



(51) International Patent Classification:
C07K 16/28 (2006.01) *A61K 39/395* (2006.01)
C07K 16/30 (2006.01)

(21) International Application Number:
PCT/US2010/032208

(22) International Filing Date:
23 April 2010 (23.04.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/172,099 23 April 2009 (23.04.2009) US

(71) Applicant (for all designated States except US): THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institutes of Health, 6011 Executive Boulevard, Suite 325, MSC 7660, Bethesda, Maryland 20892-7660 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): RADER, Christoph [US/US]; 17705 Prince Edward Drive, Olney, Maryland 20832 (US). BASKAR, Sivasubramanian [US/US]; 9910 Heather Glen, Elicott City, Maryland 21042 (US).

(74) Agents: COCHRAN, Christine M. et al.; LEYDIG, VOIT & MAYER, LTD., Two Prudential Plaze, Suite 4900, 180 N. Stetson Avenue, Chicago, Illinois 60601 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: ANTI-HUMAN ROR1 ANTIBODIES

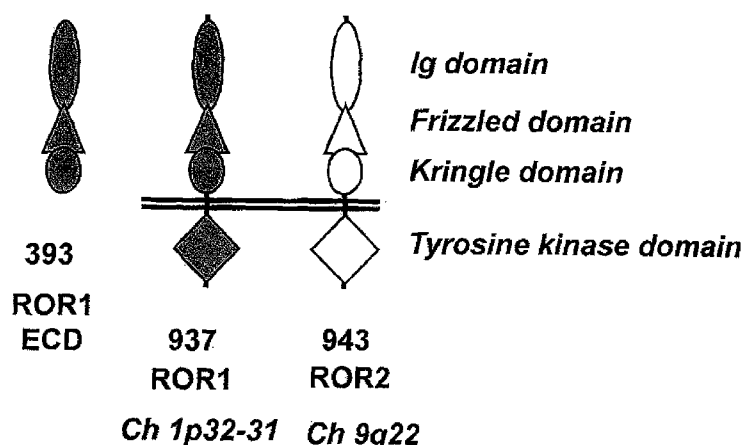


Figure 1

(57) Abstract: The invention relates to antibodies having specificity for human ROR1, compositions thereof, and methods for using such antibodies, including in the diagnosis and treatment of disorders associated with aberrant ROR1 expression.

WO 2010/124188 A1



Published:

— with international search report (Art. 21(3))

— with sequence listing part of description (Rule 5.2(a))

ANTI-HUMAN ROR1 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 61/172,099 filed April 23, 2009 which is incorporated by reference.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 8,967 Byte ASCII (Text) file named "706266ST25.TXT," created on March 26, 2010.

BACKGROUND OF THE INVENTION

[0003] Antibody therapies and diagnostics have been developed for use in treating a wide range of conditions including autoimmune diseases or disorders, infectious diseases, and cancers. Such therapies are useful but also can be associated with undesirable immunogenicity and can damage healthy cells and tissues.

[0004] B-cell chronic lymphocytic leukemia (B-CLL) and mantle cell lymphoma (MCL) are two incurable forms of B-cell lymphoma with a combined incidence of new cases that exceeds 18,000 patients per year in the United States alone. Antibody therapies that have been developed for B cell lymphomas, which include rituximab, a chimeric monoclonal antibody (mAb), and alemtuzumab, a humanized mAb. However, the target antigens for both of these drugs (CD20 and CD52, respectively) are expressed not only in malignant B cells but also in normal B cells, and CD52 is ubiquitously expressed on a variety of normal cells of the immune system. Therefore, immunosuppression can be a concern with these antibody therapies. Currently in the United States and Europe, there is no commercial therapeutic antibody that specifically recognizes an antigen present on malignant B cells, but not on normal B cells.

[0005] There is a desire for additional therapeutic and diagnostic antibodies having good efficacy and that exhibit minimal binding and/or damage to non-diseased cells.

BRIEF SUMMARY OF THE INVENTION

[0006] The invention provides an isolated antibody with specificity for the extracellular domain of receptor tyrosine kinase-like orphan receptor 1 (ROR 1), which is selectively expressed on the surface of malignant cells, including B-cell tumors and other cancers.

[0007] In particular, the invention provides an isolated antibody having specificity for human ROR1 and having (a) a heavy chain with at least 90% identity to a sequence selected from the group consisting of SEQ ID NO: 1, (b) a light chain with at least 90% identity to a sequence selected from the group consisting of SEQ ID NO: 2; or (c) both a heavy chain of (a) and a light chain of (b).

[0008] The invention also provides an isolated antibody having specificity for human ROR1 and having at least one CDR that includes a sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 11, SEQ ID NO: 13, and SEQ ID NO: 15. In other embodiments, the isolated antibody can include one or more variants of the foregoing CDRs which have 1, 2 or 3 amino acid substitutions, insertions, or deletions.

[0009] The invention further provides a pharmaceutical composition comprising an antibody of the invention and a pharmaceutically acceptable carrier.

[0010] In addition, the invention provides a method of treating a disease or condition associated with elevated expression of ROR1 (e.g., a B-cell lymphoma, renal cell carcinoma, colon cancer, or breast cancer) by administering a therapeutically effective amount of an isolated antibody of the invention or a pharmaceutical composition thereof to a subject in need thereof.

[0011] The antibodies and compositions of the invention can also be used in diagnostic methods to detect cells with altered levels of ROR1, e.g., in a sample or in a subject.

BRIEF DESCRIPTION OF THE DRAWING(S)

[0012] Figure 1 is a schematic that depicts the Ig-, Frizzled-, and Kringle-like domains of ROR1, receptor tyrosine kinase-like orphan receptor 2 (ROR2), and an ROR1-derived extracellular domain fragment (ROR1-ECD), as well as the transmembrane and intracellular tyrosine kinase domains of ROR1 and ROR2.

[0013] Figure 2A is a schematic that depicts ROR-ECD.

[0014] Figure 2B is a schematic that depicts ROR1-ECD fused to a human Fc domain (Fc-ROR1).

[0015] Figure 3 is a graph depicting the results of a BIACORE binding analysis using the indicated concentrations of mouse monoclonal antibody (mAb) 2A2 in terms of response (RU) versus time (seconds).

[0016] Figure 4 is a pair of histogram panels depicting the results of fluorescence activated cell sorting (FACS) analysis evaluating mAb 2A2 binding to peripheral blood mononuclear cells (PBMC) taken from B-CLL patients (first panel) and cultured JeKo-1 cells (second panel) in terms of the number of events versus fluorescence intensity for (A) 1 μ g/mL (~6.5 nM) mAb 2A2, (B) 0.1 μ g/mL (~650 pM) mAb 2A2, (C) 0.01 μ g/mL (~65 pM) mAb 2A2, and (D) 0.001 μ g/mL (~6.5 pM) mAb 2A2, with 1 μ g/mL polyclonal mouse IgG (solid black histogram) and goat anti-mouse IgG polyclonal antibody conjugated to fluorescein isothiocyanate (FITC) (broken line histogram) as controls.

[0017] Figure 5 is a list of the amino acid sequences corresponding to the mAb 2A2 variable region heavy chain (VH) (SEQ ID NO: 1), light chain (VL) (SEQ ID NO: 2), VH framework regions FR1-FR4 (SEQ ID NOs: 3, 5, 7, and 9), VH complementarity determining regions CDR1-3 (SEQ ID NOs: 4, 6, and 8), VL FR1-FR4 (SEQ ID NOs: 10, 12, 14, and 16), and VL CDR1-CDR3 (SEQ ID NOs: 11, 13, and 15) regions.

[0018] Figure 6 is a list of DNA coding sequences corresponding to the "original" VH (SEQ ID NO: 17) and VL (SEQ ID NO: 19) cDNAs isolated from mAb 2A2 and their respective VH (SEQ ID NO: 18) and VL (SEQ ID NO: 20) coding sequences optimized for expression in a mammalian system.

[0019] Figure 7A is a schematic that depicts mAb 2A2, including its constant and variable regions (no fill), and a chimeric antibody (ch2A2), that included human constant regions (dark fill) and mouse mAb 2A2 variable region (no fill).

[0020] Figure 7B is a graph that depicts the results of ELISA studies comparing mAb 2A2 and ch2A2 binding to ROR1 ECD (as well as binding to ROR2) as a function of antibody concentration.

[0021] Figure 8 is a pair of histogram panels depicting the results of FACS analysis evaluating mAb 2A2 (first panel) and ch2A2 (second panel) binding to JeKo-1 cells.

[0022] Figure 9 is a graph that depicts the results of a flow cytometry assay comparing dsFv 2A2-PE38 immunotoxin and mAb 2A2 with respect to the ability to bind to JeKo-1

cells in terms of fluorescence intensity of labeling antibody as a function of dsFv 2A2-PE38 and mAb 2A2 concentration.

[0023] Figure 10 is a graph that depicts the results of a cytotoxicity assay in terms of cytotoxicity % (of cells) as a function of dsFv 2A2-PE38 concentration.

[0024] Figure 11 is a dot plot depicting the results of apoptosis analysis in terms of annexin V and propidium iodide signal for 2 μ M dsFv 2A2-PE38 as applied to JeKo-1 cells.

[0025] Figure 12 is a schematic depiction of ELISA of mAb 2A2 binding to various hROR-1 extracellular domain constructs, with murine ROR1 as a control.

DETAILED DESCRIPTION OF THE INVENTION

[0026] Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is a conserved embryonic protein whose expression becomes progressively reduced during embryonic development in mammals. The intact protein, including its extracellular domain, does not appear to be significantly expressed in normal, adult mammalian tissues. In particular, studies have not identified significant expression of ROR1 on the cell surface of normal adult human tissues, including normal B cells. Baskar et al., *Clin. Cancer Res.*, 14: 396-404 (2008), DaneshManesh et al., *Int. J. Cancer*, 123: 1190-1195 (2008), and Fukuda et al., *Proc. Nat'l. Acad. Sci. USA*, 105: 3047-3052 (2008). However, ROR1 is expressed on the cell surface of malignant B-cells, including B-cell chronic lymphocytic leukemia (B-CLL) and mantle cell lymphoma (MCL). It has also been reported that ROR1 is expressed in certain other cancer cell lines including Burkett's lymphoma, renal cell carcinoma, colon cancer, and breast cancer. U.S. Patent Application Publ. 2007/0207510. Therefore, ROR1 can be considered a selective marker for these cancers. The invention provides an antibody to this selective marker.

[0027] In particular, the invention provides an antibody having specificity for ROR1, comprising (a) a heavy chain having at least 90% identity to SEQ ID NO: 1; (b) a light chain variable domain having at least 90% sequence identity to SEQ ID NO: 2; or (c) both a heavy chain of (a) and a light chain of (b). In a preferred embodiment, the antibody comprises both a heavy chain of (a) and a light chain of (b).

[0028] The antibody can be an isolated antibody having specificity for human ROR1, wherein the antibody comprises a heavy chain having at least 90% identity to a sequence such as SEQ ID NO: 1. In other embodiments, the percentage identity can be at least 91%, at

least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or even 100%. In preferred embodiments, the heavy chain has at least 95% identity to SEQ ID NO: 1. In more preferred embodiments, the heavy chain has 100% identity to SEQ ID NO: 1.

[0029] The antibody can be an isolated antibody having specificity for human ROR1, wherein the antibody comprises a light chain having at least 90% identity to a sequence such as SEQ ID NO: 2. In other embodiments, the percentage identity can be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or even 100%. In preferred embodiments, the light chain has at least 95% identity to SEQ ID NO: 2. In more preferred embodiments, the light chain has 100% identity to SEQ ID NO: 2.

[0030] In some embodiments, the antibody can comprise any heavy chain as described above, in combination with any suitable light chain, such as those described above. Likewise, the antibody can comprise any of the light chains as described above in combination with any suitable heavy chain, such as those described above. For example, in preferred embodiments, the antibody comprises a heavy chain having at least 90% identity to SEQ ID NO: 1 and a light chain having at least 90% identity to SEQ ID NO: 2. In a preferred embodiment, the antibody comprises the heavy chain of SEQ ID NO: 1 and the light chain of SEQ ID NO: 2.

[0031] Percent (%) identity of peptide sequences can be calculated, for example, as $100 \times [(\text{identical positions})/\min(\text{TG}_A, \text{TG}_B)]$, where TG_A and TG_B are the sum of the number of residues and internal gap positions in peptide sequences A and B in the alignment that minimizes TG_A and TG_B . See, e.g., Russell et al., *J. Mol Biol.*, 244: 332–350 (1994).

[0032] The antibody of the invention can be any antibody including a full length antibody or an antibody fragment. The antibody can be polyclonal, monoclonal, recombinant, chimeric, or humanized. Furthermore, the antibody can be of any isotype including without limitation IgA, IgD, IgE, IgG, or IgM. Thus, for example, the antibody can be any IgA such as IgA1 or IgA2, or any IgG such as IgG1, IgG2, IgG3, IgG4, or synthetic IgG. The antibody can also be any antibody fragment having specificity for the extracellular domain of human ROR1, such as F(ab)₂, Fv, scFv, IgGΔCH₂, F(ab')₂, scFv2CH₃, F(ab), VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)₂, a diabody, and a bivalent antibody. The antibody

can be any modified or synthetic antibody, including, but not limited to, non-depleting IgG antibodies, T-bodies, or other Fc or Fab variants of antibodies.

[0033] In addition to a heavy chain as described above, the antibody of the invention can further comprise a light chain selected from a Fab library using sequential naive chain shuffling. Likewise, in addition to a light chain as described above, the antibody of the invention can further comprise a heavy chain selected from a Fab library using sequential naive chain shuffling.

[0034] In some embodiments, the invention provides an isolated antibody, having specificity for human ROR1, comprising at least one CDR having a sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 11, SEQ ID NO: 13, and SEQ ID NO: 15. The invention also provides an isolated antibody with specificity for ROR1 comprising at least one or more variants of the foregoing CDR sequences, which include 1, 2, or 3 substitutions, insertions, deletions, or combinations thereof in a sequence selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 11, SEQ ID NO: 13, and SEQ ID NO: 15. For example, a recombinant chimeric or humanized antibody (or fragment thereof) can include one, two, three, four, five, or all six of the foregoing CDR sequences.

[0035] In some embodiments, the invention provides an antibody with avidity for ROR1 of about 10 μ M or less, 5 μ M or less, 2 μ M or less, 1 μ M or less, 500 nM or less, 400 nM or less, 300 nM or less, or 200 nM or less. The invention also provides an antibody with avidity for ROR1 of about 100 nM or less, about 75 nM or less, about 50 nM or less, about 25 nM or less, about 10 nM or less, or about 5 nM or less. The invention further provides an antibody with avidity for ROR1 of about 1 nM or less, about 800 pM or less, about 700 pM or less, about 600 pM or less, about 500 pM or less, about 400 pM or less, about 300 pM or less, about 200 pM or less, or about 100 pM or less. Avidity can be measured using art-known techniques, such as ELISA or BIACORE.

[0036] The antibody of the invention can be produced by any suitable technique, for example, using any suitable eukaryotic or non-eukaryotic expression system. In certain embodiments, the antibody is produced using a mammalian expression system. In some embodiments, the heavy chain can be encoded by a DNA sequence such as SEQ ID NO: 17 or SEQ ID NO: 18, while the light chain can be encoded by a DNA sequence such as SEQ ID NO: 19 or SEQ ID NO: 20.

[0037] The antibody of the invention can be produced using a suitable non-eukaryotic expression system such as a bacterial expression system. Bacterial expression systems can be used to produce fragments such as a F(ab)₂, Fv, scFv, IgGΔCH₂, F(ab')₂, scFv2CH₃, F(ab), VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)₂, and diabodies. Techniques for altering DNA coding sequences to produce such fragments are known in the art.

[0038] The antibody of the invention can be conjugated to a synthetic molecule using any type of suitable conjugation. Recombinant engineering and incorporated selenocysteine (e.g., as described in International Application Publication WO/2008/122039) can be used to conjugate a synthetic molecule. Other methods of conjugation can include covalent coupling to native or engineered lysine side-chain amines or cysteine side-chain thiols. See, e.g., Wu et al., *Nat. Biotechnol.*, 23: 1137-1146 (2005). The synthetic molecule can be any molecule such as one targeting a tumor. Of course, it will be understood that the synthetic molecule also can be a protein or an antibody.

[0039] Synthetic molecules include therapeutic agents such as cytotoxic, cytostatic, or antiangiogenic agents and radioisotopes. A cytotoxic agent can be a plant, fungal, or bacterial molecule (e.g., a protein toxin). A therapeutic agent can be a maytansinoid (e.g., maytansinol or DM1 maytansinoid), a taxane, or a calicheamicin. Therapeutic agents include vincristine and prednisone. A therapeutic agent can be an antimetabolite (e.g., an antifolate such as methotrexate, a fluoropyrimidine such as 5-fluorouracil, cytosine arabinoside, or an analogue of purine or adenosine); an intercalating agent (for example, an anthracycline such as doxorubicin, daunomycin, epirubicin, idarubicin, mitomycin-C, dactinomycin, or mithramycin); a platinum derivative (e.g., cisplatin or carboplatin); an alkylating agent (e.g., nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide nitrosoureas or thiotepa); an antimitotic agent (e.g., a vinca alkaloid like vincristine or taxoid such as paclitaxel or docetaxel); a topoisomerase inhibitor (for example, etoposide and teniposide, amsacrine, topotecan); a cell cycle inhibitor (for example, a flavopyridol); or a microtubule agent (e.g., an epothilone, discodermolide analog, or eleutherobin analog). A therapeutic agent can be a proteasome inhibitor or a topoisomerase inhibitor such as bortezomib, amsacrine, etoposide, etoposide phosphate, teniposide, or doxorubicin. Therapeutic radioisotopes include yttrium (⁹⁰Y), lutetium (¹⁷⁷Lu), actinium (²²⁵Ac), praseodymium, astatine (²¹¹At), rhenium (¹⁸⁶Re), bismuth (²¹²Bi or ²¹³Bi), and rhodium

(¹⁸⁸Rh). Antiangiogenic agents include linomide, bevacuzimab, angiostatin, and razoxane.

The synthetic molecule can be another antibody such as rituximab or bevacuzimab.

[0040] A synthetic molecule can also be a label. Labels can be useful in diagnostic applications and can include, for example, contrast agents. A contrast agent can be a radioisotope label such as iodine (¹³¹I or ¹²⁵I), indium (¹¹¹In), technetium (⁹⁹Tc), phosphorus (³²P), carbon (¹⁴C), tritium (³H), other radioisotope (e.g., a radioactive ion) or a therapeutic radioisotope listed above. Additionally, contrast agents can include radiopaque materials, magnetic resonance imaging (MRI) agents, ultrasound imaging agents, and any other contrast agents suitable for detection by a device that images an animal body. A synthetic molecule can also be a fluorescent label, a biologically active enzyme label, a luminescent label, or a chromophore label.

[0041] In some embodiments, the antibody can also have specificity for one or more antigens in addition to ROR1. For example, the antibody of the invention can be engineered (e.g., as a bivalent diabody or a conjugated Fab dimer or trimer) to have specificity for ROR1 and another tumor antigen, e.g., an antigen associated with B-CLL, MCL, Burkett's lymphoma, renal cell carcinoma, colon cancer (e.g., colon adenocarcinoma), or breast cancer (e.g., breast adenocarcinoma). The antibody can be engineered to have specificity for ROR1 and an antigen that promotes activation or targeting of cytotoxic effector cells.

[0042] The invention further provides eukaryotic or non-eukaryotic cells that have been recombinantly engineered to produce an antibody of the invention. The eukaryotic or non-eukaryotic cells can be used as an expression system to produce the antibody of the invention. In another embodiment, the invention provides ROR1 targeted immune cells that are engineered to recombinantly express an ROR1 specific antibody of the invention. For example, the invention provides a T-cell engineered to express an antibody of the invention (e.g., an scFv, scFv-Fc, (scFv)₂), which is linked to a synthetic molecule with the following domains: a spacer or hinge region (e.g., a CD28, CD28 or IgG hinge), a transmembrane region (e.g., a transmembrane canonical domain), and an intracellular T-cell receptor (TCR) signaling domain, thereby forming a T-body (or chimeric antigen receptor (CAR)). Intracellular TCR signaling domains that can be included in a T-body (or CAR) include, but are not limited to, CD3ζ, FcR-γ, and Syk-PTK signaling domains as well as the CD28, 4-1BB, and CD134 co-signaling domains. Methods for constructing T-cells expressing a T-

body (or CAR) are known in the art. See, e.g., Marcu-Malina et al., *Expert Opinion on Biological Therapy*, Vol. 9, No. 5, posted online April 16 (2009).

[0043] The invention provides a method of inhibiting cells that express ROR1 (ROR1 cells) by contacting the cells with an antibody of the invention. The antibody can be a naked (unconjugated) antibody or an antibody conjugated to a synthetic molecule, e.g., a cytotoxic, cytostatic, or antiangiogenic agent or a radioisotope. The method can be used to inhibit ROR1 cells *in vitro* or in a subject (i.e., *in vivo*). The contacted ROR1 cells be in, for example, a cell culture or animal model of a disorder associated with elevated levels of ROR1. The method is useful, for example, to measure and/or rank (relative to another antibody) the antibody's inhibitory activity for a specific ROR1 cell type. Inhibiting ROR1 cells can include blocking or reducing the activity or growth of ROR1 cells. Inhibiting can also include the killing of ROR1 cells. While the method is not bound by or limited to any mechanism of action, inhibitory activity can be mediated by blocking ROR1-mediated signaling or by blocking the signaling of an ROR1 associated receptor. Inhibitory activity can also be mediated by recruitment of immune system effectors that attack ROR1 cells, e.g., by activating constituents of the antibody-dependent cell-mediated cytotoxicity (ADCC) or complement systems.

[0044] The invention also provides a method of treating a subject that has, is suspected to have, or is at risk for a disorder associated with elevated levels of ROR1. Generally, the method includes administering a therapeutically effective amount of an isolated antibody of the invention to the subject. The antibody can be any anti-ROR1 antibody of the invention as described above. Thus, the antibody can be chimeric, humanized, synthetic, F(ab)₂, Fv, scFv, IgGΔCH₂, F(ab')₂, scFv2CH₃, F(ab), VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, or (scFv)₂. In some embodiments, the method includes administering an IgG, an scFv, a dsFv, a F(ab')₂, a diabody, or a bivalent antibody. The administered antibody can be conjugated to a synthetic molecule described above, e.g., a cytotoxic, cytostatic, or antiangiogenic agent or a therapeutic radioisotope. An exemplary cytotoxic agent is Pseudomonas exotoxin A (PE38). Disorders that can be treated include, for example, B-CLL and MCL. Other disorders associated with elevated ROR1 that can be treated include Burkett's lymphoma, renal cell carcinoma, colon cancer (e.g., colon adenocarcinoma), and breast cancer (e.g., breast adenocarcinoma).

[0045] The invention also provides a method of treating a subject that has, is suspected to have, or is at risk for a disorder associated with elevated levels of ROR1 by adoptive transfer of the genetically engineered T-cells described herein, which express an antibody of the invention as a T-body (or CAR) that selectively binds ROR1. Recombinant technology can be used to introduce T-body (or CAR) encoding genetic material into any suitable T-cells, e.g., central memory T-cells from the subject to be treated. The T-cells carrying the genetic material can be expanded (e.g., in the presence of cytokines). The genetically engineered T-cells are transferred, typically by infusion, to the patient. The transferred T-cells of the invention can then mount an immune response against ROR1 expressing cells in the subject. The adoptive transfer method can be used, for example, to treat subjects that have or are suspected to have B-CLL, MCL, Burkett's lymphoma, renal cell carcinoma, colon cancer (e.g., colon adenocarcinoma), or breast cancer (e.g., breast adenocarcinoma).

[0046] In some embodiments, the foregoing methods of treatment can further include co-administering a second therapeutic agent for the disorder associated with elevated ROR1. For example, when the disorder to be treated involves an ROR1-expressing cancer, the method can further include co-administration of a cytotoxic, cystostatic, or antiangiogenic agent suitable for treating the cancer. If the cancer is a B-cell lymphoma, the method can further include, for example, co-administration of rituximab, alemtuzumab, or a CHOP chemotherapeutic regimen.

[0047] The terms "treat," "treating," "treatment," and "therapeutically effective" used herein do not necessarily imply 100% or complete treatment. Rather, there are varying degrees of treatment of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive method can provide any amount of any level of treatment. Furthermore, the treatment provided by the inventive method can include the treatment of one or more conditions or symptoms of the disease being treated.

[0048] In another embodiment, the invention provides method of detecting in a test sample an altered level of ROR1 (e.g., cell surface ROR1), for example, relative to a control. Generally, the method includes contacting an antibody of the invention to the test sample and determining the amount of antibody that selectively binds to material (e.g., cells) in the sample to thereby determine the level of ROR1 in the test sample. A test sample can be from a cell culture or from a test subject, e.g., a plasma or a tissue sample from a subject that has, is suspected to have, or is at risk for a disease or condition associated with elevated ROR1 in

a subject. A control level desirably corresponds to the ROR1 level detected using the same antibody in a corresponding sample(s) from one or more control cultures or subjects.

Methods of using the antibody of the invention to determine ROR1 levels can include any immunoassay such as immuno- (Western) blotting, enzyme-linked immunosorbent assay (ELISA), and flow cytometry, e.g., fluorescence-activated cell sorting (FACS) analysis.

[0049] The method of detection can be used to screen for the presence of a disorder associated with elevated ROR1. The method includes obtaining a sample from a test subject in need of screening, e.g., a subject that has, is suspected to have, or is at risk for a disorder associated with elevated ROR1. The level of ROR1 (e.g., the amount or concentration) in the sample is measured using an antibody of the invention, and the level in the sample is compared to a control level of ROR1. The control level represents, for example, the mean level (e.g., the amount or concentration) in sample(s) from one or, preferably, multiple control group subjects that do not have a disorder associated with elevated ROR1. Alternatively, the control level can correspond to the level or mean level of ROR1 in one or more samples taken from the test subject at one or more prior times, when the test subject did not have or did not exhibit, a condition associated with elevated ROR1. A significantly higher level of ROR1 in the test sample relative to the control level is indicative of a disorder associated with elevated ROR1 in the subject.

[0050] In subjects such as humans, where cell surface ROR1 expression is largely restricted to embryonic development, a control level of ROR1 can be zero or none. Thus, in some embodiments of the method of the detection provided by the invention, any significant and detectable amount of ROR1 in a test sample can be indicative of a disorder associated with elevated ROR1 in the subject.

[0051] Additionally, the method of detection can be used to monitor the progress of a disorder associated with elevated ROR1. The method includes obtaining a sample from a subject in need of screening, e.g., a subject having been diagnosed or suspected to have a disorder associated with elevated ROR1. The level of ROR1 in the sample is measured using an antibody of the invention, and the level in the sample is compared to a control level corresponding to the level or mean level of ROR1 in one or more samples taken from the test subject at one or more prior times. Levels of ROR1 that are significantly elevated or decreased relative to control indicate that the subject's disorder is deteriorating or improving, respectively.

[0052] The foregoing method of detection can be used to screen for the presence or to monitor the progress of disorders including, for example, B-CLL, MCL, Burkett's lymphoma, renal cell carcinoma, colon cancer (e.g., colon adenocarcinoma), and breast cancer (e.g., breast adenocarcinoma).

[0053] The invention provides a method for screening a subject for an altered level of ROR1. Generally, the method includes administering to the subject an antibody of the invention that is conjugated to a label (e.g., a contrast agent), imaging the subject in a manner suitable for detecting the label, and determining whether a region in the subject has an altered density or concentration of label as compared to the background level of label in proximal tissue. Alternatively, the method includes determining whether there is an altered density or concentration of label in a region as compared to the density or concentration of label previously detected in the same region of the subject. Methods of imaging a subject can include x-ray imaging, x-ray computed tomography (CT) imaging (e.g., CT angiography (CTA) imaging), magnetic resonance (MR) imaging, magnetic resonance angiography (MRA), nuclear medicine, ultrasound (US) imaging, optical imaging, elastography, infrared imaging, microwave imaging, and the like, as appropriate for detecting the label conjugated to the antibody. In a preferred embodiment, the subject has, is suspected to have, or is at risk for an ROR1-expressing tumor, such as B-CLL, MCL, Burkett's lymphoma, renal cell carcinoma, tumor of the colon (e.g., colon adenocarcinoma), or breast tumor (e.g., breast adenocarcinoma), and the method is used to screen for or detect the presence of the tumor. In another embodiment, the method can be used to monitor the size or density of an ROR1-expressing tumor over time, e.g., during a course of treatment.

[0054] The invention also provides a pharmaceutical composition comprising an antibody as described above and a pharmaceutically acceptable carrier. Pharmaceutical compositions can be prepared from any of the antibodies described herein. An exemplary composition includes a chimeric antibody having SEQ ID NO: 1 (heavy chain) and/or SEQ ID NO: 2 (light chain). Another exemplary composition comprises a humanized antibody having one, two, three, four, five, or six CDRs selected from the group consisting of SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO: 11, SEQ ID NO: 13, and SEQ ID NO: 15. Still another exemplary pharmaceutical composition includes a dsFv fragment, which comprises the sequence of SEQ ID NO: 1 with a glycine to cysteine substitution at position 44 (heavy

chain) and/or the sequence of SEQ ID NO: 2 with a glycine to cysteine substitution at position 100 (light chain).

[0055] The composition of the invention comprises a carrier for the antibody, desirably a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier can be any suitable pharmaceutically acceptable carrier. The term “pharmaceutically acceptable carrier” as used herein means one or more compatible solid or liquid fillers, diluents, other excipients, or encapsulating substances which are suitable for administration into a human or veterinary patient (e.g., a physiologically acceptable carrier or a pharmacologically acceptable carrier). The term “carrier” denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The pharmaceutically acceptable carrier can be co-mingled with one or more of the active components, e.g., a hybrid molecule, and with each other, when more than one pharmaceutically acceptable carrier is present in the composition in a manner so as not to substantially impair the desired pharmaceutical efficacy. “Pharmaceutically acceptable” materials typically are capable of administration to a patient without the production of significant undesirable physiological effects such as nausea, dizziness, rash, or gastric upset. It is, for example, desirable for a composition comprising a pharmaceutically acceptable carrier not to be immunogenic when administered to a human patient for therapeutic purposes.

[0056] The pharmaceutical composition can contain suitable buffering agents, including, for example, acetic acid in a salt, citric acid in a salt, boric acid in a salt, and phosphoric acid in a salt. The pharmaceutical compositions also optionally can contain suitable preservatives, such as benzalkonium chloride, chlorobutanol, parabens, and thimerosal.

[0057] The pharmaceutical composition can be presented in unit dosage form and can be prepared by any suitable method, many of which are well known in the art of pharmacy. Such methods include the step of bringing the antibody of the invention into association with a carrier that constitutes one or more accessory ingredients. In general, the composition is prepared by uniformly and intimately bringing the active agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

[0058] A composition suitable for parenteral administration conveniently comprises a sterile aqueous preparation of the inventive composition, which preferably is isotonic with the blood of the recipient. This aqueous preparation can be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile

injectable preparation also can be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed, such as synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid can be used in the preparation of injectables. Carrier formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA.

[0059] The delivery systems useful in the context of the invention include time-released, delayed release, and sustained release delivery systems such that the delivery of the inventive composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. The inventive composition can be used in conjunction with other therapeutic agents or therapies. Such systems can avoid repeated administrations of the inventive composition, thereby increasing convenience to the subject and the physician, and may be particularly suitable for certain compositions of the invention.

[0060] Many types of release delivery systems are available and known to those of ordinary skill in the art. Suitable release delivery systems include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are lipids including sterols such as cholesterol, cholesterol esters, and fatty acids or neutral fats such as mono-di-and tri-glycerides; hydrogel release systems; sytastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the active composition is contained in a form within a matrix such as those described in U.S. Patents 4,452,775, 4,667,014, 4,748,034, and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patents 3,832,253 and 3,854,480. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

[0061] The term “subject” is used herein, for example, in connection with therapeutic and diagnostic methods, to refer to human or animal subjects. Animal subjects include, but are not limited to, animal models, such as, mammalian models of conditions or disorders associated with elevated ROR1 expression such as B-CLL, MCL, Burkett's lymphoma, renal cell carcinoma, colon cancer, (e.g., colon adenocarcinoma), and breast cancer (e.g., breast adenocarcinoma).

[0062] The invention also provides kits suitable for carrying out the methods of the invention. Typically, a kit comprises two or more components required for performing a therapeutic or detection method of the invention. Kit components include, but are not limited to, one or more antibody of the invention, appropriate reagents, and/or equipment.

[0063] A kit can comprise an antibody of the invention and an immunoassay buffer suitable for detecting ROR1 (e.g. by ELISA or FACS). The kit may also contain one or more microtiter plates, standards, assay diluents, wash buffers, adhesive plate covers, and/or instructions for carrying out a method of the invention using the kit. The kit can include an antibody of the invention bound to a substrate (e.g., a multi-well plate or a chip), which is suitably packaged and useful to detect ROR1. In some embodiments, the kit includes an antibody of the invention that is conjugated to a label, such as, a fluorescent label, a biologically active enzyme label, a luminescent label, or a chromophore label. The kit can further include reagents for visualizing the conjugated antibody, e.g., a substrate for the enzyme. In some embodiments, the kit includes an antibody of the invention that is conjugated to a contrast agent and, optionally, one or more reagents or pieces of equipment useful for imaging the antibody in a subject.

[0064] Generally the antibody of the invention in a kit is suitably packaged, e.g., in a vial, pouch, ampoule, and/or any container appropriate for a therapeutic or detection method. Kit components can be provided as concentrates (including lyophilized compositions), which may be further diluted prior to use or they can be provided at the concentration of use. When the antibody of the invention for use *in vivo*, single dosages may be provided in sterilized containers having the desired amount and concentration of agents.

[0065] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0066] This example demonstrates the preparation of monoclonal antibodies with specificity for ROR1.

[0067] Mice were immunized with a fusion protein consisting of the human Fc domain and extracellular domain of human ROR1 (Fc-ROR1). As depicted in Figure 1, the extracellular domain of ROR1 includes an immunoglobulin (Ig) domain, a Frizzled domain, and a Kringle domain, which span about 393 amino acids of the amino-terminal portion of ROR1. A nucleic acid sequence encoding the ROR1 extracellular domain was recombinantly linked to a nucleic acid sequence encoding human Fc1 to form a recombinant construct that then was expressed in HEK 293 cells. The produced Fc-ROR1 fusion protein (depicted in Figure 2B) was harvested and purified by ion exchange chromatography and gel filtration or by Protein A affinity chromatography. The results of a Coomassie-stained protein gel analysis of purified Fc-ROR1 under non-reducing and reducing conditions are shown in Figure 2C.

[0068] Mice were immunized with the Fc-ROR1, and antibody-producing cells were immortalized to produce hybridomas. Supernatants from twenty hybridomas were screened for specific ROR1 binding. An ELISA plate was coated with human ROR1-ECD (50 ng per well overnight at 4 °C), then blocked with 3% BSA-PBS (room temperature for 2 hours), and subsequently contacted with dilutions of affinity purified of mouse mAb (room temperature for 2 hours). After washing (with PBS-Tween 20), horseradish peroxidase (HRP)-conjugated goat anti-mouse (1:2000 dilution) was added (1 hour at room temperature) and washed. The bound antibody was detected using the peroxidase substrate ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid). Four monoclonal antibodies (mAb 2A2, 2D11, 1A1, and 1A7) were identified by ELISA as specifically binding ROR1.

[0069] The four specific mAbs were further characterized as IgG using goat anti-human IgG polyclonal antibodies (pAbs) conjugated to HRP. Flow cytometry was used to evaluate the ability of each of the four antibodies to bind to cells expressing ROR1. The mAb 2A2 antibody was also further characterized by surface plasmon resonance (BIAcore, GE Healthcare, Piscataway, NJ). The results of the foregoing experiments and also the antibody expression yield for two hybridomas are summarized in Table 1.

Table 1

| mAb | Isotype | ELISA | Flow Cytometry | BIACORE | mAb Expression |
|------------|---------------------|--------------|-----------------------|----------------|-----------------------|
| 2A2 | mouse IgG1 κ | +++ | +++ | +++ | 10 mg/L |
| 2D11 | mouse IgG1 κ | ++ | ++ | not determined | 23 mg/L |
| 1A1 | mouse IgG1 κ | ++ | + | not determined | not determined |
| 1A7 | mouse IgG1 κ | ++ | + | not determined | not determined |

[0070] The foregoing results demonstrate the isolation of four mAbs with specificity for the extracellular domain of human ROR1.

EXAMPLE 2

[0071] This example further demonstrates the desirable binding properties of mAb 2A2 for ROR1.

[0072] Hybridoma 2A2 of Example 1 was grown in CELLINE Disposable Bioreactors (BD Biosciences, San Jose, CA) using animal component-free BD Cell MAb Medium. Expressed mAb 2A2 was purified by Protein G affinity chromatography.

[0073] The avidity of mAb 2A2 for ROR1-ECD was determined using surface plasmon resonance in a BIACORE X100 instrument (GE Healthcare, Piscataway, NJ). Human ROR1-ECD was coupled to a BIACORE CM5 chip at 1600 resonance units. MAb 2A2 was diluted in HBS-EP buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, and 0.005% surfactant P20, pH 7.4; GE Healthcare) to various concentrations (from about 200 nM to about 12.5 nM) and injected sequentially. As depicted by the extended, flat tail in Figure 3, antibody binding was very stable at all concentrations tested. Association (k-on) and dissociation (k-off) rate constants were calculated based on a 1:1 Langmuir binding model using BIAEVALUATION software (GE Healthcare). The equilibrium dissociation constant ($K_d = k\text{-off}/k\text{-on}$) was calculated, and the avidity of mAb 2A2 was determined to be 100 pM.

[0074] Fluorescence-activated cell sorting (FACS) analysis was performed to test the ability of mAb 2A2 to bind to peripheral blood mononuclear cells (PBMC) from normal patients, to PBMC from a B-CLL patient, and to the human ROR1-expressing MCL cell line JeKo-1. Antibody binding was detected using goat anti-mouse IgG polyclonal antibody conjugated to fluorescein isothiocyanate (FITC). Results for antibody concentrations of from about 0.001 $\mu\text{g/mL}$ to about 1 $\mu\text{g/mL}$ (about 6.5 pM to about 6.5 nM), secondary antibody

alone, and polyclonal mouse IgG control are depicted in the histograms of Figure 4. Control mouse IgG (black-filled histogram) did not show a significant shift relative to secondary antibody alone (broken line histogram). mAb 2A2 did exhibit significant binding to JeKo-1 (Figure 4, second panel) and to primary B-CLL cells present in the PBMC sample from a representative patient (Figure 4, first panel). Binding to each of these cell populations was observed using mAb 2A2 concentrations as low as 1 ng/mL and 10 ng/mL, respectively (see Figure 4).

[0075] Similar FACS analyses of mAb 2A2 binding to normal B cells from healthy donors were negative.

[0076] The foregoing results demonstrate that mAb 2A2 has good avidity for its antigen and can be used to specifically distinguish (i) tumor cells obtained from lymphoma patients from (ii) normal B-cells taken from healthy subjects.

EXAMPLE 3

[0077] This example demonstrates the identification of mAb 2A2 variable domain coding and amino acid sequences.

[0078] The variable domain encoding sequences of heavy (VH) and light (VL) chain of mAb 2A2 were RT-PCR amplified from hybridoma 2A2 total RNA using published primer sequences (Morrison, S.L., in *Current Protocols in Immunology*, Suppl. 47, pp. 2.12.1-2.12.17, John Wiley and Sons, 2002). The cDNAs were cloned and analyzed by DNA sequencing. VH and VL chain sequences were independently confirmed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of the tryptic digests of the heavy and light chain polypeptides, which were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The amino acid sequences and cDNA sequences of the VH and VL chains of mAb 2A2 are depicted in Figures 5 and 6 ("original sequences"), respectively.

EXAMPLE 4

[0079] This example demonstrates the construction and characterization of a chimeric human-mouse antibody with desirable binding properties for ROR1.

[0080] Codon optimization of mAb 2A2 VH and VL DNA sequences (SEQ ID NO: 17 and SEQ ID NO: 19, respectively) produced the sequences of SEQ ID NO: 18 and SEQ ID NO: 20, respectively, which are depicted in Figure 6. Optimized sequences were used to

generate a chimeric antibody vector, generally according to the method described in Morrison, S.L., in *Current Protocols in Immunology*, Coligan et al. Eds., Suppl. 47, pp. 2.12.1-2.12.17, John Wiley and Sons, 2002. The optimized VH and VL sequences as well as human C κ L segments were cloned into the pIgG mammalian expression vector which is described in Rader et al., *FASEB J.*, 16:2000-2002 (2002). The resulting plasmids were transfected and expressed in HEK 293F cells to produce chimeric mouse/human 2A2 IgG1 (ch2A2) as described in Hofer et al., *J. Immunol. Methods*, 318: 75-87 (2007). The chimeric ch2A2 antibody is schematically depicted in Figure 7A with mouse and human antibody domains shown by light and dark fills, respectively.

[0081] Ch2A2 and mouse mAb 2A2 were evaluated by ELISA for their ability to bind to ROR1-ECD and a commercial ROR2-Fc (R&D Systems, Minneapolis, MN). ELISA was performed as described in Example 1, with chimeric ch2A2 being detected using HRP-conjugated goat anti-human kappa antibody. ELISA results are depicted in Figure 7B. While neither ch2A2 nor mAb 2A2 bound to the ROR2-Fc control, ch2A2 retained virtually all of the mAb 2A2 binding affinity for the extracellular domain of ROR1 (see Figure 7B).

[0082] Ch2A2 was also compared to mAb 2A2 by FACS for its ability to bind to the human ROR1-expressing MCL cell line JeKo-1 and to PBMC from B-CLL patients. A FACS analysis was carried out as described in Example 2 above, except that mAb 2A2 (1 μ g/ml) and ch2A2 (1 μ g/ml) were detected using allophycocyanin (APC) conjugated goat anti-mouse IgG (Fc) and APC conjugated goat anti-human IgG (Fc), respectively. The results of the FACS analysis for JeKo-1 cells are depicted in Figure 8. Control mouse IgG did not show a significant shift relative to secondary antibody alone (black-filled histogram). The results indicate that mAb 2A2 and ch2A2 at 1 μ g/ml similarly bind to JeKo-1 (see Figure 8).

[0083] Similar results were obtained by FACS analysis comparing mAb 2A2 and ch2A2 binding to PBMC from B-CLL patients.

[0084] The foregoing data demonstrate the generation of chimeric mouse/human antibody of the invention with conserved specificity and affinity for the extracellular domain of ROR1, including native ROR1 expressed on the cell surface of malignant B-cells.

EXAMPLE 5

[0085] This example demonstrates the construction and characterization of a disulfide stabilized fragment (dsFv) of mAb 2A2 fused to an immunotoxin.

[0086] A dsFv fragment of mAb 2A2 (dsFv 2A2) was generated and fused to a 38-kDa fragment of *Pseudomonas* exotoxin A (PE38) generally according to methods described in Pastan et al., *Methods Mol. Biol.*, 248: 503-518 (2004). The original VH and VL coding sequences of mAb 2A2 (see Figure 6) were altered to introduce a glycine to cysteine substitution at positions 44 and 100 of SEQ ID NO: 1 and SEQ ID NO: 2, respectively (substituted residues are underlined in Figure 5). The altered VH coding sequence was subcloned in-frame with a PE38 coding sequence in a pRB98 vector carrying a chloramphenicol resistance gene (the vector is described in Kreitman et al., in *Drug Targeting*, Francis et al., Eds., Vol. 25, pp. 215–226, Humana Press Inc, Totowa, N.J., 2000). Altered VH and VL chains were separately expressed in *E. coli*, and the resulting proteins were harvested and solubilized. The VH and VL were refolded together to form dsFv 2A2-PE38 fusion immunotoxin, which was purified by Q-Sepharose ion-exchange chromatography as described in Pastan et al., *supra*, 2004.

[0087] Recombinant dsFv 2A2-PE38 immunotoxin was evaluated by flow cytometry and compared to mouse mAb 2A2 for its ability to bind to the human ROR1-expressing mantle cell lymphoma cell line JeKo-1. JeKo-1 cell binding by mAb 2A2 was detected using a goat anti-mouse IgG polyclonal antibody (pAb) conjugated to APC (Jackson ImmunoResearch Laboratories, West Grove, PA) at 1:300 dilution. JeKo-1 cell binding of dsFv 2A2-PE38 was detected using rabbit anti-*Pseudomonas* exotoxin A pAb (1:100 dilution) (Sigma-Aldrich, St. Louis, MO) as a secondary antibody and goat anti-rabbit IgG pAb conjugated to Cy5 (1:300 dilution) (Jackson ImmunoResearch Laboratories) as a tertiary antibody. Flow cytometry results are depicted in Figure 9A. The results demonstrate that, despite the inherent monovalency of the recombinant dsFv 2A2-PE38 immunotoxin, its binding to native cell surface ROR1 was detectable at concentrations of less than 1 $\mu\text{g/mL}$ (Figure 9).

[0088] An analysis of dsFv 2A2-PE38 immunotoxin binding to PBMC from B-CLL patients showed similar results. Additionally, ELISA experiments demonstrated that dsFv 2A2-PE38 immunotoxin retains binding specificity for the extracellular domain of human ROR1.

[0089] The foregoing example provides a recombinant immunotoxin conjugated antibody of the invention, which is based on mAb 2A2 and which has conserved binding specificity for ROR1, including native ROR1 expressed on the cell surface of malignant B-cells.

EXAMPLE 6

[0090] This example demonstrates dsFv 2A2-PE38 mediated cytotoxicity of ROR1 expressing cells.

[0091] JeKo-1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal calf serum and incubated for 51 hours at 37 °C in a 96-well tissue culture plate with various doses (0-100 µg/mL) of the dsFv 2A2-PE38 immunotoxin prepared in Example 5. The cells were subsequently analyzed by flow cytometry using annexin V and propidium iodide to stain apoptotic and dead cells, respectively. The percentage of cells that were positive for both annexin V and propidium iodide is shown as cytotoxicity on the y-axis, as a function of the concentration of dsFv 2A2-PE38, of Figure 10. Figure 11 further demonstrates that the cytotoxicity of dsFv 2A2-PE38 (2 µM) included not only cell death (necrosis) as evidenced by propidium iodide staining, but also extensive apoptosis, as evidenced by annexin V staining.

[0092] The data confirmed the ability of dsFv 2A2-PE38 to effect dose-dependent killing of JeKo-1 cells at microgram concentrations or less.

EXAMPLE 7

[0093] This example demonstrates epitope mapping of the interaction between mAb 2A2 and ROR1 using ELISA.

[0094] Recombinant proteins containing one, two, or three of the three extracellular domains of human ROR1 (d1-Ig, d2-Frizzled, and d3-Kringle) as shown in Figure 12 were coated on an ELISA plate. Each of the proteins was genetically fused to a human Fc region; a protein containing the three extracellular domains without the Fc region was also prepared. Cross-reactivity to mouse ROR1 was determined by coating recombinant Fc-mouse ROR1 protein on the ELISA plate. Binding of mouse mAb 2A2 to the different ROR1 proteins was detected using HRP-conjugated donkey anti-mouse IgG polyclonal antibodies. The reactivity was scored “++” for strong binding, “+” for moderate binding, and “-” for no detectable binding. As shown in Figure 12, strong binding was detected for Fc-hROR1, which contained all three extracellular domains, as well as hROR1ECD, which contained the

extracellular domains without an Fc region, and Fc-hROR1(d1d2), which contained the Ig and Frizzled domains. Moderate binding was detected for Fc-hROR(d1), which contained the Ig domain only. No binding was detected for Fc-hROR1(d2d3), Fc-hROR1(d2), or Fc-hROR(d3).

[0095] These results show that the binding epitope for the 2A2-ROR1 interaction is likely in the Ig domain, but is assisted either directly or indirectly by the Frizzled domain of ROR1.

[0096] The foregoing results demonstrate the dose-dependent killing of ROR1 expressing cells by an immunotoxin-conjugated antibody of the invention.

[0097] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0098] The use of the terms “a” and “an” and “the” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing” are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0099] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as

specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIMS:

1. An isolated antibody having specificity for the extracellular domain of human receptor tyrosine kinase-like orphan receptor 1 (ROR1), wherein the antibody comprises:
 - (a) a heavy chain variable domain having at least 90% sequence identity to SEQ ID NO: 1; or
 - (b) a light chain variable domain having at least 90% sequence identity to SEQ ID NO: 2; or
 - (c) both a heavy chain of (a) and a light chain of (b).
2. The antibody of claim 1, wherein the antibody comprises a heavy chain variable domain having at least 90% sequence identity to SEQ ID NO: 1.
3. The antibody of claim 2, wherein the heavy chain variable domain has at least 95% sequence identity to SEQ ID NO: 1.
4. The antibody of claim 2, wherein the heavy chain variable domain has a sequence consisting of SEQ ID NO: 1.
5. The antibody of claim 2, wherein the heavy chain variable domain has a sequence consisting of SEQ ID NO: 1 with a glycine to cysteine substitution at position 44.
6. The antibody of claim 1, wherein the antibody comprises a light chain having at least 90% identity to SEQ ID NO: 2.
7. The antibody of claim 6, the light chain variable domain has at least 95% identity SEQ ID NO: 2.
8. The antibody of claim 6, wherein the light chain variable domain has a sequence consisting of SEQ ID NO: 2.
9. The antibody of claim 6, wherein the light chain variable domain has a sequence consisting of SEQ ID NO: 2 with a glycine to cysteine substitution at position 100.

10. The antibody of any one of claims 1-9, wherein the antibody comprises both a heavy chain of (a) and a light chain of (b).
11. The antibody of claim 10, wherein the heavy chain variable domain has a sequence consisting of SEQ ID NO: 1 and the light chain variable domain has a sequence consisting of SEQ ID NO: 2.
12. The antibody of claim 10, wherein the heavy chain variable domain has a sequence consisting of SEQ ID NO: 1 with a glycine to cysteine substitution at position 44, and the light chain variable domain has a sequence consisting of SEQ ID NO: 2 with a glycine to cysteine substitution at position 100.
13. The antibody of claim 10, wherein the antibody comprises at least one CDR having a sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, and SEQ ID NO: 15.
14. An isolated antibody having specificity for the extracellular domain of human receptor tyrosine kinase-like orphan receptor 1 (ROR1), comprising at least one CDR having a sequence selected from the group consisting of SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 13, and SEQ ID NO: 15.
15. The antibody of any one of claims 1-14, wherein the antibody is selected from the group consisting of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, IgM, F(ab)₂, Fv, scFv, IgGΔCH2, F(ab')₂, scFv2CH3, F(ab), VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)₂, a non-depleting IgG, a diabody, and a bivalent antibody.
16. The antibody of claim 15, wherein the antibody is an IgG selected from the group consisting of IgG1, IgG2, IgG3, IgG4, and synthetic IgG.
17. The antibody of claim 15, wherein the antibody is a Fab.
18. The antibody of claim 15, wherein the antibody is a dsFv.
19. The antibody of any one of claims 1-18, wherein the antibody is conjugated to a synthetic molecule.

20. The antibody of claim 19, wherein the antibody is a T-body and the synthetic molecule comprises a transmembrane region and an intracellular T-cell receptor (TCR) signaling domain.
21. The antibody of claim 19, wherein the synthetic molecule is a label.
22. The antibody of claim 19, wherein the synthetic molecule is a cytotoxic agent or a therapeutic radioisotope.
23. A pharmaceutical composition comprising a therapeutically effective amount of an isolated antibody of any one of claims 1-22 and a pharmaceutically acceptable carrier.
24. A kit comprising the isolated antibody of any one of claims 1-22.
25. The kit of claim 24, further comprising one or more immunoassay buffers.
26. The kit of claim 24 or 25, wherein the antibody is conjugated to a label.
27. A method of killing or inhibiting the growth of cells expressing ROR1 in a subject, which method comprises administering a therapeutically effective amount of an isolated antibody of any one of claims 1-22 to a subject in need thereof, thereby killing or inhibiting the growth of cell expressing ROR-1 in the subject.
28. A method of treating a disease or condition associated with elevated expression of ROR1 in a subject, the method comprising administering a therapeutically effective amount of an antibody of any one of claims 1-22 to a subject in need thereof, thereby treating a disease or condition associated with elevated expression of ROR1 in the subject.
29. The method of claim 28, wherein the disease or condition is a B-cell cancer, renal cell carcinoma, colon cancer, colorectal cancer, or breast cancer.
30. The method of claim 28, wherein the disease or condition is B-cell lymphocytic leukemia (B-CLL) or mantle cell lymphoma (MCL).

31. The method of any one claims 27-30, wherein the antibody is selected from the group consisting of an F(ab)₂, Fv, scFv, IgGΔCH₂, F(ab')₂, scFv₂CH₃, F(ab), VL, VH, scFv₄, scFv₃, scFv₂, dsFv, Fv, (scFv)₂, and a synthetic IgG.

32. The method of claim 31, wherein the antibody is conjugated to a synthetic molecule.

33. The antibody of claim 32, wherein the antibody is a T-body and the synthetic molecule comprises a transmembrane region and an intracellular T-cell receptor (TCR) signaling domain.

34. The method of claim 32, wherein the synthetic molecule is a cytotoxic agent or a radioisotope.

35. The method of any one claims 27-32, wherein the method further comprises co-administering a pharmaceutical composition comprising a second therapeutic agent to kill or inhibit the growth of cells expressing ROR1 or to treat the disease or condition associated with elevated expression of ROR1 in the subject.

36. A method of detecting an altered ROR1 level in a test sample, which method comprises:

- (a) obtaining a test sample from a subject;
- (b) contacting the sample with an isolated antibody of any one of claims 1-22;
- (c) determining the level ROR1 in the test sample; and
- (d) comparing the level of ROR1 in the test sample to a control level of ROR-1 to thereby determine whether the ROR1 level in the test sample is altered relative to the control level of ROR1.

37. The method of claim 36, wherein a level of ROR1 in the subject that is greater than the control level is indicative of a disease or condition associated with elevated expression of ROR1 in the subject.

38. The method of claim 37, wherein the disease or condition associated with elevated expression of ROR1 is a B-cell cancer, renal cell carcinoma, colon cancer, colorectal cancer, or breast cancer.

39. The method of claim 37, wherein the disease or condition is B-cell lymphocytic leukemia (B-CLL) or mantle cell lymphoma (MCL).

40. A method of detecting an ROR1-expressing tumor in a subject, which method comprises:

(a) administering the antibody of claim 20 to a subject that has, is suspected to have, or is at risk for an ROR1-expressing tumor; and

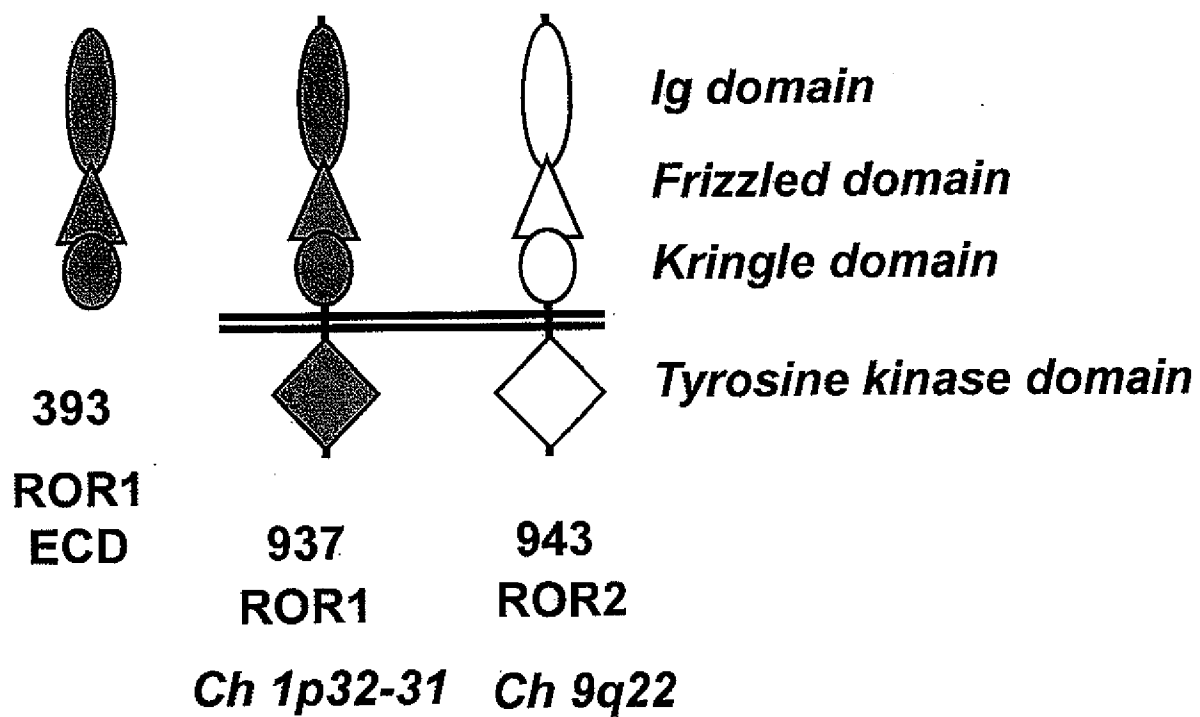
(b) imaging the subject for a region of altered conjugated label density or concentration, wherein the density or concentration is relative to (i) background in proximal tissue or (ii) the density or concentration previously detected in the same region of the subject, such that the existence of a region of altered conjugated label density or concentration is an indication of the presence an ROR1-expressing tumor in the subject.

41. A method of detecting an ROR1-expressing B-cell tumor in a subject, which method comprises:

(a) administering the antibody of claim 20 to a subject that has, is suspected to have, or is at risk for a B-cell tumor; and

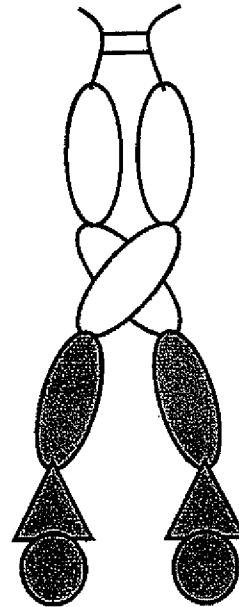
(b) imaging the subject for a region of altered conjugated label density or concentration, wherein the density or concentration is relative to (i) background in proximal tissue or (ii) the density or concentration previously detected in the same region of the subject, such that the existence of a region of altered conjugated label density or concentration is an indication of the presence an ROR1-expressing B-cell tumor tumor in the subject.

42. The method of claim 41, wherein the ROR1-expressing B-cell tumor is a B-cell lymphocytic leukemia (B-CLL) or a mantle cell lymphoma (MCL) tumor.

**Figure 1**

**ROR1-ECD**

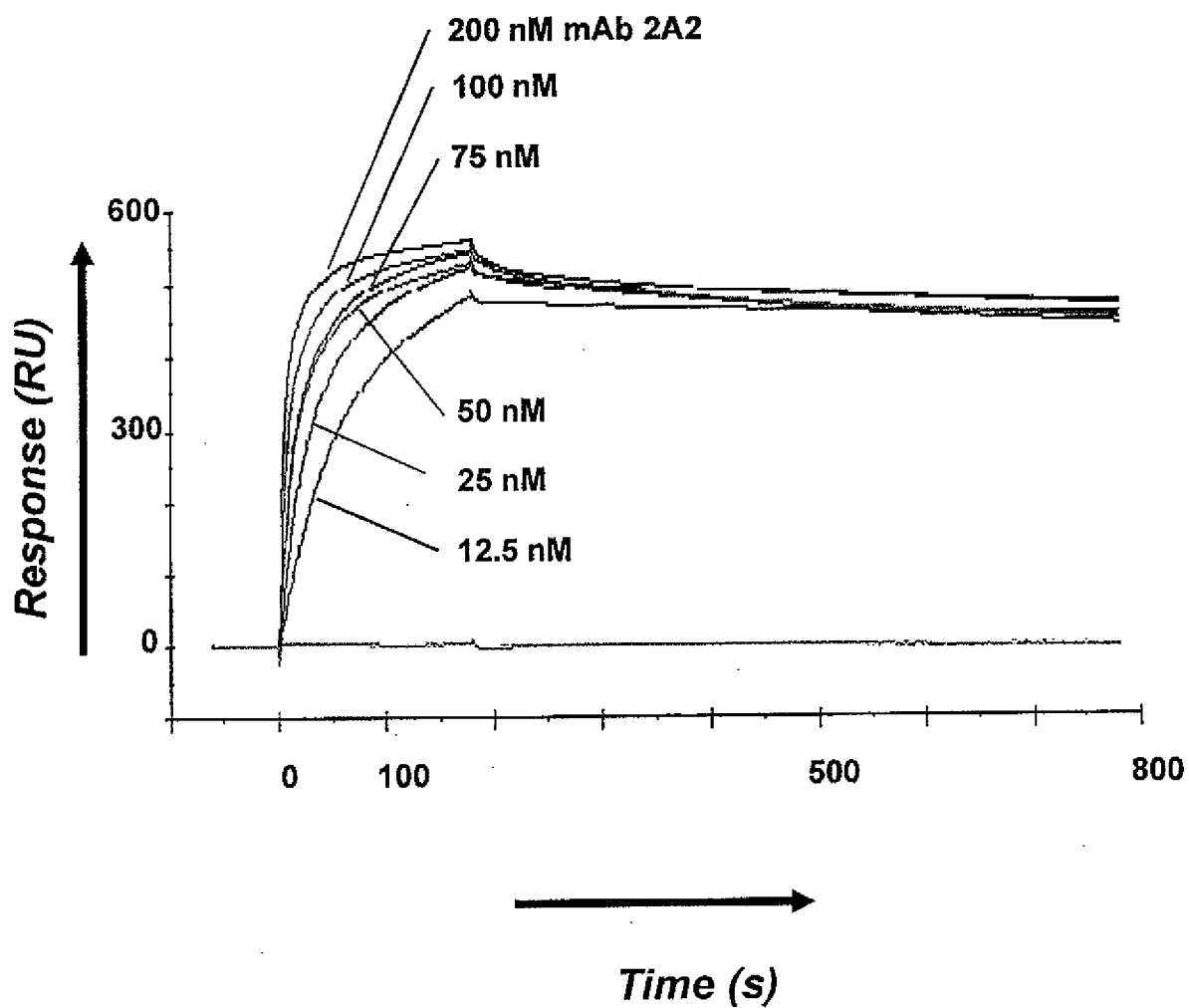
~ 50 kDa

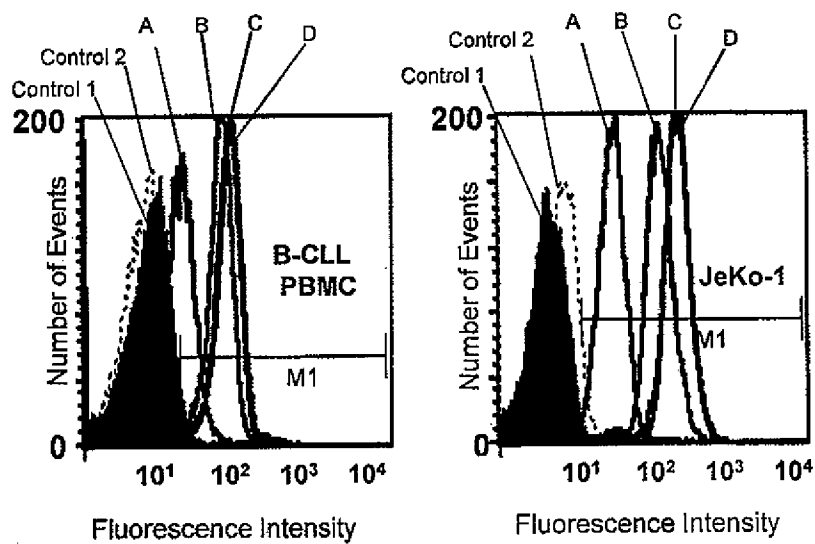
Figure 2A**Fc-ROR1**

~150 kDa (dimer)

~ 75 kDa (monomer)

Figure 2B

**Figure 3**



Control 1 - 1 $\mu\text{g/mL}$ (IgG) polyclonal mouse IgG
Control 2 - goat anti-mouse IgG polyclonal antibody-FITC
A - 1 $\mu\text{g/mL}$ (~ 6.5 nM) mAb 2A2
B - 0.1 $\mu\text{g/mL}$ (~ 650 pM) mAb 2A2
C - 0.01 $\mu\text{g/mL}$ (~ 65 pM) mAb 2A2
D - 0.001 $\mu\text{g/mL}$ (~ 6.5 pM) mAb 2A2

Figure 4

mAb 2A2 (VH)

1 QVQLQQSGAELVRPGASVTLSCKASGYTFSDYEMHWVIQT
41 PVHGLEWIG AIDPETGGTAYNQKFKGKAILTADKSSSTAY
81 MELRSLTSEDSAVYYCTGYDYDSFTYWGQGTLLTVSA (SEQ ID NO:1)

- **FR1** QVQLQQSGAELVRPGASVTLSCKASGYTFS (SEQ ID NO:3)
- **CDR1** DYEMH (SEQ ID NO:4)
- **FR2** WVIQTPVHGLEWIG (SEQ ID NO:5)
- **CDR2** AIDPETGGTAYNQKFKG (SEQ ID NO:6)
- **FR3** KAILTADKSSSTAYMELRSLTSEDSAVYYCTG (SEQ ID NO:7)
- **CDR3** YYDYDSFTY (SEQ ID NO:8)
- **FR4** WGQGTLLTVSA (SEQ ID NO:9)

mAb 2A2 (VL)

1 DIVMTQSQKIMSTTVGDRVSITCKASQNVDAAVAWYQQKP
41 GQSPKLLIYSASNRYTGVPDRFTGSGSGTDFTLTISNMQS
81 EDLADYFCQQYDIYPYTFGGGTKLEIK (SEQ ID NO:2)

- **FR1** DIVMTQSQKIMSTTVGDRVSITC (SEQ ID NO:10)
- **CDR1** KASQNVDAAVA (SEQ ID NO:11)
- **FR2** WYQQKPGQSPKLLIY (SEQ ID NO:12)
- **CDR2** SASNRYT (SEQ ID NO:13)
- **FR3** GVPDRFTGSGSGTDFTLTISNMQSEDLADYFC (SEQ ID NO:14)
- **CDR3** QQYDIYPYT (SEQ ID NO:15)
- **FR4** FGGGTKLEIK (SEQ ID NO:16)

Figure 5

2A2 VH (heavy chain variable domain)

Original DNA sequence:

CAGGTTCAACTGCAGCAGTCTGGGGCTGAGCTGGTGAGGCCTGGGGCTTCAGTGACG
CTGTCCTGCAAGGCTTCGGGCTACACATTTTCTGACTATGAAATGCACTGGGTGATT
CAGACACCTGTGCATGGCCTGGAATGGATTGGAGCTATTGATCCTGAAACTGGTGGT
ACTGCCTACAATCAGAAGTTCAAGGGCAAGGCCATACTGACTGCAGACAAATCCTCC
AGCACAGCCTACATGGAGCTCCGCAGCCTGACATCTGAGGACTCTGCCGTCTATTAC
TGTACAGGCTACTATGATTACGACTCGTTTACTTACTGGGGCCAAGGACTCTGGTC
ACTGTCTCTGCA (SEQ ID NO: 17)

Optimized DNA sequence for mammalian expression:

CAAGTGCAGCTTCAACAGTCTGGGGCAGAACTCGTAAGACCAGGAGCATCAGTGACA
CTGTCTTGTAAGCCTCCGGCTATACCTTCTCTGACTACGAGATGCATTGGGTCATC
CAGACACCAAGTACATGGGCTCGAATGGATAGGAGCCATAGATCCAGAGACAGGCGGA
ACAGCATAACAACCAGAAGTTCAAAGGCAAGGCCATTCTCACAGCGGACAAGAGCAGT
AGCACCGCTTACATGGAGTTGCGATCCCTGACCAGTGAGGACTCTGCAGTCTACTAT
TGTACAGGGTACTATGACTACGACTCATTACATATTGGGGGCAGGGTACCTTGGTG
ACTGTCTCCGCT (SEQ ID NO: 18)

2A2 VL (kappa light chain variable domain)

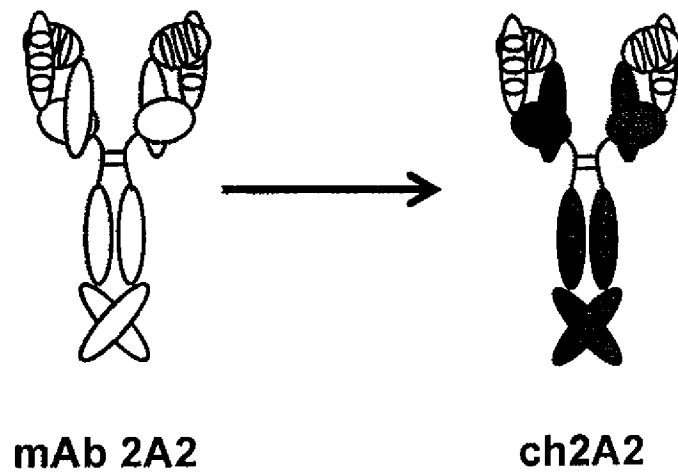
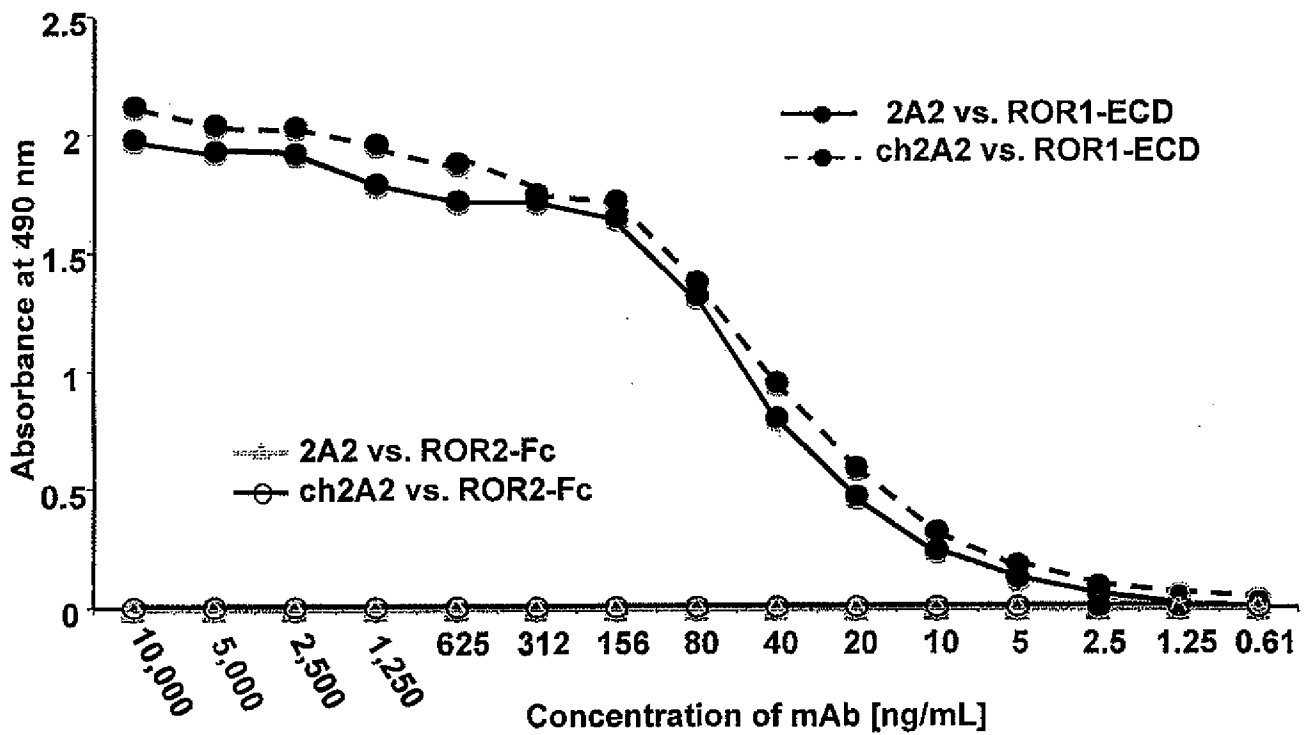
Original DNA sequence:

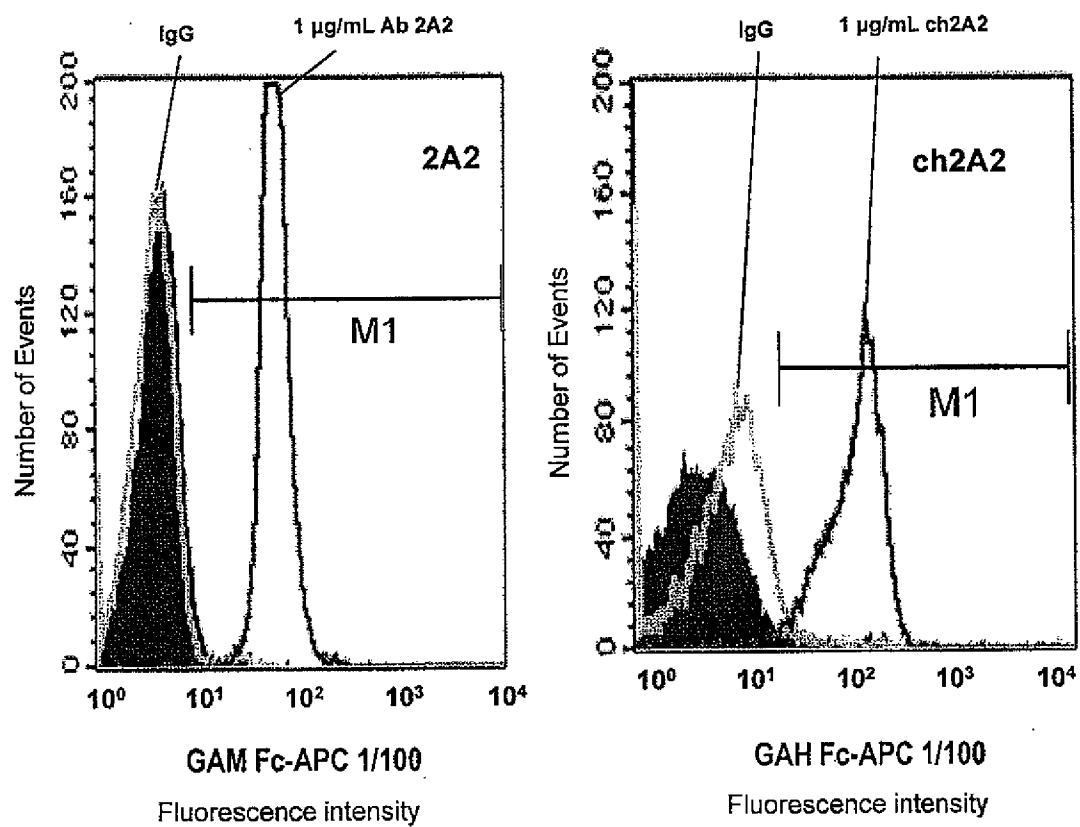
GACATTGTGATGACCCAGTCTCAAAAAATCATGTCCACAACAGTGGGAGACAGGGTC
AGCATCACCTGCAAGGCCAGTCAGAATGTGGATGCTGCTGTAGCCTGGTATCAACAG
AAACCAGGACAATCTCCTAAACTACTGATTTACTCAGCATCCAATCGGTACACTGGA
GTCCCTGATCGCTTTCACAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATTAGC
AATATGCAGTCTGAAGACCTGGCAGATTATTTCTGTCAGCAATATGACATCTATCCG
TACACGTTCCGGAGGGGGGACCAAGCTGGAAATAAAA (SEQ ID NO: 19)

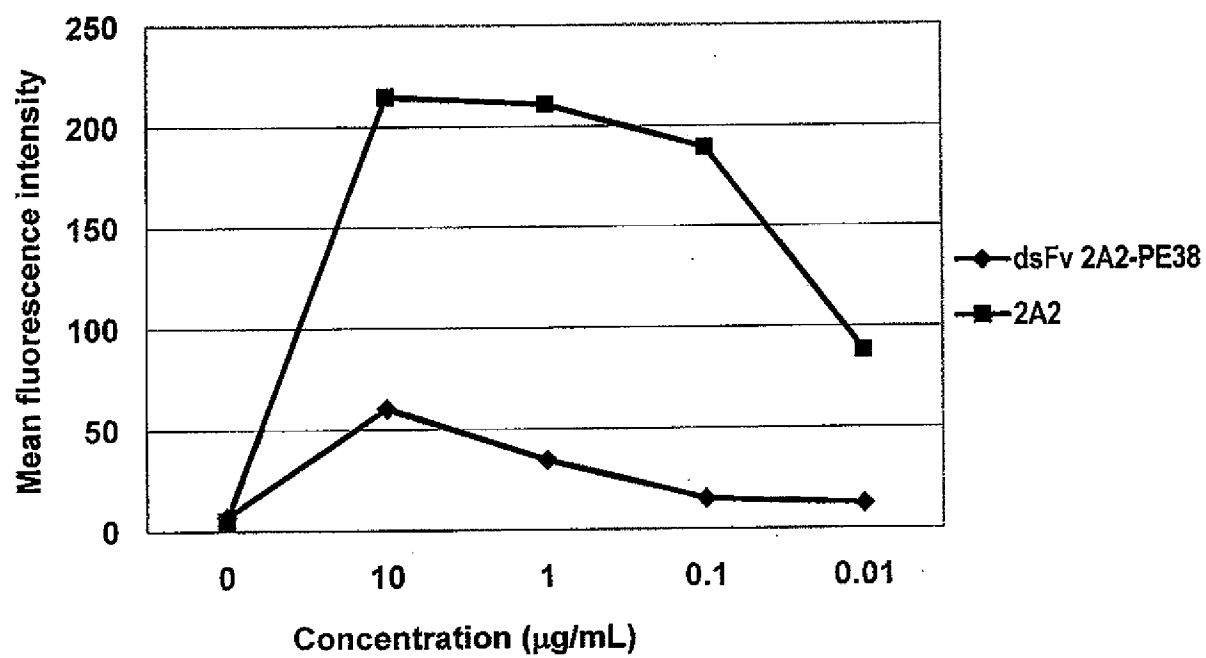
Optimized DNA sequence for mammalian expression:

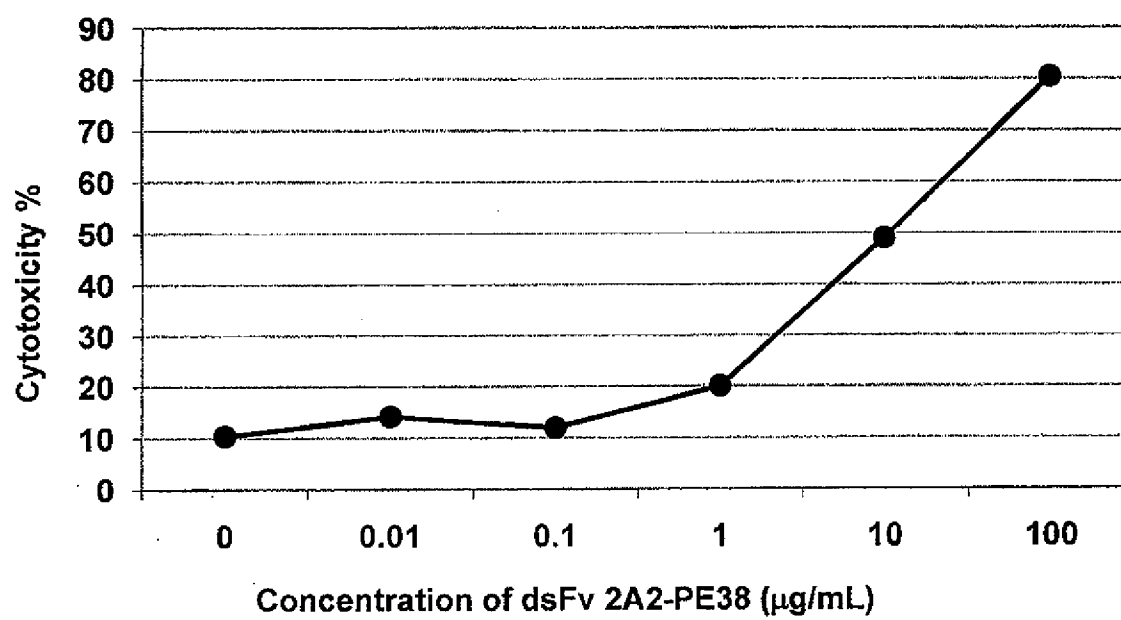
GATATAGTGATGACGCAGTCCCAGAAGATCATGTCCACGACCGTCGGGGATCGGGTC
AGTATAACATGTAAGGCATCCCAGAACGTGGACGCGGCCGTGGCTTGGTATCAACAG
AAACCCGGTCAATCCCCAAAGCTCCTCATCTACTCTGCGAGCAATAGATATACCGGT
GTGCCTGATAGGTTACCCGGAAGCGGATCCGGAACAGATTTACCCCTGACTATCAGC
AATATGCAATCCGAGGACTTGGCTGACTACTTTTGCCAGCAATACGACATATACCCC
TACACCTTCGGCGGAGGCACAAAGCTCGAGATAAAG (SEQ ID NO: 20)

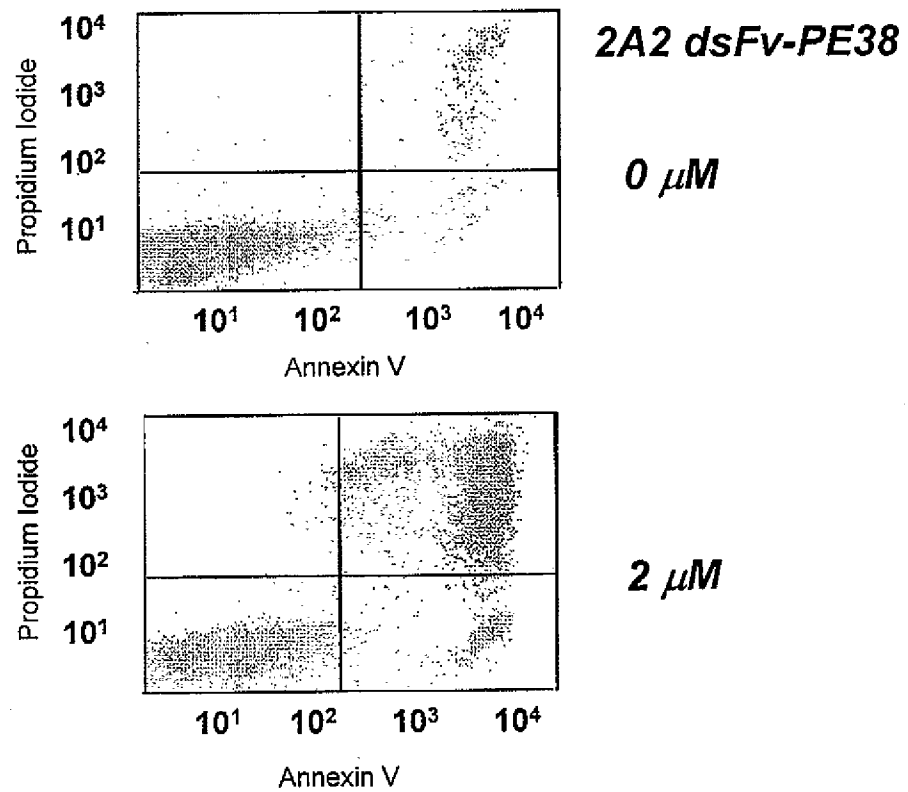
Figure 6

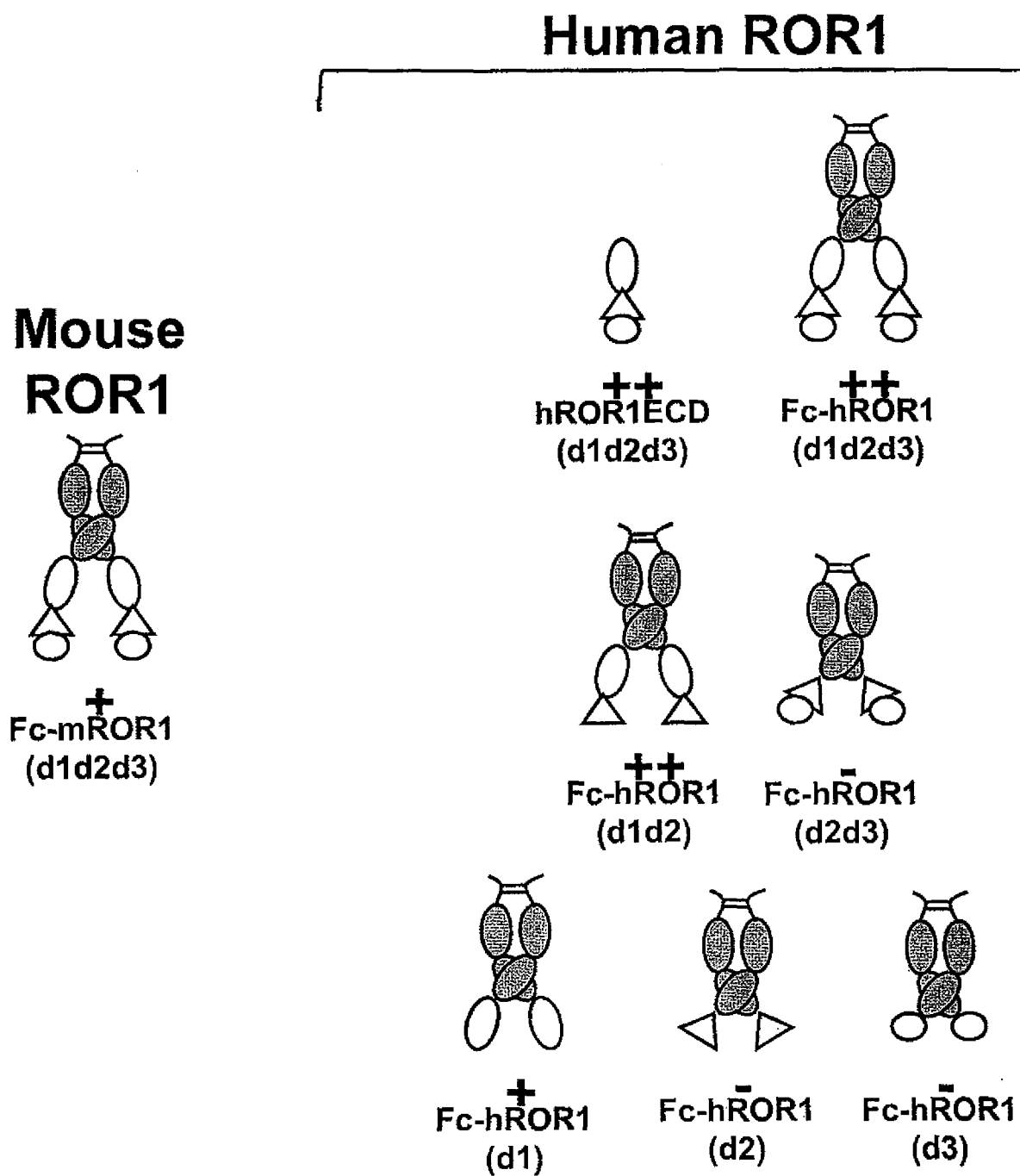
**Figure 7A****Figure 7B**

**Figure 8**

**Figure 9**

**Figure 10**

**Figure 11**

**Figure 12**

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/032208

| A. CLASSIFICATION OF SUBJECT MATTER INV. C07K16/28 C07K16/30 A61K39/395 ADD. | | |
|---|--|--|
| 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 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 practical, search terms used) EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | BASKAR SIVASUBRAMANIAN ET AL: "Targeting Human B Cell Chronic Lymphocytic Leukemia With a Monoclonal Antibody Specific for the Receptor Tyrosine Kinase ROR1" JOURNAL OF IMMUNOTHERAPY, LIPPINCOTT WILLIAMS & WILKINS, HAGERSTOWN, MD, US, vol. 31, no. 9, 1 January 2008 (2008-01-01), page 969, XP009135123 ISSN: 1524-9557 * abstract ----- -/-- | 1-42 |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input checked="" type="checkbox"/> 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 document 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 |
| 24 June 2010 | | 06/07/2010 |
| Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016 | | Authorized officer: Bernhardt, Wiebke |

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2010/032208

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | <p>BROOME H ELIZABETH ET AL: "Detection of Minimal Residual Disease in Chronic Lymphocytic Leukemia with Monoclonal Antibodies Specific for CD5, CD10, CD19, and ROR1"</p> <p>BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 112, no. 11, 9 December 2008 (2008-12-09), page 724, XP009135125</p> <p>ISSN: 0006-4971</p> <p>* abstract</p> | 1-42 |
| X | <p>WO 2007/146957 A2 (IRM LLC [BM]; WARMUTH MARKUS [US]; FOSTER KARIN [US] IRM LLC; WARMUTH) 21 December 2007 (2007-12-21)</p> <p>pg. 2-3, examples, claims</p> | 1-42 |
| X | <p>WO 2007/051077 A2 (UNIV CALIFORNIA [US]; KIPPS THOMAS J [US]; FUKUDA TETSUYA [JP]; ENDO T) 3 May 2007 (2007-05-03)</p> <p>pg. 1-13, examples, claims</p> | 1-42 |
| X | <p>LITTLE M ET AL: "Of mice and men: hybridoma and recombinant antibodies"</p> <p>IMMUNOLOGY TODAY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB LNKD-</p> <p>DOI:10.1016/S0167-5699(00)01668-6, vol. 21, no. 8, 1 August 2000 (2000-08-01), pages 364-370, XP004215163</p> <p>ISSN: 0167-5699</p> <p>pg. 367, fig. 3</p> | 1, 14, 15 |
| X | <p>DAVIES J ET AL: "Affinity improvement of single antibody VH domains: residues in all three hypervariable regions affect antigen binding"</p> <p>IMMUNOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS BV, NL LNKD-</p> <p>DOI:10.1016/S1380-2933(96)00045-0, vol. 2, no. 3, 1 September 1996 (1996-09-01), pages 169-179, XP004070292</p> <p>ISSN: 1380-2933</p> <p>* abstract</p> | 14 |
| X | <p>HOLT L J ET AL: "Domain antibodies: proteins for therapy"</p> <p>TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB LNKD-</p> <p>DOI:10.1016/J.TIBTECH.2003.08.007, vol. 21, no. 11, 1 November 2003 (2003-11-01), pages 484-490, XP004467495</p> <p>ISSN: 0167-7799</p> <p>* abstract</p> | 14 |

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2010/032208

| C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | |
|--|---|-----------------------|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| A | <p>BASKAR SIVASUBRAMANIAN ET AL: "Unique cell surface expression of receptor tyrosine kinase ROR1 in human B-cell chronic lymphocytic leukemia." CLINICAL CANCER RESEARCH : AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH 15 JAN 2008 LNKD-PUBMED:18223214, vol. 14, no. 2, 15 January 2008 (2008-01-15), pages 396-404, XP009135221 ISSN: 1078-0432 abstract pg. 398, left-hand col. - pg. 399, left-hand col.</p> | 1-42 |
| A | <p>CARSON DENNIS A ET AL: "Restricted expression of the orphan tyrosine kinase receptor ROR1 in chronic lymphocytic leukemia" BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 104, no. 11 Part 1, 1 November 2004 (2004-11-01), page 221A, XP009100203 ISSN: 0006-4971 * abstract</p> | 1-42 |
| A | <p>FUKUDA TETSUYA ET AL: "Immune therapy for chronic lymphocytic leukemia induces the antibody response against a novel tumor-associated antigen, the orphan tyrosine kinase receptor ROR1" BLOOD, AMERICAN SOCIETY OF HEMATOLOGY, US, vol. 106, no. 11, Part 1, 1 November 2005 (2005-11-01), page 834A, XP009104045 ISSN: 0006-4971 * abstract</p> | 1-42 |
| A | <p>FUKUDA TETSUYA ET AL: "Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal antigen and receptor for Wnt5a" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 105, no. 8, February 2008 (2008-02), pages 3047-3052, XP009135222 ISSN: 0027-8424 the whole document</p> | 1-42 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2010/032208

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| WO 2007146957 A2 | 21-12-2007 | NONE | |
| WO 2007051077 A2 | 03-05-2007 | EP 1948680 A2 | 30-07-2008 |