] Except as stated immediately above, nothing that has been stated or illustrated is intended or should be interpreted to cause a dedication of any component, step, feature, object, benefit, advantage, or equivalent to the public, regardless of whether it is or is not recited in the claims.

[0087] It will be understood that the terms and expressions used herein have the ordinary meaning as is accorded to such terms and expressions with respect to their corresponding respective areas of inquiry and study except where specific meanings have otherwise been set forth herein. Relational terms such as first and second and the like may be used solely to distinguish one entity or action from another without necessarily requiring or implying any actual such relationship or order between such entities or actions. The terms "comprises," "comprising," or any other variation thereof, are intended to cover a non-exclusive inclusion, such that a process, method, article, or apparatus that comprises a list of elements does not include only those elements but may include other elements not expressly listed or inherent to such process, method, article, or apparatus. An element proceeded by "a" or "an" does not, without further constraints, preclude the existence of additional identical elements in the process, method, article, or apparatus that comprises the element.

[0088] The Abstract of the Disclosure is provided to allow the reader to quickly ascertain the nature of the technical disclosure. It is submitted with the understanding that it will not be used to interpret or limit the scope or meaning of the claims. In addition, in the foregoing Detailed Description, it can be seen that various features are grouped together in various implementations. This is for purposes of streamlining the disclosure and is not to be interpreted as reflecting an intention that the claimed implementations require more features than are expressly recited in each claim. Rather, as the following claims reflect, inventive subject matter lies in less than all features of a single disclosed implementation. Thus, the following claims are hereby incorporated into the Detailed Description, with each claim standing on its own as a separately claimed subject matter.

[0089] While various implementations have been described, the description is intended to be exemplary, rather than limiting and it will be apparent to those of ordinary skill in the art that many more implementations and implementations are possible that are within the scope of the implementations. Although many possible combinations of features are shown in the accompanying figures and discussed in this detailed description, many other combinations of the disclosed features are possible. Any feature of any implementation may be used in

combination with or substituted for any other feature or element in any other implementation unless specifically restricted. Therefore, it will be understood that any of the features shown and/or discussed in the present disclosure may be implemented together in any suitable combination. Accordingly, the implementations are not to be restricted except in the light of the attached claims and their equivalents. Also, various modifications and changes may be made within the scope of the attached claims.

WHAT IS CLAIMED IS:

1. An anti-cluster of differentiation-22 heavy-chain variable domain antibody (anti-CD22 VHH antibody) comprising an amino acid sequence as set forth in at least one of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, and combinations thereof.

2. The anti-CD22 VHH antibody of claim 1, wherein a complex of the anti-CD22 VHH antibody and a CD22 antigen has a dissociation constant (K_D) between 10^{-7} M and 10^{-8} M.

3. The anti-CD22 VHH antibody of claim 1, wherein the anti-CD22 VHH antibody has a molecular weight between 15 kilodaltons (kDa) and 17 kDa.

4. The anti-CD22 VHH antibody of claim 1, wherein the anti-CD22 VHH antibody comprises a Camelidae VHH antibody.

5. A formulation, the formulation comprising:

an anti-CD22 VHH antibody comprising an amino acid sequence as set forth in at least one of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, and combinations thereof; and

at least one of a therapeutic agent and a diagnostic agent conjugated to the anti-CD22 VHH antibody.

6. The formulation of claim 5, wherein a complex of the anti-CD22 VHH antibody and a CD22 antigen has a dissociation constant (K_D) between 10^{-7} M and 10^{-8} M.

7. The formulation of claim 5, wherein the anti-CD22 VHH antibody comprises a Camelidae VHH antibody and has a molecular weight between 15 kilodaltons (kDa) and 17 kDa.

8. The formulation of claim 5, wherein the B-cell malignancy comprises at least one of non-Hodgkin's lymphoma (NHL), hairy cell leukemia (HCL), Burkitt's lymphoma, acute lymphoblastic leukemia (ALL), and combinations thereof.

9. The formulation of claim 5, wherein the therapeutic agent comprises at least one of a radionuclide, an immunomodulator, an anti-angiogenic agent, a pro-apoptotic agent, a cytokine, a chemokine, a drug, a toxin, a hormone, a small interfering (siRNA), an enzyme, and combinations thereof.

10. The formulation of claim 9, wherein the drug comprises at least one of 5-fluorouracil, aplidin, azaribine, anastrozole, anthracyclines, bendamustine, bleomycin, bortezomib, bryostatin-1, busulfan, calicheamycin, camptothecin, carboplatin, 10-hydroxycamptothecin, carmustine, celecoxib, chlorambucil, cisplatinum, Cox-2 inhibitors, irinotecan, carboplatin, cladribine, camptothecans, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunorubicin, doxorubicin, 2-pyrrolinodoxorubicine (2P-DOX), pro-2-pyrrolinodoxorubicine (pro-2P-DOX), cyano-morpholino doxorubicin, doxorubicin glucuronide, epirubicin glucuronide, estramustine, epipodophyllotoxin, estrogen receptor binding agents, etoposide (VP16), etoposide glucuronide, etoposide phosphate, floxuridine (FUdR), 3′,5′-O-dioleoyl-FudR (FUdR-dO), fludarabine, flutamide, farnesyl-protein transferase inhibitors, gemcitabine, hydroxyurea,

idarubicin, ifosfamide, L-asparaginase, lenolidamide, leucovorin, lomustine, mechlorethamine, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, navelbine, nitrosourea, plicomycin, procarbazine, paclitaxel, pentostatin, raloxifene, semustine, streptozocin, tamoxifen, paclitaxel, temazolomide, transplatinum, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, vinorelbine, vinblastine, vincristine, a vinca alkaloid, a tyrophostin, canertinib, dasatinib, erlotinib, gefitinib, imatinib, lapatinib, leflunomide, nilotinib, pazopanib, semaxinib, sorafenib, sunitinib, sutent, vatalanib, ibrutinib, and combinations thereof.

11. The formulation of claim 9, wherein the toxin comprises at least one of ricin, abrin, alpha toxin, saporin, ribonuclease (RNase), onconase, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, Pseudomonas endotoxin, and combinations thereof.

12. The formulation of claim 9, wherein the immunomodulator comprises at least one of a cytokine, a stem cell growth factor, a lymphotoxin, a hematopoietic factor, a colony stimulating factor (CSF), an interleukin (IL), erythropoietin, thrombopoietin, tumor necrosis factor (TNF), granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), interferon-α, interferon-β, interferon-γ, interferon-λ, transforming growth factor-alpha (TGF-α), transforming growth factor-beta (TGF-β), interleukin-1 (IL-1), interleukin-1α (IL-1α), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-7 (IL-7), interleukin-8 (IL-8), interleukin-9 (IL-9), interleukin-10 (IL-10), interleukin-11 (IL-11), interleukin-12 (IL-12), interleukin-13 (IL-13), interleukin-14 (IL-14), interleukin-15 (IL-

15), interleukin-16 (IL-16), interleukin-17 (IL-17), interleukin-18 (IL-18), interleukin-21 (IL-21), interleukin-23 (IL-23), interleukin-25 (IL-25), Leukemia inhibitory factor (LIF), Fms-like tyrosine kinase 3 (FLT-3), angiostatin, thrombospondin, endostatin, lymphotoxin, and combinations thereof.

13. The formulation of claim 9, wherein the radionuclide comprises at least one of ^{110}In, ^{111}In, ^{177}Lu, ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y,^{89}Zr, ^{94}mTc, ^{94}Tc, ^{99}Tc, ^{120}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{154}Gd, ^{155}Gd, ^{156}Gd, ^{157}Gd, ^{158}Gd, ^{32}F, ^{11}C, ^{13}N, ^{15}O,^{186}Re, ^{188}Re, ^{51}Mn, ^{52}Mn, ^{55}Co, ^{72}As, ^{75}Br, ^{76}Br, ^{82}Rb, ^{83}Sr, ^{99}Mo, ^{105}Rh, ^{149}Pm, ^{169}Er, ^{194}Ir, ^{58}Co, ^{80}Br, ^{99}Tc, ^{103}Rh, ^{109}Pt, ^{119}Sb, ^{125}I, ^{189}mOs, ^{192}Ir, ^{219}Rn, ^{215}Po, ^{221}Fr, ^{255}Fm, ^{11}C, ^{13}N, ^{15}O, ^{75}Br, ^{198}Au, ^{199}Au, ^{224}Ac, ^{77}Br, ^{113}mIn, ^{95}Ru, ^{97}Ru, ^{103}Ru, ^{105}Ru, ^{107}Hg, ^{203}Hg, ^{121}Te, ^{122}Te, ^{227}Th, ^{125}Te, ^{165}Tm, ^{167}Tm, ^{168}Tm, ^{197}Pt, ^{109}Pd, ^{142}Pr, ^{143}Pr, ^{161}Tb, ^{57}Co, ^{58}Co, ^{51}Cr, ^{59}Fe, ^{75}Se, ^{201}Tl, ^{76}Br, ^{169}Yb, and combinations thereof.

14. The formulation of claim 5, wherein the diagnostic agent comprises at least one of a radionuclide, a contrast agent, a fluorescent agent, a chemiluminescent agent, a bioluminescent agent, a paramagnetic ion, an enzyme, a photoactive diagnostic agent, and combinations thereof.

15. The formulation of claim 14, wherein the radionuclide comprises at least one of ^{110}In, ^{111}In, ^{177}Lu, ^{18}F, ^{52}Fe, ^{62}Cu, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{86}Y, ^{90}Y,^{89}Zr, ^{94}mTc, ^{94}Tc, ^{99}Tc, ^{120}I, ^{123}I, ^{124}I, ^{125}I, ^{131}I, ^{154}Gd, ^{155}Gd, ^{156}Gd, ^{157}Gd, ^{158}Gd, ^{32}F, ^{11}C, ^{13}N, ^{15}O,^{186}Re, ^{188}Re, ^{51}Mn, ^{52}Mn, ^{55}Co, ^{72}As, ^{75}Br, ^{76}Br, ^{82}Rb, ^{83}Sr, ^{99}Mo, ^{105}Rh, ^{149}Pm, ^{169}Er, ^{194}Ir, ^{58}Co, ^{80}Br, ^{99}Tc, ^{103}Rh, ^{109}Pt, ^{119}Sb, ^{125}I, ^{189}mOs, ^{192}Ir, ^{219}Rn, ^{215}Po, ^{221}Fr, ^{255}Fm, ^{11}C, ^{13}N, ^{15}O, ^{75}Br, ^{198}Au, ^{199}Au, ^{224}Ac, ^{77}Br, ^{113}mIn, ^{95}Ru, ^{97}Ru, ^{103}Ru, ^{105}Ru, ^{107}Hg, ^{203}Hg, ^{121}Te, ^{122}Te, ^{227}Th, ^{1}

^{25}Te, ^{165}Tm, ^{167}Tm, ^{168}Tm, ^{197}Pt, ^{109}Pd, ^{142}Pr, ^{143}Pr, ^{161}Tb, ^{57}Co, ^{58}Co, ^{51}Cr, ^{59}Fe, ^{75}Se, ^{201}Tl, ^{76}Br, ^{169}Yb, and combinations thereof.

16. The formulation of claim 14, wherein the paramagnetic ion comprises at least one of chromium (III), manganese (II), iron (III), iron (II), cobalt (II), nickel (II), copper (II), neodymium (III), samarium (III), ytterbium (III), gadolinium (III), vanadium (II), terbium (III), dysprosium (III), holmium (III), erbium (III), and combinations thereof.

17. The formulation of claim 14, wherein the fluorescent agent comprises at least one of fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde, fluorescamine, and combinations thereof.

18. The formulation of claim 14, wherein the chemiluminescent agent comprises at least one of luminol, isoluminol, an aromatic acridinium ester, an imidazole, an acridinium salt, an oxalate ester, and combinations thereof

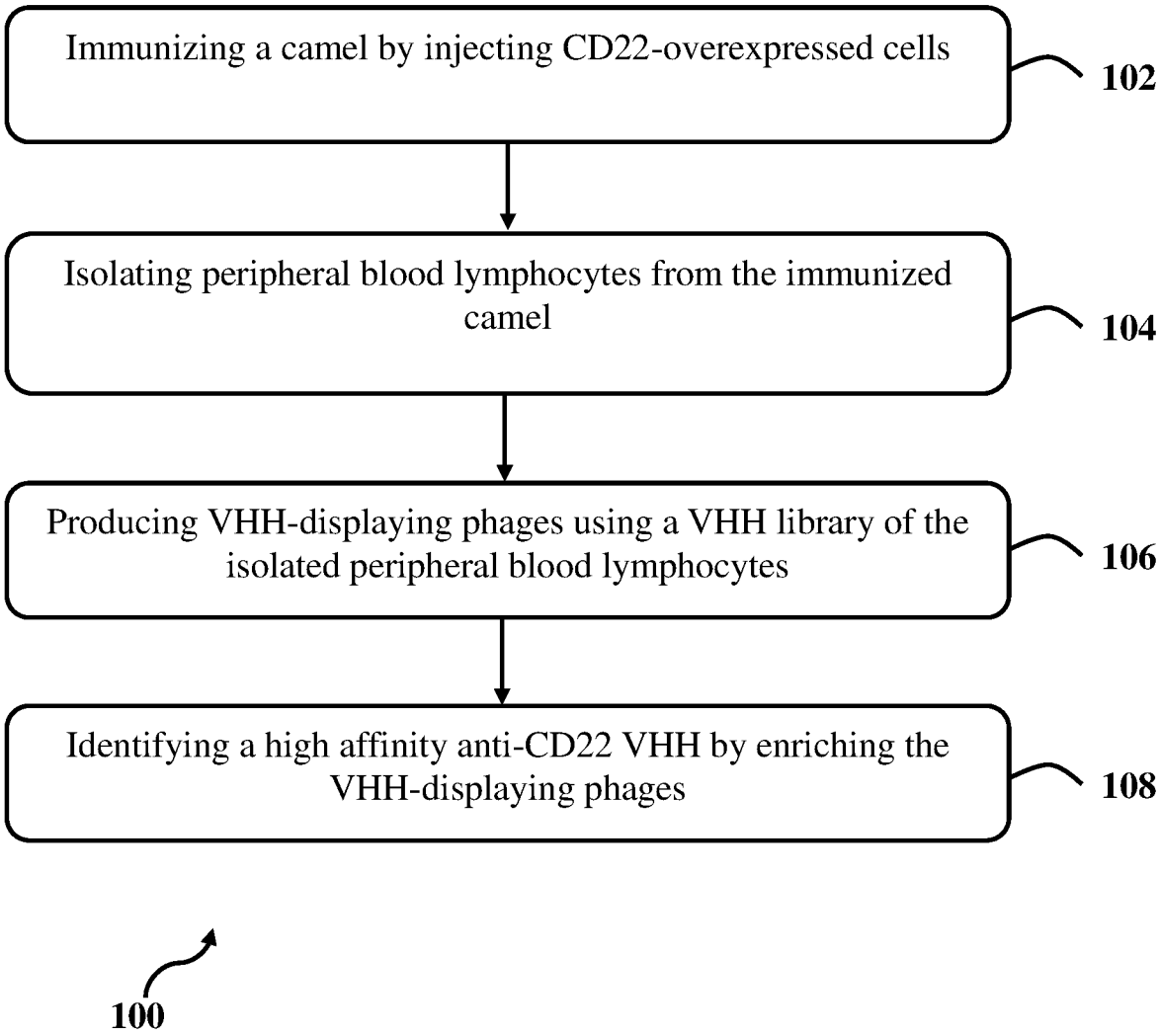
19. The formulation of claim 14, wherein the bioluminescent agent comprises at least one of luciferin, luciferase, aequorin, and combinations thereof.

20. A method for treating a B-cell malignancy, the method comprising:

administering a formulation comprising:

an anti-CD22 VHH antibody comprising an amino acid sequence as set forth in at least one of SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7, SEQ ID NO. 8, and combinations thereof; and

at least one of a therapeutic agent and a diagnostic agent conjugated to the anti-CD22 VHH antibody.


Immunizing a camel by injecting CD22-overexpressed cells
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Isolating peripheral blood lymphocytes from the immunized camel
104
Producing VHH-displaying phages using a VHH library of the isolated peripheral blood lymphocytes
106
Identifying a high affinity anti-CD22 VHH by enriching the VHH-displaying phages
108
100

FIG. 1

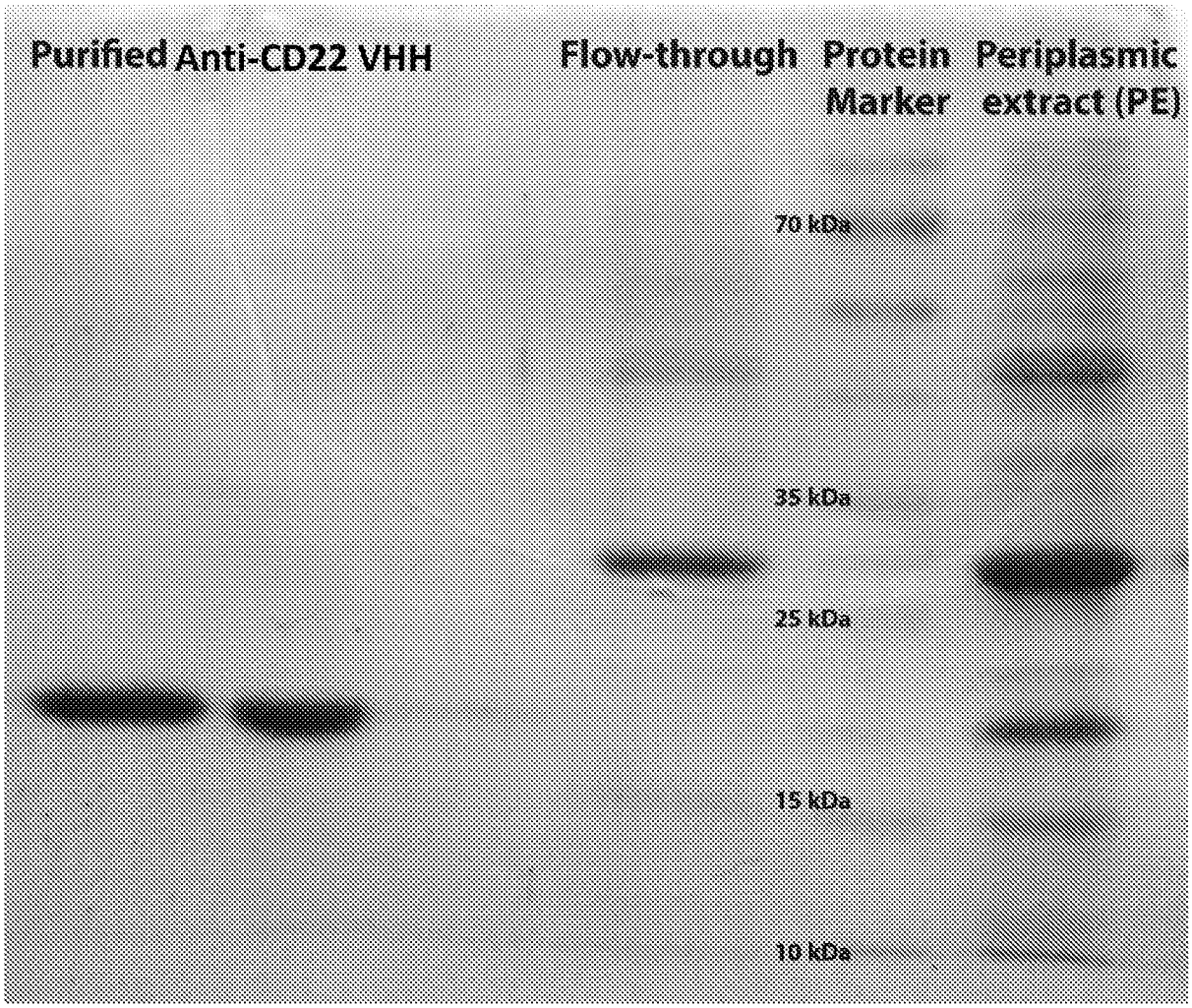
Purified Anti-CD22 VHH
Flow-through
Protein Marker
Periplasmic extract (PE)
70 kDa
35 kDa
25 kDa
15 kDa
10 kDa

FIG. 2A

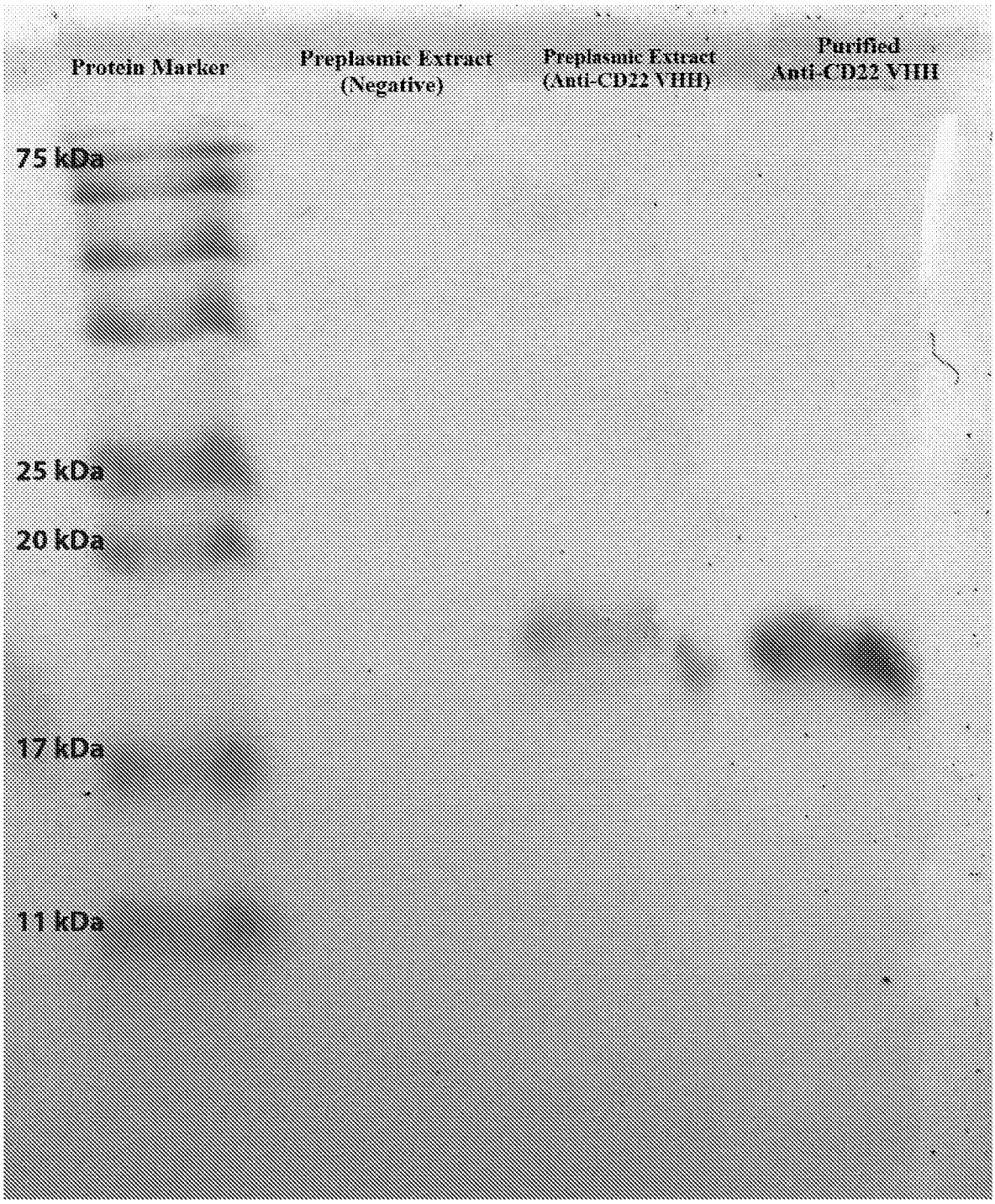
Protein Marker
Preplasmic Extract (Negative)
Preplasmic Extract (Anti-CD22 VHH)
Purified Anti-CD22 VHH
75 kDa
25 kDa
20 kDa
17 kDa
11 kDa

FIG. 2B

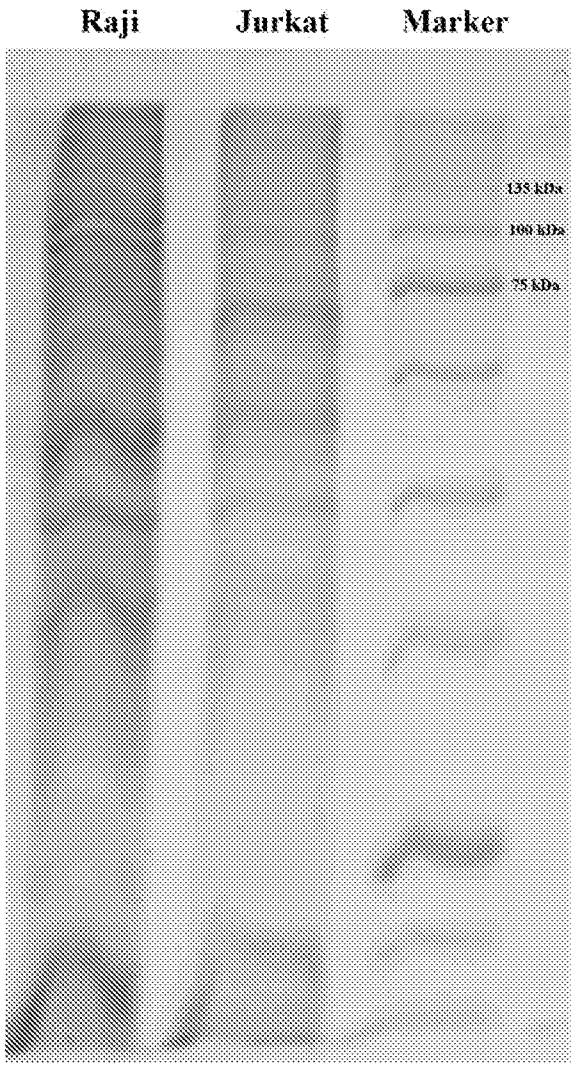

FIG. 3A

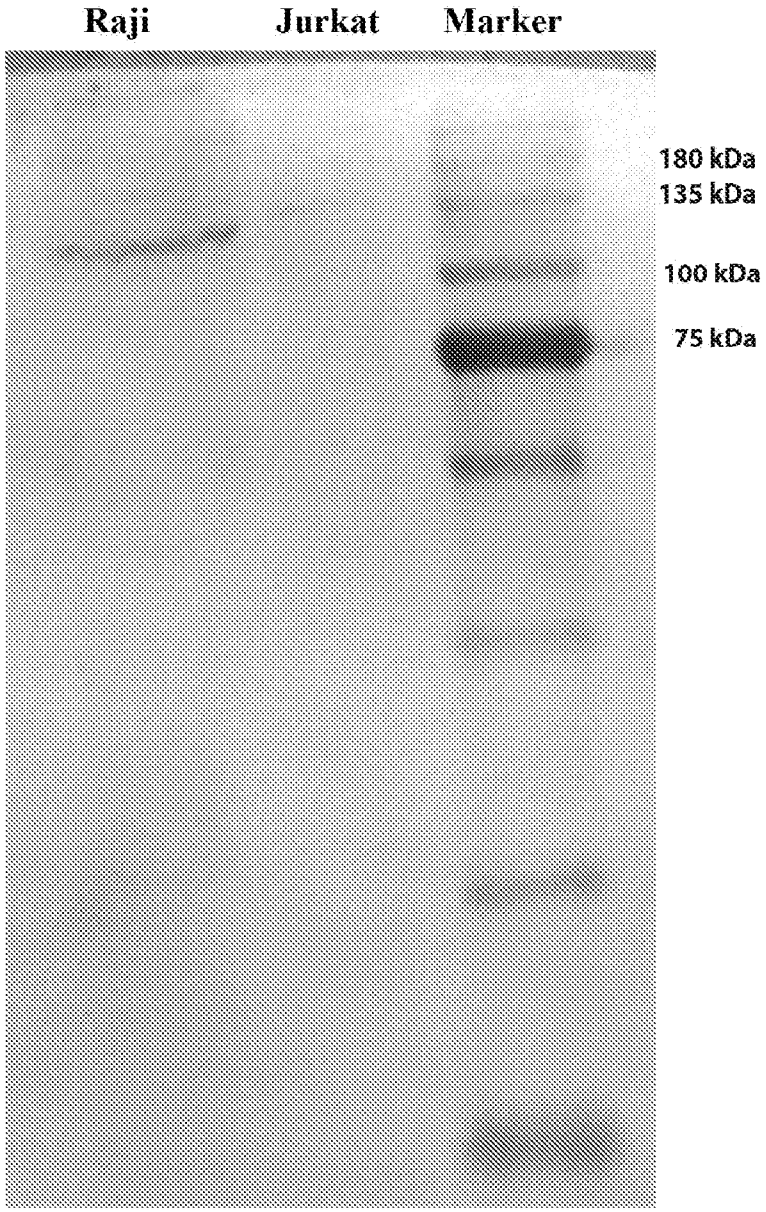

FIG. 3B

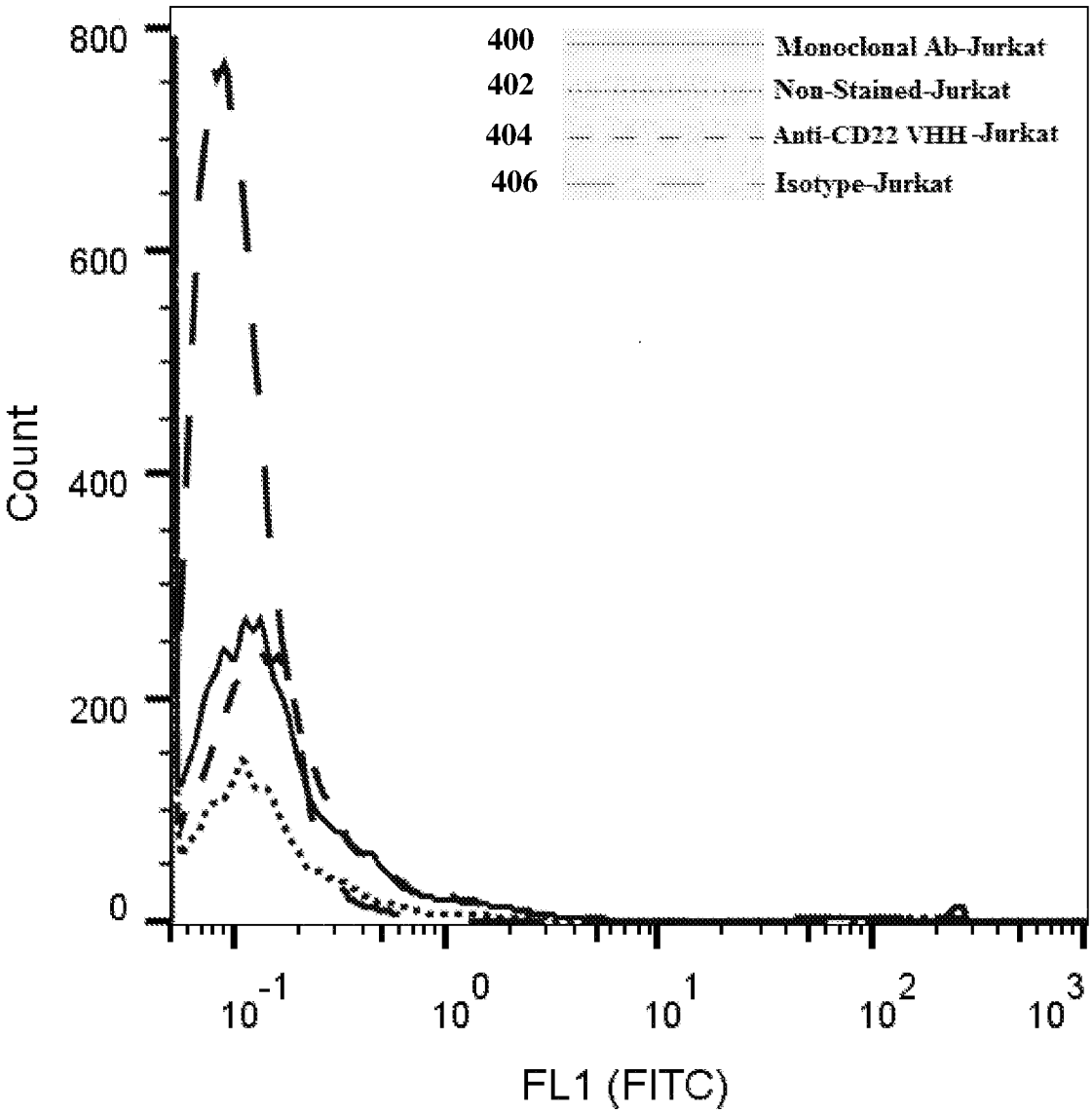
400 Monoclonal Ab-Jurkat
402 Non-Stained-Jurkat
404 Anti-CD22 VHH-Jurkat
406 Isotype-Jurkat
800
600
400
200
0
Count
10^-1
10^0
10^1
10^2
10^3
FL1 (FITC)

FIG. 4A

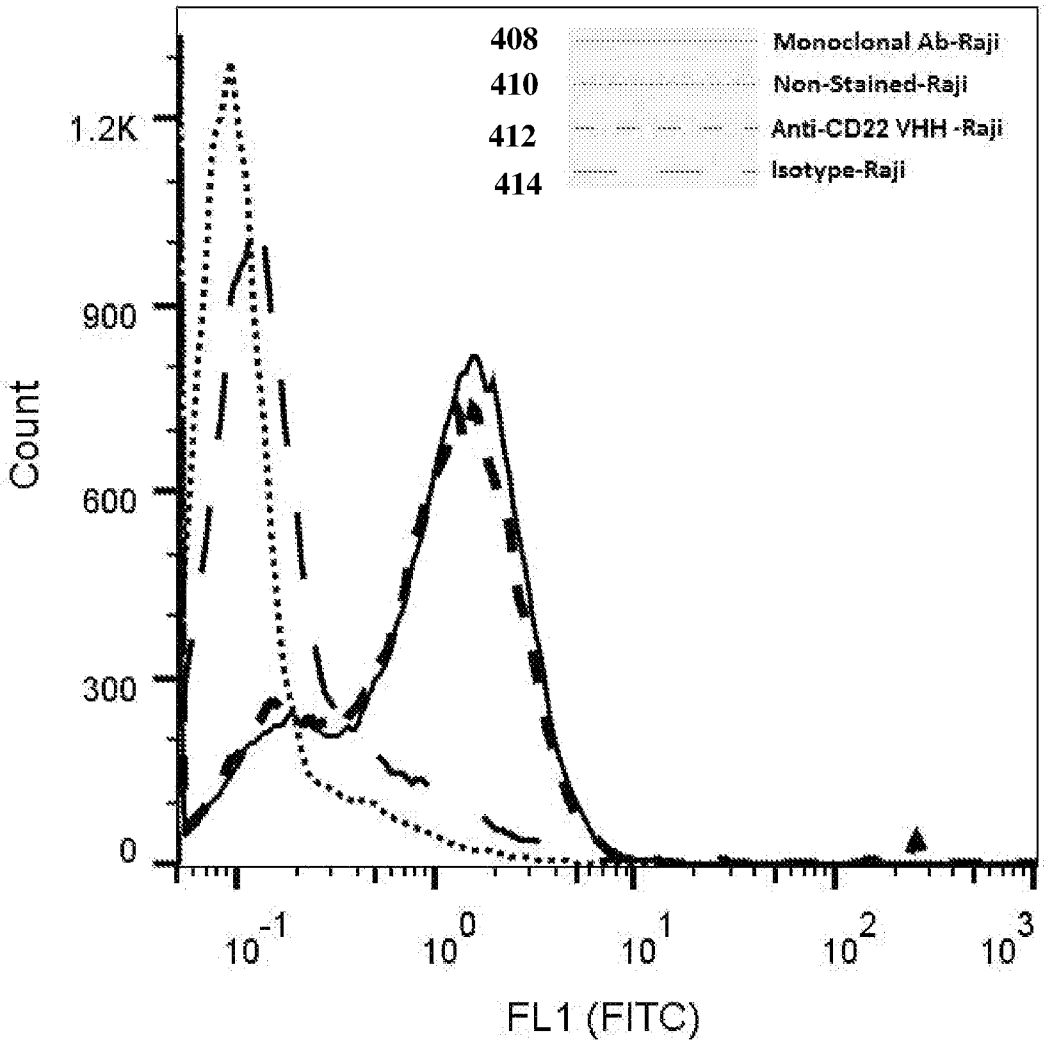
408
410
412
414
Monoclonal Ab-Raji
Non-Stained-Raji
Anti-CD22 VHH -Raji
Isotype-Raji
1.2K
900
600
300
0
Count
10^-1
10^0
10^1
10^2
10^3
FL1 (FITC)

FIG. 4B

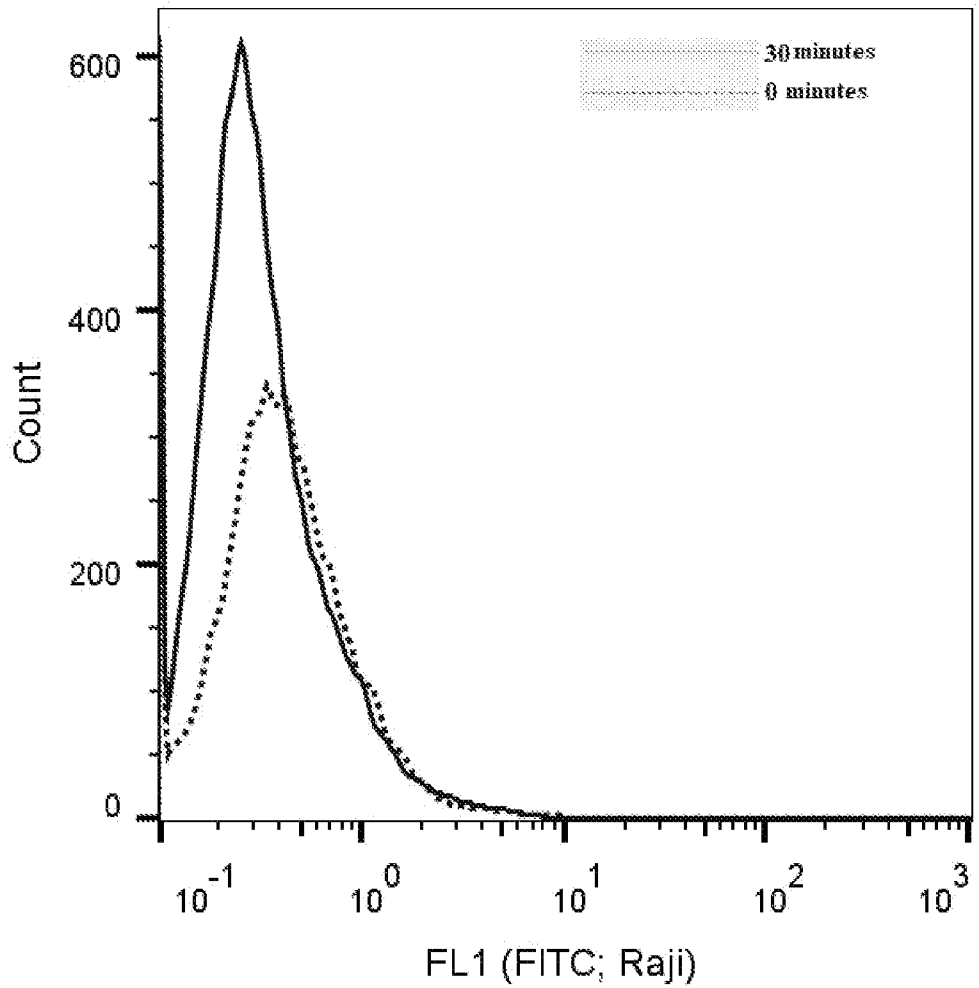
30 minutes
0 minutes
600
400
200
0
Count
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
FL1 (FITC; Raji)

FIG. 5A

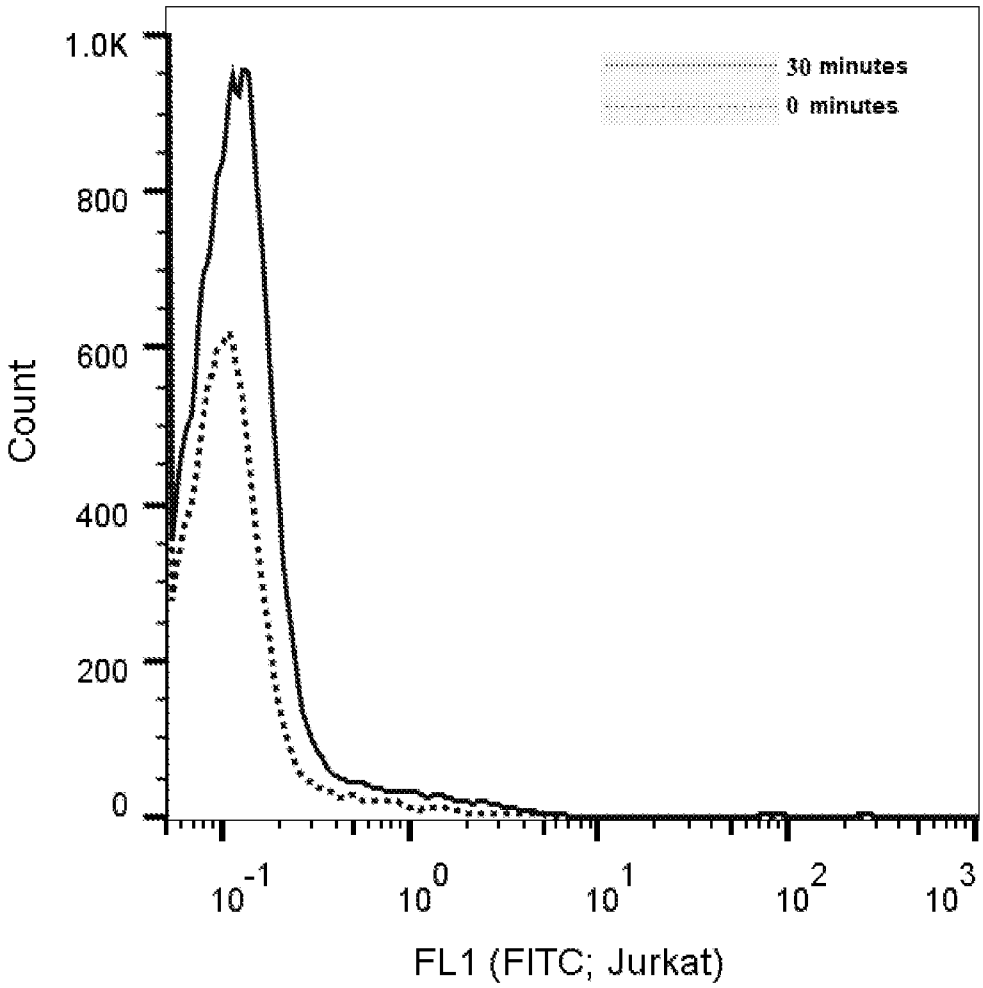
30 minutes
0 minutes
1.0K
800
600
400
200
0
Count
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
FL1 (FITC; Jurkat)

FIG. 5B

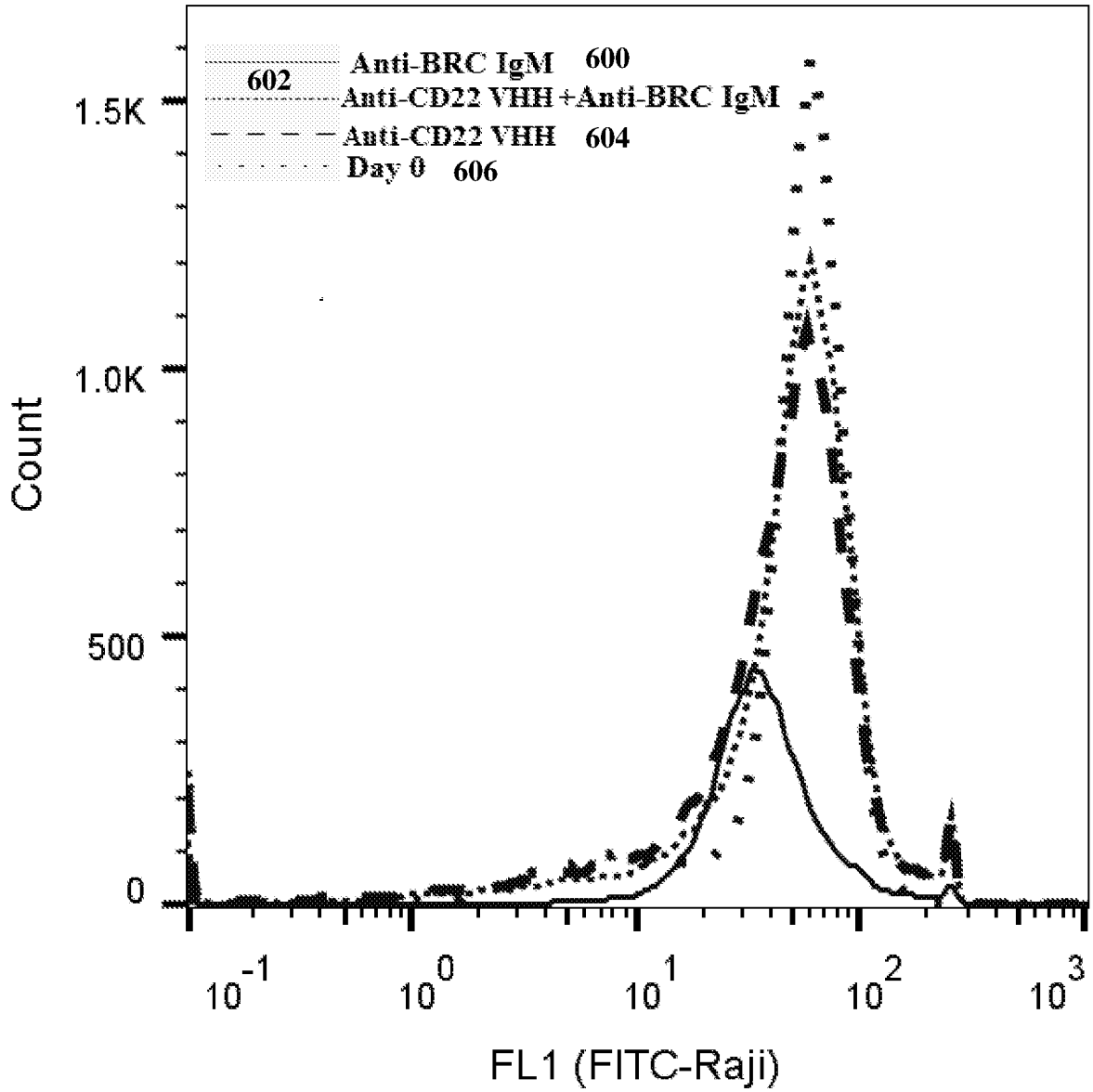
602
Anti-BRC IgM 600
Anti-CD22 VHH +Anti-BRC IgM
Anti-CD22 VHH 604
Day 0 606
1.5K
1.0K
500
0
Count
10-1
100
101
102
103
FL1 (FITC-Raji)

FIG. 6A

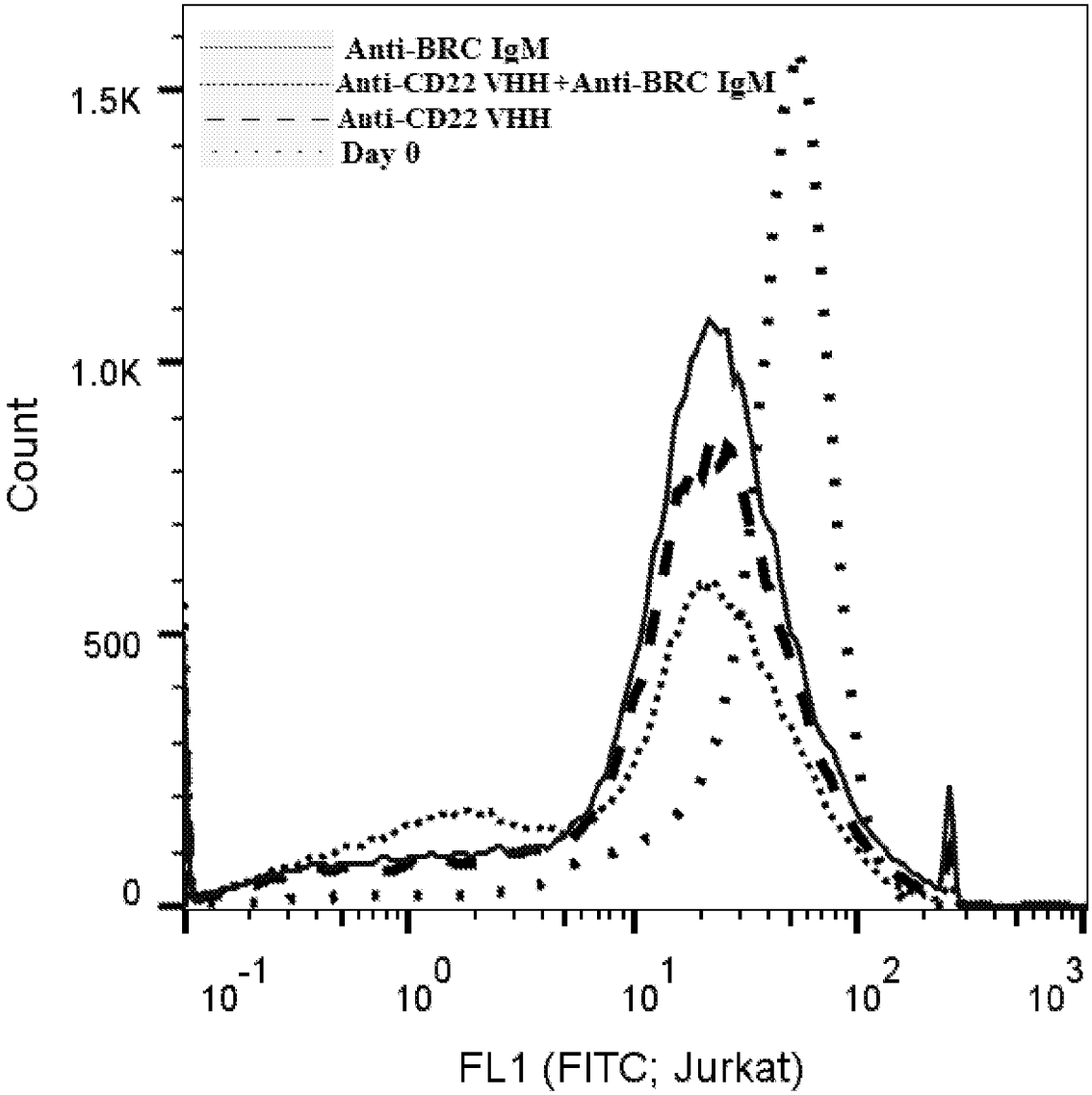
Anti-BRC IgM
Anti-CD22 VHH+Anti-BRC IgM
Anti-CD22 VHH
Day 0
1.5K
1.0K
500
0
Count
10^-1
10^0
10^1
10^2
10^3
FL1 (FITC; Jurkat)

FIG. 6B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2018/057092

A. CLASSIFICATION OF SUBJECT MATTER
C07K16/00,A61K39/395 Version=2018.01

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K, 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)

TotalPatent One, IPO Internal Database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US20120183472A1 IMMUNOMEDICS INC, 07-19-2012 (July 19, 2012) Abstract, claims 1-3	1-19
Y	US20050112060A1 IDEC PHARMA CORP 05-26-2005 (May 26, 2005) Abstract, claims 1-4	1-19

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14-12-2018	14-12-2018

Name and mailing address of the ISA/	Authorized officer
Indian Patent Office Plot No.32, Sector 14,Dwarka,New Delhi-110075 Facsimile No.	P Jyothish Kumar Telephone No. +91-1125300200

International application No.
PCT/IB2018/057092

Citation	Pub.Date	Family	Pub.Date
US 20120183472 A1	19-07-2012	AU 4829600 A	21-11-2000
		AU 774044 B2	17-06-2004
		CA 2373618 A1	16-11-2000
		EP 1178826 A1	13-02-2002
		JP 2002544173 A	24-12-2002
		US 2002071807 A1	13-06-2002
		US 6306393 B1	23-10-2001
		US 7837995 B2	23-11-2010
		US 7910103 B2	22-03-2011
		US 7939073 B2	10-05-2011
		US 8105596 B2	31-01-2012
		WO 0067795 A1	16-11-2000
US 20050112060 A1	26-05-2005	AU 2001268363 B2	17-08-2006
		AU 6836301 A	02-01-2002
		CA 2411102 A1	27-12-2001
		EP 1299128 A2	09-04-2003
		EP 2052742 A1	29-04-2009
		JP 2004512262 A	22-04-2004
		US 6846476 B2	25-01-2005
		WO 0197858 A2	27-12-2001

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2018/057092

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 20
because they relate to subject matter not required to be searched by this Authority, namely:
The subject matter of claim 20 relates to a method for treating a B-cell malignancy, which does not require an international search by the International Searching Authority in accordance with PCT Article 17 (2) (a) (i) and Rule 39.1 (iv).

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
The subject-matter claimed in claims 1-19 lacks unity of invention (a posteriori) as per Rule 13.2

The present invention contains following Groups of Inventions:

Group 1: Claims 1-19 (partially) relates to an anti-cluster of differentiation-22 heavy-chain variable domain antibody (anti-CD22 V

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Continuation of Observations where unity of invention is lacking(Box III)

H H antibody) comprising an amino acid SEQ ID No: 1, and its corresponding formulation.

Groups 2-8: Claims 1-19 (partially) relates to an anti-cluster of differentiation-22 heavy-chain variable domain antibody (anti-CD22 V H H antibody) comprising an amino acid SEQ ID No: 2, and its corresponding formulation (group 2), SEQ ID No: 3, and its corresponding formulation (group 3)…. SEQ ID No: 8, and its corresponding formulation (group 8).

The claims of the said application lack unity of invention (posteriori) as claim 1 mentions "comprising an amino acid sequence as set forth in at least one of SEQ ID No: 1, SEQ ID No 2, SEQ ID No: 3, SEQ ID No: 4, SEQ ID No: 5, SEQ ID No: 6, SEQ ID No: 7, SEQ ID No: 8, and combinations thereof". So, any one sequence may be selected that entails a whole library of heavy-chain variable domain antibodies.

The above said 8 separate groups of inventions listed above do not relate to a single general inventive concept because they lack special technical feature linking all the groups. An anti-CD22 V H H antibody for the same purpose is already known in the art (US20120183472A1 or US20050112060A1). In view of the prior art, the provision of such antibodies is a matter of routine procedures. Therefore, the feature "An anti-CD22 V H H antibody " cannot be considered as a special technical feature which could provide for a common unifying concept for different antibodies generated in the application. Therefore, claims 1-19 lacks unity of invention as per Rule 13.2.