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(54) Title: ANTI-RORI ANTIBODIES

(57) Abstract: The invention provides antibodies which bind to the extracellular domain of the Tyrosine- protein kinase transmembrane receptor ROR1. Nucleic acid molecules encoding the antibodies, expression vectors, host cells and methods for expressing the antibodies are also provided. The antibodies may be used for the treatment of cancer, including non-small cell lung carcinoma, B-cell chronic lymphocytic leukemia and colon cancer.

ANTI-ROR1 ANTIBODIES

[0001] .This patent application claims the benefit of U.S. Provisional Patent Application Nos. 61/388,694 filed on October 1, 2010 and 61/482,554 filed on May 4, 2011, both of which are incorporated by reference in their entirety.

INTRODUCTION

[0002] The present disclosure relates generally to the fields of immunology and molecular biology. More specifically, provided herein are antibodies and other therapeutic proteins directed against the Tyrosine-protein kinase transmembrane receptor ROR1, nucleic acids encoding such antibodies and therapeutic proteins, methods for preparing monoclonal antibodies and other therapeutic proteins, and methods for the treatment of diseases, such as cancers mediated by the Tyrosine-protein kinase transmembrane receptor ROR1 expression/activity and/or associated with abnormal expression/activity of ligands therefore.

BACKGROUND

[0003] The tyrosine-protein kinase transmembrane receptor ROR1 is a cell surface receptor that belongs to the ROR subfamily of cell surface proteins. It shows strong homology to the tyrosine kinase domain of growth factor receptors, in particular the Trk family [Reddy et al. (1997) Genomics 41(2):283-5] and modulates neurite growth in the central nervous system. So far two isoforms have been reported (SWISS-PROT entry; Q01973) and the nucleotide sequence encoding this protein is found at accession number NM_005012.

[0004] According to SWISS-PROT, the tyrosine-protein kinase transmembrane receptor ROR1 is expressed strongly in human heart, lung, and kidney, but weakly in the CNS. The short isoform (missing amino acids 1–549 of the long isoform) is strongly expressed in fetal and adult CNS and in a variety of human cancers, including those originating from CNS or PNS neuroectoderm. The tyrosine-protein kinase transmembrane receptor ROR1 (described as ROR1 henceforward) is expressed at high levels during early embryonic development. The expression levels drop strongly around day 16 and there are only very low levels in adult tissues. Over-expression of the ROR1 gene has recently been reported in B-cell chronic lymphocytic leukemia [Basker et al. (2008) Clin Cancer Res. 14(2):396-404].

[0005] BRIEF DESCRIPTION OF THE DRAWINGS

[0006] **Figure 1** depicts (a) the alignment of the nucleotide sequences of the light chain CDR1 regions of A1 (SEQ ID NO:181); A2, A3, A4, A5, A7, A9 and A12 (SEQ ID NO:182); A6 (SEQ ID NO:183); A10 (SEQ ID NO:185) and A13 (SEQ ID NO:187) with nucleotides 813-848 of the mouse germline V_K 14-111 nucleotide sequence (SEQ ID NO: 243); the alignments of the nucleotide sequences of the light chain CDR1 regions of A8 (SEQ ID NO:184) and A11 (SEQ ID NO:186)with nucleotides 594-629 of the mouse germline V_k 17-121 nucleotide sequence (SEQ ID NO:255) and the alignment of the light chain CDR1 region of A14 (SEQ ID NO:188) with nucleotides 430-465 of the mouse germline V_k 8-21 nucleotide sequence (SEQ ID NO:260), while (b) shows the alignment of the light chain CDR1 region of A14 (SEQ ID NO:189) with nucleotides 421-471 of the mouse germline V_k 8-21 nucleotide sequence (SEQ ID NO: 261).

[0007] **Figure 2** depicts (a) the alignment of the nucleotide sequences of the light chain CDR2 regions of A1, A2, A9 and A10 (SEQ ID NO:210); A3 (SEQ ID NO:212); A4 (SEQ ID NO:213); A5 (SEQ ID NO:215); A6 (SEQ ID NO:217); A7 (SEQ ID NO:219); A12 (SEQ ID NO:225) and A13 (SEQ ID NO:226) with nucleotides 879-908 of the mouse germline V_K 14-111 nucleotide sequence (SEQ ID NO:245); the alignments of the nucleotide sequences of the light chain CDR2 regions of A8 (SEQ ID NO:221) and A11 (SEQ ID NO:223) with nucleotides 660-689 of the mouse germline V_k 17-121 nucleotide sequence (SEQ ID NO:257) and the alignment of the light chain CDR2 region of A14 (SEQ ID NO:227) with nucleotides 502-534 of the mouse germline V_k 8-21 nucleotide sequence (SEQ ID NO:262), while (b) shows the alignment of the nucleotide sequences of the light chain CDR2 regions of A1, A2, A3, A9, A10, A12 and A13 (SEQ ID NO:209); A4 (SEQ ID NO:214); A5 (SEQ ID NO:216); A6 (SEQ ID NO:218) and A7 (SEQ ID NO:220) with nucleotides 879-908 of the mouse germline V_K 14-111 nucleotide sequence (SEQ ID NO:244); the alignments of the nucleotide sequences of the light chain CDR2 regions of A8 (SEQ ID NO:222) and A11 (SEQ ID NO:224) with nucleotides 660-689 of the mouse germline V_k 17-121 nucleotide sequence (SEQ ID NO:258) and the alignment of the light chain CDR2 region of A14 (SEQ ID NO:228) with nucleotides 514-537of the mouse germline V_k 8-21 nucleotide sequence (SEQ ID NO:263).

[0008] **Figure 3** depicts the alignment of the nucleotide sequences of the light chain CDR3 regions of A1, A3, A4, A6, A9, A10, A12 and A13 (SEQ ID NO:234); A2 (SEQ ID NO:235); A5 (SEQ ID NO:236) and A7 (SEQ ID NO:237) with nucleotides 1008-1034 of the mouse germline V_K 14-111 nucleotide sequence (SEQ ID NO:246); the alignments of the nucleotide sequences of the light chain CDR3 regions of A8 (SEQ ID NO:238) and A11 (SEQ ID NO:239) with nucleotides 789-815 of the mouse germline V_k 17-121 nucleotide sequence (SEQ ID NO:259) and the alignment of the light

chain CDR2 region of A14 (SEQ ID NO:240) with nucleotides 634-658 of the mouse germline V_k 8-21 nucleotide sequence (SEQ ID NO:264).

[0009] Figure 4 depicts (a) the alignment of the nucleotide sequences of the heavy chain CDR1 regions of A14 (SEQ ID NO:97) with nucleotides 86263-86299 of the mouse germline V_H 1-9 nucleotide sequence (SEQ ID NO:265); the alignments of the nucleotide sequences of the heavy chain CDR1 regions of A2 (SEQ ID NO:80); A12 and A13 (SEQ ID NO:82); A10 (SEQ ID NO:93) and A11 (SEQ ID NO:95) with nucleotides 926028-926063 of the mouse germline V_H 5-12 nucleotide sequence (SEQ ID NO:247) and the alignment of the heavy chain CDR1 region of A1 (SEQ ID NO:78); A3 and A8 (SEQ ID NO:81); A4 (SEQ ID NO:83); A5 (SEQ ID NO:85); A6 (SEQ ID NO:87); A7 (SEQ ID NO:89) and A9 (SEQ ID NO:91) with nucleotides 983326-983361 of the mouse germline V_H 5-6 nucleotide sequence (SEQ ID NO:251) while, (b) shows the alignment of the nucleotide sequences of the heavy chain CDR1 regions of A14 (SEQ ID NO:98) with nucleotides 86263-86299 of the mouse germline V_H 1-9 nucleotide sequence (SEQ ID NO:256); the alignments of the nucleotide sequences of the heavy chain CDR1 regions of A2 (SEQ ID NO:81); A12 and A13 (SEQ ID NO:79); A10 (SEQ ID NO:94) and A11 (SEQ ID NO:96) with nucleotides 926022-926063 of the mouse germline V_H 5-12 nucleotide sequence (SEQ ID NO:248) and the alignment of the heavy chain CDR1 region of A1, A3, A8 (SEQ ID NO:79); A4 (SEQ ID NO:84); A5 (SEQ ID NO:86); A6 (SEQ ID NO:88); A7 (SEQ ID NO:90) and A9 (SEQ ID NO:92) with nucleotides 983320-983361 of the mouse germline V_H 5-6 nucleotide sequence (SEQ ID NO:252).

[0010] Figure 5 depicts (a) the alignment of the nucleotide sequences of the heavy chain CDR2 regions of A14 (SEQ ID NO:147) with nucleotides 86341-86370 of the mouse germline V_H 1-9 nucleotide sequence (SEQ ID NO:266); the alignments of the nucleotide sequences of the heavy chain CDR2 regions of A2 and A13 (SEQ ID NO:126); A12 (SEQ ID NO:135); A10 (SEQ ID NO:142) and A11 (SEQ ID NO:144) with nucleotides 926106-926135 of the mouse germline V_H 5-12 nucleotide sequence (SEQ ID NO:1249) and the alignment of the heavy chain CDR2 region of A1 (SEQ ID NO:124); A3 (SEQ ID NO:128); A4 (SEQ ID NO:130); A5 (SEQ ID NO:132); A6 (SEQ ID NO:135); A7 (SEQ ID NO:136); A8 (SEQ ID NO:138) and A9 (SEQ ID NO:140) with nucleotides 983404-983433 of the mouse germline V_H 5-6 nucleotide sequence (SEQ ID NO:253), while (b) shows the alignment of the nucleotide sequences of the heavy chain CDR2 regions of A14 (SEQ ID NO:148) with nucleotides 86341-86391 of the mouse germline V_H 1-9 nucleotide sequence (SEQ ID NO:267); the alignments of the nucleotide sequences of the heavy chain CDR2 regions of A2 and A13 (SEQ ID NO:127); A12 (SEQ ID NO:146); A10 (SEQ ID NO:143) and A11 (SEQ ID NO:242) with nucleotides 926106-926156 of the mouse germline V_H 5-12 nucleotide sequence (SEQ ID

NO:250) and the alignment of the heavy chain CDR2 region of A1 (SEQ ID NO:125); A3 (SEQ ID NO:129); A4 (SEQ ID NO:131); A5 (SEQ ID NO:133); A6 (SEQ ID NO:134); A7 (SEQ ID NO:137); A8 (SEQ ID NO:139) and A9 (SEQ ID NO:141) with nucleotides 983404-983454 of the mouse germline V_H 5-6 nucleotide sequence (SEQ ID NO:254).

[0011] Figure 6 depicts the flow cytometry analysis of ROR1 monoclonal antibodies, indicating the specific binding of those antibodies to the human lung adenocarcinoma cell line, A549.

[0012] Figure 7 depicts the flow cytometry analysis of ROR1_A1, ROR1_A3, ROR1_A8 and ROR1_A14 monoclonal antibodies, indicating the specific binding of those antibodies to the human cancer cell lines including CALU1, H358, PANC-1, H226, H69, A549 and HT-29.

[0013] Figure 8 depicts the flow cytometry analysis of ROR1 chimeric and humanised monoclonal antibodies, indicating the specific binding of those antibodies to the human lung adenocarcinoma cell line, A549 (a) and human colon adenocarcinoma cell line, HT-29 (b).

[0014] Figure 9 depicts the internalization of ROR1_A11 by the human colon adenocarcinoma cell line, HT29, at intervals 0 min, 15 min, 60 min and 120 min.

[0015] Figure 10 depicts the internalisation of ROR1_A1 and ROR1_A3 monoclonal antibodies by the ZAP assay to the human epidermoid lung carcinoma cell line, CALU1.

[0016] Figure 11 depicts the internalisation of ROR1_A1 and ROR1_A3 monoclonal antibodies by the ZAP assay to the human pancreatic carcinoma cell line, PANC1.

[0017] Figure 12 depicts the internalisation of ROR1_A1 chimeric and humanised monoclonal antibodies by the ZAP assay to the human lung adenocarcinoma cell line, A549.

[0018] Figure 13 depicts the internalisation of ROR1_A1 chimeric and humanised monoclonal antibodies by the ZAP assay to the human colon adenocarcinoma cell line, HT29.

[0019] Figure 14 depicts the amino acid and nucleotide sequences of the variable heavy chain and variable light chain of antibodies of the invention, including CDRs and antigen binding regions, as well as the ROR1 sequence.

[0020] Figure 15 depicts a summary of the amino acids and nucleic acids encoding the antigen binding regions, such as CDRs, of the heavy and light chain variable regions of the invention.

BRIEF SUMMARY OF THE INVENTION

[0021] The present disclosure provides antibodies directed against the ROR1, nucleic acids encoding such antibodies and therapeutic proteins, host cells comprising such nucleic acids encoding the antibodies of the invention, methods for preparing anti-ROR1, and methods for the treatment of diseases, such as the ROR1 mediated disorders, e.g. human cancers, including non-small cell lung carcinoma, B-cell chronic lymphocytic leukemia and colon cancer.

[0022] In some embodiments, an antibody that specifically binds to the extracellular domain of ROR1 (SEQ ID NO:272) and is internalized by a cell expressing ROR1 is provided, the antibody encompassing a heavy chain variable region encompassing a first vhCDR encompassing SEQ ID NO:275; a second vhCDR encompassing SEQ ID NO: 277; and a third vhCDR encompassing SEQ ID NO:279; and a light chain variable region encompassing a first vlCDR encompassing SEQ ID NO:281; a second vlCDR encompassing SEQ ID NO: 283; and a third vlCDR encompassing SEQ ID NO:285.

[0023] In some embodiments, the antibodies of the invention further encompass a covalently attached moiety. In some embodiments, the moiety is a drug. In other embodiments, the drug is selected from the group a maytansinoid, a dolastatin, an auristatin, a trichothecene, a calicheamicin, a CC1065 and derivatives thereof.

[0024] In some embodiments, a nucleic acid encoding a heavy chain of any of the disclosed antibodies of the invention are provided. In some embodiments a nucleic acid encoding a light chain of the disclosed antibodies of the invnetion are provided.

[0025] In some embodiments a host cell containing the nucleic acid(s) encoding the heavy or light chain or both of the antibodies of the invention are provided wherien the host cell is grown under conidtions wherein the nucleic acid(s) is expressed. In other embodiments, a method of recovering one or more antibodies of the invention are provided.

[0026] In some embodiments a method of treating cancer is provided, wherein a patient in need thereof is adminstered an antibody or antibodies of the invention which bind to ROR1 (SEQ ID NO:272) and wherein such antibody or antibodies of the invention are internalized, the antibody encopassing a heavy chain variable region encompassing a first vhCDR

comprising SEQ ID NO:275; a second vh CDR encompassing SEQ ID NO: 277; and a third vhCDR encompassing SEQ ID NO: 279 and a light chain variable region encompassing a first vlCDR encompassing Seq ID NO: 281, a second vlCDR encompassing SEQ ID NO: 283 and a third vlCDR encompassing SEQ ID NO: 285 and a covalently attached drug conjugate.

[0027] In some embodiments, the cancer is selected from the group of non-small cell lung carcinoma, B-cell chronic lymphocytic leukemia and colon cancer.

[0028] In some embodiments, an antibody comprising a variable heavy chain region selected from the group consisting of SEQ ID NOs:1-14 or a sequence that is about 90% identical to one of SEQ ID NOs:1-14 is provided. In other embodiments an antibody comprising a variable light chain region selected from the group consisting of SEQ ID NOs:29-42 or a sequence that is about 90% identical to one of SEQ ID NOs:29-42 is provided.

DETAILED DESCRIPTION OF THE INVENTION

Introduction

[0029] The present disclosure relates to isolated antibodies which bind specifically to the extracellular domain of the ROR1 receptor with high affinity as outlined herein. As discussed herein, the ROR1 receptor is reported to have two isoforms, one of which is missing the first 549 amino acids ("the short form") of the "long form". In some embodiments, the antibodies discussed herein bind to the extracellular domain of the short form.

[0030] In addition, the ROR1 antibodies of the present invention are internalized when contacted with cells expressing the ROR1 receptor. As discussed herein, the ROR1 receptor is overexpressed and/or differentially expressed on certain cancer cells, including but not limited to, tumors of non-small cell lung carcinoma, B-cell chronic lymphocytic leukemia and colon cancer.

[0031] As such, when the ROR1 antibodies of the present invention are conjugated to drugs (sometimes referred to herein as "antibody-drug conjugates" or "ADCs"), the internalization of these ADC molecules into cancer cells results in cell death and thus tumor treatment.

[0032] The present invention provides antibodies that possess particular structural features such as CDR regions with particular amino acid sequences. As described herein, 14 different

sets of CDRs, e.g. 14 different antibodies, exhibit binding to ROR1. In addition, as described herein, each CDR shows similarity to other identified CDRs, and thus antibodies comprising consensus CDRs are provided, as is more fully described below.

[0033] Thus, the disclosure provides isolated antibodies (which, as outlined below, includes a wide variety of well known antibody structures, derivatives, mimetics and conjugates), nucleic acids encoding these antibodies, host cells used to make the antibodies, methods of making the antibodies, and pharmaceutical compositions comprising the antibodies and optionally a pharmaceutical carrier.

ROR1 Proteins

[0034] Accordingly, the present invention provides isolated anti-ROR1 antibodies that specifically bind the extracellular domain of a human ROR1 protein. By "human ROR1" or "human ROR1 antigen" refers to the protein of SEQ ID NO:272 or a functional fraction such as the extracellular domain, as defined herein. In general, ROR1 possesses a short intracytoplasmic tail, a transmembrane domain, and an extracellular domain. In specific embodiments, the antibodies of the invention bind to the extracellular part of the ROR1 protein, and in some embodiments, to the extracellular domain of the shorter version of ROR1.

[0035] The antibodies of the invention may, in certain cases, cross-react with the ROR1 from species other than human. For example, to facilitate clinical testing, the antibodies of the invention may cross react with murine or primate ROR1 molecules. Alternatively, in certain embodiments, the antibodies may be completely specific for one or more human ROR1 and may not exhibit species or other types of non-human cross-reactivity.

Antibodies

[0036] The present invention provides anti-ROR1 antibodies, generally therapeutic and/or diagnostic antibodies as described herein. Antibodies that find use in the present invention can take on a number of formats as described herein, including traditional antibodies as well as antibody derivatives, fragments and mimetics, described below. Essentially, the invention provides antibody structures that contain a set of 6 CDRs as defined herein (including small numbers of amino acid changes as described below).

[0037] "Antibody" as used herein includes a wide variety of structures, as will be appreciated by those in the art, that at a minimum contain a set of 6 CDRs as defined herein; including, but not limited to traditional antibodies (including both monoclonal and polyclonal antibodies), humanized and/or chimeric antibodies, antibody fragments, engineered antibodies (e.g. with amino acid modifications as outlined below), multispecific antibodies (including bispecific antibodies), and other analogs known in the art and discussed herein.

[0038] Traditional antibody structural units typically comprise a tetramer. Each tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one "light" (typically having a molecular weight of about 25 kDa) and one "heavy" chain (typically having a molecular weight of about 50-70 kDa). Human light chains are classified as kappa and lambda light chains. Heavy chains are classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. IgG has several subclasses, including, but not limited to IgG1, IgG2, IgG3, and IgG4. IgM has subclasses, including, but not limited to, IgM1 and IgM2. Thus, "isotype" as used herein is meant any of the subclasses of immunoglobulins defined by the chemical and antigenic characteristics of their constant regions. The known human immunoglobulin isotypes are IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM1, IgM2, IgD, and IgE. It should be understood that therapeutic antibodies can also comprise hybrids of any combination of isotypes and/or subclasses.

[0039] In many embodiments, IgG isotypes are used in the present invention, with IgG1 finding particular use in a number of applications.

[0040] The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. In the variable region, three loops are gathered for each of the V domains of the heavy chain and light chain to form an antigen-binding site. Each of the loops is referred to as a complementarity-determining region (hereinafter referred to as a "CDR"), in which the variation in the amino acid sequence is most significant. "Variable" refers to the fact that certain segments of the variable region differ extensively in sequence among antibodies. Variability within the variable region is not evenly distributed. Instead, the V regions consist of relatively invariant stretches called

framework regions (FRs) of 15-30 amino acids separated by shorter regions of extreme variability called "hypervariable regions" that are each 9-15 amino acids long or longer.

[0041] Each VH and VL is composed of three hypervariable regions ("complementary determining regions," "CDRs") and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4.

[0042] The hypervariable region generally encompasses amino acid residues from about amino acid residues 24-34 (LCDR1; "L" denotes light chain), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable region and around about 31-35B (HCDR1; "H" denotes heavy chain), 50-65 (HCDR2), and 95-102 (HCDR3) in the heavy chain variable region; Kabat et al., SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991) and/or those residues forming a hypervariable loop (e.g. residues 26-32 (LCDR1), 50-52 (LCDR2) and 91-96 (LCDR3) in the light chain variable region and 26-32 (HCDR1), 53-55 (HCDR2) and 96-101 (HCDR3) in the heavy chain variable region; Chothia and Lesk (1987) J. Mol. Biol. 196:901-917. Specific CDRs of the invention are described below

[0043] In some cases, defining the exact CDR region is difficult. The CDRs of the antibodies of the present invention have been identified in two different ways, in general a "longer" CDR and a "shorter" CDR. For example, as shown in Figure **XX, SEQ ID NO:57 and SEQ ID NO:58 both describe the first CDR of the heavy chain of the first antibody (A1).

[0044] Throughout the present specification, the Kabat numbering system is generally used when referring to a residue in the variable domain (approximately, residues 1-107 of the light chain variable region and residues 1-113 of the heavy chain variable region) (e.g, Kabat et al., supra (1991)).

[0045] The CDRs contribute to the formation of the antigen-binding, or more specifically, epitope binding site of antibodies. "Epitope" refers to a determinant that interacts with a specific antigen binding site in the variable region of an antibody molecule known as a paratope. Epitopes are groupings of molecules such as amino acids or sugar side chains and usually have specific structural characteristics, as well as specific charge characteristics. A single antigen may have more than one epitope. In the present invention, the exact epitope is

not determinative; rather, the ability of the antibodies of the invention to bind to the ROR1 receptor and be internalized is important.

[0046] The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Kabat et al. collected numerous primary sequences of the variable regions of heavy chains and light chains. Based on the degree of conservation of the sequences, they classified individual primary sequences into the CDR and the framework and made a list thereof (see SEQUENCES OF IMMUNOLOGICAL INTEREST, 5th edition, NIH publication, No. 91-3242, E.A. Kabat et al., entirely incorporated by reference).

[0047] In the IgG subclass of immunoglobulins, there are several immunoglobulin domains in the heavy chain. By "immunoglobulin (Ig) domain" herein is meant a region of an immunoglobulin having a distinct tertiary structure. Of interest in the present invention are the heavy chain domains, including, the constant heavy (CH) domains and the hinge domains. In the context of IgG antibodies, the IgG isotypes each have three CH regions. Accordingly, "CH" domains in the context of IgG are as follows: "CH1" refers to positions 118-220 according to the EU index as in Kabat. "CH2" refers to positions 237-340 according to the EU index as in Kabat, and "CH3" refers to positions 341-447 according to the EU index as in Kabat.

[0048] Another type of Ig domain of the heavy chain is the hinge region. By "hinge" or "hinge region" or "antibody hinge region" or "immunoglobulin hinge region" herein is meant the flexible polypeptide comprising the amino acids between the first and second constant domains of an antibody. Structurally, the IgG CH1 domain ends at EU position 220, and the IgG CH2 domain begins at residue EU position 237. Thus for IgG the antibody hinge is herein defined to include positions 221 (D221 in IgG1) to 236 (G236 in IgG1), wherein the numbering is according to the EU index as in Kabat. In some embodiments, for example in the context of an Fc region, the lower hinge is included, with the "lower hinge" generally referring to positions 226 or 230.

[0049] Of particular interest in the present invention are the Fc regions. By "Fc" or "Fc region" or "Fc domain" as used herein is meant the polypeptide comprising the constant region of an antibody excluding the first constant region immunoglobulin domain and in some cases, part of the hinge. Thus Fc refers to the last two constant region immunoglobulin

domains of IgA, IgD, and IgG, the last three constant region immunoglobulin domains of IgE and IgM, and the flexible hinge N-terminal to these domains. For IgA and IgM, Fc may include the J chain. For IgG, the Fc domain comprises immunoglobulin domains Cγ2 and Cγ3 (Cγ2 and Cγ3) and the lower hinge region between Cγ1 (Cγ1) and Cγ2 (Cγ2). Although the boundaries of the Fc region may vary, the human IgG heavy chain Fc region is usually defined to include residues C226 or P230 to its carboxyl-terminus, wherein the numbering is according to the EU index as in Kabat. In some embodiments, as is more fully described below, amino acid modifications are made to the Fc region, for example to alter binding to one or more FcγR receptors or to the FcRn receptor.

[0050] In some embodiments, the antibodies are full length. By "full length antibody" herein is meant the structure that constitutes the natural biological form of an antibody, including variable and constant regions, including one or more modifications as outlined herein.

[0051] Alternatively, the antibodies can be a variety of structures, including, but not limited to, antibody fragments, monoclonal antibodies, bispecific antibodies, minibodies, domain antibodies, synthetic antibodies (sometimes referred to herein as "antibody mimetics"), chimeric antibodies, humanized antibodies, antibody fusions (sometimes referred to as "antibody conjugates"), and fragments of each, respectively. Structures that rely on the use of a set of CDRs are included within the definition of "antibody".

[0052] In one embodiment, the antibody is an antibody fragment. Specific antibody fragments include, but are not limited to, (i) the Fab fragment consisting of VL, VH, CL and CH1 domains, (ii) the Fd fragment consisting of the VH and CH1 domains, (iii) the Fv fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward et al., 1989, Nature 341:544-546, entirely incorporated by reference) which consists of a single variable, (v) isolated CDR regions, (vi) F(ab')2 fragments, a bivalent fragment comprising two linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al., 1988, Science 242:423-426, Huston et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:5879-5883, entirely incorporated by reference), (viii) bispecific single chain Fv (WO 03/11161, hereby incorporated by reference) and (ix) "diabodies" or "triabodies", multivalent or multispecific fragments constructed by gene

fusion (Tomlinson et. al., 2000, Methods Enzymol. 326:461-479; WO94/13804; Holliger et al., 1993, Proc. Natl. Acad. Sci. U.S.A. 90:6444-6448, all entirely incorporated by reference).

Chimeric and Humanized Antibodies

[0053] In some embodiments, the antibody can be a mixture from different species, e.g. a chimeric antibody and/or a humanized antibody. That is, in the present invention, the CDR sets can be used with framework and constant regions other than those specifically described by sequence herein.

[0054] In particular, the A1 antibody herein is shown as both the murine form and the humanized form (huA1).

[0055] In general, both "chimeric antibodies" and "humanized antibodies" refer to antibodies that combine regions from more than one species. For example, "chimeric antibodies" traditionally comprise variable region(s) from a mouse (or rat, in some cases) and the constant region(s) from a human. "Humanized antibodies" generally refer to non-human antibodies that have had the variable-domain framework regions swapped for sequences found in human antibodies. Generally, in a humanized antibody, the entire antibody, except the CDRs, is encoded by a polynucleotide of human origin or is identical to such an antibody except within its CDRs. The CDRs, some or all of which are encoded by nucleic acids originating in a non-human organism, are grafted into the beta-sheet framework of a human antibody variable region to create an antibody, the specificity of which is determined by the engrafted CDRs. The creation of such antibodies is described in, e.g., WO 92/11018, Jones, 1986, Nature 321:522-525, Verhoeyen et al., 1988, Science 239:1534-1536, all entirely incorporated by reference. "Backmutation" of selected acceptor framework residues to the corresponding donor residues is often required to regain affinity that is lost in the initial grafted construct (US 5530101; US 5585089; US 5693761; US 5693762; US 6180370; US 5859205; US 5821337; US 6054297; US 6407213, all entirely incorporated by reference). The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region, typically that of a human immunoglobulin, and thus will typically comprise a human Fc region. Humanized antibodies can also be generated using mice with a genetically engineered immune system. Roque et al., 2004, Biotechnol. Prog. 20:639-654, entirely incorporated by reference. A variety of techniques and methods for

humanizing and reshaping non-human antibodies are well known in the art (See Tsurushita & Vasquez, 2004, Humanization of Monoclonal Antibodies, Molecular Biology of B Cells, 533-545, Elsevier Science (USA), and references cited therein, all entirely incorporated by reference). Humanization methods include but are not limited to methods described in Jones et al., 1986, Nature 321:522-525; Riechmann et al.,1988; Nature 332:323-329; Verhoeyen et al., 1988, Science, 239:1534-1536; Queen et al., 1989, Proc Natl Acad Sci, USA 86:10029-33; He et al., 1998, J. Immunol. 160: 1029-1035; Carter et al., 1992, Proc Natl Acad Sci USA 89:4285-9, Presta et al., 1997, Cancer Res. 57(20):4593-9; Gorman et al., 1991, Proc. Natl. Acad. Sci. USA 88:4181-4185; O'Connor et al., 1998, Protein Eng 11:321-8, all entirely incorporated by reference. Humanization or other methods of reducing the immunogenicity of nonhuman antibody variable regions may include resurfacing methods, as described for example in Roguska et al., 1994, Proc. Natl. Acad. Sci. USA 91:969-973, entirely incorporated by reference. In one embodiment, the parent antibody has been affinity matured, as is known in the art. Structure-based methods may be employed for humanization and affinity maturation, for example as described in USSN 11/004,590. Selection based methods may be employed to humanize and/or affinity mature antibody variable regions, including but not limited to methods described in Wu et al., 1999, J. Mol. Biol. 294:151-162; Baca et al., 1997, J. Biol. Chem. 272(16):10678-10684; Rosok et al., 1996, J. Biol. Chem. 271(37): 22611-22618; Rader et al., 1998, Proc. Natl. Acad. Sci. USA 95: 8910-8915; Krauss et al., 2003, Protein Engineering 16(10):753-759, all entirely incorporated by reference. Other humanization methods may involve the grafting of only parts of the CDRs, including but not limited to methods described in USSN 09/810,510; Tan et al., 2002, J. Immunol. 169:1119-1125; De Pascalis et al., 2002, J. Immunol. 169:3076-3084, all entirely incorporated by reference.

[0056] In one embodiment, the antibodies of the invention can be multispecific antibodies, and notably bispecific antibodies, also sometimes referred to as "diabodies". These are antibodies that bind to two (or more) different antigens, or different epitopes on the same antigen. Diabodies can be manufactured in a variety of ways known in the art (Holliger and Winter, 1993, Current Opinion Biotechnol. 4:446-449, entirely incorporated by reference), e.g., prepared chemically or from hybrid hybridomas.

[0057] In one embodiment, the antibody is a minibody. Minibodies are minimized antibody-like proteins comprising a scFv joined to a CH3 domain. Hu et al., 1996, Cancer Res. 56:3055-3061, entirely incorporated by reference. In some cases, the scFv can be joined to the Fc region, and may include some or the entire hinge region. It should be noted that minibodies are included within the definition of "antibody" despite the fact it does not have a full set of CDRs.

[0058] The antibodies of the present invention are generally isolated or recombinant. "Isolated," when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Thus an isolated antibody is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g. an isolated antibody that specifically binds to the ROR1 is substantially free of antibodies that specifically bind antigens other than the ROR1). Thus, an "isolated" antibody is one found in a form not normally found in nature (e.g. non-naturally occuring).

[0059] In some embodiments, the antibodies of the invention are recombinant proteins, isolated proteins or substantially pure proteins. An "isolated" protein is unaccompanied by at least some of the material with which it is normally associated in its natural state, for example constituting at least about 5%, or at least about 50% by weight of the total protein in a given sample. It is understood that the isolated protein may constitute from 5 to 99.9% by weight of the total protein content depending on the circumstances. For example, the protein may be made at a significantly higher concentration through the use of an inducible promoter or high expression promoter, such that the protein is made at increased concentration levels. In the case of recombinant proteins, the definition includes the production of an antibody in a wide variety of organisms and/or host cells that are known in the art in which it is not naturally produced. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An "isolated antibody," refers to an antibody which is substantially free of other antibodies having different antigenic specificities. For instance, an isolated antibody that specifically binds to ROR1 is substantially free of antibodies that specifically bind antigens other than ROR1.

[0060] Isolated monoclonal antibodies, having different specificities, can be combined in a well defined composition. Thus for example, the A1 to A14 antibodies of the invention can optionally and individually be included or excluded in a formulation, as is further discussed below.

[0061] The anti-ROR1 antibodies of the present invention specifically bind ROR1 ligands (e.g. the extracellular domain of the human ROR1 proteins of SEQ ID NO:**ROR1). "Specific binding" or "specifically binds to" or is "specific for" a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

[0062] Specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a K_D for an antigen or epitope of at least about 10^{-4} M, at least about 10^{-5} M, at least about 10^{-6} M, at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, alternatively at least about 10^{-10} M, at least about 10^{-11} M, at least about $10^{-1}2$ M, or greater, where K_D refers to a dissociation rate of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope. However, in the present invention, when administering ADCs of the ROR1 antibodies of the invention, what is important is that the KD is sufficient to allow internalization and thus cell death without significant side effects.

[0063] Also, specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a K_A or K_a for an antigen or epitope of at least 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for the epitope relative to a control, where KA or Ka refers to an association rate of a particular antibody-antigen interaction.

[0064] Standard assays to evaluate the binding ability of the antibodies toward the ROR1 can be done on the protein or cellular level and are known in the art, including for example, ELISAs, Western blots, RIAs, BIAcore® assays and flow cytometry analysis. Suitable assays are described in detail in the Examples. The binding kinetics (e.g. binding affinity) of the

antibodies also can be assessed by standard assays known in the art, such as by Biacore® system analysis. To assess binding to Raji or Daudi B cell tumor cells, Raji (ATCC Deposit No. CCL-86) or Daudi (ATCC Deposit No. CCL-213) cells can be obtained from publicly available sources, such as the American Type Culture Collection, and used in standard assays, such as flow cytometric analysis.

ROR1 Antibodies

[0065] The present invention provides ROR1 antibodies that specifically bind the extracellular domain human ROR1 (SEQ ID NO:*xx) and are internalized when contacted with cells expressing ROR1 on the cell surface. These antibodies are referred to herein either as "anti-ROR1" antibodies or, for ease of description, "ROR1 antibodies".

[0066] The ROR1 antibodies are internalized upon contact with cells, particularly tumor cells, that express ROR1 on the surface. That is, ROR1 antibodies as defined herein that also comprise drug conjugates are internalized by tumor cells, resulting in the release of the drug and subsequent cell death, allowing for treatment of cancers that exhibit ROR1 expression. Internalization in this context can be measured in several ways. In one embodiment, the ROR1 antibodies of the invention are contacted with cells, such as a cell line as outlined herein, using standard assays such as MAbZap and HuZap. In these in vitro assay embodiments, the ROR1 antibodies of the invention are added, along with an anti-ROR1 antibody comprising a toxin; for example, the ROR1 antibody may be murine or humanized and the anti-ROR1 antibody can be anti-murine or anti-humanized and contain a toxin such as saporin. Upon formation of the [ROR1 antibody of the invention]-[anti-ROR1 antibody-drug conjugate] complex, the complex is internalized and the drug (e.g. saporin) is released, resulting in cell death. Only upon internalization does the drug get released, and thus cells remain viable in the absence of internalization. As outlined below, without being bound by theory, in therapeutic applications, the anti-ROR1 antibody contains the toxin, and upon internalization, the bond between the antibody and the toxin is cleaved, releasing the toxin and killing the cell.

[0067] In one embodiment, the antibody of the invention is an A1 antibody. By "A1 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few

additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A1	SEQ ID NOs
variable heavy CDR1	57, 58
variable heavy CDR2	99, 100
variable heavy CDR3	149
variable light CDR1	173
variable light CDR2	190, 191
variable light CDR3	229

[0068] In addition, the A1 antibody has been "humanized"; the humanized antibody is referred to herein as "huA1" or equivalents. Thus, included within the definition of "A1" antibody is the huA1 antibody.

[0069] As will be appreciated by those in the art, given a set of CDRs, any number of antibodies can be generated, using different framework and Fc regions, as is more fully described herein for each of A1 to A14.

[0070] In one embodiment, the antibody of the invention is an A2 antibody. By "A2 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A2	SEQ ID NOs
variable heavy CDR1	59, 60
variable heavy CDR2	101, 102
variable heavy CDR3	150
variable light CDR1	179
variable light CDR2	190, 191
variable light CDR3	229

[0071] In one embodiment, the antibody of the invention is an A3 antibody. By "A3 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A3	SEQ ID NOs

variable heavy CDR1	58, 61
variable heavy CDR2	103, 104
variable heavy CDR3	149
variable light CDR1	179
variable light CDR2	190, 192
variable light CDR3	229

[0072] In one embodiment, the antibody of the invention is an A4 antibody. By "A4 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A4	SEQ ID NOs
variable heavy CDR1	62, 63
variable heavy CDR2	105, 106
variable heavy CDR3	151
variable light CDR1	179
variable light CDR2	193, 194
variable light CDR3	229

[0073] In one embodiment, the antibody of the invention is an A5 antibody. By "A5 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A5	SEQ ID NOs
variable heavy CDR1	64, 65
variable heavy CDR2	107, 108
variable heavy CDR3	152
variable light CDR1	179
variable light CDR2	195, 196
variable light CDR3	229

[0074] In one embodiment, the antibody of the invention is an A6 antibody. By "A6 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A6	SEQ ID NOs
variable heavy CDR1	66, 67
variable heavy CDR2	109, 110
variable heavy CDR3	153
variable light CDR1	179
variable light CDR2	197, 198
variable light CDR3	229

[0075] In one embodiment, the antibody of the invention is an A7 antibody. By "A7 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A7	SEQ ID NOs
variable heavy CDR1	68, 69
variable heavy CDR2	111, 112
variable heavy CDR3	154
variable light CDR1	179
variable light CDR2	199, 200
variable light CDR3	230

[0076] In one embodiment, the antibody of the invention is an A8 antibody. By "A8 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A8	SEQ ID NOs
variable heavy CDR1	58, 61
variable heavy CDR2	113, 114
variable heavy CDR3	155
variable light CDR1	180
variable light CDR2	201, 202
variable light CDR3	231

[0077] In one embodiment, the antibody of the invention is an A9 antibody. By "A9 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few

additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A9	SEQ ID NOs
variable heavy CDR1	70, 71
variable heavy CDR2	115, 116
variable heavy CDR3	156
variable light CDR1	179
variable light CDR2	190, 191
variable light CDR3	229

[0078] In one embodiment, the antibody of the invention is an A10 antibody. By "A10 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A10	SEQ ID NOs
variable heavy CDR1	72, 73
variable heavy CDR2	117, 118
variable heavy CDR3	157
variable light CDR1	174
variable light CDR2	190, 191
variable light CDR3	229

[0079] In one embodiment, the antibody of the invention is an A11 antibody. By "A11 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A11	SEQ ID NOs
variable heavy CDR1	74, 75
variable heavy CDR2	119, 120
variable heavy CDR3	155
variable light CDR1	175
variable light CDR2	203, 204
variable light CDR3	232

[0080] In one embodiment, the antibody of the invention is an A12 antibody. By "A12 antibody" herein is meant an antibody comprising the following CDRs; as described herein,

the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A12	SEQ ID NOs
variable heavy CDR1	58, 61
variable heavy CDR2	110
variable heavy CDR3	158
variable light CDR1	179
variable light CDR2	190, 205
variable light CDR3	229

[0081] In one embodiment, the antibody of the invention is an A13 antibody. By "A13 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A13	SEQ ID NOs
variable heavy CDR1	58, 61
variable heavy CDR2	101, 102
variable heavy CDR3	159
variable light CDR1	176
variable light CDR2	190, 206
variable light CDR3	229

[0082] In one embodiment, the antibody of the invention is an A14 antibody. By "A14 antibody" herein is meant an antibody comprising the following CDRs; as described herein, the first SEQ ID listed in the chart below has been defined more broadly to include a few additional amino acids; in addition, as discussed below, these CDR sequences can also contain a limited number of amino acid variants:

A14	SEQ ID NOs
variable heavy CDR1	76, 77
variable heavy CDR2	122, 123
variable heavy CDR3	160
variable light CDR1	177, 178
variable light CDR2	207, 208
variable light CDR3	223

[0083] As discussed herein, in addition, the identification of the CDRs of the A1 to A14 antibodies shows that these CDRs fall into consensus sequences. Thus, "ROR1 antibodies" of the invention also include CDRs that have the following sequences. As it appears from sequence identities, the A14 CDRs are more dissimilar than the A1-A13 CDR sets. As such, the consensus sequences discussed below include both a consensus sequence based on all of the CDRs of A1-A14, and a consensus sequence based on A1-A13:

Consensus sequence	SEQ ID NO
variable heavy CDR1 (A1-A14)	275
variable heavy CDR1 (A1-A13)	276
variable heavy CDR2 (A1-A14)	277
variable heavy CDR2 (A1-A13)	278
variable heavy CDR3 (A1-A14)	279
variable heavy CDR3 (A1-A13)	280
variable light CDR1 (A1-A14)	281
variable light CDR1 (A1-A13)	282
variable light CDR2 (A1-A14)	283
variable light CDR2 (A1-A13)	284
variable light CDR3 (A1-A14)	285
variable light CDR3 (A1-A13)	286

[0084] Thus, at a minimum, an A1-A14 antibody is defined by the set of CDRs it contains. In addition, given that each of these antibodies can bind to the ROR1 and there is significant identity between the CDRs as outlined herein, the CDRs as well as the V_H and V_K sequences can be "mixed and matched" to create other anti-ROR1 binding molecules of the invention. The ROR1 binding of such "mixed and matched" antibodies can be tested using the binding assays described above and in the Examples (e.g. ELISAs or Biacore® assays). Preferably, when V_H and V_K chains are mixed and matched, a V_H sequence from a particular V_H/V_K pairing is replaced with a structurally similar V_H sequence. Likewise, preferably a V_K sequence from a particular V_H/V_K pairing is replaced with a structurally similar V_K sequence. Accordingly, the invention specifically includes every possible combination of CDRs of the heavy and light chains of each of A1-A14.

[0085] Thus, at a minimum, an A1-A14 antibody is defined by the set of CDRs it contains. In addition to including the CDRs, the A1-A14 antibodies can include additional sequences, including framework and constant regions. As for the CDRs, as outlined below, the variable

heavy and light chains, the Fc regions and/or the framework regions can comprise variants from the parent antibody sequence as outlined herein.

[0086] Disclosed herein are also variable heavy and light chains that comprise the CDR sets of the invention, as well as full length heavy and light chains (e.g. comprising constant regions as well). As will be appreciated by those in the art, the CDR sets of the invention can be incorporated into murine, humanized or human constant regions (including framework regions). As shown for A1 and huA1, the amino acid identity between the murine and human sequences is about 90%. Accordingly, the present invention provides variable heavy and light chains that are at least about 90%-99% identical to the SEQ IDs disclosed herein, with 90, 91, 92, 93, 94, 95, 96, 97, 98 and 99% all finding use in the present invention.

[0087]

Antibodies that Bind to the Same Epitope as the ROR1 Antibodies of the Invention

[0088] In another embodiment, the invention provides antibodies that bind to the same epitope on the human ROR1 as any of the ROR1 monoclonal antibodies of the invention (i.e. antibodies that have the ability to cross-compete for binding to the ROR1 with any of the monoclonal antibodies of the invention). In preferred embodiments, the reference antibody for cross-competition studies can be any or all of the A1-A14 ROR1 antibodies as defined herein. Such cross-competing antibodies can be identified based on their ability to cross-compete with any or all of the A1-A14 antibodies,

Antibody Modifications

[0089] The present invention further provides variant antibodies, sometimes referred to as "antibody derivatives" or "antibody analogs" as well. That is, there are a number of modifications that can be made to the antibodies of the invention, including, but not limited to, amino acid modifications in the CDRs (affinity maturation), amino acid modifications in the Fc region, glycosylation variants, covalent modifications of other types (e.g. for attachment of drug conjugates, etc.

[0090] By "variant" herein is meant a polypeptide sequence that differs from that of a parent polypeptide by virtue of at least one amino acid modification. In this case, the parent polypeptide is either the full length variable heavy or light chains, listed in Figure ****.

Amino acid modifications can include substitutions, insertions and deletions, with the former being preferred in many cases.

[0091] In general, variants can include any number of modifications, as long as the function of the antibody is still present, as described herein. That is, any of antibodies A1-A14, for example, the antibody should still specifically bind to human ROR1. Similarly, if amino acid variants are generated with the Fc region, for example, the variant antibodies should maintain the required receptor binding functions for the particular application or indication of the antibody.

[0092] "Variants" in this case can be made in either the listed CDR sequences, the framework or Fc regions of the antibody.

[0093] However, in general, from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions are generally utilized as often the goal is to alter function with a minimal number of modifications. In some cases, there are from 1 to 5 modifications (e.g. individual amino acid substitutions, insertions or deletions), with from 1-2, 1-3 and 1-4 also finding use in many embodiments. The number of modifications can depend on the size of the region being modified; for example, in general, fewer modifications are desired in CDR regions. However, as shown herein, the CDRs of the A1-A14 antibodies herein are similar, such that a number of amino acid changes can be made and preserve binding.

[0094] It should be noted that the number of amino acid modifications may be within functional domains: for example, it may be desirable to have from 1-5 modifications in the Fc region of wild-type or engineered proteins, as well as from 1 to 5 modifications in the Fv region, for example. A variant polypeptide sequence will preferably possess at least about 80%, 85%, 90%, 95% or up to 98 or 99% identity to the parent sequences (e.g. the variable regions, the constant regions, and/or the heavy and light chain sequences A1-A14). It should be noted that depending on the size of the sequence, the percent identity will depend on the number of amino acids.

[0095] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with another amino acid. For example, the substitution S100A refers to a variant polypeptide in which the serine at position 100 is replaced with alanine. By "amino acid insertion" or "insertion" as used herein

is meant the addition of an amino acid at a particular position in a parent polypeptide sequence. By “amino acid deletion” or “deletion” as used herein is meant the removal of an amino acid at a particular position in a parent polypeptide sequence.

[0096] By “parent polypeptide”, “parent protein”, “precursor polypeptide”, or “precursor protein” as used herein is meant an unmodified polypeptide that is subsequently modified to generate a variant. In general, the parent polypeptides herein are A1-A14 (including huA1) Accordingly, by “parent antibody” as used herein is meant an antibody that is modified to generate a variant antibody.

[0097] By “wild type" or "WT” or "native" herein is meant an amino acid sequence or a nucleotide sequence that is found in nature, including allelic variations. A WT protein, polypeptide, antibody, immunoglobulin, IgG, etc. has an amino acid sequence or a nucleotide sequence that has not been intentionally modified.

[0098] By “variant Fc region” herein is meant an Fc sequence that differs from that of a wild-type Fc sequence by virtue of at least one amino acid modification. Fc variant may refer to the Fc polypeptide itself, compositions comprising the Fc variant polypeptide, or the amino acid sequence.

[0099] In some embodiments, one or more amino acid modifications are made in one or more of the CDRs of the antibody (any of A1 to A14, including huA1). In general, only 1 or 2 or 3 amino acids are substituted in any single CDR, and generally no more than from 4, 5, 6, 7, 8 9 or 10 changes are made within a set of CDRs. However, it should be appreciated that any combination of no substitutions, 1, 2 or 3 substitutions in any CDR can be independently and optionally combined with any other substitution. In addition, as is shown herein, the A1-A13 CDRs share strong identity; for example, as shown in Figure 14, the vh CDR1 region of A1 and A2 only differ by 1 amino acid; similarly, the A1 and A11 vh CDR regions differ by 3 amino acids.

[00100] In some cases, amino acid modifications in the CDRs are referred to as “affinity maturation”. An "affinity matured" antibody is one having one or more alteration(s) in one or more CDRs which results in an improvement in the affinity of the antibody for antigen, compared to a parent antibody which does not possess those alteration(s). In some

cases, although rare, it may be desirable to decrease the affinity of an antibody to its antigen, but this is generally not preferred.

[00101] Affinity maturation can be done to increase the binding affinity of the antibody for the antigen by at least about 10% to 50-100-150% or more, or from 1 to 5 fold as compared to the "parent" antibody. Preferred affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by known procedures. See, for example, Marks et al., 1992, Biotechnology 10:779-783 that describes affinity maturation by variable heavy chain (VH) and variable light chain (VL) domain shuffling. Random mutagenesis of CDR and/or framework residues is described in: Barbas, et al. 1994, Proc. Nat. Acad. Sci, USA 91:3809-3813; Shier et al., 1995, Gene 169:147-155; Yelton et al., 1995, J. Immunol. 155:1994-2004; Jackson et al., 1995, J. Immunol. 154(7):3310-9; and Hawkins et al, 1992, J. Mol. Biol. 226:889-896, for example.

[00102] Alternatively, amino acid modifications can be made in one or more of the CDRs of the antibodies of the invention that are "silent", e.g. that do not significantly alter the affinity of the antibody for the antigen. These can be made for a number of reasons, including optimizing expression (as can be done for the nucleic acids encoding the antibodies of the invention).

[00103] Thus, included within the definition of the CDRs and antibodies of the invention are variant CDRs and antibodies; that is, the antibodies of the invention can include amino acid modifications in one or more of the CDRs of A1-A14. In addition, as outlined below, amino acid modifications can also independently and optionally be made in any region outside the CDRs, including framework and constant regions as described herein.

[00104] In some embodiments, the anti-ROR1 antibodies of the invention are composed of a variant Fc domain. As is known in the art, the Fc region of an antibody interacts with a number of Fc receptors and ligands, imparting an array of important functional capabilities referred to as effector functions. These Fc receptors include, but are not limited to, (in humans) FcγRI (CD64) including isoforms FcγRIa, FcγRIb, and FcγRIc; FcγRII (CD32), including isoforms FcγRIIa (including allotypes H131 and R131), FcγRIIb (including FcγRIIb-1 and FcγRIIb-2), and FcγRIIc; and FcγRIII (CD16), including isoforms FcγRIIIa (including allotypes V158 and F158, correlated to antibody-dependent cell

cytotoxicity (ADCC)) and FcγRIIIb (including allotypes FcγRIIIb-NA1 and FcγRIIIb-NA2), FcRn (the neonatal receptor), C1q (complement protein involved in complement dependent cytotoxicity (CDC)) and FcRn (the neonatal receptor involved in serum half-life). Suitable modifications can be made at one or more positions as is generally outlined, for example in US Patent Application 11/841,654 and references cited therein, US 2004/013210, US 2005/0054832, US 2006/0024298, US 2006/0121032, US 2006/0235208, US 2007/0148170, USSN 12/341,769, US Patent No. 6,737,056, US Patent No. 7,670,600, US Patent No. 6,086,875 all of which are expressly incorporated by reference in their entirety, and in particular for specific amino acid substitutions that increase binding to Fc receptors.

[00105] In addition to the modifications outlined above, other modifications can be made. For example, the molecules may be stabilized by the incorporation of disulphide bridges linking the VH and VL domains (Reiter et al., 1996, Nature Biotech. 14:1239-1245, entirely incorporated by reference).

[00106] In addition, modifications at cysteines are particularly useful in antibody-drug conjugate (ADC) applications, further described below. In some embodiments, the constant region of the antibodies can be engineered to contain one or more cysteines that are particularly "thiol reactive", so as to allow more specific and controlled placement of the drug moiety. See for example US Patent No. 7,521,541, incorporated by reference in its entirety herein.

[00107] In addition, there are a variety of covalent modifications of antibodies that can be made as outlined below.

[00108] Covalent modifications of antibodies are included within the scope of this invention, and are generally, but not always, done post-translationally. For example, several types of covalent modifications of the antibody are introduced into the molecule by reacting specific amino acid residues of the antibody with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

[00109] Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues may also be derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate,

N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole and the like.

[00110] Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1M sodium cacodylate at pH 6.0.

[00111] Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

[00112] Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

[00113] The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using 125I or 131I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

[00114] Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'—N=C=N--R'), where R and R' are optionally different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

[00115] Derivatization with bifunctional agents is useful for crosslinking antibodies to a water-insoluble support matrix or surface for use in a variety of methods, in addition to methods described below. Commonly used crosslinking agents include, e.g., 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis (succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cynomolgusogen bromide-activated carbohydrates and the reactive substrates described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440, all entirely incorporated by reference, are employed for protein immobilization.

[00116] Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

[00117] Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, pp. 79-86 [1983], entirely incorporated by reference), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[00118] In addition, as will be appreciated by those in the art, labels (including fluorescent, enzymatic, magnetic, radioactive, etc. can all be added to the antibodies (as well as the other compositions of the invention).

Glycosylation

[00119] Another type of covalent modification is alterations in glycosylation. In some embodiments, the antibodies disclosed herein can be modified to include one or more engineered glycoforms. By "engineered glycoform" as used herein is meant a carbohydrate composition that is covalently attached to the antibody, wherein the carbohydrate

composition differs chemically from that of a parent antibody. Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. For example, an aglycoslated antibody can be made (i.e. the antibody that lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in US Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*, and can be accomplished by removing the asparagine at position 297.

[00120] A preferred form of engineered glycoform is afucosylation, which has been shown to be correlated to an increase in ADCC function, presumably through tighter binding to the FcγRIIIa receptor. In this context, "afucosylation" means that the majority of the antibody produced in the host cells is substantially devoid of fucose, e.g. 90-95-98% of the generated antibodies do not have appreciable fucose as a component of the carbohydrate moiety of the antibody (generally attached at N297 in the Fc region). Defined functionally, afucosylated antibodies generally exhibit at least a 50% or higher affinity to the FcγRIIIa receptor.

[00121] Engineered glycoforms may be generated by a variety of methods known in the art (Umaña et al., 1999, Nat Biotechnol 17:176-180; Davies et al., 2001, Biotechnol Bioeng 74:288-294; Shields et al., 2002, J Biol Chem 277:26733-26740; Shinkawa et al., 2003, J Biol Chem 278:3466-3473; US 6,602,684; USSN 10/277,370; USSN 10/113,929; PCT WO 00/61739A1; PCT WO 01/29246A1; PCT WO 02/31140A1; PCT WO 02/30954A1, all entirely incorporated by reference; (POTELLIGENT® technology [Biowa, Inc., Princeton, NJ]; GlycoMAb® glycosylation engineering technology [Glycart Biotechnology AG, Zürich, Switzerland]). Many of these techniques are based on controlling the level of fucosylated and/or bisecting oligosaccharides that are covalently attached to the Fc region, for example by expressing an IgG in various organisms or cell lines, engineered or otherwise (for example Lec-13 CHO cells or rat hybridoma YB2/0 cells, by regulating

enzymes involved in the glycosylation pathway (for example FUT8 [α1,6-fucosyltranserase] and/or β1-4- N-acetylglucosaminyltransferase III [GnTIII]), or by modifying carbohydrate(s) after the IgG has been expressed. For example, the "sugar engineered antibody" or "SEA technology" of Seattle Genetics functions by adding modified saccharides that inhibit fucosylation during production; see for example US/2009/0317869, hereby incorporated by reference in its entirety. "Engineered glycoform"typically refers to the different carbohydrate or oligosaccharide as compared to the antibody made in the absence of the glycosylation technology; thus an antibody can include an engineered glycoform.

[00122] Alternatively, engineered glycoform may refer to the IgG variant that comprises the different carbohydrate or oligosaccharide. As is known in the art, glycosylation patterns can depend on both the sequence of the protein (e.g., the presence or absence of particular glycosylation amino acid residues, discussed below), or the host cell or organism in which the protein is produced. Particular expression systems are discussed below.

[00123] Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[00124] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the antibody amino acid sequence is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the target polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

[00125] Another means of increasing the number of carbohydrate moieties on the antibody is by chemical or enzymatic coupling of glycosides to the protein. These procedures are advantageous in that they do not require production of the protein in a host cell that has glycosylation capabilities for N- and O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330 and in Aplin and Wriston, 1981, CRC Crit. Rev. Biochem., pp. 259-306, both entirely incorporated by reference.

[00126] Removal of carbohydrate moieties present on the starting antibody (e.g. post-translationally) may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin et al., 1987, Arch. Biochem. Biophys. 259:52 and by Edge et al., 1981, Anal. Biochem. 118:131, both entirely incorporated by reference. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura et al., 1987, Meth. Enzymol. 138:350, entirely incorporated by reference, including removal of fucose residues using a fucosidase enzyme as is known in the art. Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin et al., 1982, J. Biol. Chem. 257:3105, entirely incorporated by reference. Tunicamycin blocks the formation of protein-N-glycoside linkages.

[00127] Another type of covalent modification of the antibody comprises linking the antibody to various nonproteinaceous polymers, including, but not limited to, various polyols such as polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in, for example, 2005-2006 PEG Catalog from Nektar Therapeutics (available at the Nektar website) US Patents 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337, all entirely incorporated by reference. In addition, as is known in the art, amino

acid substitutions may be made in various positions within the antibody to facilitate the addition of polymers such as PEG. See for example, U.S. Publication No. 2005/0114037A1, entirely incorporated by reference.

[00128] In additional embodiments, for example in the use of the antibodies of the invention for diagnostic or detection purposes, the antibodies may comprise a label. By "labeled" herein is meant that a compound has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes; b) magnetic, electrical, thermal; and c) colored or luminescent dyes; although labels include enzymes and particles such as magnetic particles as well. Preferred labels include, but are not limited to, fluorescent lanthanide complexes (including those of Europium and Terbium), and fluorescent labels including, but not limited to, quantum dots, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade Blue, Texas Red, the Alexa dyes, the Cy dyes, and others described in the 6th Edition of the Molecular Probes Handbook by Richard P. Haugland, hereby expressly incorporated by reference.

Antibody-Drug Conjugates

[00129] In some embodiments, the anti-ROR1 antibodies of the invention are conjugated with drugs to form antibody-drug conjugates (ADCs). In general, ADCs are used in oncology applications, where the use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents allows for the targeted delivery of the drug moiety to tumors, which can allow higher efficacy, lower toxicity, etc. An overview of this technology is provided in Ducry et al., Bioconjugate Chem., 21:5-13 (2010), Carter et al., Cancer J. 14(3):154 (2008) and Senter, Current Opin. Chem. Biol. 13:235-244 (2009), all of which are hereby incorporated by reference in their entirety.

[00130] Thus the invention provides anti-ROR1 antibodies conjugated to drugs. Generally, conjugation is done by covalent attachment to the antibody, as further described below, and generally relies on a linker, often a peptide linkage (which, as described below, may be designed to be sensitive to cleavage by proteases at the target site or not). In addition, as described above, linkage of the linker-drug unit (LU-D) can be done by attachment to

cysteines within the antibody. As will be appreciated by those in the art, the number of drug moieties per antibody can change, depending on the conditions of the reaction, and can vary from 1:1 to 10:1 drug:antibody. As will be appreciated by those in the art, the actual number is an average.

[00131] Thus the invention provides anti-ROR1 antibodies conjugated to drugs. As described below, the drug of the ADC can be any number of agents, including but not limited to cytotoxic agents such as chemotherapeutic agents, growth inhibitory agents, toxins (for example, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (that is, a radioconjugate) are provided. In other embodiments, the invention further provides methods of using the ADCs.

[00132] Drugs for use in the present invention include cytotoxic drugs, particularly those which are used for cancer therapy. Such drugs include, in general, DNA damaging agents, anti-metabolites, natural products and their analogs. Exemplary classes of cytotoxic agents include the enzyme inhibitors such as dihydrofolate reductase inhibitors, and thymidylate synthase inhibitors, DNA intercalators, DNA cleavers, topoisomerase inhibitors, the anthracycline family of drugs, the vinca drugs, the mitomycins, the bleomycins, the cytotoxic nucleosides, the pteridine family of drugs, diynenes, the podophyllotoxins, dolastatins, maytansinoids, differentiation inducers, and taxols.

[00133] Members of these classes include, for example, taxol. methotrexate, methopterin, dichloromethotrexate, 5-fluorouracil, 6-mercaptopurine, cytosine arabinoside, melphalan, leurosine, leurosideine, actinomycin, daunorubicin, doxorubicin, mitomycin C, mitomycin A, caminomycin, aminopterin, tallysomycin, podophyllotoxin and podophyllotoxin derivatives such as etoposide or etoposide phosphate, vinblastine, vincristine, vindesine, taxanes including taxol, taxotere retinoic acid, butyric acid, N8-acetyl spermidine, camptothecin, calicheamicin, esperamicin, ene-diynes, duocarmycin A, duocarmycin SA, calicheamicin, camptothecin, maytansinoids (including DM1), monomethylauristatin E (MMAE), monomethylauristatin F (MMAF), and maytansinoids (DM4) and their analogues.

[00134] Toxins may be used as antibody-toxin conjugates and include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as

geldanamycin (Mandler et al (2000) J. Nat. Cancer Inst. 92(19):1573-1581; Mandler et al (2000) Bioorganic & Med. Chem. Letters 10:1025-1028; Mandler et al (2002) Bioconjugate Chem. 13:786-791), maytansinoids (EP 1391213; Liu et al., (1996) Proc. Natl. Acad. Sci. USA 93:8618-8623), and calicheamicin (Lode et al (1998) Cancer Res. 58:2928; Hinman et al (1993) Cancer Res. 53:3336-3342). Toxins may exert their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition.

[00135] Conjugates of an anti-ROR1 antibody and one or more small molecule toxins, such as a maytansinoids, dolastatins, auristatins, a trichothecene, calicheamicin, and CC1065, and the derivatives of these toxins that have toxin activity, are contemplated.

[00136] Maytansinoids

[00137] Maytansine compounds suitable for use as maytansinoid drug moieties are well known in the art, and can be isolated from natural sources according to known methods, produced using genetic engineering techniques (see Yu et al (2002) PNAS 99:7968-7973), or maytansinol and maytansinol analogues prepared synthetically according to known methods. As described below, drugs may be modified by the incorporation of a functionally active group such as a thiol or amine group for conjugation to the antibody.

[00138] Exemplary maytansinoid drug moieties include those having a modified aromatic ring, such as: C-19-dechloro (U.S. Pat. No. 4,256,746) (prepared by lithium aluminum hydride reduction of ansamytocin P2); C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (U.S. Pat. Nos. 4,361,650 and 4,307,016) (prepared by demethylation using Streptomyces or Actinomyces or dechlorination using LAH); and C-20-demethoxy, C-20-acyloxy (--OCOR), +/-dechloro (U.S. Pat. No. 4,294,757) (prepared by acylation using acyl chlorides) and those having modifications at other positions

[00139] Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (U.S. Pat. No. 4,424,219) (prepared by the reaction of maytansinol with H2S or P2S5); C-14-alkoxymethyl(demethoxy/CH2OR) (U.S. Pat. No. 4,331,598); C-14-hydroxymethyl or acyloxymethyl (CH2OH or CH2OAc) (U.S. Pat. No. 4,450,254) (prepared from Nocardia); C-15-hydroxy/acyloxy (U.S. Pat. No. 4,364,866) (prepared by the conversion of maytansinol by Streptomyces); C-15-methoxy (U.S. Pat. Nos. 4,313,946 and 4,315,929) (isolated from Trewia nudlflora); C-18-N-demethyl (U.S. Pat. Nos.

4,362,663 and 4,322,348) (prepared by the demethylation of maytansinol by Streptomyces); and 4,5-deoxy (U.S. Pat. No. 4,371,533) (prepared by the titanium trichloride/LAH reduction of maytansinol).

[00140] Of particular use are DM1 (disclosed in US Patent No. 5,208,020, incorporated by reference) and DM4 (disclosed in US Patent No. 7,276,497, incorporated by reference). See also a number of additional maytansinoid derivatives and methods in 5,416,064, WO/01/24763, 7,303,749, 7,601,354, USSN 12/631,508, WO02/098883, 6,441,163, 7,368,565, WO02/16368 and WO04/1033272, all of which are expressly incorporated by reference in their entirety.

[00141] ADCs containing maytansinoids, methods of making same, and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020; 5,416,064; 6,441,163 and European Patent EP 0 425 235 B1, the disclosures of which are hereby expressly incorporated by reference. Liu et al., Proc. Natl. Acad. Sci. USA 93:8618-8623 (1996) described ADCs comprising a maytansinoid designated DM1 linked to the monoclonal antibody C242 directed against human colorectal cancer. The conjugate was found to be highly cytotoxic towards cultured colon cancer cells, and showed antitumor activity in an in vivo tumor growth assay.

[00142] Chari et al., Cancer Research 52:127-131 (1992) describe ADCs in which a maytansinoid was conjugated via a disulfide linker to the murine antibody A7 binding to an antigen on human colon cancer cell lines, or to another murine monoclonal antibody TA.1 that binds the HER-2/neu oncogene. The cytotoxicity of the TA.1-maytansonoid conjugate was tested in vitro on the human breast cancer cell line SK-BR-3, which expresses 3x105 HER-2 surface antigens per cell. The drug conjugate achieved a degree of cytotoxicity similar to the free maytansinoid drug, which could be increased by increasing the number of maytansinoid molecules per antibody molecule. The A7-maytansinoid conjugate showed low systemic cytotoxicity in mice.

Auristatins and Dolastatins

[00143] In some embodiments, the ADC comprises an anti-ROR1 antibody conjugated to dolastatins or dolostatin peptidic analogs and derivatives, the auristatins (U.S. Pat. Nos. 5,635,483; 5,780,588). Dolastatins and auristatins have been shown to interfere with

microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al (2001) Antimicrob. Agents and Chemother. 45(12):3580-3584) and have anticancer (U.S. Pat. No. 5,663,149) and antifungal activity (Pettit et al (1998) Antimicrob. Agents Chemother. 42:2961-2965). The dolastatin or auristatin drug moiety may be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172).

[00144] Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties DE and DF, disclosed in "Senter et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented Mar. 28, 2004 and described in United States Patent Publication No. 2005/0238648, the disclosure of which is expressly incorporated by reference in its entirety.

[00145] An exemplary auristatin embodiment is MMAE (shown in Figure 10 wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody drug conjugate; see US Patent No. 6,884,869 expressly incorporated by reference in its entirety).

[00146] Another exemplary auristatin embodiment is MMAF, shown in Figure 10 wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody drug conjugate (US 2005/0238649, 5,767,237 and 6,124,431, expressly incorporated by reference in their entirety):

[00147] Additional exemplary embodiments comprising MMAE or MMAF and various linker components (described further herein) have the following structures and abbreviations (wherein Ab means antibody and p is 1 to about 8):

[00148] Typically, peptide-based drug moieties can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (see E. Schroder and K. Lubke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry. The auristatin/dolastatin drug moieties may be prepared according to the methods of: U.S. Pat. No. 5,635,483; U.S. Pat. No. 5,780,588; Pettit et al (1989) J. Am. Chem. Soc. 111:5463-5465; Pettit et al (1998) Anti-Cancer Drug Design 13:243-277; Pettit, G. R., et al. Synthesis, 1996, 719-725; Pettit et al (1996) J. Chem. Soc. Perkin Trans. 1 5:859-863; and Doronina (2003) Nat Biotechnol 21(7):778-784.

Calicheamicin

[00149] In other embodiments, the ADC comprises an antibody of the invention conjugated to one or more calicheamicin molecules. For example, Mylotarg is the first commercial ADC drug and utilizes calicheamicin γ1 as the payload (see US Patent No. 4,970,198, incorporated by reference in its entirety). Additional calicheamicin derivatives are described in US Patent Nos. 5,264,586, 5,384,412, 5,550,246, 5,739,116, 5,773,001, 5,767,285 and 5,877,296, all expressly incorporated by reference. The calicheamicin family of antibiotics are capable of producing double-stranded DNA breaks at sub-picomolar concentrations. For the preparation of conjugates of the calicheamicin family, see U.S. Pat. Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, 5,877,296 (all to American Cyanamid Company). Structural analogues of calicheamicin which may be used include, but are not limited to, γ1I, α2I, α2I, N-acetyl- γ1I, PSAG and θI1 (Hinman et al., Cancer Research 53:3336-3342 (1993), Lode et al., Cancer Research 58:2925-2928 (1998) and the aforementioned U.S. patents to American Cyanamid). Another anti-tumor drug that the antibody can be conjugated is QFA which is an antifolate. Both calicheamicin and QFA have intracellular sites of action and do not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody mediated internalization greatly enhances their cytotoxic effects.

Duocarmycins

[00150] CC-1065 (see 4,169,888, incorporated by reference) and duocarmycins are members of a family of antitumor antibiotics utilized in ADCs. These antibiotics appear to work through sequence-selectively alkylating DNA at the N3 of adenine in the minor groove, which initiates a cascade of events that result in apoptosis.

[00151] Important members of the duocarmycins include duocarmycin A (US Patent No. 4,923,990, incorporated by reference) and duocarmycin SA (U.S. Pat. No. 5,101,038, incorporated by reference), and a large number of analogues as described in US Patent Nos. 7,517,903, 7,691,962, 5,101,038; 5,641,780; 5,187,186; 5,070,092; 5,070,092; 5,641,780; 5,101,038; 5,084,468, 5,475,092, 5,585,499, 5,846,545, WO2007/089149, WO2009/017394A1, 5,703,080, 6,989,452, 7,087,600, 7,129,261, 7,498,302, and 7,507,420, all of which are expressly incorporated by reference.

Other Cytotoxic Agents

[00152] Other antitumor agents that can be conjugated to the antibodies of the invention include BCNU, streptozoicin, vincristine and 5-fluorouracil, the family of agents known collectively LL-E33288 complex described in U.S. Pat. Nos. 5,053,394, 5,770,710, as well as esperamicins (U.S. Pat. No. 5,877,296).

[00153] Enzymatically active toxins and fragments thereof which can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from Pseudomonas aeruginosa), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolaca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, for example, WO 93/21232 published Oct. 28, 1993.

[00154] The present invention further contemplates an ADC formed between an antibody and a compound with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease such as a deoxyribonuclease; DNase).

[00155] For selective destruction of the tumor, the antibody may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At211, I131, I125, Y90, Re186, Re188, Sm153, Bi212, P32, Pb212 and radioactive isotopes of Lu.

[00156] The radio- or other labels may be incorporated in the conjugate in known ways. For example, the peptide may be biosynthesized or may be synthesized by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as Tc99m or I123, Re186, Re188 and In111 can be attached via a cysteine residue in the peptide. Yttrium-90 can be attached via a lysine residue. The IODOGEN method (Fraker et al (1978) Biochem. Biophys. Res. Commun. 80: 49-57 can be used to incorporate Iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes other methods in detail.

[00157] For compositions comprising a plurality of antibodies, the drug loading is represented by p, the average number of drug molecules per Antibody. Drug loading may

range from 1 to 20 drugs (D) per Antibody. The average number of drugs per antibody in preparation of conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of Antibody-Drug-Conjugates in terms of p may also be determined.

[00158] In some instances, separation, purification, and characterization of homogeneous Antibody-Drug-conjugates where p is a certain value from Antibody-Drug-Conjugates with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis. In exemplary embodiments, p is 2, 3, 4, 5, 6, 7, or 8 or a fraction thereof

[00159] The generation of Antibody-drug conjugate compounds can be accomplished by any technique known to the skilled artisan. Briefly, the Antibody-drug conjugate compounds can include an anti-ROR1 antibody as the Antibody unit, a drug, and optionally a linker that joins the drug and the binding agent.

[00160] A number of different reactions are available for covalent attachment of drugs and/or linkers to binding agents. This is can be accomplished by reaction of the amino acid residues of the binding agent, for example, antibody molecule, including the amine groups of lysine, the free carboxylic acid groups of glutamic and aspartic acid, the sulfhydryl groups of cysteine and the various moieties of the aromatic amino acids. A commonly used non-specific methods of covalent attachment is the carbodiimide reaction to link a carboxy (or amino) group of a compound to amino (or carboxy) groups of the antibody. Additionally, bifunctional agents such as dialdehydes or imidoesters have been used to link the amino group of a compound to amino groups of an antibody molecule.

[00161] Also available for attachment of drugs to binding agents is the Schiff base reaction. This method involves the periodate oxidation of a drug that contains glycol or hydroxy groups, thus forming an aldehyde which is then reacted with the binding agent. Attachment occurs via formation of a Schiff base with amino groups of the binding agent. Isothiocyanates can also be used as coupling agents for covalently attaching drugs to binding agents. Other techniques are known to the skilled artisan and within the scope of the present invention

[00162] In some embodiments, an intermediate, which is the precursor of the linker, is reacted with the drug under appropriate conditions. In other embodiments, reactive groups are

used on the drug and/or the intermediate. The product of the reaction between the drug and the intermediate, or the derivatized drug, is subsequently reacted with an anti-ROR1 antibody of the invention under appropriate conditions.

[00163] It will be understood that chemical modifications may also be made to the desired compound in order to make reactions of that compound more convenient for purposes of preparing conjugates of the invention. For example a functional group e.g. amine, hydroxyl, or sulfhydryl, may be appended to the drug at a position which has minimal or an acceptable effect on the activity or other properties of the dru

[00164] **Linker Units**

[00165] Typically, the antibody-drug conjugate compounds comprise a Linker unit between the drug unit and the antibody unit. In some embodiments, the linker is cleavable under intracellular or extracellular conditions, such that cleavage of the linker releases the drug unit from the antibody in the appropriate environment. For example, solid tumors that secrete certain proteases may serve as the target of the cleavable linker; in other embodiments, it is the intracellular proteases that are utilized. In yet other embodiments, the linker unit is not cleavable and the drug is released, for example, by antibody degradation in lysosomes.

[00166] In some embodiments, the linker is cleavable by a cleaving agent that is present in the intracellular environment (for example, within a lysosome or endosome or caveolea). The linker can be, for example, a peptidyl linker that is cleaved by an intracellular peptidase or protease enzyme, including, but not limited to, a lysosomal or endosomal protease. In some embodiments, the peptidyl linker is at least two amino acids long or at least three amino acids long or more.

[00167] Cleaving agents can include,without limitation, cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside target cells (see, e.g., Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123). Peptidyl linkers that are cleavable by enzymes that are present in ROR1-expressing cells. For example, a peptidyl linker that is cleavable by the thiol-dependent protease cathepsin-B, which is highly expressed in cancerous tissue, can be used (e.g., a Phe-Leu or a Gly-Phe-Leu-Gly linker (SEQ ID NO: X)). Other examples of such

linkers are described, e.g., in U.S. Pat. No. 6,214,345, incorporated herein by reference in its entirety and for all purposes

[00168] In some embodiments, the peptidyl linker cleavable by an intracellular protease is a Val-Cit linker or a Phe-Lys linker (see, e.g., U.S. Pat. No. 6,214,345, which describes the synthesis of doxorubicin with the val-cit linker).

[00169] In other embodiments, the cleavable linker is pH-sensitive, that is, sensitive to hydrolysis at certain pH values. Typically, the pH-sensitive linker hydrolyzable under acidic conditions. For example, an acid-labile linker that is hydrolyzable in the lysosome (for example, a hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, orthoester, acetal, ketal, or the like) may be used. (See, e.g., U.S. Pat. Nos. 5,122,368; 5,824,805; 5,622,929; Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123; Neville et al., 1989, Biol. Chem. 264:14653-14661.) Such linkers are relatively stable under neutral pH conditions, such as those in the blood, but are unstable at below pH 5.5 or 5.0, the approximate pH of the lysosome. In certain embodiments, the hydrolyzable linker is a thioether linker (such as, e.g., a thioether attached to the therapeutic agent via an acylhydrazone bond (see, e.g., U.S. Pat. No. 5,622,929).

[00170] In yet other embodiments, the linker is cleavable under reducing conditions (for example, a disulfide linker). A variety of disulfide linkers are known in the art, including, for example, those that can be formed using SATA (N-succinimidyl-5-acetylthioacetate), SPDP (N-succinimidyl-3-(2-pyridyldithio)propionate), SPDB (N-succinimidyl-3-(2-pyridyldithio)butyrate) and SMPT (N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene)- , SPDB and SMPT. (See, e.g., Thorpe et al., 1987, Cancer Res. 47:5924-5931; Wawrzynczak et al., In Immunoconjugates: Antibody Conjugates in Radioimagery and Therapy of Cancer (C. W. Vogel ed., Oxford U. Press, 1987. See also U.S. Pat. No. 4,880,935.)

[00171] In other embodiments, the linker is a malonate linker (Johnson et al., 1995, Anticancer Res. 15:1387-93), a maleimidobenzoyl linker (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1299-1304), or a 3'-N-amide analog (Lau et al., 1995, Bioorg-Med-Chem. 3(10):1305-12).

[00172] In yet other embodiments, the linker unit is not cleavable and the drug is released by antibody degradation. (See U.S. Publication No. 2005/0238649 incorporated by reference herein in its entirety and for all purposes).

[00173] In many embodiments, the linker is self-immolative. As used herein, the term "self-immolative Spacer" refers to a bifunctional chemical moiety that is capable of covalently linking together two spaced chemical moieties into a stable tripartite molecule. It will spontaneously separate from the second chemical moiety if its bond to the first moiety is cleaved. See for example, WO 2007059404A2, WO06110476A2, WO05112919A2, WO2010/062171, WO09/017394, WO07/089149, WO 07/018431, WO04/043493 and WO02/083180, which are directed to drug-cleavable substrate conjugates where the drug and cleavable substrate are optionally linked through a self-immolative linker and which are all expressly incorporated by reference.

[00174] Often the linker is not substantially sensitive to the extracellular environment. As used herein, "not substantially sensitive to the extracellular environment," in the context of a linker, means that no more than about 20%, 15%, 10%, 5%, 3%, or no more than about 1% of the linkers, in a sample of antibody-drug conjugate compound, are cleaved when the antibody-drug conjugate compound presents in an extracellular environment (for example, in plasma).

[00175] Whether a linker is not substantially sensitive to the extracellular environment can be determined, for example, by incubating with plasma the antibody-drug conjugate compound for a predetermined time period (for example, 2, 4, 8, 16, or 24 hours) and then quantitating the amount of free drug present in the plasma.

[00176] In other, non-mutually exclusive embodiments, the linker promotes cellular internalization. In certain embodiments, the linker promotes cellular internalization when conjugated to the therapeutic agent (that is, in the milieu of the linker-therapeutic agent moiety of the antibody-drug conjugate compound as described herein). In yet other embodiments, the linker promotes cellular internalization when conjugated to both the auristatin compound and the anti-ROR1 antibodies of the invention.

[00177] A variety of exemplary linkers that can be used with the present compositions and methods are described in WO 2004-010957, U.S. Publication No. 2006/0074008, U.S.

Publication No. 20050238649, and U.S. Publication No. 2006/0024317 (each of which is incorporated by reference herein in its entirety and for all purposes).

Drug Loading

[00178] Drug loading is represented by p and is the average number of Drug moieties per antibody in a molecule. Drug loading ("p") may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more moieties (D) per antibody, although frequently the average number is a fraction or a decimal. Generally, drug loading of from 1 to 4 is frequently useful, and from 1 to 2 is also useful. ADCs of the invention include collections of antibodies conjugated with a range of drug moieties, from 1 to 20. The average number of drug moieties per antibody in preparations of ADC from conjugation reactions may be characterized by conventional means such as mass spectroscopy and, ELISA assay.

[00179] The quantitative distribution of ADC in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous ADC where p is a certain value from ADC with other drug loadings may be achieved by means such as electrophoresis.

[00180] For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in the exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In certain embodiments, higher drug loading, e.g. p>5, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the drug loading for an ADC of the invention ranges from 1 to about 8; from about 2 to about 6; from about 3 to about 5; from about 3 to about 4; from about 3.1 to about 3.9; from about 3.2 to about 3.8; from about 3.2 to about 3.7; from about 3.2 to about 3.6; from about 3.3 to about 3.8; or from about 3.3 to about 3.7. Indeed, it has been shown that for certain ADCs, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5. See US 2005-0238649 A1 (herein incorporated by reference in its entirety).

[00181] In certain embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for

example, lysine residues that do not react with the drug-linker intermediate or linker reagent, as discussed below. Generally, antibodies do not contain many free and reactive cysteine thiol groups which may be linked to a drug moiety; indeed most cysteine thiol residues in antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarbonylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

[00182] The loading (drug/antibody ratio) of an ADC may be controlled in different ways, e.g., by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, (iii) partial or limiting reductive conditions for cysteine thiol modification, (iv) engineering by recombinant techniques the amino acid sequence of the antibody such that the number and position of cysteine residues is modified for control of the number and/or position of linker-drug attachements (such as thioMab or thioFab prepared as disclosed herein and in WO2006/034488 (herein incorporated by reference in its entirety)).

[00183] It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate or linker reagent followed by drug moiety reagent, then the resulting product is a mixture of ADC compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass spectroscopy and separated by HPLC, e.g. hydrophobic interaction chromatography.

[00184] In some embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

Methods of Determining Cytotoxic Effect of ADCs

[00185] Methods of determining whether a Drug or Antibody-Drug conjugate exerts a cytostatic and/or cytotoxic effect on a cell are known. Generally, the cytotoxic or cytostatic activity of an Antibody Drug conjugate can be measured by: exposing mammalian cells expressing a target protein of the Antibody Drug conjugate in a cell culture medium;

culturing the cells for a period from about 6 hours to about 5 days; and measuring cell viability. Cell-based in vitro assays can be used to measure viability (proliferation), cytotoxicity, and induction of apoptosis (caspase activation) of the Antibody Drug conjugate.

[00186] For determining whether an Antibody Drug conjugate exerts a cytostatic effect, a thymidine incorporation assay may be used. For example, cancer cells expressing a target antigen at a density of 5,000 cells/well of a 96-well plated can be cultured for a 72-hour period and exposed to 0.5 μCi of ^{3}H-thymidine during the final 8 hours of the 72-hour period. The incorporation of ^{3}H-thymidine into cells of the culture is measured in the presence and absence of the Antibody Drug conjugate.

[00187] For determining cytotoxicity, necrosis or apoptosis (programmed cell death) can be measured. Necrosis is typically accompanied by increased permeability of the plasma membrane; swelling of the cell, and rupture of the plasma membrane. Apoptosis is typically characterized by membrane blebbing, condensation of cytoplasm, and the activation of endogenous endonucleases. Determination of any of these effects on cancer cells indicates that an Antibody Drug conjugate is useful in the treatment of cancers.

[00188] Cell viability can be measured by determining in a cell the uptake of a dye such as neutral red, trypan blue, or ALAMAR™ blue (see, e.g., Page *et al.*, 1993, Intl. J. Oncology 3:473-476). In such an assay, the cells are incubated in media containing the dye, the cells are washed, and the remaining dye, reflecting cellular uptake of the dye, is measured spectrophotometrically. The protein-binding dye sulforhodamine B (SRB) can also be used to measure cytoxicity (Skehan et al., 1990, J. Natl. Cancer Inst. 82:1107-12).

[00189] Alternatively, a tetrazolium salt, such as MTT, is used in a quantitative colorimetric assay for mammalian cell survival and proliferation by detecting living, but not dead, cells (see, e.g., Mosmann, 1983, J. Immunol. Methods 65:55-63).

[00190] Apoptosis can be quantitated by measuring, for example, DNA fragmentation. Commercial photometric methods for the quantitative in vitro determination of DNA fragmentation are available. Examples of such assays, including TUNEL (which detects incorporation of labeled nucleotides in fragmented DNA) and ELISA-based assays, are described in Biochemica, 1999, no. 2, pp. 34-37 (Roche Molecular Biochemicals).

[00191] Apoptosis can also be determined by measuring morphological changes in a cell. For example, as with necrosis, loss of plasma membrane integrity can be determined by measuring uptake of certain dyes (e.g., a fluorescent dye such as, for example, acridine orange or ethidium bromide). A method for measuring apoptotic cell number has been described by Duke and Cohen, Current Protocols in Immunology (Coligan et al. eds., 1992, pp. 3.17.1-3.17.16). Cells also can be labeled with a DNA dye (e.g., acridine orange, ethidium bromide, or propidium iodide) and the cells observed for chromatin condensation and margination along the inner nuclear membrane. Other morphological changes that can be measured to determine apoptosis include, e.g., cytoplasmic condensation, increased membrane blebbing, and cellular shrinkage.

[00192] The presence of apoptotic cells can be measured in both the attached and "floating" compartments of the cultures. For example, both compartments can be collected by removing the supernatant, trypsinizing the attached cells, combining the preparations following a centrifugation wash step (e.g., 10 minutes at 2000 rpm), and detecting apoptosis (e.g., by measuring DNA fragmentation). (See, e.g., Piazza et al., 1995, Cancer Research 55:3110-16).

[00193] *In vivo*, the effect of a therapeutic composition of the anti-ROR1 antibody of the invention can be evaluated in a suitable animal model. For example, xenogenic cancer models can be used, wherein cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice (Klein *et al.*, 1997, Nature Medicine 3: 402-408). Efficacy can be measured using assays that measure inhibition of tumor formation, tumor regression or metastasis, and the like.

[00194] The therapeutic compositions used in the practice of the foregoing methods can be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material that when combined with the therapeutic composition retains the anti-tumor function of the therapeutic composition and is generally non-reactive with the patient's immune system. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's Pharmaceutical Sciences 16th Edition, A. Osal., Ed., 1980).

Methods for producing the antibdies of the invention

[00195] The present invention further provides methods for producing the disclosed anti-ROR1 antibodies. These methods encompass culturing a host cell containing isolated nucleic acid(s) encoding the antibodies of the invention. As will be appreciated by those in the art, this can be done in a variety of ways, depending on the nature of the antibody. In some embodiments, in the case where the antibodies of the invention are full length traditional antibodies, for example, a heavy chain variable region and a light chain variable region under conditions such that an antibody is produced and can be isolated.

[00196] The variable heavy and light chains of antibodies A1-A14 are disclosed herein (both protein and nucleic acid sequences); as will be appreciated in the art, these can be easily augmented to produce full length heavy and light chains. That is, having provided the DNA fragments encoding V_H and V_K segments as outlined herein, these DNA fragments can be further manipulated by standard recombinant DNA techniques, for example, to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes, or to a scFv gene. In these manipulations, a V_K- or V_H-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

[00197] The isolated DNA encoding the V_H region can be converted to a full-length heavy chain gene by operatively linking the V_H-encoding DNA to another DNA molecule encoding heavy chain constant regions (C_H1, C_H2 and C_H3). The sequences of murine heavy chain constant region genes are known in the art [see e.g. Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, US Department of Health and Human Services, NIH Publication No. 91-3242] and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region, but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the V_H-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain C_H1 constant region.

[00198] The isolated DNA encoding the VL / VK region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the V_L-encoding DNA to another DNA molecule encoding the light chain constant region, C_L. The sequences of murine light chain constant region genes are known in the art [see, e.g. Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, US Department of Health and Human Services, NIH Publication No. 91-3242] and DNA fragments encompassing these regions can be obtained by standard PCR amplification. In preferred embodiments, the light chain constant region can be a kappa or lambda constant region.

[00199] To create a scFv gene, the V_H- and V_L / V_K-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, e.g. encoding the amino acid sequence $(Gly_4\text{-}Ser)_3$, such that the V_H and V_L / V_K sequences can be expressed as a contiguous single-chain protein, with the V_L / V_K and V_H regions joined by the flexible linker [see e.g. Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, (1990) *Nature* 348:552-554].

[00200] In general, nucleic acids are provided which encode the antibodies of the invention. Such polynucleotides encode for both the variable and constant regions of each of the heavy and light chains, although other combinations are also contemplated by the present invention in accordance with the compositions described herein. The present invention also contemplates oligonucleotide fragments derived from the disclosed polynucleotides and nucleic acid sequences complementary to these polynucleotides.

[00201] The polynucleotides can be in the form of RNA or DNA. Polynucleotides in the form of DNA, cDNA, genomic DNA, nucleic acid analogs, and synthetic DNA are within the scope of the present invention. The DNA may be double-stranded or single-stranded, and if single stranded, may be the coding (sense) strand or non-coding (anti-sense) strand. The coding sequence that encodes the polypeptide may be identical to the coding sequence provided herein or may be a different coding sequence, which sequence, as a result of the redundancy or degeneracy of the genetic code, encodes the same polypeptides as the DNA provided herein.

[00202] In some embodiments, nucleic acid(s) encoding the antibodies of the invention are incorporated into expression vectors, which can be extrachromosomal or designed to integrate into the genome of the host cell into which it is introduced. Expression vectors can contain any number of appropriate regulatory sequences (including, but not limited to, transcriptional and translational control sequences, promoters, ribosomal binding sites, enhancers, origins of replication, etc.) or other components (selection genes, etc.), all of which are operably linked as is well known in the art. In some cases two nucleic acids are used and each put into a different expression vector (e.g. heavy chain in a first expression vector, light chain in a second expression vector), or alternatively they can be put in the same expression vector. It will be appreciated by those skilled in the art that the design of the expression vector(s), including the selection of regulatory sequences may depend on such factors as the choice of the host cell, the level of expression of protein desired, etc.

[00203] In general, the nucleic acids and/or expression can be introduced into a suitable host cell to create a recombinant host cell using any method appropriate to the host cell selected (e.g., transformation, transfection, electroporation, infection), such that the nucleic acid molecule(s) are operably linked to one or more expression control elements (e.g., in a vector, in a construct created by processes in the cell, integrated into the host cell genome). The resulting recombinant host cell can be maintained under conditions suitable for expression (e.g. in the presence of an inducer, in a suitable non-human animal, in suitable culture media supplemented with appropriate salts, growth factors, antibiotics, nutritional supplements, etc.), whereby the encoded polypeptide(s) are produced. In some cases, the heavy chains are produced in one cell and the light chain in another.

[00204] Mammalian cell lines available as hosts for expression are known in the art and include many immortalized cell lines available from the American Type Culture Collection (ATCC), Manassas, VA including but not limited to Chinese hamster ovary (CHO) cells, HEK 293 cells, NSO cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (e.g., Hep G2), and a number of other cell lines. Non-mammalian cells including but not limited to bacterial, yeast, insect, and plants can also be used to express recombinant antibodies. In some embodiments, the antibodies can be produced in transgenic animals such as cows or chickens.

[00205] General methods for antibody molecular biology, expression, purification, and screening are well known, for example, see US Patent Nos. 4,816,567, 4,816,397, 6,331,415 and 7,923,221, as well as Antibody Engineering, edited by Kontermann & Dubel, Springer, Heidelberg, 2001 and 2010 Hayhurst & Georgiou, 2001, Curr Opin Chem Biol 5:683-689; Maynard & Georgiou, 2000, Annu Rev Biomed Eng 2:339-76; and Morrison, S. (1985) Science 229:1202.

Pharmaceutical Compositions

[00206] In another aspect, the present invention provides a composition, e.g. a pharmaceutical composition, containing one or a combination of ROR1 antibodies, or antigen-binding portion(s) thereof, of the present invention, formulated together with a pharmaceutically acceptable carrier. Such compositions may include one or a combination of (e.g. two or more different) antibodies, or immunoconjugates or bispecific molecules of the invention. For example, a pharmaceutical composition of the invention can comprise a combination of antibodies (or immunoconjugates or bispecifics) that bind to different epitopes on the target antigen or that have complementary activities.

[00207] Pharmaceutical compositions of the invention also can be administered in combination therapy, i.e. combined with other agents. For example, the combination therapy can include an anti- antibody of the present invention combined with at least one other anti-tumor agent, or an anti-inflammatory or immunosuppressant agent. Examples of therapeutic agents that can be used in combination therapy are described in greater detail below in the section on uses of the antibodies of the invention.

[00208] As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Preferably, the carrier is suitable for intravenous, intramuscular, subcutaneous, parenteral, spinal or epidermal administration (e.g. by injection or infusion). Depending on the route of administration, the active compound, i.e. antibody, immunoconjugate, or bispecific molecule, may be coated in a material to protect the compound from the action of acids and other natural conditions that may inactivate the compound.

[00209] The pharmaceutical compounds of the invention may include one or more pharmaceutically acceptable salts. A "pharmaceutically acceptable salt" refers to a salt that retains the desired biological activity of the parent compound and does not impart any undesired toxicological effects [see, e.g. Berge, S.M., *et al.* (1977) *J. Pharm. Sci.* 66:1-19]. Examples of such salts include acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N'-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like.

[00210] A pharmaceutical composition of the invention also may include a pharmaceutically acceptable anti-oxidant. Examples of pharmaceutically acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[00211] Examples of suitable aqueous and nonaqueous carriers that may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[00212] These compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms

may be ensured both by sterilization procedures, supra, and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[00213] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00214] Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

[00215] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those

enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[00216] The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated, and the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the composition which produces a therapeutic effect. Generally, out of 100 per cent, this amount will range from about 0.01 per cent to about 99 per cent of active ingredient, preferably from about 0.1 per cent to about 70 per cent, most preferably from about 1 per cent to about 30 per cent of active ingredient in combination with a pharmaceutically acceptable carrier.

[00217] Dosage regimens are adjusted to provide the optimum desired response (e.g. a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[00218] For administration of the antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 0.3 mg/kg body weight, 1 mg/kg body weight, 3 mg/kg body weight, 5 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. An exemplary treatment

regime entails administration once per week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months or once every three to 6 months. Preferred dosage regimens for an anti-ROR1 antibody of the invention include 1 mg/kg body weight or 3 mg/kg body weight via intravenous administration, with the antibody being given using one of the following dosing schedules: (i) every four weeks for six dosages, then every three months; (ii) every three weeks; (iii) 3 mg/kg body weight once followed by 1 mg/kg body weight every three weeks.

[00219] In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibody is usually administered on multiple occasions. Intervals between single dosages can be, for example, weekly, monthly, every three months or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to the target antigen in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1-1000 μg /ml and in some methods about 25-300 μg /ml.

[00220] Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human antibodies show the longest half life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[00221] Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular

patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the ester, salt or amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[00222] A "therapeutically effective dosage" of an anti-ROR1 antibody of the invention preferably results in a decrease in severity of disease symptoms, an increase in frequency and duration of disease symptom-free periods, or a prevention of impairment or disability due to the disease affliction. For example, for the treatment of the ROR1 mediated tumors, a "therapeutically effective dosage" preferably inhibits cell growth or tumor growth by at least about 20%, more preferably by at least about 40%, even more preferably by at least about 60%, and still more preferably by at least about 80% relative to untreated subjects. The ability of a compound to inhibit tumor growth can be evaluated in an animal model system predictive of efficacy in human tumors. Alternatively, this property of a composition can be evaluated by examining the ability of the compound to inhibit cell growth, such inhibition can be measured *in vitro* by assays known to the skilled practitioner. A therapeutically effective amount of a therapeutic compound can decrease tumor size, or otherwise ameliorate symptoms in a subject. One of ordinary skill in the art would be able to determine such amounts based on such factors as the subject's size, the severity of the subject's symptoms, and the particular composition or route of administration selected.

[00223] A composition of the present invention can be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Preferred routes of administration for antibodies of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. The phrase "parenteral administration" as used herein means modes of administration other than

enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

[00224] Alternatively, an antibody of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically.

[00225] The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art [see, e.g. *Sustained and Controlled Release Drug Delivery Systems* (1978) J.R. Robinson, ed., Marcel Dekker, Inc., N.Y].

[00226] Therapeutic compositions can be administered with medical devices known in the art. For example, in a preferred embodiment, a therapeutic composition of the invention can be administered with a needleless hypodermic injection device, such as the devices disclosed in US Patent Nos. 5,399,163; 5,383,851; 5,312,335; 5,064,413; 4,941,880; 4,790,824; or 4,596,556. Examples of well-known implants and modules useful in the present invention include: US Patent No. 4,487,603, which discloses an implantable micro-infusion pump for dispensing medication at a controlled rate; US Patent No. 4,486,194, which discloses a therapeutic device for administering medicants through the skin; US Patent No. 4,447,233, which discloses a medication infusion pump for delivering medication at a precise infusion rate; US Patent No. 4,447,224, which discloses a variable flow implantable infusion apparatus for continuous drug delivery; US Patent No. 4,439,196, which discloses an osmotic drug delivery system having multi-chamber compartments; and US Patent No. 4,475,196, which discloses an osmotic drug delivery system. These patents are incorporated herein by reference. Many other such implants, delivery systems, and modules are known to those skilled in the art.

[00227] In certain embodiments, the monoclonal antibodies of the invention can be formulated to ensure proper distribution *in vivo*. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, e.g. US Patents 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery [see, e.g. V.V. Ranade (1989) *J. Clin. Pharmacol.* 29:685]. Exemplary targeting moieties include folate or biotin (see, e.g. US Patent 5,416,016.); mannosides [Umezawa *et al.* (1988) *Biochem. Biophys. Res. Commun.* 153:1038]; antibodies [P.G. Bloeman *et al.* (1995) *FEBS Lett.* 357:140; M. Owais *et al.* (1995) *Antimicrob. Agents Chemother.* 39:180]; surfactant protein A receptor [Briscoe *et al.* (1995) *Am. J. Physiol.* 1233:134]; p120 [Schreier *et al.* (1994) *J. Biol. Chem.* 269:9090]; see also K. Keinanen; M.L. Laukkanen (1994) *FEBS Lett.* 346:123; J.J. Killion; I.J. Fidler (1994) *Immunomethods* 4:273.

Uses and Methods

[00228] The antibodies, antibody compositions and methods of the present invention have numerous *in vitro* and *in vivo* diagnostic and therapeutic utilities involving the diagnosis and treatment of ROR1 mediated disorders.

[00229] In some embodiments, these molecules can be administered to cells in culture, *in vitro* or *ex vivo*, or to human subjects, e.g. *in vivo*, to treat, prevent and to diagnose a variety of disorders. As used herein, the term "subject" is intended to include human and non-human animals. Non-human animals include all vertebrates, e.g. mammals and non-mammals, such as non-human primates, sheep, dogs, cats, cows, horses, chickens, amphibians, and reptiles. Preferred subjects include human patients having disorders mediated by ROR1 activity. The methods are particularly suitable for treating human patients having a disorder associated with the aberrant ROR1 expression. When antibodies to ROR1 are administered together with another agent, the two can be administered in either order or simultaneously.

[00230] Given the specific binding of the antibodies of the invention for ROR1, the antibodies of the invention can be used to specifically detect ROR1 expression on the surface of cells and, moreover, can be used to purify ROR1 via immunoaffinity purification.

[00231] Furthermore, given the expression of ROR1 on tumor cells, the antibodies, antibody compositions and methods of the present invention can be used to treat a subject with a tumorigenic disorder, e.g. a disorder characterized by the presence of tumor cells expressing ROR1 including, for example non-small cell lung carcinoma, B-cell chronic lymphocytic leukemia and colon cancer. ROR1 has been demonstrated to be internalised on antibody binding as illustrated in Examples 5 and 7 below, thus enabling the antibodies of the invention to be used in any payload mechanism of action e.g. an ADC approach, radioimmunoconjugate, or ADEPT approach.

[00232] In one embodiment, the antibodies (e.g. monoclonal antibodies, antibody fragments, Nanobodies, multispecific and bispecific molecules and compositions, etc.) of the invention can be used to detect levels of ROR1, or levels of cells which contain ROR1 on their membrane surface, which levels can then be linked to certain disease symptoms. Alternatively, the antibodies, generally administered as ADCs, can be used to inhibit or block ROR1 function which, in turn, can be linked to the prevention or amelioration of certain disease symptoms, thereby implicating the ROR1 as a mediator of the disease. This can be achieved by contacting a sample and a control sample with the anti-ROR1 antibody under conditions that allow for the formation of a complex between the antibody and ROR1. Any complexes formed between the antibody and the ROR1 are detected and compared in the sample and the control.

[00233] In another embodiment, the antibodies (e.g. monoclonal antibodies, multispecific and bispecific molecules and compositions) of the invention can be initially tested for binding activity associated with therapeutic or diagnostic use *in vitro*. For example, compositions of the invention can be tested using the flow cytometric assays described in the Examples below.

[00234] The antibodies (e.g. monoclonal antibodies, multispecific and bispecific molecules, immunoconjugates and compositions) of the invention have additional utility in therapy and diagnosis of ROR1 related diseases. For example, the monoclonal antibodies, the

multispecific or bispecific molecules and the immunoconjugates can be used to elicit *in vivo* or *in vitro* one or more of the following biological activities: to inhibit the growth of and/or kill a cell expressing ROR1; to mediate phagocytosis or ADCC of a cell expressing ROR1 in the presence of human effector cells, or to block ROR1 ligand binding to ROR1.

[00235] In a particular embodiment, the antibodies (e.g. monoclonal antibodies, multispecific and bispecific molecules and compositions) are used *in vivo* to treat, prevent or diagnose a variety of ROR1-related diseases. Examples of ROR1-related diseases include, among others, human cancer tissues representing non-small cell lung carcinoma, B-cell chronic lymphocytic leukemia and colon cancer.

[00236] Suitable routes of administering the antibody compositions (e.g. monoclonal antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention *in vivo* and *in vitro* are well known in the art and can be selected by those of ordinary skill. For example, the antibody compositions can be administered by injection (e.g. intravenous or subcutaneous). Suitable dosages of the molecules used will depend on the age and weight of the subject and the concentration and/or formulation of the antibody composition.

[00237] As previously described, the anti-ROR1 antibodies of the invention can be co-administered with one or other more therapeutic agents, e.g. a cytotoxic agent, a radiotoxic agent or an immunosuppressive agent. The antibody can be linked to the agent (as an immunocomplex) or can be administered separate from the agent. In the latter case (separate administration), the antibody can be administered before, after or concurrently with the agent or can be co-administered with other known therapies, e.g. an anti-cancer therapy, e.g. radiation. Such therapeutic agents include, among others, anti-neoplastic agents such as doxorubicin (adriamycin), cisplatin bleomycin sulfate, carmustine, chlorambucil, and cyclophosphamide hydroxyurea which, by themselves, are only effective at levels which are toxic or subtoxic to a patient. Cisplatin is intravenously administered as a 100 mg/kg dose once every four weeks and adriamycin is intravenously administered as a 60-75 mg/ml dose once every 21 days. Other agents suitable for co-administration with the antibodies of the invention include other agents used for the treatment of cancers, e.g. non-small cell lung carcinoma, B-cell chronic lymphocytic leukemia or colon cancer, such as Avastin®, 5FU and gemcitabine. Co-administration of the anti-ROR1 antibodies or antigen binding fragments

thereof, of the present invention with chemotherapeutic agents provides two anti-cancer agents which operate via different mechanisms which yield a cytotoxic effect to human tumor cells. Such co-administration can solve problems due to development of resistance to drugs or a change in the antigenicity of the tumor cells which would render them unreactive with the antibody.

[00238] Target-specific effector cells, e.g. effector cells linked to compositions (e.g. monoclonal antibodies, multispecific and bispecific molecules) of the invention can also be used as therapeutic agents. Effector cells for targeting can be human leukocytes such as macrophages, neutrophils or monocytes. Other cells include eosinophils, natural killer cells and other IgG- or IgA-receptor bearing cells. If desired, effector cells can be obtained from the subject to be treated. The target-specific effector cells can be administered as a suspension of cells in a physiologically acceptable solution. The number of cells administered can be in the order of 10^8-10^9, but will vary depending on the therapeutic purpose. In general, the amount will be sufficient to obtain localization at the target cell, e.g. a tumor cell expressing ROR1, and to affect cell killing by, e.g. phagocytosis. Routes of administration can also vary.

[00239] Therapy with target-specific effector cells can be performed in conjunction with other techniques for removal of targeted cells. For example, anti-tumor therapy using the compositions (e.g. monoclonal antibodies, multispecific and bispecific molecules) of the invention and/or effector cells armed with these compositions can be used in conjunction with chemotherapy. Additionally, combination immunotherapy may be used to direct two distinct cytotoxic effector populations toward tumor cell rejection. For example, anti-ROR1 antibodies linked to anti-Fc-gamma RI or anti-CD3 may be used in conjunction with IgG- or IgA-receptor specific binding agents.

[00240] Bispecific and multispecific molecules of the invention can also be used to modulate FcγR or FcγR levels on effector cells, such as by capping and elimination of receptors on the cell surface. Mixtures of anti-Fc receptors can also be used for this purpose.

[00241] The compositions (e.g. monoclonal antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention which have complement binding sites, such as portions from IgG1, -2, or -3 or IgM which bind complement, can also be used in the

presence of complement. In one embodiment, *ex vivo* treatment of a population of cells comprising target cells with a binding agent of the invention and appropriate effector cells can be supplemented by the addition of complement or serum containing complement. Phagocytosis of target cells coated with a binding agent of the invention can be improved by binding of complement proteins. In another embodiment target cells coated with the compositions (e.g. monoclonal antibodies, multispecific and bispecific molecules) of the invention can also be lysed by complement. In yet another embodiment, the compositions of the invention do not activate complement.

[00242] The compositions (e.g. monoclonal antibodies, multispecific and bispecific molecules and immunoconjugates) of the invention can also be administered together with complement. In certain embodiments, the instant disclosure provides compositions comprising antibodies, multispecific or bispecific molecules and serum or complement. These compositions can be advantageous when the complement is located in close proximity to the antibodies, multispecific or bispecific molecules. Alternatively, the antibodies, multispecific or bispecific molecules of the invention and the complement or serum can be administered separately.

[00243] Also within the scope of the present invention are kits comprising the antibody compositions of the invention (e.g. monoclonal antibodies, bispecific or multispecific molecules, or immunoconjugates) and instructions for use. The kit can further contain one or more additional reagents, such as an immunosuppressive reagent, a cytotoxic agent or a radiotoxic agent, or one or more additional antibodies of the invention (e.g. an antibody having a complementary activity which binds to an epitope in the ROR1 antigen distinct from the first antibody).

[00244] Accordingly, patients treated with antibody compositions of the invention can be additionally administered (prior to, simultaneously with, or following administration of an antibody of the invention) with another therapeutic agent, such as a cytotoxic or radiotoxic agent, which enhances or augments the therapeutic effect of the antibodies.

[00245] In other embodiments, the subject can be additionally treated with an agent that modulates, e.g. enhances or inhibits, the expression or activity of Fcγ or Fcγ receptors by, for example, treating the subject with a cytokine. Preferred cytokines for administration

during treatment with the multispecific molecule include of granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-γ (IFN-γ), and tumor necrosis factor (TNF).

[00246] The compositions (e.g. antibodies, multispecific and bispecific molecules) of the invention can also be used to target cells expressing FcγR or ROR1, for example, for labeling such cells. For such use, the binding agent can be linked to a molecule that can be detected. Thus, the invention provides methods for localizing *ex vivo* or *in vitro* cells expressing Fc receptors, such as FcγR, or ROR1. The detectable label can be, e.g. a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor.

[00247] In a particular embodiment, the invention provides methods for detecting the presence of the ROR1 antigen in a sample, or measuring the amount of the ROR1 antigen, comprising contacting the sample, and a control sample, with a monoclonal antibody, or an antigen binding portion thereof, which specifically binds to ROR1, under conditions that allow for formation of a complex between the antibody or portion thereof and ROR1. The formation of a complex is then detected, wherein a difference complex formation between the sample compared to the control sample is indicative the presence of the ROR1 antigen in the sample.

[00248] In other embodiments, the invention provides methods for treating an ROR1 mediated disorder in a subject, e.g. human cancers, including non-small cell lung carcinoma, B-cell chronic lymphocytic leukemia and colon cancer.

[00249] All references cited in this specification, including without limitation all papers, publications, patents, patent applications, presentations, texts, reports, manuscripts, brochures, books, internet postings, journal articles, periodicals, product fact sheets, and the like, one hereby incorporated by reference into this specification in their entireties. The discussion of the references herein is intended to merely summarize the assertions made by their authors and no admission is made that any reference constitutes prior art and Applicants' reserve the right to challenge the accuracy and pertinence of the cited references.

[00250] Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes

and modifications may be made thereto without departing from the spirit or scope of the dependant claims.

[00251] The present invention is further illustrated by the following examples which should not be construed as further limiting.

EXAMPLES

EXAMPLE 1: Construction of a Phage-Display Library

[00252] A recombinant protein composed of the extracellular domain of ROR1 (SEQ ID NO: 272) was eurkaryotically synthesized by standard recombinant methods and used as antigen for immunization.

Immunization and mRNA isolation

[00253] A phage display library for identification of the ROR1-binding molecules was constructed as follows. A/J mice (Jackson Laboratories, Bar Harbor, Me.) were immunized intraperitoneally with the recombinant ROR1 antigen (the extracellular domain), using 100 µg protein in Freund's complete adjuvant, on day 0, and with 100 µg antigen on day 28. Test bleeds of mice were obtained through puncture of the retro-orbital sinus. If, by testing the titers, they were deemed high by ELISA using the biotinylated ROR1 antigen immobilized via neutravidin (Reacti-Bind™) NeutrAvidin(TM)-Coated Polystyrene Plates, Pierce, Rockford, Ill.), the mice were boosted with 100 µg of protein on day 70, 71 and 72, with subsequent sacrifice and splenectomy on day 77. If titers of antibody were not deemed satisfactory, mice were boosted with 100 µg antigen on day 56 and a test bleed taken on day 63. If satisfactory titers were obtained, the animals were boosted with 100 µg of antigen on day 98, 99, and 100 and the spleens harvested on day 105.

[00254] The spleens were harvested in a laminar flow hood and transferred to a petri dish, trimming off and discarding fat and connective tissue. The spleens were macerated quickly with the plunger from a sterile 5 cc syringe in the presence of 1.0 ml of solution D (25.0 g guanidine thiocyanate (Boehringer Mannheim, Indianapolis, Ind.), 29.3 ml sterile water, 1.76 ml 0.75 M sodium citrate pH 7.0, 2.64 ml 10% sarkosyl (Fisher Scientific, Pittsburgh, Pa.), 0.36 ml 2-mercaptoethanol (Fisher Scientific, Pittsburgh, Pa.). This spleen suspension was pulled through an 18 gauge needle until all cells were lysed and the viscous solution was transferred to a microcentrifuge tube. The petri dish was washed with 100 µl of solution D to recover any remaining spleen. This suspension was then pulled through a 22 gauge needle an additional 5-10 times.

[00255] The sample was divided evenly between two microcentrifuge tubes and the following added, in order, with mixing by inversion after each addition: 50 µl 2 M sodium acetate pH 4.0, 0.5 ml water-saturated phenol (Fisher Scientific, Pittsburgh, Pa.), 100 µl chloroform/isoamyl alcohol 49:1 (Fisher Scientific, Pittsburgh, Pa.). The solution was vortexed for 10 sec and incubated on ice for 15 min. Following centrifugation at 14 krpm for 20 min at 2-8°C, the aqueous phase was transferred to a fresh tube. An equal volume of water saturated phenol:chloroform:isoamyl alcohol (50:49:1) was added, and the tube vortexed for ten seconds. After 15 min incubation on ice, the sample was centrifuged for 20 min at 2-8°C, and the aqueous phase transferred to a fresh tube and precipitated with an equal volume of isopropanol at -20°C for a minimum of 30 min. Following centrifugation at 14 krpm for 20 min at 4°C, the supernatant was aspirated away, the tubes briefly spun and all traces of liquid removed from the RNA pellet.

[00256] The RNA pellets were each dissolved in 300 µl of solution D, combined, and precipitated with an equal volume of isopropanol at -20°C for a minimum of 30 min. The sample was centrifuged 14 krpm for 20 min at 4°C, the supernatant aspirated as before, and the sample rinsed with 100 µl of ice-cold 70% ethanol. The sample was again centrifuged 14 krpm for 20 min at 4°C, the 70% ethanol solution aspirated, and the RNA pellet dried in vacuo. The pellet was resuspended in 100 µl of sterile diethyl pyrocarbonate-treated water. The concentration was determined by A260 using an absorbance of 1.0 for a concentration of 40 µg/ml. The RNAs were stored at -80°C.

Preparation of Complementary DNA (cDNA)

[00257] The total RNA purified from mouse spleens as described above was used directly as template for cDNA preparation. RNA (50 µg) was diluted to 100 µL with sterile water, and 10 µL of 130 ng/µL oligo dT12 (synthesized on Applied Biosystems Model 392 DNA synthesizer) was added. The sample was heated for 10 min at 70°C, then cooled on ice. Forty µL 5* first strand buffer was added (Gibco/BRL, Gaithersburg, Md.), along with 20 µL 0.1 M dithiothreitol (Gibco/BRL, Gaithersburg, Md.), 10 µL 20 mM deoxynucleoside triphosphates (dNTP's, Boehringer Mannheim, Indianapolis, Ind.), and 10 µL water on ice. The sample was then incubated at 37°C for 2 min. Ten µL reverse transcriptase (Superscript™) II, Gibco/BRL, Gaithersburg, Md.) was added and incubation was continued at 37°C for 1 hr. The cDNA products were used directly for polymerase chain reaction (PCR).

Amplification of Antibody Genes by PCR

[00258] To amplify substantially all of the H and L chain genes using PCR, primers were chosen that corresponded to substantially all published sequences. Because the nucleotide sequences

of the amino termini of H and L contain considerable diversity, 33 oligonucleotides were synthesized to serve as 5' primers for the H chains, and 29 oligonucleotides were synthesized to serve as 5' primers for the kappa L chains as described in US 6,555,310. The constant region nucleotide sequences for each chain required only one 3' primer for the H chains and one 3' primer for the kappa L chains.

[00259] A 50 µL reaction was performed for each primer pair with 50 µmol of 5' primer, 50 µmol of 3' primer, 0.25 µL Taq DNA Polymerase (5 units/µL, Boehringer Mannheim, Indianapolis, Ind.), 3 µL cDNA (prepared as described), 5 µL 2 mM dNTP's, 5 µL 10*Taq DNA polymerase buffer with MgCl2 (Boehringer Mannheim, Indianapolis, Ind.), and H_2O to 50 µL. Amplification was done using a GeneAmp(R) 9600 thermal cycler (Perkin Elmer, Foster City, Calif.) with the following thermocycle program: 94°C for 1 min; 30 cycles of 94°C for 20 sec, 55°C for 30 sec, and 72°C for 30 sec, 72°C for 6 min; 4°C.

[00260] The dsDNA products of the PCR process were then subjected to asymmetric PCR using only a 3' primer to generate substantially only the anti-sense strand of the target genes. A 100 µL reaction was done for each dsDNA product with 200 µmol of 3' primer, 2 µL of ds-DNA product, 0.5 µL Taq DNA Polymerase, 10 µL 2 mM dNTP's, 10 µL 10*Taq DNA polymerase buffer with $MgCl_2$ (Boehringer Mannheim, Indianapolis, Ind.), and H_2O to 100 µL. The same PCR program as that described above was used to amplify the single-stranded (ss)-DNA.

Purification of Single-Stranded DNA by High Performance Liquid Chromatography and Kinasing Single-Stranded DNA

[00261] The H chain ss-PCR products and the L chain single-stranded PCR products were ethanol precipitated by adding 2.5 volumes ethanol and 0.2 volumes 7.5 M ammonium acetate and incubating at -20°C for at least 30 min. The DNA was pelleted by centrifuging in an Eppendorf centrifuge at 14 krpm for 10 min at 2-8°C. The supernatant was carefully aspirated, and the tubes were briefly spun a 2nd time. The last drop of supernatant was removed with a pipette. The DNA was dried in vacuo for 10 min on medium heat. The H chain products were pooled in 210 µL water and the L chain products were pooled separately in 210 µL water. The single-stranded DNA was purified by high performance liquid chromatography (HPLC) using a Hewlett Packard 1090 HPLC and a Gen-Pak™) FAX anion exchange column (Millipore Corp., Milford, Mass.). The gradient used to purify the single-stranded DNA is shown in Table 1, and the oven temperature was 60°C. Absorbance was monitored at 260 nm. The single-stranded DNA eluted from the HPLC was collected in 0.5 min fractions. Fractions containing single-stranded DNA were ethanol precipitated, pelleted and dried as described above. The dried DNA pellets were pooled in 200 µL sterile water.

Table 1 - HPLC gradient for purification of ss-DNA

Time (min)	%A	%B	%C	Flow (ml/min)
0	70	30	0	0.75
2	40	60	0	0.75
17	15	85	0	0.75
18	0	100	0	0.75
23	0	100	0	0.75
24	0	0	100	0.75
28	0	0	100	0.75
29	0	100	0	0.75
34	0	100	0	0.75
35	70	30	0	0.75

Buffer A is 25 mM Tris, 1 mM EDTA, pH 8.0

Buffer B is 25 mM Tris, 1 mM EDTA, 1 M NaCl, pH 8.0

Buffer C is 40 mM phosphoric acid

[00262] The single-stranded DNA was 5'-phosphorylated in preparation for mutagenesis. Twenty-four µL 10* kinase buffer (United States Biochemical, Cleveland, Ohio), 10.4 µL 10 mM adenosine-5'-triphosphate (Boehringer Mannheim, Indianapolis, Ind.), and 2 µL polynucleotide kinase (30 units/µL, United States Biochemical, Cleveland, Ohio) was added to each sample, and the tubes were incubated at 37°C for 1 hr. The reactions were stopped by incubating the tubes at 70°C for 10 min. The DNA was purified with one extraction of Tris equilibrated phenol (pH>8.0, United States Biochemical, Cleveland, Ohio):chloroform:isoamyl alcohol (50:49:1) and one extraction with chloroform:isoamyl alcohol (49:1). After the extractions, the DNA was ethanol precipitated and pelleted as described above. The DNA pellets were dried, then dissolved in 50 µL sterile water. The concentration was determined by measuring the absorbance of an aliquot of the DNA at 260 nm using 33 µg/ml for an absorbance of 1.0. Samples were stored at -20°C.

Preparation of Uracil Templates Used in Generation of Spleen Antibody Phage Libraries

[00263] One ml of *E. coli* CJ236 (BioRAD, Hercules, Calif.) overnight culture was added to 50 ml 2*YT in a 250 ml baffled shake flask. The culture was grown at 37°C to OD600=0.6, inoculated with 10 µl of a 1/100 dilution of BS45 vector phage stock (described in US 6,555,310) and growth continued for 6 hr. Approximately 40 ml of the culture was centrifuged at 12 krpm for 15 min at 4°C. The supernatant (30 ml) was transferred to a fresh centrifuge tube and incubated at room

temperature for 15 min after the addition of 15 µl of 10 mg/ml RNaseA (Boehringer Mannheim, Indianapolis, Ind.). The phages were precipitated by the addition of 7.5 ml of 20% polyethylene glycol 8000 (Fisher Scientific, Pittsburgh, Pa.)/3.5M ammonium acetate (Sigma Chemical Co., St. Louis, Mo.) and incubation on ice for 30 min. The sample was centrifuged at 12 krpm for 15 min at 2-8°C. The supernatant was carefully discarded, and the tube briefly spun to remove all traces of supernatant. The pellet was resuspended in 400 µl of high salt buffer (300 mM NaCl, 100 mM Tris pH 8.0, 1 mM EDTA), and transferred to a 1.5 ml tube.

[00264] The phage stock was extracted repeatedly with an equal volume of equilibrated phenol:chloroform:isoamyl alcohol (50:49:1) until no trace of a white interface was visible, and then extracted with an equal volume of chloroform:isoamyl alcohol (49:1). The DNA was precipitated with 2.5 volumes of ethanol and 1/5 volume 7.5 M ammonium acetate and incubated 30 min at -20°C. The DNA was centrifuged at 14 krpm for 10 min at 4°C, the pellet washed once with cold 70% ethanol, and dried in vacuo. The uracil template DNA was dissolved in 30 µl sterile water and the concentration determined by A260 using an absorbance of 1.0 for a concentration of 40 µg/ml. The template was diluted to 250 ng/µL with sterile water, aliquoted and stored at -20°C.

Mutagenesis of Uracil Template with ss-DNA and Electroporation into E. coli to Generate Antibody Phage Libraries

[00265] Antibody phage display libraries were generated by simultaneously introducing single-stranded heavy and light chain genes onto a phage display vector uracil template. A typical mutagenesis was performed on a 2 µg scale by mixing the following in a 0.2 ml PCR reaction tube: 8 µl of (250 ng/µL) uracil template, 8 µL of 10* annealing buffer (200 mM Tris pH 7.0, 20 mM MgCl2, 500 mM NaCl), 3.33 µl of kinased single-stranded heavy chain insert (100 ng/µL), 3.1 µl of kinased single-stranded light chain insert (100 ng/µL), and sterile water to 80 µl. DNA was annealed in a GeneAmp(R) 9600 thermal cycler using the following thermal profile: 20 sec at 94°C, 85°C for 60 sec, 85°C to 55°C ramp over 30 min, hold at 55°C for 15 min. The DNA was transferred to ice after the program finished. The extension/ligation was carried out by adding 8 µl of 10* synthesis buffer (5 mM each dNTP, 10 mM ATP, 100 mM Tris pH 7.4, 50 mM MgCl2, 20 mM DTT), 8 µL T4 DNA ligase (1 U/µL, Boehringer Mannheim, Indianapolis, Ind.), 8 µL diluted T7 DNA polymerase (1 U/µL, New England BioLabs, Beverly, Mass.) and incubating at 37°C for 30 min. The reaction was stopped with 300 µL of mutagenesis stop buffer (10 mM Tris pH 8.0, 10 mM EDTA). The mutagenesis DNA was extracted once with equilibrated phenol (pH>8):chloroform:isoamyl alcohol (50:49:1), once with chloroform:isoamyl alcohol (49:1), and the DNA was ethanol precipitated at -20°C for at least 30 min. The DNA was pelleted and the supernatant carefully removed as described

above. The sample was briefly spun again and all traces of ethanol removed with a pipetman. The pellet was dried in vacuo. The DNA was resuspended in 4 µL of sterile water.

[00266] One µL of mutagenesis DNA (500 ng) was transferred into 40 µl electrocompetent *E. coli*DH12S (Gibco/BRL, Gaithersburg, Md.) using electroporation. The transformed cells were mixed with approximately 1.0 ml of overnight XL-1 cells which were diluted with 2*YT broth to 60% the original volume. This mixture was then transferred to a 15-ml sterile culture tube and 9 ml of top agar added for plating on a 150-mm LB agar plate. Plates were incubated for 4 hr at 37°C and then transferred to 20°C overnight. First round antibody phage were made by eluting phage off these plates in 10 ml of 2*YT, spinning out debris, and taking the supernatant. These samples are the antibody phage display libraries used for selecting antibodies against the ROR1. Efficiency of the electroporations was measured by plating 10 µl of a 10^{-4} dilution of suspended cells on LB agar plates, follow by overnight incubation of plates at 37°C. The efficiency was calculated by multiplying the number of plaques on the 10^{-4} dilution plate by 106. Library electroporation efficiencies are typically greater than $1*10^{7}$ phages under these conditions.

Transformation of E. coli by Electroporation

[00267] Electrocompetent *E. coli* cells were thawed on ice. DNA was mixed with 40 L of these cells by gently pipetting the cells up and down 2-3 times, being careful not to introduce an air bubble. The cells were transferred to a Gene Pulser cuvette (0.2 cm gap, BioRAD, Hercules, Calif.) that had been cooled on ice, again being careful not to introduce an air bubble in the transfer. The cuvette was placed in the E. coli Pulser (BioRAD, Hercules, Calif.) and electroporated with the voltage set at 1.88 kV according to the manufacturer's recommendation. The transformed sample was immediately resuspended in 1 ml of 2*YT broth or 1 ml of a mixture of 400 µl 2*YT/600 µl overnight XL-1 cells and processed as procedures dictated.

Plating M13 Phage or Cells Transformed with Antibody Phage-Display Vector Mutagenesis Reaction

[00268] Phage samples were added to 200 µL of an overnight culture of E. coli XL1-Blue when plating on 100 mm LB agar plates or to 600 µL of overnight cells when plating on 150 mm plates in sterile 15 ml culture tubes. After adding LB top agar (3 ml for 100 mm plates or 9 ml for 150 mm plates, top agar stored at 55°C (see, Appendix A1, Sambrook et al., supra.), the mixture was evenly distributed on an LB agar plate that had been pre-warmed (37°C-55°C) to remove any excess moisture on the agar surface. The plates were cooled at room temperature until the top agar solidified. The plates were inverted and incubated at 37°C as indicated.

EXAMPLE 2: Preparation of Biotinylated Tyrosine-Protein Kinase Transmembrane Receptor ROR1 and Biotinylated Antibodies

[00269] The concentrated recombinant ROR1 antigen (full length extracellular domain) was extensively dialyzed into BBS (20 mM borate, 150 mM NaCl, 0.1% NaN_3, pH 8.0). After dialysis, 1 mg of the ROR1 (1 mg/ml in BBS) was reacted with a 15 fold molar excess of biotin-XX-NHS ester (Molecular Probes, Eugene, Oreg., stock solution at 40 mM in DMSO). The reaction was incubated at room temperature for 90 min and then quenched with taurine (Sigma Chemical Co., St. Louis, Mo.) at a final concentration of 20 mM. The biotinylation reaction mixture was then dialyzed against BBS at 2-8°C. After dialysis, the biotinylated ROR1 was diluted in panning buffer (40 mM Tris, 150 mM NaCl, 20 mg/ml BSA, 0.1% Tween 20, pH 7.5), aliquoted, and stored at -80°C until needed.

[00270] Antibodies were reacted with 3-(N-maleimidylpropionyl)biocytin (Molecular Probes, Eugene, Oreg.) using a free cysteine located at the carboxy terminus of the heavy chain. Antibodies were reduced by adding DTT to a final concentration of 1 mM for 30 min at room temperature. Reduced antibody was passed through a Sephadex G50 desalting column equilibrated in 50 mM potassium phosphate, 10 mM boric acid, 150 mM NaCl, pH 7.0. 3-(N-maleimidylpropionyl)-biocytin was added to a final concentration of 1 mM and the reaction allowed to proceed at room temperature for 60 min. Samples were then dialyzed extensively against BBS and stored at 2-8°C.

Preparation of Avidin Magnetic Latex

[00271] The magnetic latex (Estapor, 10% solids, Bangs Laboratories, Fishers, Ind.) was thoroughly resuspended and 2 ml aliquoted into a 15 ml conical tube. The magnetic latex was suspended in 12 ml distilled water and separated from the solution for 10 min using a magnet (PerSeptive Biosystems, Framingham, Mass.). While maintaining the separation of the magnetic latex with the magnet, the liquid was carefully removed using a 10 ml sterile pipette. This washing process was repeated an additional three times. After the final wash, the latex was resuspended in 2 ml of distilled water. In a separate 50 ml conical tube, 10 mg of avidin-HS (NeutrAvidin, Pierce, Rockford, Ill.) was dissolved in 18 ml of 40 mM Tris, 0.15 M sodium chloride, pH 7.5 (TBS). While vortexing, the 2 ml of washed magnetic latex was added to the diluted avidin-HS and the mixture mixed an additional 30 sec. This mixture was incubated at 45°C for 2 hr, shaking every 30 min. The avidin magnetic latex was separated from the solution using a magnet and washed three times with 20 ml BBS as described above. After the final wash, the latex was resuspended in 10 ml BBS and stored at 4°C.

[00272] Immediately prior to use, the avidin magnetic latex was equilibrated in panning buffer (40 mM Tris, 150 mM NaCl, 20 mg/ml BSA, 0.1% Tween 20, pH 7.5). The avidin magnetic latex needed for a panning experiment (200 µl/sample) was added to a sterile 15 ml centrifuge tube and brought to 10 ml with panning buffer. The tube was placed on the magnet for 10 min to separate the latex. The solution was carefully removed with a 10 ml sterile pipette as described above. The magnetic latex was resuspended in 10 ml of panning buffer to begin the second wash. The magnetic latex was washed a total of 3 times with panning buffer. After the final wash, the latex was resuspended in panning buffer to the starting volume.

[00273] **EXAMPLE 3: Selection of Recombinant Polyclonal Antibodies to Tyrosine-Protein Kinase Transmembrane Receptor ROR1 Antigen**

[00274] Binding reagents that specifically bind to the ROR1 were selected from the phage display libraries created from hyperimmunized mice as described in Example 1.

Panning

[00275] First round antibody phage were prepared as described in Example 1 using BS45 uracil template. Electroporations of mutagenesis DNA were performed yielding phage samples derived from different immunized mice. To create more diversity in the recombinant polyclonal library, each phage sample was panned separately.

[00276] Before the first round of functional panning with the biotinylated ROR1 antigen, antibody phage libraries were selected for phage displaying both heavy and light chains on their surface by panning with 7F11-magnetic latex (as described in Examples 21 and 22 of US 6,555,310). Functional panning of these enriched libraries was performed in principle as described in Example 16 of US 6,555,310. Specifically, 10 µL of $1*10^{-6}$ M biotinylated ROR1 antigen was added to the phage samples (approximately $1*10^{-8}$ M final concentration of the ROR1), and the mixture allowed to come to equilibrium overnight at 2-8°C.

[00277] After reaching equilibrium, samples were panned with avidin magnetic latex to capture antibody phage bound to the ROR1. Equilibrated avidin magnetic latex (Example 1), 200 µL latex per sample, was incubated with the phage for 10 min at room temperature. After 10 min, approximately 9 ml of panning buffer was added to each phage sample, and the magnetic latex separated from the solution using a magnet. After a ten minute separation, unbound phage was carefully removed using a 10 ml sterile pipette. The magnetic latex was then resuspended in 10 ml of panning buffer to begin the second wash. The latex was washed a total of three times as described above. For each wash, the tubes were in contact with the magnet for 10 min to separate unbound

phage from the magnetic latex. After the third wash, the magnetic latex was resuspended in 1 ml of panning buffer and transferred to a 1.5 mL tube. The entire volume of magnetic latex for each sample was then collected and resuspended in 200 µl 2*YT and plated on 150 mm LB plates as described in Example 1 to amplify bound phage. Plates were incubated at 37°C for 4 hr, then overnight at 20°C.

[00278] The 150 mm plates used to amplify bound phage were used to generate the next round of antibody phage. After the overnight incubation, second round antibody phage were eluted from the 150 mm plates by pipetting 10 mL of 2*YT media onto the lawn and gently shaking the plate at room temperature for 20 min. The phage samples were then transferred to 15 ml disposable sterile centrifuge tubes with a plug seal cap, and the debris from the LB plate pelleted by centrifuging the tubes for 15 min at 3500 rpm. The supernatant containing the second round antibody phage was then transferred to a new tube.

[00279] A second round of functional panning was set up by diluting 100 µL of each phage stock into 900 µL of panning buffer in 15 ml disposable sterile centrifuge tubes. The biotinylated ROR1 antigen was then added to each sample as described for the first round of panning, and the phage samples incubated for 1 hr at room temperature. The phage samples were then panned with avidin magnetic latex as described above. The progress of panning was monitored at this point by plating aliquots of each latex sample on 100 mm LB agar plates to determine the percentage of kappa positives. The majority of latex from each panning (99%) was plated on 150 mm LB agar plates to amplify the phage bound to the latex. The 100 mm LB agar plates were incubated at 37°C for 6-7 hr, after which the plates were transferred to room temperature and nitrocellulose filters (pore size 0.45 mm, BA85 Protran, Schleicher and Schuell, Keene, N.H.) were overlaid onto the plaques.

[00280] Plates with nitrocellulose filters were incubated overnight at room temperature and then developed with a goat anti-mouse kappa alkaline phosphatase conjugate to determine the percentage of kappa positives as described below. Phage samples with lower percentages (<70%) of kappa positives in the population were subjected to a round of panning with 7F11-magnetic latex before performing a third functional round of panning overnight at 2-8°C using the biotinylated ROR1 antigen at approximately $2*10^{-9}$ M. This round of panning was also monitored for kappa positives. Individual phage samples that had kappa positive percentages greater than 80% were pooled and subjected to a final round of panning overnight at 2-8°C at $5*10^{-9}$ M. The ROR1 antibody genes contained within the eluted phage from this fourth round of functional panning were subcloned into the expression vector, pBRncoH3.

[00281] The subcloning process was done generally as described in Example 18 of US 6,555,310. After subcloning, the expression vector was electroporated into DH10B cells and the mixture grown overnight in 2*YT containing 1% glycerol and 10 μg/ml tetracycline. After a second round of growth and selection in tetracycline, aliquots of cells were frozen at -80°C. as the source for the ROR1 polyclonal antibody production. Monoclonal antibodies were selected from these polyclonal mixtures by plating a sample of the mixture on LB agar plates containing 10 μg/ml tetracycline and screening for antibodies that recognized the ROR1.

Expression and Purification of Recombinant Antibodies Against Tyrosine-Protein Kinase Transmembrane Receptor ROR1

[00282] A shake flask inoculum was generated overnight from a -70°C cell bank in an Innova 4330 incubator shaker (New Brunswick Scientific, Edison, N.J.) set at 37°C, 300 rpm. The inoculum was used to seed a 20 L fermentor (Applikon, Foster City, Calif.) containing defined culture medium [Pack et al. (1993) Bio/Technology 11: 1271-1277] supplemented with 3 g/L L-leucine, 3 g/L L-isoleucine, 12 g/L casein digest (Difco, Detroit, Mich.), 12.5 g/L glycerol and 10 μg/ml tetracycline. The temperature, pH and dissolved oxygen in the fermentor were controlled at 26°C, 6.0-6.8 and 25% saturation, respectively. Foam was controlled by addition of polypropylene glycol (Dow, Midland, Mich.). Glycerol was added to the fermentor in a fed-batch mode. Fab expression was induced by addition of L(+)-arabinose (Sigma, St. Louis, Mo.) to 2 g/L during the late logarithmic growth phase. Cell density was measured by optical density at 600 nm in an UV-1201 spectrophotometer (Shimadzu, Columbia, Md.). Following run termination and adjustment of pH to 6.0, the culture was passed twice through an M-210B-EH Microfluidizer (Microfluidics, Newton, Mass.) at 17,000 psi. The high pressure homogenization of the cells released the Fab into the culture supernatant.

[00283] The first step in purification was expanded bed immobilized metal affinity chromatography (EB-IMAC). Streamline™ chelating resin (Pharmacia, Piscataway, N.J.) was charged with 0.1 M $NiCl_2$ and was then expanded and equilibrated in 50 mM acetate, 200 mM NaCl, 10 mM imidazole, 0.01% NaN_3, pH 6.0 buffer flowing in the upward direction. A stock solution was used to bring the culture homogenate to 10 mM imidazole, following which it was diluted two-fold or higher in equilibration buffer to reduce the wet solids content to less than 5% by weight. It was then loaded onto the Streamline column flowing in the upward direction at a superficial velocity of 300 cm/hr. The cell debris passed through unhindered, but the Fab was captured by means of the high affinity interaction between nickel and the hexahistidine tag on the Fab heavy chain. After washing,

the expanded bed was converted to a packed bed and the Fab was eluted with 20 mM borate, 150 mM NaCl, 200 mM imidazole, 0.01% NaN_3, pH 8.0 buffer flowing in the downward direction.

[00284] The second step in the purification used ion-exchange chromatography (IEC). Q Sepharose FastFlow resin (Pharmacia, Piscataway, N.J.) was equilibrated in 20 mM borate, 37.5 mM NaCl, 0.01% NaN_3, pH 8.0. The Fab elution pool from the EB-IMAC step was diluted four-fold in 20 mM borate, 0.01% NaN_3, pH 8.0 and loaded onto the IEC column. After washing, the Fab was eluted with a 37.5-200 mM NaCl salt gradient. The elution fractions were evaluated for purity using an Xcell II™ SDS-PAGE system (Novex, San Diego, Calif.) prior to pooling. Finally, the Fab pool was concentrated and diafiltered into 20 mM borate, 150 mM NaCl, 0.01% NaN_3, pH 8.0 buffer for storage. This was achieved in a Sartocon Slice™ system fitted with a 10,000 MWCO cassette (Sartorius, Bohemia, N.Y.). The final purification yields were typically 50%. The concentration of the purified Fab was measured by UV absorbance at 280 nm, assuming an absorbance of 1.6 for a 1 mg/ml solution.

EXAMPLE 4: Structural Characterization of Monoclonal Antibodies to Tyrosine-Protein Kinase Transmembrane Receptor ROR1

[00285] The cDNA sequences encoding the heavy and light chain variable regions of the ROR1_A1, ROR1_A2, ROR1_A3, ROR1_A4, ROR1_A5, ROR1_A6, ROR1_A7, ROR1_A8, ROR1_A9, ROR1_A10, ROR1_A11, ROR1_A12, ROR1_A13 and ROR1_A14 monoclonal antibodies were obtained using standard PCR techniques and were sequenced using standard DNA sequencing techniques.

[00286] The antibody sequences may be mutagenized to revert back to germline residues at one or more residues.

[00287] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A1 are shown in SEQ ID NO:43 and 29, respectively.

[00288] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A1 are shown in SEQ ID NO:15 and 1, respectively.

[00289] The nucleotide and amino acid sequences of the light chain variable region of humanized ROR1_A1 are shown in SEQ ID NO:271 and 270, respectively.

[00290] The nucleotide and amino acid sequences of the heavy chain variable region of humanized ROR1_A1 are shown in SEQ ID NO:269 and 268, respectively.

[00291] Comparison of the ROR1_A1 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A1 light chain utilizes a V_K segment from murine germline V_K 14-111. Further analysis of the ROR1_A1 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs:173, 190 or 191 and 229, respectively. The alignments of the ROR1_A1 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequences are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00292] Comparison of the ROR1_A1 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A1 heavy chain utilizes a V_H segment from murine germline V_H 5-6. Further analysis of the ROR1_A1 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 57 or 58, 99 or 100 and 149, respectively. The alignments of the ROR1_A1 CDR1 and CDR2 V_H sequences to the germline V_H 5-6 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00293] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A2 are shown in SEQ ID NO:44 and 30, respectively.

[00294] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A2 are shown in SEQ ID NO:16 and 2, respectively.

[00295] Comparison of the ROR1_A2 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A2 light chain utilizes a V_K segment from murine germline V_K 14-111. Further analysis of the ROR1_A2 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions shown in SEQ ID NOs: 179, 190 or 191 and 229, respectively. The alignments of the ROR1_A2 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00296] Comparison of the ROR1_A2 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A2 heavy chain utilizes a V_H segment from murine germline V_H 5-12. Further analysis of the ROR1_A2 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 59 or 60, 101 or 102 and 150, respectively. The alignments of the ROR1_A2 CDR1 and CDR2 V_H sequence to the germline V_H 5-12 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00297] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A3 are shown in SEQ ID NO:45 and 31, respectively.

[00298] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A3 are shown in SEQ ID NO:17 and 3, respectively.

[00299] Comparison of the ROR1_A3 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A3 light chain utilizes a V_K segment from murine germline V_K 14-111 Further analysis of the ROR1_A3 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 179, 190 or 192 and 229, respectively. The alignments of the ROR1_A3 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00300] Comparison of the ROR1_A3 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A3 heavy chain utilizes a V_H segment from murine germline V_H 5-6 Further analysis of the ROR1_A3 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 58 or 61, 103 or 104 and 149, respectively. The alignments of the ROR1_A3 CDR1 and CDR2 V_H sequence to the germline V_H 5-6 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00301] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A4 are shown in SEQ ID NO:46 and 32, respectively.

[00302] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A4 are shown in SEQ ID NO:18 and 4, respectively.

[00303] Comparison of the ROR1_A4 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A4 light chain utilizes a V_K segment from murine germline V_K 14-111.Further analysis of the ROR1_A4 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 179, 193 or 194 and 229, respectively. The alignments of the ROR1_A4 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00304] Comparison of the ROR1_A4 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A4

heavy chain utilizes a V_H segment from murine germline V_H 5-6 Further analysis of the ROR1_A4 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 62 or 63, 105 or 106 and 151, respectively. The alignment ofs the ROR1_A4 CDR1 and CDR2 V_H sequence to the germline V_H 5-6 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00305] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A5 are shown in SEQ ID NO:47 and 33, respectively.

[00306] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A5 are shown in SEQ ID NO:19 and 5, respectively.

[00307] Comparison of the ROR1_A5 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A5 light chain utilizes a V_K segment from murine germline V_K 14-111. Further analysis of the ROR1_A5 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 179, 195 or 196 and 229, respectively. The alignments of the ROR1_A5 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00308] Comparison of the ROR1_A5 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A5 heavy chain utilizes a V_H segment from murine germline V_H 5-6. Further analysis of the ROR1_A5 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 64 or 65, 107 or 108 and 152, ,respectively. The alignments of the ROR1_A5 CDR1 and CDR2 V_H sequence to the germline V_H 5-6 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00309] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A6 are shown in SEQ ID NO:48 and 34, respectively.

[00310] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A6 are shown in SEQ ID NO:20 and 6, respectively.

[00311] Comparison of the ROR1_A6 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A6 light chain utilizes a V_K segment from murine germline V_K 14-111. Further analysis of the ROR1_A6 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain

CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 179, 197 or 198 and 229, respectively. The alignments of the ROR1_A6 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00312] Comparison of the ROR1_A6 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A6 heavy chain utilizes a V_H segment from murine germline V_H 5-6. Further analysis of the ROR1_A6 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 66 or 67, 109 or 110 and 153, respectively. The alignments of the ROR1_A6 CDR1 and CDR2 V_H sequence to the germline V_H 5-6 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00313] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A7 are shown in SEQ ID NO:49 and 35, respectively.

[00314] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A7 are shown in SEQ ID NO:21 and 7, respectively.

[00315] Comparison of the ROR1_A7 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A7 light chain utilizes a V_K segment from murine germline V_K 14-111 Further analysis of the ROR1_A7 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 179, 199 or 200 and 230, respectively. The alignments of the ROR1_A7 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00316] Comparison of the ROR1_A7 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A7 heavy chain utilizes a V_H segment from murine germline V_H 5-6. Further analysis of the ROR1_A7 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 68 or 69, 111 or 112 and 154, respectively. The alignments of the ROR1_A7 CDR1 and CDR2 V_H sequence to the germline V_H 5-6 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00317] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A8 are shown in SEQ ID NO:50 and 36, respectively.

[00318] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A8 are shown in SEQ ID NO:22 and 8, respectively.

[00319] Comparison of the ROR1_A8 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A8 light chain utilizes a V_K segment from murine germline V_K 17-121 Further analysis of the ROR1_A8 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 180, 201 or 202 and 231, respectively. The alignments of the ROR1_A8 CDR1, CDR2 and CDR3 V_k sequences to the germline V_K 17-121 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00320] Comparison of the ROR1_A8 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A8 heavy chain utilizes a V_H segment from murine germline V_H 5-6. Further analysis of the ROR1_A8 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 58 or 61, 113 or 114 and 155, respectively. The alignments of the ROR1_A8 CDR1 and CDR2V_H sequence to the germline V_H 5-6 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00321] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A9 are shown in SEQ ID NO:51and 37, respectively.

[00322] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A9 are shown in SEQ ID NO:23 and 9, respectively.

[00323] Comparison of the ROR1_A9 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A9 light chain utilizes a V_K segment from murine germline V_K 14-111 Further analysis of the ROR1_A9 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 179, 190 or 191 and 229, respectively. The alignments of the ROR1_A9 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00324] Comparison of the ROR1_A9 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A9 heavy chain utilizes a V_H segment from murine germline V_H 5-6. Further analysis of the ROR1_A9 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 70 or 71, 115 or 116 and 156,

respectively. The alignments of the ROR1_A9 CDR1 and CDR2 V_H sequence to the germline V_H 5-6 sequence is shown in Figures 32a and 32b, and 33a and 33b.

[00325] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A10 are shown in SEQ ID NO:52 and 38, respectively.

[00326] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A10 are shown in SEQ ID NO:24 and 10, respectively.

[00327] Comparison of the ROR1_A10 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A10 light chain utilizes a V_K segment from murine germline V_K 14-111. Further analysis of the ROR1_A10 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 174, 190 or 191 and 229, respectively. The alignments of the ROR1_A10 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00328] Comparison of the ROR1_A10 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A10 heavy chain utilizes a V_H segment from murine germline V_H 5-12. Further analysis of the ROR1_A10 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 72 or 73, 117 or 118 and 157, respectively. The alignments of the ROR1_A10 CDR1 and CDR2 V_H sequence to the germline V_H II gene H17 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00329] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A11 are shown in SEQ ID NO:53 and 39, respectively.

[00330] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A11 are shown in SEQ ID NO:25 and 11, respectively.

[00331] Comparison of the ROR1_A11 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A11 light chain utilizes a V_K segment from murine germline V_K 17-121 Further analysis of the ROR1_A11 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 175, 203 or 204 and 232, respectively. The alignments of the ROR1_A11 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 17-121 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00332] Comparison of the ROR1_A11 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A11 heavy chain utilizes a V_H segment from murine germline V_H 5-12. Further analysis of the ROR1_A11 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 74 or 75, 119 or 120 and 155, respectively. The alignments of the ROR1_A11 CDR1 and CDR2 V_H sequence to the germline V_H 5-12 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00333] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A12 are shown in SEQ ID NO:54 and 40, respectively.

[00334] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A12 are shown in SEQ ID NO:26 and 12, respectively.

[00335] Comparison of the ROR1_A12 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A12 light chain utilizes a V_K segment from murine germline V_K 14-111 Further analysis of the ROR1_A12 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 179, 190 or 205 and 229, respectively. The alignments of the ROR1_A12 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00336] Comparison of the ROR1_A12 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A12 heavy chain utilizes a V_H segment from murine germline V_H 5-12. Further analysis of the ROR1_A12 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 58 or 61, 110 or 266 and 158, respectively. The alignments of the ROR1_A12 CDR1 and CDR2 V_H sequence to the germline V_H 5-12 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00337] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A13 are shown in SEQ ID NO:55 and 41, respectively.

[00338] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A13 are shown in SEQ ID NO:27 and 13, respectively.

[00339] Comparison of the ROR1_A13 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A13

light chain utilizes a V_K segment from murine germline V_K 14-111. Further analysis of the ROR1_A13 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 176, 190 or 206 and 229, respectively. The alignments of the ROR1_A13 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 14-111 sequence are shown in Figures 29a, 30a and 30b, and 31, respectively.

[00340] Comparison of the ROR1_A13 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A13 heavy chain utilizes a V_H segment from murine germline V_H 5-12. Further analysis of the ROR1_A13 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 58 or 61, 101 or 102 and 159, respectively. The alignments of the ROR1_A13 CDR1 and CDR2 V_H sequence to the germline V_H 5-12 sequence are shown in Figures 32a and 32b, and 33a and 33b.

[00341] The nucleotide and amino acid sequences of the light chain variable region of ROR1_A14 are shown in SEQ ID NO:56 and 42, respectively.

[00342] The nucleotide and amino acid sequences of the heavy chain variable region of ROR1_A14 are shown in SEQ ID NO:28 and 14, respectively.

[00343] Comparison of the ROR1_A14 light chain immunoglobulin sequence to the known murine germline immunoglobulin light chain sequences demonstrated that the ROR1_A14 light chain utilizes a V_K segment from murine germline V_K 8-21. Further analysis of the ROR1_A14 V_K sequence using the Kabat system of CDR region determination led to the delineation of the light chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 177 or 178, 207 or 208 and 233, respectively. The alignments of the ROR1_A14 CDR1, CDR2 and CDR3 V_K sequences to the germline V_K 8-21 sequence are shown in Figures 29a and 29b, 30a and 30b, and 31, respectively.

[00344] Comparison of the ROR1_A14 heavy chain immunoglobulin sequence to the known murine germline immunoglobulin heavy chain sequences demonstrated that the ROR1_A14 heavy chain utilizes a V_H segment from murine germline V_H 1-9. Further analysis of the ROR1_A14 V_H sequence using the Kabat system of CDR region determination led to the delineation of the heavy chain CDR1, CDR2 and CDR3 regions as shown in SEQ ID NOs: 76 or 77, 122 or 123 and 160, respectively. The alignments of the ROR1_A14 CDR1 and CDR2 V_H sequence to the germline V_H 1-9 sequence are shown in Figures 32a and 32b, and 33a and 33b.

EXAMPLE 5: Immunohistochemistry Using Monoclonal Antibodies to Tyrosine-Protein Kinase Transmembrane Receptor ROR1

[00345] Using the following Reference Protocol, ROR_A1 was used in IHC experiments at 20ug/ml. Under these conditions significant staining was observed in pancreatic cancer and lung cancer for tissue sections prepared as FFPE or frozen formats. The same conditions were used to test binding of ROR1_A1 and ROR1_A8 on normal human tissues

Deparaffinisation and Rehydration

[00346] Slides were heated for 2hr at 60°C in 50 ml Falcons in a water bath with no buffer. Each Falcon had one slide or two slides back-to back with long gel loading tip between them to prevent slides from sticking to each other. Slides were deparaffinised in EZ-DeWax (BioGenex, CA, USA) for 5 min in black slide rack, then rinsed well with the same DeWax solution using 1 ml pipette, then washed with water. Slides were placed in a coplin jar filled with water until the pressure cooker was ready; the water was changed a couple of times.

Antigen Retrieval

[00347] Water was exchanged for antigen retrieval solution = 1 x citrate buffer, pH 6 (DAKO). Antigen was retrieved by the pressure cooker method. The slides in the plastic coplin jar in antigen retrieval solution were placed into a pressure cooker which was then heated up to position 6 (the highest setting). 15-20 min into the incubation, the temperature was reduced to position 3 and left at that (when the temperature inside the pressure cooker was 117°C) for another 20-25 min. Then the hob was switched off and the cooker was placed onto the cold hob and the pressure was released by carefully moving the handle into the position between "open" and "closed". The whole system was left to release the pressure and to cool down for another 20 min. The lid was opened and samples taken out to rest on the bench. The slides were washed 1x5min with PBS-3T (0.5 L PBS + 3 drops of Tween-20) and the slides were placed in PBS.

Staining

[00348] After antigen retrieval, slides were mounted in the Shandon Coverplate system. Trapping of air bubbles between the slide and plastic coverplate was prevented by placing the coverplate into the coplin jar filled with PBS and gently sliding the slide with tissue sections into the coverplate. The slide was pulled out of the coplin jar while holding it tightly together with the coverplate. The assembled slide was placed into the rack, letting PBS trapped in the funnel and between the slide and coverplate to run through. Slides were washed with 2x2 ml (or 4x1 ml) PBS-3T and 1x2 ml PBS, waiting until all PBS had gone through the slide and virtually no PBS was left in the funnel

[00349] Endogenous peroxide blockade was performed using peroxidase blocking reagent (S2001, DAKO). 1-4 drops of peroxide solution was used per slide and incubated for 5 minutes. The slides were rinsed with water and then once with 2 ml PBS-3T and once with 2 ml PBS; it was important to wait until virtually no liquid was left in the funnel before adding a new portion of wash buffer.

[00350] The primary antibody was diluted with an Antibody diluent reagent (DAKO). Optimal dilution was determined to be 0.5μg/ml. 50-200 μl of diluted primary antibody was applied to each section and/or tissue microarray; taking care to cover the whole tissue. The slide was gently tapped to distribute the antibody evenly over the section or a pipette tip was used over the top of the section. The slide was incubated for 45 min in a moist chamber at room temperature. Slides were washed with 2x2 ml (or 4x1 ml) PBS-3T and then 1x2 ml PBS, waiting until all PBS had gone through the slide and virtually no PBS was left in the funnel. The corresponding donkey anti-goat IgG:HRP (OBT1500P, 1 mg/ml, Serotec) was applied at 1:1000 and incubated for 35 min at room temperature. The slides were washed as above. The DAB substrate was made up in dilution buffer; 2 ml containing 2 drops of substrate was enough for 10 slides. The DAB reagent was applied to the slides by applying a few drops at a time. All of the DAB was distributed between the slides. The slides were incubated for 10 min. The slides were washed 1x2 ml (or 2x1 ml) with PBS-3T and 1x2 ml (or 2x1 ml) with PBS, waiting until all PBS had gone through the slide and virtually no PBS was left in the funnel. Hematoxylin (DAKO) was applied; 1 ml was enough for 10 slides and slides were incubated for 1 min at room temperature. The funnels of the Shandon Coverplate system were filled with 2 ml of water and let to run through. When slides were clear of the excess of hematoxylin, the system was disassembled, tissue sections and/or arrays were washed with water from the wash bottle and placed into a black slide rack. Tissues were rehydrated by incubating in EZ-DeWax for 5 min and then in 95% ethanol for 2-5 min. Slides were left to dry on the bench at room temperature and then mounted in mounting media and covered with coverslip.

[00351] The following normal tissues showed no evidence of specific staining with ROR1_A1, in correlation with proteomics and mRNA analyses : Lung, pancreas, liver, heart, lymph node, spleen, colon, skin, brain, kidney, stomach, bladder, skeletal muscle, breast, ovary, prostate, cervix, tonsil, spinal cord, retina (N=2 for each tissue). However, the other antibody, ROR1_A8 showed staining in multiple normal tissues, particularly on endothelial cells and fibroblasts in discordance with other analysis methods, suggesting that this antibody has cross reactivity to another protein/s.

EXAMPLE 6: Specificity of Monoclonal Antibodies to Tyrosine-Protein Kinase Transmembrane Receptor ROR1. Determined by Flow Cytometry Analysis

[00352] The specificity of antibodies against the ROR1 selected in Example 2 was tested by flow cytometry. To test the ability of the antibodies to bind to the cell surface ROR1 protein, the antibodies were incubated with the ROR1-expressing cells. Cells were washed in FACS buffer (DPBS, 2% FBS),centrifuged and resuspended in 100µl of the diluted primary ROR1 antibody (also diluted in FACS buffer). The antibody-cell line complex was incubated on ice for 60 min and then washed twice with FACS buffer as described above. The cell-antibody pellet was resuspended in 100µl of the diluted secondary antibody (also diluted in FACS buffer) and incubated on ice for 60 min on ice. The pellet was washed as before and resuspended in 200µl FACS buffer. The samples were loaded onto the BD FACScanto II flow sytometer and the data analyzed using the BD FACSdiva software.

[00353] The results of the flow cytometry analysis demonstrated that 14 monoclonal antibodies designated ROR1_A1, ROR1_A2, ROR1_A3, ROR1_A4, ROR1_A5, ROR1_A6, ROR1_A7, ROR1_A8, ROR1_A9, ROR1_A10, ROR1_A11, ROR1_A12, ROR1_A13 and ROR1_A14 bound effectively to the cell-surface human ROR1 expressed in A549 cells, from human lung adenocarinoma (Figure 34). Further analysis using the above procedure demonstrated ROR1_A1, ROR1_A3, ROR1_A8 and ROR1_A14 bound effectively to the cell-surface human ROR1 expressed in CALU1, H358, PANC-1, H226, H69 and HT-29 cells (Figure 35).

EXAMPLE 7: Specificity of Chimeric and Humanised Monoclonal Antibodies to Tyrosine-Protein Kinase Transmembrane Receptor ROR1. Determined by Flow Cytometry Analysis

[00354] The specificity of chimeric and humanised ROR1_A1 antibody was tested by flow cytometry. To test the ability of the antibodies to bind to the cell surface ROR1 protein, the antibodies were incubated with the ROR1-expressing cells, A549 from human lung adenocarcinoma and HT-29, from human colon adenocarcinoma. Cells were washed in FACS buffer (DPBS, 2% FBS),centrifuged and resuspended in 100µl of the diluted primary ROR1 antibody (also diluted in FACS buffer). The antibody-cell line complex was incubated on ice for 60 min and then washed twice with FACS buffer as described above. The cell-antibody pellet was resuspended in 100µl of the diluted secondary antibody (also diluted in FACS buffer) and incubated on ice for 60 min on ice. The pellet was washed as before and resuspended in 200µl FACS buffer. The samples were loaded onto the BD FACScanto II flow sytometer and the data analyzed using the BD FACSdiva software.

[00355] The results of the flow cytometry analysis demonstrated that chimeric and humanized ROR1_A1 antibodies bound effectively to the cell-surface human ROR1 (Figures 36a and 36b).

EXAMPLE 8: Internalization of ROR1_A11 by HT29 cells.

[00356] ROR1_A11 was shown to be internalized by HT29 cells (human colon adenocarcinoma cell line) upon binding to the cells using an immunofluorescence microscopy assay. The immunofluorescence microscopy assay showed internalization of ROR1_A11 through binding of an anti-human IgG secondary antibody conjugated to fluorescein isothiocyanate (GamK-FITC).

[00357] The immunofluorescence microscopy assay was conducted as follows HT29 cells were incubated at 37°C for 12 hr for cells to adhere to each other. ROR1_A11 and secondary antibody conjugated to fluorescein isothiocyanate were serially diluted, washed with FACS buffer (PBS, 2% FBS) and then added to the culture media. The media was then washed again with FACS buffer (PBS, 2% FBS) and incubated at 37 °C, after which 200 µl 2% PFA was added. Coverslips were mounted using a 9µl aqeous mountaing media and the cells were then visualized at regular time intervals using Leica fluorescent microscope. Figure 37 shows surface binding of ROR1_A11/ secondary antibody FITC conjugate complex to HT29 cells after 0 min, 15 min, 60 min and 120 min. The complete internalization of ROR1_A11 was observed after 120 min.

EXAMPLE 9: Internalization of ROR1_A1 and ROR1_A3 monoclonal antibodies by CALU1 and PANC1 cells.

[00358] ROR1_A1 and ROR1_A3 antibodies were shown to be internalized by CALU1 cells (human epidermoid lung carcinoma cell line) and PANC1 cells (human pancreatic carcinoma cell line) upon binding to the cells using MabZAP assays. The MabZAP antibodies were bound to the primary antibodies. Next, the MabZAP complex was internalized by the cells. The entrance of Saporin into the cells resulted in protein synthesis inhibition and eventual cell death

[00359] The MabZAP assay was conducted as follows. Each of the cells was seeded at a density of 5x103 cells per well. The anti-ROR1 monoclonal antibodies or an isotype control human IgG were serially diluted then added to the cells. The MabZAP were then added at a concentration of 50 µg/ml and the plates allowed to incubate for 48 and 72 hours. Cell viability in the plates was detected by CellTiter-Glo® Luminescent Cell Viability Assay kit (Promega, G7571) and the plates were read at 490nM by a Luminomitor (Tuner BioSystems, Sunnyvale, CA). The data was analyzed by Prism (Graphpad). Cell death was proportional to the concentration of ROR1_A1 and ROR1_A3 antibodies. Figures 38 and 39 show that the anti-ROR1 monoclonal antibodies were efficiently

internalized by both CALU1 and PANC1 cells, as compared to the anti-human IgG isotype control antibody.

EXAMPLE 10: Internalization of Chimeric and Humanised ROR1_A1 by A549 and HT-29 cells.

[00360] Chimeric and humanised ROR1_A1 antibodies were shown to be internalized by A549 cells (human lung adenocarcinoma cell line) and HT29 cells (human colon adenocarcinoma cell line) upon binding to the cells using MabZAP and HuZAP assays. The MabZAP / HuZAP antibodies were bound to the primary antibodies. Next, the MabZAP / HuZAP complex was internalized by the cells. The entrance of Saporin into the cells resulted in protein synthesis inhibition and eventual cell death.

[00361] The MabZAP / HuZAP assay was conducted as follows. Each of the cells was seeded at a density of 5x103 cells per well. The anti-ROR1 chimeric and humanised monoclonal antibodies or an isotype control human IgG were serially diluted then added to the cells. The MabZAP / HuZAP were then added at a concentration of 50 μg/ml and the plates allowed to incubate for 48 and 72 hours. Cell viability in the plates was detected by CellTiter-Glo® Luminescent Cell Viability Assay kit (Promega, G7571) and the plates were read at 490nM by a Luminomitor (Tuner BioSystems, Sunnyvale, CA). The data was analyzed by Prism (Graphpad). Cell death was proportional to the concentration of chimeric and humanised ROR1_A1 antibodies. Figures 40 and 41 show that the anti-ROR1 chimeric and humanised monoclonal antibodies were efficiently internalized by both A549 and HT-29 cells, as compared to the anti-human IgG isotype control antibody.

WE CLAIM:

1. An antibody that specifically binds to the extracellular domain of ROR1 (SEQ ID NO:272) and is internalized by a cell expressing ROR1, said antibody comprising:

a) a heavy chain variable region comprising:

i) a first vhCDR comprising SEQ ID NO:275;

ii) a second vhCDR comprising SEQ ID NO: 277; and

iii) a third vhCDR comprising SEQ ID NO:279; and

b) a light chain variable region comprising:

i) a first vlCDR comprising SEQ ID NO:281;

ii) a second vlCDR comprising SEQ ID NO: 283; and

iii) a third vlCDR comprising SEQ ID NO:285.

2. An antibody according to claim 1 wherein said first vhCDR is selected from the group consisting of SEQ ID NOs: 57, 58, 59, 60, 58, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76 and 77, or a sequence that has 1, 2 or 3 amino acid variants as compared to SEQ ID NOs: 57, 58, 59, 60, 58, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76 and 77.

3. An antibody according to claim 1 wherein said second vhCDR is selected from the group consisting of SEQ ID NOs: 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 11, 112, 113, 114, 115, 116, 117, 118, 119, 120, 266, 122 and 123 or a sequence that has 1, 2 or 3 amino acid variants as compared to SEQ ID NOs: 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 11, 112, 113, 114, 115, 116, 117, 118, 119, 120, 266, 122 and 123.

4. An antibody according to claim 1 wherein said third vhCDR is selected from the group consisting of SEQ ID NOs: 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159 and 160 or a sequence that has 1, 2 or 3 amino acid variants as compared to SEQ ID NOs: 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159 and 160.

5. An antibody according to claim 1 wherein said first vlCDR is selected from the group consisting of SEQ ID NOs: 173, 179, 180, 174, 175, 176, 177 and 178 or a sequence that has 1, 2 or 3 amino acid variants as compared to SEQ ID NOs: 173, 179, 180, 174, 175, 176, 177 and 178.

6. An antibody according to claim 1 wherein said second vlCDR is selected from the group consisting of SEQ ID NOs: 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207 and 208 or a sequence that has 1, 2 or 3 amino acid variants as compared to SEQ ID NOs: 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207 and 208.

7. An antibody according to claim 1 wherein said third vlCDR is selected from the group consisting of SEQ ID NOs: 229, 230, 231, 232 and 233 or a sequence that has 1, 2 or 3 amino acid variants as compared to SEQ ID NOs: 229, 230, 231, 232 and 233.

8. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:58;

ii) a second vh CDR comprising SEQ ID NO: 99;

iii) a third vh CDR comprising SEQ ID NO:149; and

b) a light chain comprising

i) a first vl CDR comprising SEQ ID NO:173;

ii) a second vl CDR comprising SEQ ID NO: 190; and

iii) a third vl CDR comprising SEQ ID NO: 229.

9. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:60;

ii) a second vh CDR comprising SEQ ID NO:101;

iii) a third vh CDR comprising SEQ ID NO: 150; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:179;

ii) a second vl CDR comprising SEQ ID NO:190;

iii) a third vl CDR comprising SEQ ID NO:229.

10. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:58;

ii) a second vh CDR comprising SEQ ID NO:103;

iii) a third vh CDR comprising SEQ ID NO:149; and

b) a light chain comprising

i) a first vl CDR comprising SEQ ID NO:179;

ii) a second vl CDR comprising SEQ ID NO:190;

iii) a third vl CDR comprising SEQ ID NO:229.

11. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:63;

ii) a second vh CDR comprising SEQ ID NO:105;

iii) a third vh CDR comprising SEQ ID NO:149; and

b) a light chain comprising:

i) a first vl CDR comprising SEQ ID NO:179;

ii) a second vl CDR comprising SEQ ID NO:194;

iii) a third vl CDR comprising SEQ ID NO:229.

12. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:65;

ii) a second vh CDR comprising SEQ ID NO:107;

iii) a third vh CDR comprising SEQ ID NO: 152; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:179;

ii) a second vl CDR comprising SEQ ID NO: 196;

iii) a third vl CDR comprising SEQ ID NO:229.

13. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:67;

ii) a second vh CDR comprising SEQ ID NO:110;

iii) a third vh CDR comprising SEQ ID NO: 153; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:179;

ii) a second vl CDR comprising SEQ ID NO:198 ;

iii) a third vl CDR comprising SEQ ID NO:229.

14. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:69;

ii) a second vh CDR comprising SEQ ID NO:111;

iii) a third vh CDR comprising SEQ ID NO: 154; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:179;

ii) a second vl CDR comprising SEQ ID NO:200;

iii) a third vl CDR comprising SEQ ID NO:230.

15. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:58;

ii) a second vh CDR comprising SEQ ID NO:113;

iii) a third vh CDR comprising SEQ ID NO: 155; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:180;

ii) a second vl CDR comprising SEQ ID NO:202;

iii) a third vl CDR comprising SEQ ID NO:231.

16. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:71;

ii) a second vh CDR comprising SEQ ID NO:115;

iii) a third vh CDR comprising SEQ ID NO: 156; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:179;

ii) a second vl CDR comprising SEQ ID NO:190;

iii) a third vl CDR comprising SEQ ID NO:229.

17. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:73;

ii) a second vh CDR comprising SEQ ID NO:117;

iii) a third vh CDR comprising SEQ ID NO: 157; and

b) a light chain comprising

i) a first vl CDR comprising SEQ ID NO:174;

ii) a second vl CDR comprising SEQ ID NO:190;

iii) a third vl CDR comprising SEQ ID NO:229.

18. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:69;

ii) a second vh CDR comprising SEQ ID NO:111;

iii) a third vh CDR comprising SEQ ID NO: 154; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:179;

ii) a second vl CDR comprising SEQ ID NO:200;

iii) a third vl CDR comprising SEQ ID NO:230.

19. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:75;

ii) a second vh CDR comprising SEQ ID NO:119;

iii) a third vh CDR comprising SEQ ID NO: 155; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:175;

ii) a second vl CDR comprising SEQ ID NO:204;

iii) a third vl CDR comprising SEQ ID NO:232.

20. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:58;

ii) a second vh CDR comprising SEQ ID NO:110;

iii) a third vh CDR comprising SEQ ID NO: 158; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:179;

ii) a second vl CDR comprising SEQ ID NO:190

iii) a third vl CDR comprising SEQ ID NO:229.

21. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:58;

ii) a second vh CDR comprising SEQ ID NO:101;

iii) a third vh CDR comprising SEQ ID NO: 159; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:176;

ii) a second vl CDR comprising SEQ ID NO:190;

iii) a third vl CDR comprising SEQ ID NO:229.

22. An antibody according to claim 1 comprising:

a) a heavy chain comprising:

i) a first vh CDR comprising SEQ ID NO:77;

ii) a second vh CDR comprising SEQ ID NO:122;

iii) a third vh CDR comprising SEQ ID NO: 160; and

b) a light chain comprising"

i) a first vl CDR comprising SEQ ID NO:177;

ii) a second vl CDR comprising SEQ ID NO:208;

iii) a third vl CDR comprising SEQ ID NO:233.

23. An antibody according to claim 1 comprising:

a) a heavy chain variable region of SEQ ID NO: 268, comprising amino acids 22-144 and

b) a light chain variable region of SEQ ID NO: 270, comprising amino acids 23-124.

24. An antibody according to claim 1 comprising:

a) a heavy chain at least 95% identical to amino acids 22-144 of SEQ ID NO: 268 and

b) a light chain at least 95% identical to amino acids 23-124 of SEQ ID NO: 270.

25. An antibody according to any previous claim further comprising a covalently attached moiety.

26. An antibody according to claim 25 wherein said moiety is a drug.

27. An antibody according to claim (26 above) wherein said drug is selected from the group consisting of a maytansinoid, a dolastatin, an auristatin, a trichothecene, a calicheamicin, a CC1065 and derivatives thereof.

28. A nucleic acid encoding a heavy chain of any of the antibodies of any of the previous claims.

29. A nucleic acid encoding a light chain of any of the antibodies of any of the previous claims.

30. A host cell containing the nucleic acids of claim 28 and 29.

31. A method of making an antibody comprising culturing a host cell according to claim 30 under conditions where the antibody is expressed.

32. A method according to claim 31 further comprising recovering said antibody.

33. A method of treating cancer comprising administering to a patient in need thereof an antibody that specifically binds to the extracellular domain of ROR1 (SEQ ID NO:272) and is internalized by a cell expressing ROR1, said antibody comprising:

a) a heavy chain variable region comprising:

i) a first vhCDR comprising SEQ ID NO:275;

ii) a second vhCDR comprising SEQ ID NO: 277; and

iii) a third vhCDR comprising SEQ ID NO:279; and

b) a light chain variable region comprising:

i) a first vlCDR comprising SEQ ID NO:281;

ii) a second vlCDR comprising SEQ ID NO: 283; and

iii) a third vlCDR comprising SEQ ID NO:285; and

c) a covalently attached drug conjugate.

34. A method according to claim 33 wherein said cancer is selected from the group consisting of non-small cell lung carcinoma, B-cell chronic lymphocytic leukemia and colon cancer.

VK CDR1 Alignments

A1

```
SEQ ID No: 181    aaggcgagtcaggacattaatagctatttaaactgg
SEQ ID No: 243    aaggcgagtcaggacattaatagctatttaagctgg
                  ******************************** ****
```

A2

```
SEQ ID No: 182    aaggcgagtcaggacattaatagctatttaagctgg
SEQ ID No: 243    aaggcgagtcaggacattaatagctatttaagctgg
                  ************************************
```

A3, A4, A5, A7, A9 and A12

```
SEQ ID No: 182    aaggcgagtcaggacattaatagctatttaagctgg
SEQ ID No: 243    aaggcgagtcaggacattaatagctatttaagctgg
                  ************************************
```

A6

```
SEQ ID No: 183    aaggcgagtcaagacattaatagctatttaagctgg
SEQ ID No: 243    aaggcgagtcaggacattaatagctatttaagctgg
                  *********** ************************
```

A10

```
SEQ ID No: 185    aaggcgagtcaggacatttatagctatttaagctgg
SEQ ID No: 243    aaggcgagtcaggacattaatagctatttaagctgg
                  ****************** ******************
```

A13

```
SEQ ID No: 187    aagtcgagtcaggacattaatagctatttaagttgg
SEQ ID No: 243    aaggcgagtcaggacattaatagctatttaagctgg
                  *** ***************************** ***
```

A8

```
SEQ ID No: 184    ataaccaacactgatattgatgatgctatgaactgg
SEQ ID No: 255    ataaccagcactgatattgatgatgatatgaactgg
                  ******* ***************** **********
```

A11

```
SEQ ID No: 186    atgaccagcactgatattgatgatgctctgaactgg
SEQ ID No: 255    ataaccagcactgatattgatgatgatatgaactgg
                  ** ********************** * ********
```

A14

```
SEQ ID No: 188    cagagtctgctcaacagtagaacccgaaagaactac
SEQ ID No: 260    cagagtctgctcaacagtagaacccgaaagaactac
                  ************************************
```

FIGURE 1a

A14

```
SEQ ID No: 189    aaatccagtcagagtctgctcaacagtagaacccgaaagaactacttggct
SEQ ID No: 261    aaatccagtcagagtctgctcaacagtagaacccgaaagaactacttggct
                  ***************************************************
```

FIGURE 1b

VK CDR2 Alignments

A1, A2, A9 and A10
SEQ ID No: 210 accctgatctatcgtgcaaacagattggta
SEQ ID No: 245 accctgatctatcgtgcaaacagattggta
****

A3
SEQ ID No: 212 accctgatccatcgtgcaaacagattggta
SEQ ID No: 245 accctgatctatcgtgcaaacagattggta
********* **********

A4
SEQ ID No: 213 accctgatctatcgtgcaaacaaattggta
SEQ ID No: 245 accctgatctatcgtgcaaacagattggta
********** *******

A5
SEQ ID No: 215 accctgatctatcgtgcaaagagattgata
SEQ ID No: 245 accctgatctatcgtgcaaacagattggta
********** ****** **

A6
SEQ ID No: 217 accctgacctatcgtgcaaacagattggta
SEQ ID No: 245 accctgatctatcgtgcaaacagattggta
******* **********

A7
SEQ ID No: 219 accctgatctatcgtgcaaacagattgata
SEQ ID No: 245 accctgatctatcgtgcaaacagattggta
********** **

A12
SEQ ID No: 225 accctgacccatcgtgcaaacagattggta
SEQ ID No: 245 accctgatctatcgtgcaaacagattggta
******* * **********

A13
SEQ ID No: 226 accctgatctttcgtgcaaacagattggta
SEQ ID No: 245 accctgatctatcgtgcaaacagattggta
********** **********

A8
SEQ ID No: 221 ctccttatttcagaaggcaatactcttcgt
SEQ ID No: 257 ctccttatttcagaaggcaatactcttcgt
****

A11
SEQ ID No: 223 ctccttatttcagaaggcaatagtcttcgt
SEQ ID No: 257 ctccttatttcagaaggcaatactcttcgt
********** *******

A14
SEQ ID No: 227 aaactgctgatctactggacatccactagggaa
SEQ ID No: 262 aaactgctgatctactgggcatccactagggaa
********** **********

FIGURE 2a

VK CDR2 Alignments

A1, A2, A3, A9, A10, A12 and A13

```
SEQ ID No: 209    cgtgcaaacagattggtagat
SEQ ID No: 244    cgtgcaaacagattggtagat
                  ********************
```

A4

```
SEQ ID No: 214    cgtgcaaacaaattggtagat
SEQ ID No: 244    cgtgcaaacagattggtagat
                  ********** **********
```

A5

```
SEQ ID No: 216    cgtgcaaagagattgatagat
SEQ ID No: 244    cgtgcaaacagattggtagat
                  ******** ****** *****
```

A6

```
SEQ ID No: 218    cgtgcaaacagattggtagaa
SEQ ID No: 244    cgtgcaaacagattggtagat
                  ******************
```

A7

```
SEQ ID No: 220    cgtgcaaacagattgatagat
SEQ ID No: 244    cgtgcaaacagattggtagat
                  *************** *****
```

A8

```
SEQ ID No: 222    gaaggcaatactcttcgtcct
SEQ ID No: 258    gaaggcaatactcttcgtcct
                  ********************
```

A11

```
SEQ ID No: 224    gaaggcaatagtcttcgtcct
SEQ ID No: 258    gaaggcaatactcttcgtcct
                  ********** **********
```

A14

```
SEQ ID No: 228    tggacatccactagggaatct
SEQ ID No: 263    tgggcatccactagggaatct
                  *** ****************
```

FIGURE 2b

VK CDR3 Alignments

A1, A3, A4, A6, A9, A10, A12 and A13

```
SEQ ID No: 234    ctacagtatgatgagtttccgtacacg
SEQ ID No: 246    ctacagtatgatgagtttcctcccaca
                  ********************   ***
```

A2

```
SEQ ID No: 235    ctacagtatgatgaatttccgtacacg
SEQ ID No: 246    ctacagtatgatgagtttcctcccaca
                  ************** *****   ***
```

A5

```
SEQ ID No: 236    ctacagtatgatgagtttccttacacg
SEQ ID No: 246    ctacagtatgatgagtttcctcccaca
                  **********************  ***
```

A7

```
SEQ ID No: 237    ctacagtatgatgagtttccattcacg
SEQ ID No: 246    ctacagtatgatgagtttcctcccaca
                  ********************   ***
```

A8

```
SEQ ID No: 238    ttgcaaactgataacttgcctctcacg
SEQ ID No: 259    ttgcaaagtgataacttgcctctcaca
                  ******* ******************
```

A11

```
SEQ ID No: 239    ttgcaaagtgataacttgcctctcacg
SEQ ID No: 259    ttgcaaagtgataacttgcctctcaca
                  *************************
```

A14

```
SEQ ID No: 240    aagcaatcttatgatcttccgtggacg
SEQ ID No: 264    aagcaatcttataatcttcccacagtg
                  ************ *******      *
```

FIGURE 3

VH CDR1 Alignments

```
A14
SEQ ID No: 97     gctactggctacacattcagtagttactggatagag
SEQ ID No: 265    gctactggctacacattcactggctactggatagag
                  ******************* * * ************
A2
SEQ ID No: 80     gcctctggattcactttcagtacctatgccatgtct
SEQ ID No: 247    gcctctggattcgctttcagtagctatgacatgtct
                  ************ ********* ***** *******
A12 and A13
SEQ ID No: 82     gcctctggattcactttcagtagctatgccatgtct
SEQ ID No: 247    gcctctggattcgctttcagtagctatgacatgtct
                  ************ *************** *******
A10
SEQ ID No: 93     gcctctggattcgctttcagtagctatgccatgtct
SEQ ID No: 247    gcctctggattcgctttcagtagctatgacatgtct
                  ***************************** *******
A11
SEQ ID No: 95     gcctctggattcactttcagtagatatggcatgtct
SEQ ID No: 247    gcctctggattcgctttcagtagctatgacatgtct
                  ************ ********** **** *******
A1
SEQ ID No: 78     gtctctggattcactttcagtagctatgccatgtct
SEQ ID No: 251    gcctctggattcactttcagtagctattacatgtct
                  * ************************  *******
A3 and A8
SEQ ID No: 82     gcctctggattcactttcagtagctatgccatgtct
SEQ ID No: 251    gcctctggattcactttcagtagctattacatgtct
                  ***************************  *******
A4
SEQ ID No: 83     gcctctggattcactttcagtaactatggcatgtct
SEQ ID No: 251    gcctctggattcactttcagtagctattacatgtct
                  ********************** ****  *******
A5
SEQ ID No: 85     gcctctggattcactttcagtaactatgacatgtct
SEQ ID No: 251    gcctctggattcactttcagtagctattacatgtct
                  ********************** **** ********
A6
SEQ ID No: 87     gcctctggattcactttcagtccctatgccatgtct
SEQ ID No: 251    gcctctggattcactttcagtagctattacatgtct
                  *********************  ****  *******
A7
SEQ ID No: 89     gcctctggattctctttcagtagctatgccatgtct
SEQ ID No: 251    gcctctggattcactttcagtagctattacatgtct
                  ************ **************  *******
A9
SEQ ID No: 91     gcctctggattcactttcagtagcaatgccatgtcc
SEQ ID No: 251    gcctctggattcactttcagtagctattacatgtct
                  ************************ **  ******
```

FIGURE 4a

VH CDR1 Alignments

A1
SEQ ID No: 79 ggattcactttcagtagctatgccatgtct
SEQ ID No: 252 ggattcactttcagtagctattacatgtct
********** *******

A2
SEQ ID No: 81 ggattcactttcagtacctatgccatgtct
SEQ ID No: 248 ggattcgctttcagtagctatgacatgtct
****** ********* ***** *******

A12 and A13
SEQ ID No: 79 ggattcactttcagtagctatgccatgtct
SEQ ID No: 248 ggattcgctttcagtagctatgacatgtct
****** ************** *******

A10
SEQ ID No: 94 ggattcgctttcagtagctatgccatgtct
SEQ ID No: 248 ggattcgctttcagtagctatgacatgtct
********** ******* *******

A11
SEQ ID No: 96 ggattcactttcagtagatatggcatgtct
SEQ ID No: 248 ggattcgctttcagtagctatgacatgtct
****** ********** **** *******

A3 and A8
SEQ ID No: 79 ggattcactttcagtagctatgccatgtct
SEQ ID No: 252 ggattcactttcagtagctattacatgtct
********** *******

A4
SEQ ID No: 84 ggattcactttcagtaactatggcatgtct
SEQ ID No: 252 ggattcactttcagtagctattacatgtct
********** **** *******

A5
SEQ ID No: 86 ggattcactttcagtaactatgacatgtct
SEQ ID No: 252 ggattcactttcagtagctattacatgtct
********** **** ********

A6
SEQ ID No: 88 ggattcactttcagtccctatgccatgtct
SEQ ID No: 252 ggattcactttcagtagctattacatgtct
********** **** *******

A7
SEQ ID No: 90 ggattctctttcagtagctatgccatgtct
SEQ ID No: 252 ggattcactttcagtagctattacatgtct
****** ********** *******

A9
SEQ ID No: 92 ggattcactttcagtagcaatgccatgtcc
SEQ ID No: 252 ggattcactttcagtagctattacatgtct
********** ** ******

A14
SEQ ID No: 98 ggctacacattcagtagttactggatagag
SEQ ID No: 256 ggctacacattcactggctactggatagag
********** * * **********

FIGURE 4b

VH CDR2 Alignments

A14
SEQ ID No: 147 gagattttacctggaattggtaatactaac
SEQ ID No: 266 gagattttacctggaagtggtagtactaac
****

A2 and A13
SEQ ID No: 126 ggcattaatagtaatcgtggtaccacctac
SEQ ID No: 249 tacattagtagtggtggtggtagcacctac
***** **** * ****** *******

A12
SEQ ID No: 135 gccattaatagtaatcgtggtaccacctac
SEQ ID No: 249 tacattagtagtggtggtggtagcacctac
***** **** * ****** *******

A10
SEQ ID No: 142 gccattaataatagaggtggtggcacctac
SEQ ID No: 249 tacattagtagtggtggtggtagcacctac
***** ** * * ****** ********

A11
SEQ ID No: 144 gccattaatcctaatggtggtactacctac
SEQ ID No: 249 tacattagtagtggtggtggtagcacctac
***** * ** ******** ******

A1
SEQ ID No: 124 gccattaattttaatcgtggtaccacctac
SEQ ID No: 253 gccattaatagtaatggtggtagcacctac
********* **** ****** *******

A3
SEQ ID No: 128 gccattaatattaatcgtggtaccacctac
SEQ ID No: 253 gccattaatagtaatggtggtagcacctac
********** **** ****** *******

A4
SEQ ID No: 130 gccatgaataataatggtgctagcacctac
SEQ ID No: 253 gccattaatagtaatggtggtagcacctac
***** **** ******** **********

A5
SEQ ID No: 132 gccattaatcgtaaaggtcatagtacctac
SEQ ID No: 253 gccattaatagtaatggtggtagcacctac
********* **** *** *** ******

A6
SEQ ID No: 135 gccattaatagtaatcgtggtaccacctac
SEQ ID No: 253 gccattaatagtaatggtggtagcacctac
*************** ****** *******

A7
SEQ ID No: 136 gccattaatattaatcgtggtaccccctat
SEQ ID No: 253 gccattaatagtaatggtggtagcacctac
********** **** ****** * ****

A8
SEQ ID No: 138 gccattaatcctaatggtggtagtacctac
SEQ ID No: 253 gccattaatagtaatggtggtagcacctac
********* ************ ******

A9
SEQ ID No: 140 gccattaatagtaaaggtggtggcacctac
SEQ ID No: 253 gccattaatagtaatggtggtagcacctac
************** ****** ********

FIGURE 5a

VH CDR2 Alignments

A1

```
SEQ ID No: 125    gccattaattttaatcgtggtaccacctactattcagacactgtgaagggc
SEQ ID No: 254    gccattaatagtaatggtggtagcacctactatccagacactgtgaagggc
                  *********  **** ****** ********** ****************
```

A2 and A13

```
SEQ ID No: 127    ggcattaatagtaatcgtggtaccacctactatccagacactgtgaagggc
SEQ ID No: 250    tacattagtagtggtggtggtagcacctactatccagacactgtgaagggc
                    ***** ****  * ****** ****************************
```

A12

```
SEQ ID No: 146    gccattaatagtaatcgtggtaccacctactattcagacactgtgaagggc
SEQ ID No: 250    tacattagtagtggtggtggtagcacctactatccagacactgtgaagggc
                    ***** ****  * ****** ********** ****************
```

A10

```
SEQ ID No: 143    gccattaataatagaggtggtggcacctactatccagacactgtgaggggc
SEQ ID No: 250    tacattagtagtggtggtggtagcacctactatccagacactgtgaagggc
                    ***** ** * * ****** ************************ ****
```

A11

```
SEQ ID No: 242    gccattaatcctaatggtggtactacctactatccagacactgtgaagggc
SEQ ID No: 250    tacattagtagtggtggtggtagcacctactatccagacactgtgaagggc
                    ***** *  *  ********  ****************************
```

A3

```
SEQ ID No: 129    gccattaatattaatcgtggtaccacctactattcagacactgtgaagggc
SEQ ID No: 254    gccattaatagtaatggtggtagcacctactatccagacactgtgaagggc
                  ********** **** ****** ********** ****************
```

A4

```
SEQ ID No: 131    gccatgaataataatggtgctagcacctactatccagacactgtgaagggc
SEQ ID No: 254    gccattaatagtaatggtggtagcacctactatccagacactgtgaagggc
                  ***** **** ******** ********************************
```

A5

```
SEQ ID No: 133    gccattaatcgtaaaggtcatagtacctactatccagacactgtgcagggc
SEQ ID No: 254    gccattaatagtaatggtggtagcacctactatccagacactgtgaagggc
                  ********* **** ***  *** ********************** *****
```

A6

```
SEQ ID No: 134    gccattaatagtaatcgtggtaccacctactatccagacactgtgaagggc
SEQ ID No: 254    gccattaatagtaatggtggtagcacctactatccagacactgtgaagggc
                  *************** ****** ****************************
```

A7

```
SEQ ID No: 137    gccattaatattaatcgtggtaccccctattatccagacactgtgaagggc
SEQ ID No: 254    gccattaatagtaatggtggtagcacctactatccagacactgtgaagggc
                  ********** **** ****** * *************************
```

A8

```
SEQ ID No: 139    gccattaatcctaatggtggtagtacctactatccagacactgtgaagggc
SEQ ID No: 254    gccattaatagtaatggtggtagcacctactatccagacactgtgaagggc
                  *********  ************ ****************************
```

A9

```
SEQ ID No: 141    gccattaatagtaaaggtggtggcacctactatccagacactgtgaggggc
SEQ ID No: 254    gccattaatagtaatggtggtagcacctactatccagacactgtgaagggc
                  ************** ****** ************************ ****
```

A14

```
SEQ ID No: 148    gagattttacctggaattggtaatactaactacaatgagaaattcaagggc
SEQ ID No: 267    gagattttacctggaagtggtagtactaactacaatgagaagttcaagggc
                  **************** ***** ****************** *********
```

FIGURE 5b

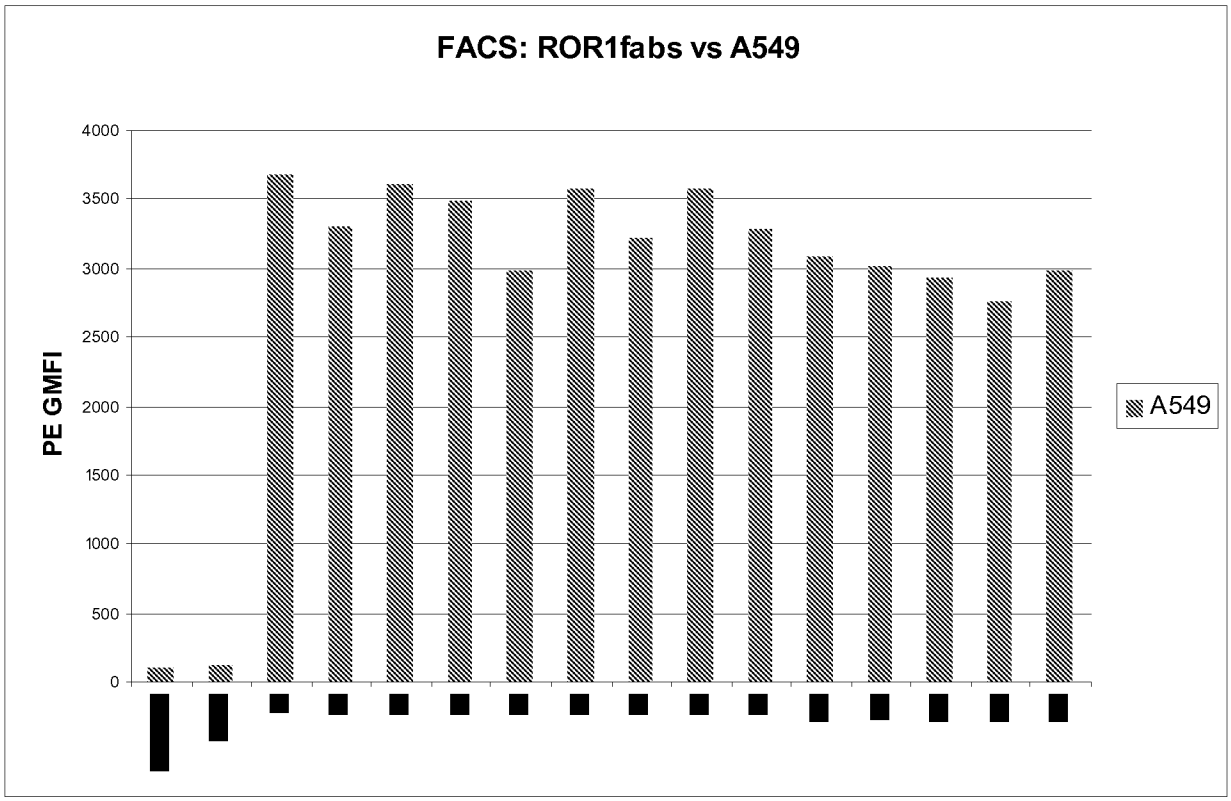

FIGURE 6

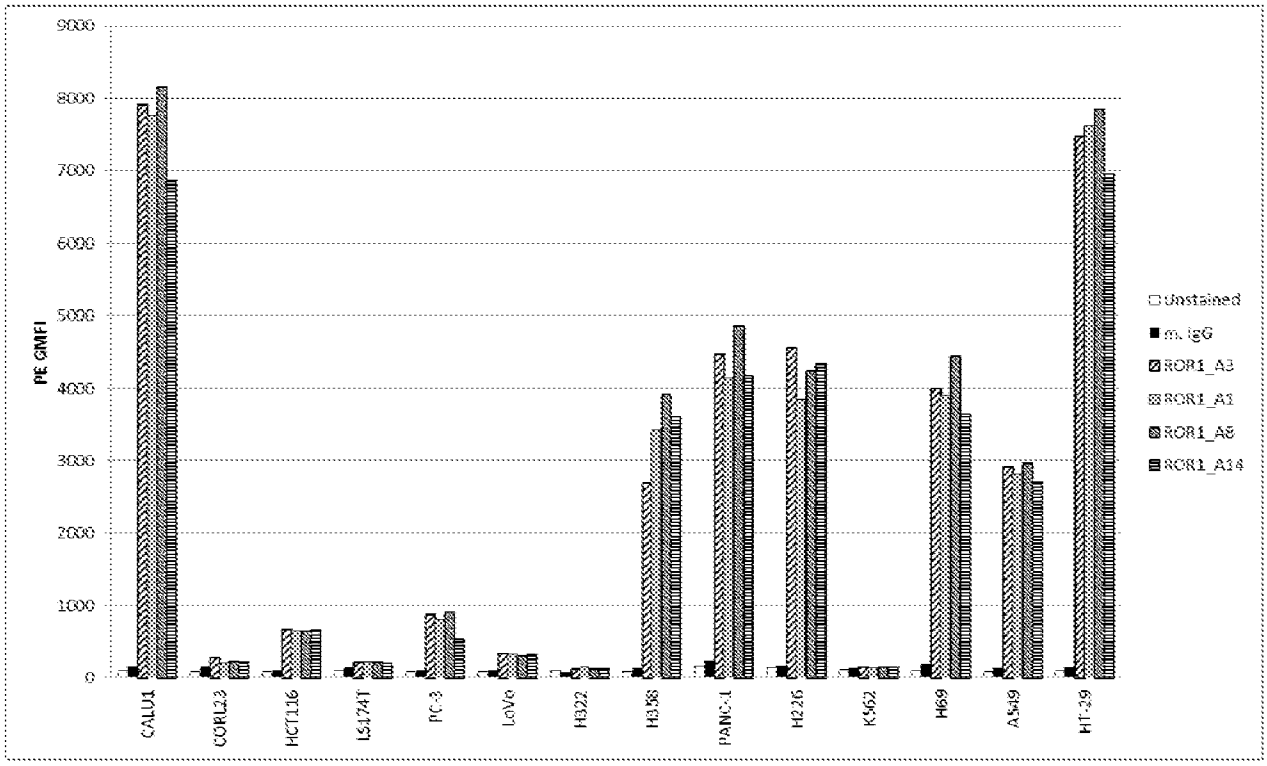

FIGURE 7

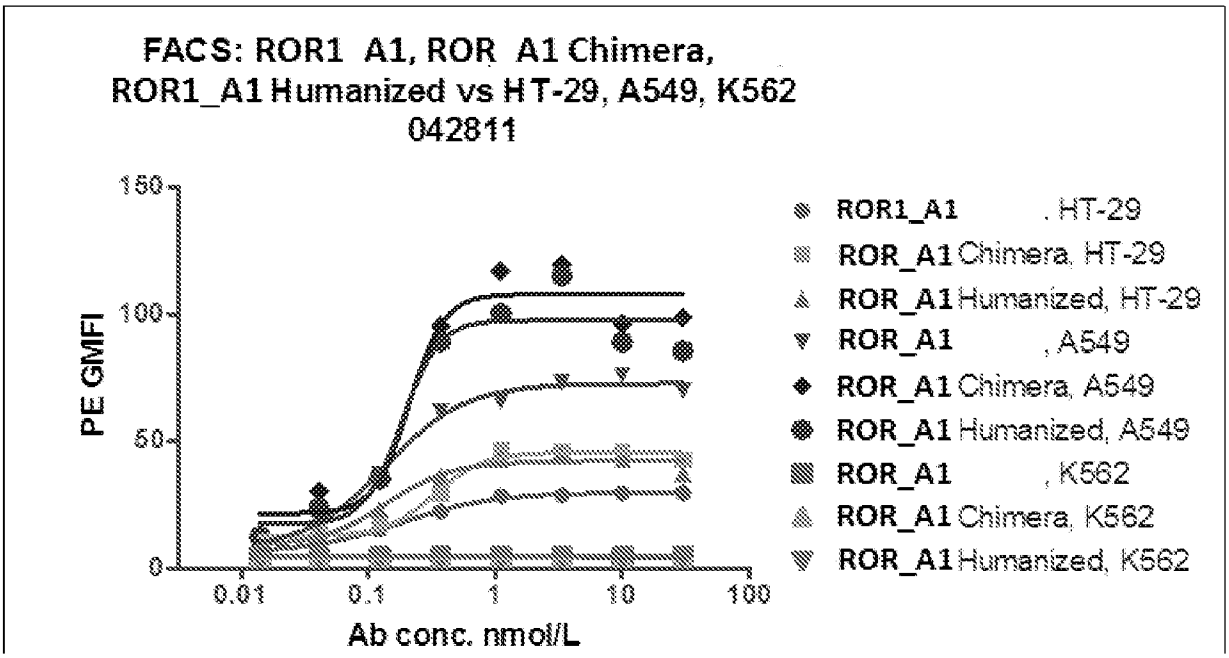

FIGURE 8a

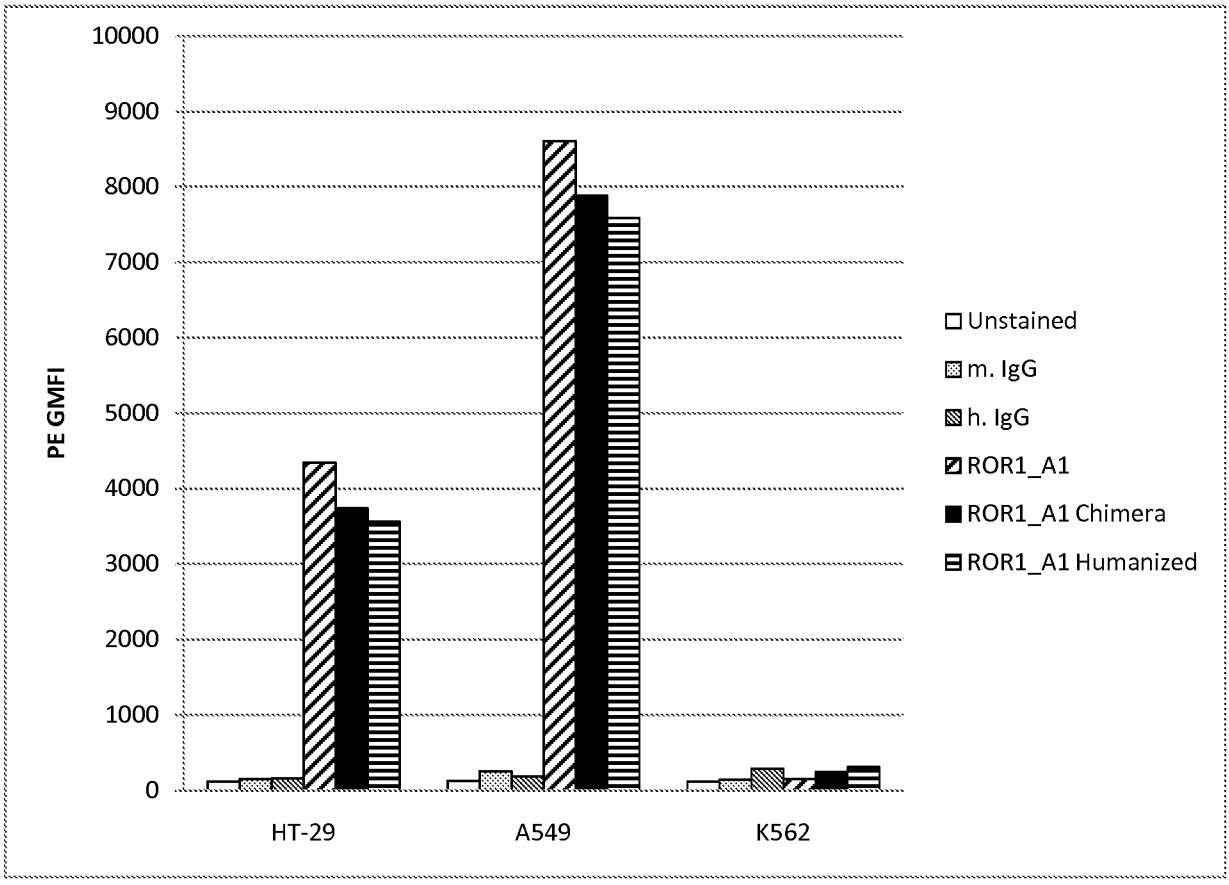

FIGURE 8b

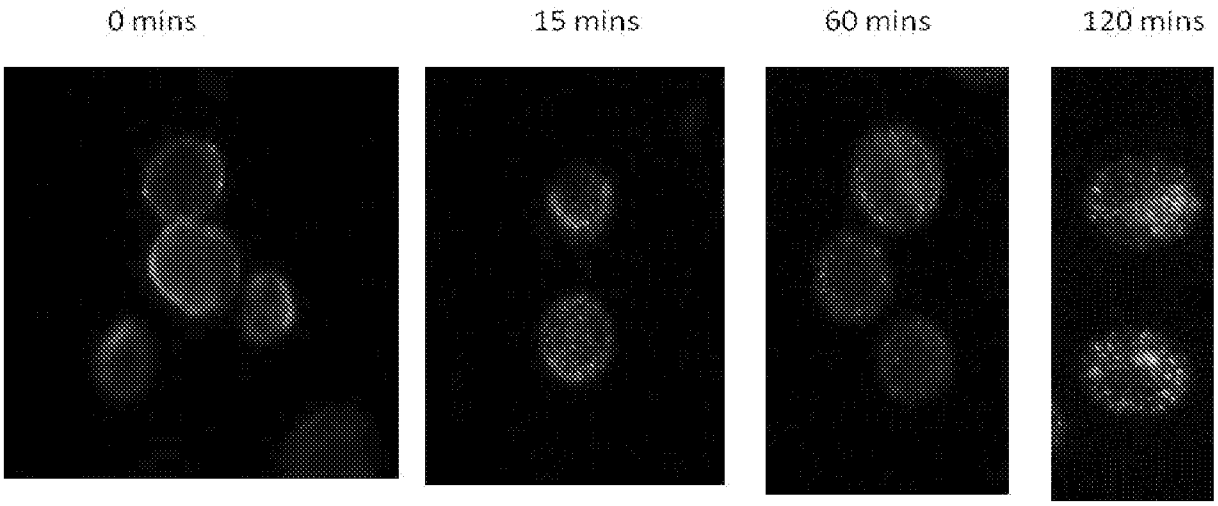

FIGURE 9

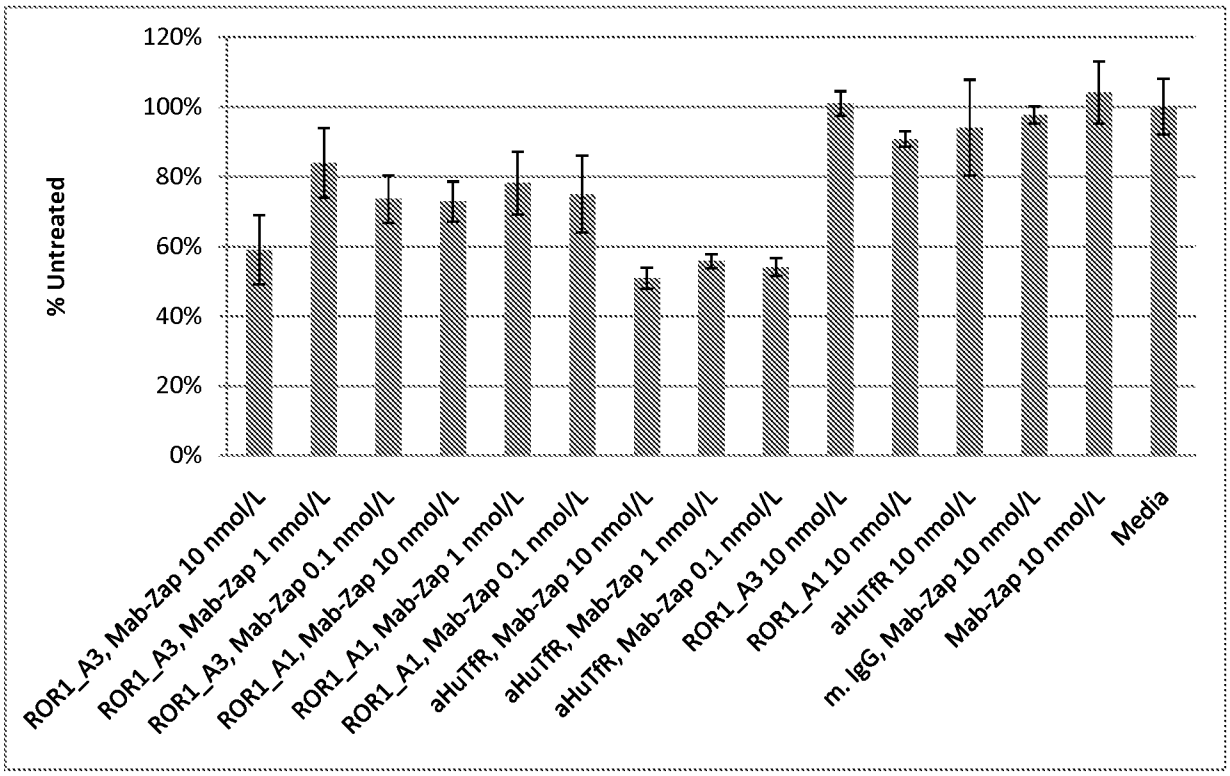

FIGURE 10

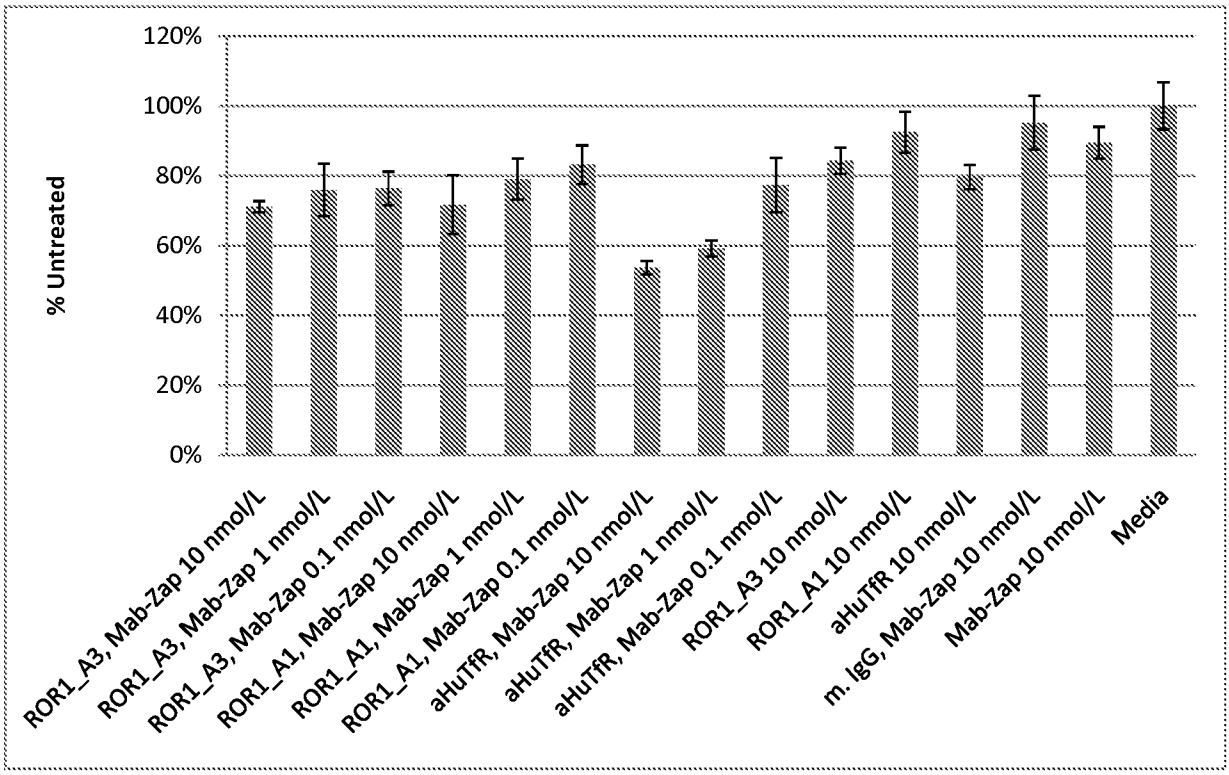
120%
100%
80%
60%
40%
20%
0%
% Untreated
ROR1_A3, Mab-Zap 10 nmol/L
ROR1_A3, Mab-Zap 1 nmol/L
ROR1_A3, Mab-Zap 0.1 nmol/L
ROR1_A1, Mab-Zap 10 nmol/L
ROR1_A1, Mab-Zap 1 nmol/L
ROR1_A1, Mab-Zap 0.1 nmol/L
aHuTfR, Mab-Zap 10 nmol/L
aHuTfR, Mab-Zap 1 nmol/L
aHuTfR, Mab-Zap 0.1 nmol/L
ROR1_A3 10 nmol/L
ROR1_A1 10 nmol/L
aHuTfR 10 nmol/L
m. IgG, Mab-Zap 10 nmol/L
Mab-Zap 10 nmol/L
Media

FIGURE 11

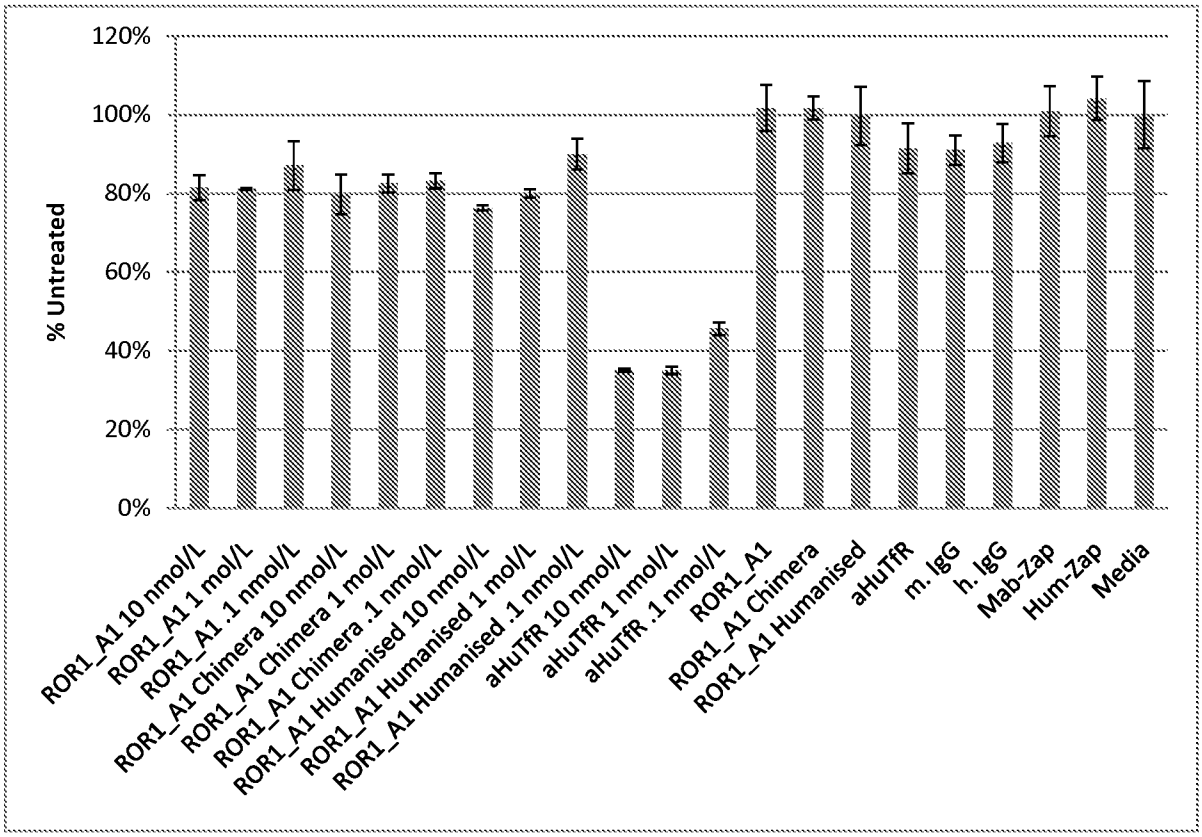

FIGURE 12

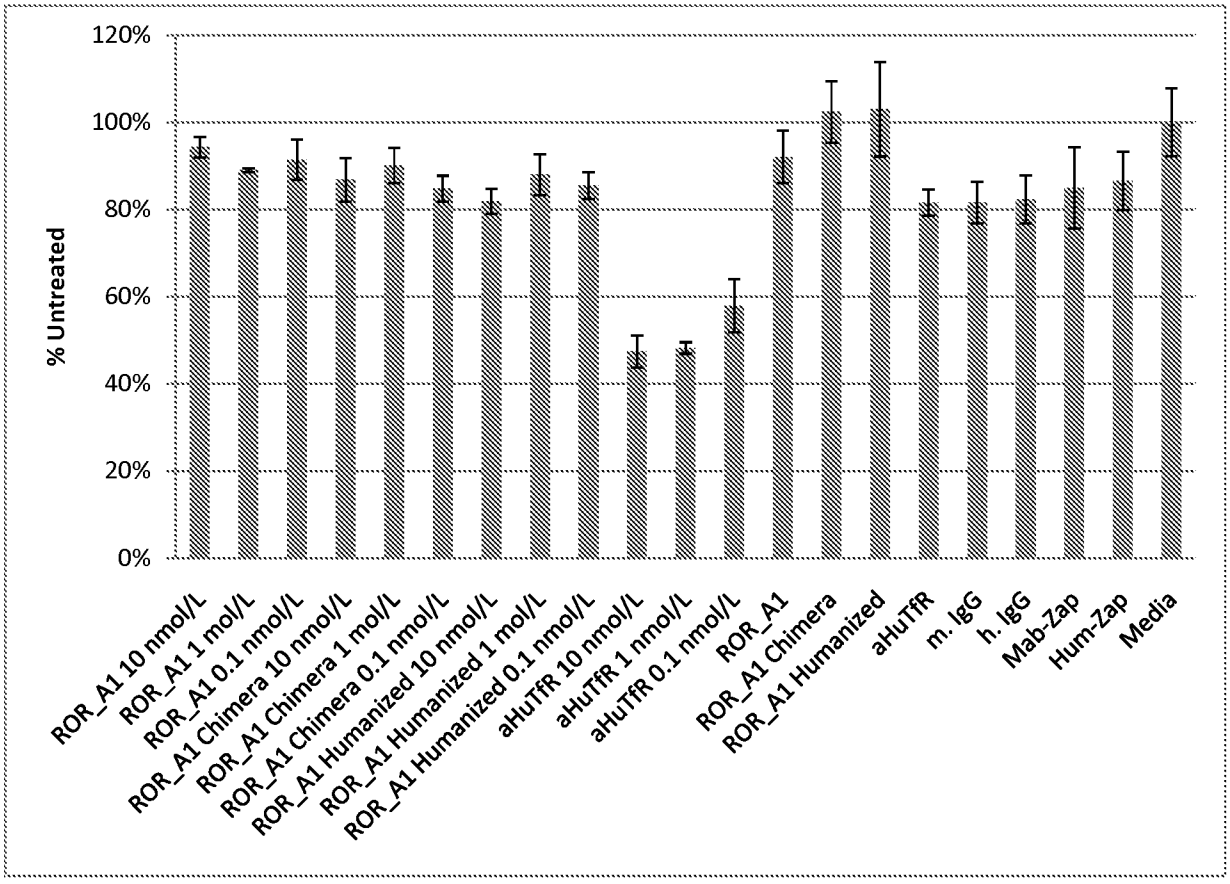

FIGURE 13

SEQ ID No.	Description	Sequence
1	VH_aa A1	MKQSTIALALLPLLFTPVAKAEVKLVESGGGLV RPGGSLKLSCAVSGFTFSSYAMSWVRQTPEKRL EWVAAINFNRGTTYYSDTVKGRFTISRDNAKNT LYLQLSSLRSEDTAFYYCSRHRYSDYDYAMDY WGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNSM VTLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPA VLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPAS STKVDKKIVPRDC
2	VH_aa A2	MKQSTIALALLPLLFTPVAKAEVQLLETGGGLV KPGGSLKLSCAASGFTFSTYAMSWVRQTPEKR LEWVAGINSNRGTTYYPDTVKGRFTISRDNAKN TLSLQMTSLRSEDTALYYCVRHRYTNYDYAMD YWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNS MVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTF PAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHP ASSTKVDKKIVPRDC
3	VH_aa A3	MKQSTIALALLPLLFTPVAKADVMLVESGGGL VKPGGSLKLSCAASGFTFSSYAMSWVRQTPEK RLEWVAAININRGTTYYSDTVKGRFTISRDNAK NTLYLQLSSLRSEDTALYYCSRHRYSDYDYAMD YWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNS MVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTF PAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHP ASSTKVDKKIVPRDC
4	VH_aa A4	MKQSTIALALLPLLFTPVAKAEVKLVESGGGLV KPGGSLKLSCAASGFTFSNYGMSWVRQTPERR LEWVAAMNNNGASTYYPDTVKGRFTISRDNAK NTLYLQMSSLRSEDTALYFCVRHNNYVDYAMD YWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNS MVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTF PAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHP ASSTKVDKKIVPRDC
5	VH_aa A5	MKQSTIALALLPLLFTPVAKAEVKLVESGGGLV KPGGSLKLSCAASGFTFSNYDMSWVRQSPEKR LEWVAAINRKGHSTYYPDTVQGRFTISRDNAK NTLYLQMSSLRSEDTALYYCVRLDDNYYFFDY WGQGTTLTVSSAKTTPPSVYPLAPGSAAQTNS MVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTF PAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHP ASSTKVDKKIVPRDC
6	VH_aa A6	MKQSTIALALLPLLFTPVAKAEVMLVESGGGL VKPGGSLKLSCAASGFTFSPYAMSWVRQTPEK RLEWVAAINSNRGTTYYPDTVKGRFTISRDNAK NTLYLQMSSLRSEDTAFYYCVRHRYNNYDYAM DYWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTN SMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHT FPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAH PASSTKVDKKIVPRDC

SEQ ID No.	Description	Sequence
7	VH_aa A7	MKQSTIALALLPLLFTPVAKAEVMLVESGGGL VKPGGSLKISCAASGFSFSSYAMSWVRQTPEKS LEWVAAININRGTPYYPDTVKGRFTISRDNAKN TLYLQMSSLRSEDTALYYCVRHRNSNNDYAMD YWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNS MVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTF PAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHP ASSTKVDKKIVPRDC
8	VH_aa A8	MKQSTIALALLPLLFTPVAKADVQVVESGGGL VKPGGSLKLSCAASGFTFSSYAMSWVRQTPEK RLEWVAAINPNGGSTYYPDTVKGRFTISRDNAK NTLYLQMSGLRSEDTALYYCARLPWSPYTLDY WGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNSM VTLGCLVKGYFPE
9	VH_aa A9	MKQSTIALALLPLLFTPVAKAEVQLVETGGDLV KPGGSLKLSCVASGFTFSSNAMSWVRQTPEKR LEWVAAINSKGGGTYYPDTVRGRFTISRDNAK NTLYLQVTSLRSEDTALYYCVSHGDNKYFYAM DYWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTN SMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHT FPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAH PASSTKVDKKIVPRDC
10	VH_aa A10	MKQSTIALALLPLLFTPVAKAEVQLVETGGGL VKPGGSLKLSCAASGFAFSSYAMSWVRQTPEK RLEWVAAINNRGGGTYYPDTVRGRFTISRDNA KNTLYLQMSSLRSADTALYYCVRHDNLNYDYA MDSWGQGTSVTVSSAKTTPPSVYPLAPGSAAQT NSMVTLGCLVKGYFPEPVTVTWNSGSLSSGVH TFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVA HPASSTKVDKKIVPRDC
11	VH_aa A11	MKQSTIALALLPLLFTPVPKAEVQLVESGGDLV KPGGSLKLSCAASGFTFSRYGMSWVRQTPEKR LEWVAAINPNGGTTYYPDTVKGRFTISRDNAKN TLFLQMTGLRSEDTALYYCARLPWSPYTLDYW GQGTSVIVSSAKTTPPSVYPLAPGSAAQTNSMV TLGCLVKGYFPEPVTVTWNSGSLSSGVHTFPAV LQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASS TKVDKKIVPRDC
12	VH_aa A12	MKQSTIALALLPLLFTPVAKAEVQLVESGGGLV KPGGSLKLSCAASGFTFSSYAMSWVRQTPEKR LEWVAAINSNRGTTYYSDTVKGRFTISRDNAKN TLYLQMSSLRSEDTAFYYCTRHRYSDYDYAMD YWGQGTSVTVSSAKTTPPSVYPLAPGSAAQTNS MVTLGCLVKGYFPEPVTVTWNSGSLSSGVHTF PAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHP ASSTKVDKKIVPRDC
13	VH_aa A13	MKQSTIALALLPLLFTPVAKAEVQLVETGGGL

SEQ ID No.	Description	Sequence
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14	VH_aa A14	MKQSTIALALLPLLFTPVAKAQVQLKQSGAEL VKPGASVKISCKATGYTFSSYWIEWVKERPGH GLEWIGEILPGIGNTNYNEKFKGKATFTADLSS KTAYMQLSSLTSEDSAVYYCASGGYSTVYWYF DVWGAGTTVTVSSAKTTPPSVYPLAPGSAAQTN SMVTLGCLVKGYFPEPVTVTWNSGSLSSGVHT FPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAH PASSTKVDKKIVPRDC
15	VH_nt A1	TTACCCACGCTTTGTACATGGAGAAAATAAA GTGAAACAAAGCACTATTGCACTGGCACTCT TACCGCTCTTATTTACCCCTGTGGCAAAAGCC GAGGTGAAGCTGGTGGAATCTGGGGGAGGC TTAGTGAGGCCTGGAGGGTCCCTGAAACTCT CCTGTGCAGTCTCTGGATTCACTTTCAGTAGC TATGCCATGTCTTGGGTTCGCCAGACTCCGG AGAAGAGGCTGGAATGGGTCGCAGCCATTAA TTTTAATCGTGGTACCACCTACTATTCAGACA CTGTGAAGGGCCGATTCACCATCTCCAGAGA CAATGCCAAGAATACCCTGTACCTGCAACTG AGCAGTCTGAGGTCTGAGGACACAGCCTTTT ATTACTGTTCAAGACACCGCTATAGTGACTAC GACTATGCTATGGACTACTGGGGTCAAGGAA CCTCAGTCACCGTCTCCTCAGCCAAAACGAC ACCCCCATCTGTCTATCCACTGGCCCCTGGA TCTGCTGCCCAAACTAACTCCATGGTGACCC TGGGATGCCTGGTCAAGGGCTATTTCCCTGA GCCAGTGACAGTGACCTGGAACTCTGGATCC CTGTCCAGCGGTGTGCACACCTTCCCAGCTG TCCTGCAGTCTGACCTCTACACTCTGAGCAG CTCAGTGACTGTCCCCTCCAGCACCTGGCCC AGCGAGACCGTCACCTGCAACGTTGCCCACC CGGCCAGCAGCACCAAGGTGGACAAGAAAAT TGTGCCCAGGGATTGTCATCATCACCATCAC CATCACTAAATGGACAGCTTAATCATTTATAA AGCT
16	VH_nt A2	AACCCTGGCGTTACCCACGCTTTGTACATGG AGAAAATAAAGTGAAACAAAGCACTATTGCA CTGGCACTCTTACCGCTCTTATTTACCCCTGT GGCAAAAGCCGAAGTGCAGCTGTTGGAGACT GGGGGAGGCTTAGTGAAGCCTGGAGGGTCC CTGAAACTCTCCTGTGCAGCCTCTGGATTCA

SEQ ID No.	Description	Sequence
		CTTTCAGTACCTATGCCATGTCTTGGGTTCGC CAGACTCCGGAGAAGAGGCTGGAGTGGGTC GCAGGCATTAATAGTAATCGTGGTACCACCT ACTATCCAGACACTGTGAAGGGCCGCTTCAC CATCTCCAGAGACAATGCCAAGAACACCCTG TCCCTGCAAATGACCAGTCTGAGGTCTGAGG ACACAGCCTTGTATTATTGTGTAAGACACCG CTATACTAACTACGACTATGCTATGGACTACT GGGGTCAAGGAACCTCAGTCACCGTCTCCTC AGCCAAAACGACACCCCCATCTGTCTATCCA CTGGCCCCTGGATCTGCTGCCCAAACTAACT CCATGGTGACCCTGGGATGCCTGGTCAAGGG CTATTTCCCTGAGCCAGTGACAGTGACCTGG AACTCTGGATCCCTGTCCAGCGGTGTGCACA CCTTCCCAGCTGTCCTGCAGTCTGACCTCTAC ACTCTGAGCAGCTCAGTGACTGTCCCCTCCA GCACCTGGCCCAGCGAGACCGTCACCTGCAA CGTTGCCCACCCGGCCAGCAGCACCAAGGTG GACAAGAAAATTGTGCCCAGGGATTGTCATC ATCACCATCACCATCACTAATTGACAGCTTAT CATCGATAAGCTTTAATGCGGTAGTTTAT
17	VH_nt A3	CGTCGTTTTACAACGTCGTGACTGGGAAAAC CCTGGCGTTACCCACGCTTTGTACATGGAGA AAATAAAGTGAAACAAAGCACTATTGCACTG GCACTCTTACCGCTCTTATTTACCCCTGTGGC AAAAGCCGATGTGATGCTGGTGGAGTCTGGG GGAGGCTTAGTGAAGCCTGGAGGGTCCCTGA AACTCTCCTGTGCAGCCTCTGGATTCACTTTC AGTAGCTATGCCATGTCTTGGGTTCGCCAGA CTCCGGAGAAGAGGCTGGAATGGGTCGCAGC CATTAATATTAATCGTGGTACCACCTACTATT CAGACACTGTGAAGGGCCGATTCACCATCTC CAGAGACAATGCCAAGAATACCCTGTACCTG CAACTGAGCAGTCTGAGGTCTGAGGACACAG CCTTGTATTACTGTTCAAGACACCGCTATAGT GACTACGACTATGCTATGGACTACTGGGGTC AAGGAACCTCAGTCACCGTCTCCTCAGCCAA AACGACACCCCCATCTGTCTATCCACTGGCC CCTGGATCTGCTGCCCAAACTAACTCCATGG TGACCCTGGGATGCCTGGTCAAGGGCTATTT CCCTGAGCCAGTGACAGTGACCTGGAACTCT GGATCCCTGTCCAGCGGTGTGCACACCTTCC CAGCTGTCCTGCAGTCTGACCTCTACACTCT GAGCAGCTCAGTGACTGTCCCCTCCAGCACC TGGCCCAGCGAGACCGTCACCTGCAACGTTG CCCACCCGGCCAGCAGCACCAAGGTGGACAA GAAAATTGTGCCCAGGGATTGTCATCATCAC CATCACCATCACTAATTGACAGCTTATCATCG

SEQ ID No.	Description	Sequence
		ATAAGCTTTAATGCGGTAGTTT
18	VH_nt A4	TTACCCACGCTTTGTACATGGAGAAAATAAA GTGAAACAAAGCACTATTGCACTGGCACTCT TACCGCTCTTATTTACCCCTGTGGCAAAAGCC GAGGTGAAGCTGGTGGAATCTGGGGGAGGC TTAGTGAAGCCTGGAGGGTCCCTGAAACTCT CCTGTGCAGCCTCTGGATTCACTTTCAGTAAC TATGGCATGTCTTGGGTTCGCCAGACTCCGG AGAGGAGGCTGGAGTGGGTCGCAGCCATGA ATAATAATGGTGCTAGCACCTACTATCCAGA CACTGTGAAGGGCCGATTCACCATCTCCAGA GACAATGCCAAGAACACCCTGTACCTGCAAA TGAGCAGTCTGAGGTCTGAGGACACAGCCTT GTATTTCTGTGTAAGACATAATAACTACGTTG ACTATGCTATGGACTATTGGGGTCAAGGAAC CTCAGTCACCGTCTCCTCAGCCAAAACGACA CCCCCATCTGTCTATCCACTGGCCCCTGGAT CTGCTGCCCAAACTAACTCCATGGTGACCCT GGGATGCCTGGTCAAGGGCTATTTCCCTGAG CCAGTGACAGTGACCTGGAACTCTGGATCCC TGTCCAGCGGTGTGCACACCTTCCCAGCTGT CCTGCAGTCTGACCTCTACACTCTGAGCAGC TCAGTGACTGTCCCCTCCAGCACCTGGCCCA GCGAGACCGTCACCTGCAACGTTGCCCACCC GGCCAGCAGCACCAAGGTGGACAAGAAAATT GTGCCCAGGGATTGTCATCATCACCATCACC ATCACTAATTGACAGCTTATCATCGATAAGCT TTAATGCGGTAGTTTATCACAGTTAAATT
19	VH_nt A5	ACCCTGGCGTTACCCACGCTTTGTACATGGA GAAAATAAAGTGAAACAAAGCACTATTGCAC TGGCACTCTTACCGCTCTTATTTACCCCTGTG GCAAAAGCCGAGGTGAAGCTGGTGGAATCTG GGGGAGGCTTAGTGAAGCCTGGAGGGTCCCT GAAACTCTCCTGTGCAGCCTCTGGATTCACTT TCAGTAACTATGACATGTCTTGGGTTCGCCA GAGTCCGGAGAAGAGGCTGGAGTGGGTCGC AGCCATTAATCGTAAAGGTCATAGTACCTACT ATCCAGACACTGTGCAGGGCCGATTCACCAT CTCCAGAGACAATGCCAAGAACACCCTGTAC CTGCAAATGAGCAGTCTGAGGTCTGAGGACA CAGCCTTGTATTACTGTGTAAGACTTGACGAT AACTACTACTTCTTTGACTACTGGGGCCAAG GCACCACTCTCACAGTCTCCTCAGCCAAAAC GACACCCCCATCTGTCTATCCACTGGCCCCT GGATCTGCTGCCCAAACTAACTCCATGGTGA CCCTGGGATGCCTGGTCAAGGGCTATTTCCC TGAGCCAGTGACAGTGACCTGGAACTCTGGA TCCCTGTCCAGCGGTGTGCACACCTTCCCAG

SEQ ID No.	Description	Sequence
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20	VH_nt A6	GGAAAACCCTGGCGTTACCCACGCTTTGTAC ATGGAGAAAATAAAGTGAAACAAAGCACTAT TGCACTGGCACTCTTACCGCTCTTATTTACCC CTGTGGCAAAAGCCGAAGTGATGCTGGTGGA GTCTGGGGGAGGCTTAGTGAAGCCTGGAGG GTCCCTGAAACTCTCCTGCGCAGCCTCTGGA TTCACTTTCAGTCCCTATGCCATGTCTTGGGT TCGCCAGACTCCGGAGAAGAGGCTGGAGTGG GTCGCAGCCATTAATAGTAATCGTGGTACCA CCTACTATCCAGACACTGTGAAGGGCCGATT CACCATCTCCAGAGACAATGCCAAGAACACC CTGTACCTGCAAATGAGCAGTCTGAGGTCTG AGGACACAGCCTTTTATTACTGTGTAAGACA CCGCTATAATAACTACGACTATGCTATGGACT ACTGGGGTCAAGGAACCTCAGTCACCGTCTC CTCAGCCAAAACGACACCCCCATCTGTCTAT CCACTGGCCCCTGGATCTGCTGCCCAAACTA ACTCCATGGTGACCCTGGGATGCCTGGTCAA GGGCTATTTCCCTGAGCCAGTGACAGTGACC TGGAACTCTGGATCCCTGTCCAGCGGTGTGC ACACCTTCCCAGCTGTCCTGCAGTCTGACCT CTACACTCTGAGCAGCTCAGTGACTGTCCCC TCCAGCACCTGGCCCAGCGAGACCGTCACCT GCAACGTTGCCCACCCGGCCAGCAGCACCAA GGTGGACAAGAAAATTGTGCCCAGGGATTGT CATCATCACCATCACCATCACTAATTGACAGC TTATCATCGATAAGCTTTAATGCGGTAGTTTA TCACAGT
21	VH_nt A7	CGTTTTACAACGTCGTGACTGGGAAAACCCT GGCGTTACCCACGCTTTGTACATGGAGAAAA TAAAGTGAAACAAAGCACTATTGCACTGGCA CTCTTACCGCTCTTATTTACCCCTGTGGCAAA AGCCGAAGTGATGCTGGTGGAGTCTGGGGGA GGCTTAGTGAAGCCTGGAGGGTCCCTGAAAA TCTCCTGTGCAGCCTCTGGATTCTCTTTCAGT AGCTATGCCATGTCTTGGGTTCGCCAGACTC CGGAGAAGAGCCTGGAATGGGTCGCAGCCAT TAATATTAATCGTGGTACCCCCTATTATCCAG ACACTGTGAAGGGCCGATTCACCATCTCCAG AGACAATGCCAAGAACACCCTGTACCTGCAA ATGAGTAGTCTGAGGTCTGAGGACACAGCCT

SEQ ID No.	Description	Sequence
		TGTATTACTGTGTAAGACACCGCAATAGTAA CAACGACTATGCTATGGACTACTGGGGTCAA GGAACCTCAGTCACCGTCTCCTCAGCCAAAA CGACACCCCCATCTGTCTATCCACTGGCCCC TGGATCTGCTGCCCAAACTAACTCCATGGTG ACCCTGGGATGCCTGGTCAAGGGCTATTTCC CTGAGCCAGTGACAGTGACCTGGAACTCTGG ATCCCTGTCCAGCGGTGTGCACACCTTCCCA GCTGTCCTGCAGTCTGACCTCTACACTCTGA GCAGCTCAGTGACTGTCCCCTCCAGCACCTG GCCCAGCGAGACCGTCACCTGCAACGTTGCC CACCCGGCCAGCAGCACCAAGGTGGACAAGA AAATTGTGCCCAGGGATTGTCATCATCACCA TCACCATCACTAATTGACAGCTTATCATCGAT AAGCTTTAAT
22	VH_nt A8	TACAACGTCGTGACTGGGAAAACCCTGGCGT TACCCACGCTTTGTACATGGAGAAAATAAAG TGAAACAAAGCACTATTGCACTGGCACTCTT ACCGCTCTTATTTACCCCTGTGGCAAAAGCC GACGTGCAGGTGGTGGAGTCTGGGGGAGGC TTAGTGAAGCCTGGAGGGTCCCTGAAACTCT CCTGTGCAGCCTCTGGATTCACTTTCAGTAG CTATGCCATGTCTTGGGTTCGCCAGACTCCG GAGAAGAGGCTGGAGTGGGTCGCAGCCATTA ATCCTAATGGTGGTAGTACCTACTATCCAGA CACTGTGAAGGGCCGATTCACCATCTCCAGA GACAATGCCAAGAACACCCTATACCTGCAAA TGAGCGGTCTGAGGTCTGAGGACACAGCCTT GTATTACTGTGCAAGACTCCCATGGTCCCCC TATACTTTGGACTACTGGGGTCAAGGAACCT CAGTCACCGTCTCCTCAGCCAAAACGACACC CCCATCTGTCTATCCACTGGCCCCTGGATCT GCTGCCCAAACTAACTCCATGGTGACCCTGG GATGCCTGGTCAAGGGCTATTTCCCTGAGC
23	VH_nt A9	TTACCCACGCTTTGTACATGGAGAAAATAAA GTGAAACAAAGCACTATTGCACTGGCACTCT TACCGCTCTTATTTACCCCTGTGGCAAAAGCC GAAGTGCAGCTTGTGGAGACTGGGGGAGACT TAGTGAAGCCTGGAGGGTCCCTGAAACTCTC CTGTGTAGCCTCTGGATTCACTTTCAGTAGCA ATGCCATGTCCTGGGTTCGCCAGACTCCGGA GAAGAGGCTGGAGTGGGTCGCAGCCATTAAT AGTAAAGGTGGTGGCACCTACTATCCAGACA CTGTGAGGGGCCGATTCACCATCTCCAGAGA CAATGCCAAGAACACCCTGTACCTGCAAGTG ACCAGTCTGAGGTCTGAGGACACAGCCTTGT ATTACTGTGTAAGCCATGGGGATAATAAGTA CTTTTATGCTATGGACTACTGGGGTCAAGGA

SEQ ID No.	Description	Sequence
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24	VH_nt A10	TTACCCACGCTTTGTACATGGAGAAAATAAA GTGAAACAAAGCACTATTGCACTGGCACTCT TACCGCTCTTATTTACCCCTGTGGCAAAAGCC GAAGTGCAGCTTGTGGAGACTGGGGGAGGCT TAGTGAAGCCTGGAGGGTCCCTGAAACTCTC CTGTGCAGCCTCTGGATTCGCTTTCAGTAGC TATGCCATGTCTTGGGTTCGCCAAACTCCGG AGAAGAGGCTGGAGTGGGTCGCAGCCATTAA TAATAGAGGTGGTGGCACCTACTATCCAGAC ACTGTGAGGGGCCGATTCACCATCTCCAGAG ACAATGCCAAGAACACCCTGTACCTGCAAAT GAGCAGCCTGAGGTCTGCGGACACAGCCTTG TATTACTGTGTGAGACATGACAATCTTAACTA TGACTATGCTATGGACTCCTGGGGTCAAGGA ACCTCAGTCACCGTCTCCTCAGCCAAAACGA CACCCCCATCTGTCTATCCACTGGCCCCTGG ATCTGCTGCCCAAACTAACTCCATGGTGACC CTGGGATGCCTGGTCAAGGGCTATTTCCCTG AGCCAGTGACAGTGACCTGGAACTCTGGATC CCTGTCCAGCGGTGTGCACACCTTCCCAGCT GTCCTGCAGTCTGACCTCTACACTCTGAGCA GCTCAGTGACTGTCCCCTCCAGCACCTGGCC CAGCGAGACCGTCACCTGCAACGTTGCCCAC CCGGCCAGCAGCACCAAGGTGGACAAGAAAA TTGTGCCCAGGGATTGTCATCATCACCATCA CCATCACTAATTGACAGCTTATCATCGATAAG CTTTAA
25	VH_nt A11	CGTCGTGACTGGGAAAACCCTGGCGTTACCC ACGCTTTGTACATGGAGAAAATAAAGTGAAA CAAAGCACTATTGCACTGGCACTCTTACCGC TCTTATTTACCCCTGTGCCAAAAGCCGAAGT GCAGCTGGTGGAGTCTGGGGGAGACTTAGTG AAGCCTGGAGGGTCCCTGAAACTCTCCTGTG CAGCCTCTGGATTCACTTTCAGTAGATATGG CATGTCTTGGGTTCGCCAGACTCCGGAGAAG AGGCTGGAGTGGGTCGCAGCCATTAATCCTA

SEQ ID No.	Description	Sequence
		ATGGTGGTACTACCTACTATCCAGACACTGT GAAGGGCCGATTCACCATCTCCCGAGACAAT GCCAAGAACACCCTGTTCCTGCAAATGACCG GTCTGAGGTCTGAGGACACAGCCTTATACTA CTGTGCAAGACTCCCATGGTCCCCCTATACTT TGGACTACTGGGGTCAAGGAACCTCAGTCAT CGTCTCCTCAGCCAAAACGACACCCCCATCT GTCTATCCACTGGCCCCTGGATCTGCTGCCC AAACTAACTCCATGGTGACCCTGGGATGCCT GGTCAAGGGCTATTTCCCTGAGCCAGTGACA GTGACCTGGAACTCTGGATCCCTGTCCAGCG GTGTGCACACCTTCCCAGCTGTCCTGCAGTC TGACCTCTACACTCTGAGCAGCTCAGTGACT GTCCCCTCCAGCACCTGGCCCAGCGAGACCG TCACCTGCAACGTTGCCCACCCGGCCAGCAG CACCAAGGTGGACAAGAAAATTGTGCCCAGG GATTGTCATCATCACCATCACCATCACTAATT GACAGCTTATCATCGATAAGCTTTAATGCGG TAGTTTATCACAGTTAAAT
26	VH_nt A12	GCGTTACCCACGCTTTGTACATGGAGAAAAT AAAGTGAAACAAAGCACTATTGCACTGGCAC TCTTACCGCTCTTATTTACCCCTGTGGCAAAA GCCGAAGTGCAGCTGGTGGAGTCTGGGGGA GGCTTAGTGAAGCCTGGAGGGTCCCTGAAAC TCTCCTGCGCAGCCTCTGGATTCACTTTCAGT AGCTATGCCATGTCTTGGGTTCGCCAGACTC CGGAGAAGAGGCTAGAGTGGGTCGCAGCCAT TAATAGTAATCGTGGTACCACCTACTATTCAG ACACTGTGAAGGGCCGATTCACCATCTCCAG AGACAATGCCAAGAACACCCTGTACCTGCAA ATGAGCAGTCTGAGGTCTGAGGACACAGCCT TCTATTACTGTACAAGACACCGCTATAGTGAC TACGACTATGCTATGGACTACTGGGGTCAAG GAACCTCAGTCACCGTCTCCTCAGCCAAAAC GACACCCCCATCTGTCTATCCACTGGCCCCT GGATCTGCTGCCCAAACTAACTCCATGGTGA CCCTGGGATGCCTGGTCAAGGGCTATTTCCC TGAGCCAGTGACAGTGACCTGGAACTCTGGA TCCCTGTCCAGCGGTGTGCACACCTTCCCAG CTGTCCTGCAGTCTGACCTCTACACTCTGAG CAGCTCAGTGACTGTCCCCTCCAGCACCTGG CCCAGCGAGACCGTCACCTGCAACGTTGCCC ACCCGGCCAGCAGCACCAAGGTGGACAAGAA AATTGTGCCCAGGGATTGTCATCATCACCAT CACCATCACTAATTGACAGCTTATCATCGATA AGCTTTAATGCGGTAGTTTATCACAGTTAAAT TGCTACG
27	VH_nt A13	GGCGTTACCCACGCTTTGTACATGGAGAAAA

SEQ ID No.	Description	Sequence
		TAAAGTGAAACAAAGCACTATTGCACTGGCA CTCTTACCGCTCTTATTTACCCCTGTGGCAAA AGCCGAAGTGCAGCTTGTGGAGACTGGGGGA GGCTTAGTGAAGCCTGGAGGGTCCCTGAAAC TCTCCTGTGCAGCCTCTGGATTCACTTTCAGT AGCTATGCCATGTCTTGGATTCGCCAGACTC CGGAGAAGAGGCTGGAGTGGGTCGCAGGCA TTAATAGTAATCGTGGTACCACCTACTATCCA GACACTGTGAAGGGCCGATTCACCATCTCCA GAGACAATGCCAAGAACACCCTGTACCTGCA AATGAACAGTCTGAGGTCTGAGGACTCAGCC TTGTATTACTGTGTAAGACACCGCTATATTGA CTACGACTATGCTATGGACTACTGGGGTCAA GGAACCTCAGTCACCGTCTCCTCAGCCAAAA CGACACCCCCATCTGTCTATCCACTGGCCCC TGGATCTGCTGCCCAAACTAACTCCATGGTG ACCCTGGGATGCCTGGTCAAGGGCTATTTCC CTGAGCCAGTGACAGTGACCTGGAACTCTGG ATCCCTGTCCAGCGGTGTGCACACCTTCCCA GCTGTCCTGCAGTCTGACCTCTACACTCTGA GCAGCTCAGTGACTGTCCCCTCCAGCACCTG GCCCAGCGAGACCGTCACCTGCAACGTTGCC CACCCGGCCAGCAGCACCAAGGTGGACAAGA AAATTGTGCCCAGGGATTGTCATCATCACCA TCACCATCACTAATTGACAGCTTATCATCGAT AAGCTTTAATGCGGTAGTT
28	VH_nt A14	GTCGTGACTGGGAAAACCCTGGCGTTACCCA CGCTTTGTACATGGAGAAAATAAAGTGAAAC AAAGCACTATTGCACTGGCACTCTTACCGCT CTTATTTACCCCTGTGGCAAAAGCCCAGGTG CAGCTTAAGCAGTCTGGGGCTGAGCTGGTGA AGCCTGGGGCCTCAGTGAAGATATCCTGCAA GGCTACTGGCTACACATTCAGTAGTTACTGG ATAGAGTGGGTAAAGGAGAGGCCTGGACATG GCCTTGAGTGGATTGGAGAGATTTTACCTGG AATTGGTAATACTAACTACAATGAGAAATTCA AGGGCAAGGCCACATTCACTGCTGATCTATC CTCCAAGACAGCCTACATGCAACTCAGCAGC CTGACATCTGAGGACTCTGCCGTCTATTACT GTGCAAGTGGGGGGTATAGTACCGTCTATTG GTATTTTGATGTCTGGGGCGCAGGGACCACG GTCACCGTCTCCTCAGCCAAAACGACACCCC CATCTGTCTATCCACTGGCCCCTGGATCTGCT GCCCAAACTAACTCCATGGTGACCCTGGGAT GCCTGGTCAAGGGCTATTTCCCTGAGCCAGT GACAGTGACCTGGAACTCTGGATCCCTGTCC AGCGGTGTGCACACCTTCCCAGCTGTCCTGC AGTCTGACCTCTACACTCTGAGCAGCTCAGT

SEQ ID No.	Description	Sequence
		GACTGTCCCCTCCAGCACCTGGCCCAGCGAG ACCGTCACCTGCAACGTTGCCCACCCGGCCA GCAGCACCAAGGTGGACAAGAAAATTGTGCC CAGGGATTGTCATCATCACCATCACCATCACT AATTTGACAGCTTTAATCATTCAATTAAGCTT TTAAT
29	VK_aa A1	MKYLLPTAAAGLLLLAAQPAMADIVMSQSPSS MYASLGERVTITCKASQDINSYLNWFQQKPGK SPKTLIYRANRLVDGVPSRFSGSGSGHDYFLTIR SLEYEDMGIYYCLQYDEFPYTFGGGTKLEIKRA DAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKD INVKWKIDGSERQNGVLNSWTDQDSKDSTYSM SSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSF NRNES
30	VK_aa A2	MKYLLPTAAAGLLLLAAQPAMADIKMTQSPSS MYASLGERVTITCKASQDINSYLSWFQQKPGKS PKTLIYRANRLVDGVPSRFSGSGSGQDYSLTISS LEYEDMGIYYCLQYDEFPYTFGGGTKLEIKRA DAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKD INVKWKIDGSERQNGVLNSWTDQDSKDSTYSM SSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSF NRNES
31	VK_aa A3	MKYLLPTAAAGLLLLAAQPAMADIQLTQSPSS MYASLGERVTIACKASQDINSYLSWFQQKPGKS PKTLIHRANRLVDGVPSRFSGSGSGQDYSLTISS LEYEDIGIYYCLQYDEFPYTFGGGTKLEIKRAD AAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDI NVKWKIDGSERQNGVLNSWTDQDSKDSTYSMS STLTLTKDEYERHNSYTCEATHKTSTSPIVKSFN RNES
32	VK_aa A4	MKYLLPTAAAGLLLLAAQPAMADILLTQSPSS MYTSLGERVTITCKASQDINSYLSWFQQKPGKS PKTLIYRANKLVDGVPSRFSGSGSGQDYSLTISS LESEDMGIYYCLQYDEFPYTFGGGTKLEIKRAD AAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDI NVKWKIDGSERQNGVLNSWTDQDSKDSTYSMS STLTLTKDEYERHNSYTCEATHKTSTSPIVKSFN RNES
33	VK_aa A5	MKYLLPTAAAGLLLLAAQPAMADIKMTQSPSS MYASLGERVTITCKASQDINSYLSWFQQKPGKS PKTLIYRAKRLIDGVPSRFSGSGSGQDYSLTISSL EYEDMGIYYCLQYDEFPYTFGGGTKLEIKRAD AAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDI NVKWKIDGSERQNGVLNSWTDQDSKDSTYSMS STLTLTKDEYERHNSYTCEATHKTSTSPIVKSFN RNES
34	VK_aa A6	MKYLLPTAAAGLLLLAAQPAMADIVMSQSPSS

SEQ ID No.	Description	Sequence
		MYASLGERVTITCKASQDINSYLSWFQQKPGKS PKTLTYRANRLVEGVPSRFSGSGSGQDYSLTISS LEYEDMGIYYCLQYDEFPYTFGGGTKLEIKRA DAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKD INVKWKIDGSERQNGVLNSWTDQDSKDSTYSM SSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSF NRNES
35	VK_aa A7	MKYLLPTAAAGLLLLAAQPAMADIVMTQSPSS MYTSLGERVTITCKASQDINSYLSWFQQKPGKS PKTLIYRANRLIDGVPSRFSGSGSGQDYSLTISSL EYEDMGIYYCLQYDEFPFTFGSGTKLEIKRADA APTVSIFPPSSEQLTSGGASVVCFLNNFYPKDIN VKWKIDGSERQNGVLNSWTDQDSKDSTYSMSS TLTLTKDEYERHNSYTCEATHKTSTSPIVKSFN RNES
36	VK_aa A8	MKYLLPTAAAGLLLLAAQPAMANIVMTQSPVS LSMAIGEKVTIRCITNTDIDDAMNWYQQKPGEP PKLLISEGNTLRPGVPSRFSSSGYGTDFVFTIEN MLSEDVADYYCLQTDNLPLTFGSGTKLAIKRA DAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKD INVKWKIDGSERQNGVMNSWTDQDSKDSTYSM SSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSF NRNES
37	VK_aa A9	MKYLLPTAAAGLLLLAAQPAMANIVMTQSPSS MYASLGERVTITCKASQDINSYLSWFQQKPGKS PKTLIYRANRLVDGVPSRFSGSGSGQDYSLTISS LEYEDMGIYYCLQYDEFPYTFGGGTKLEIKRA DAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKD INVKWKIDGSERQNGVLNSWTDQDSKDSTYSM SSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSF NRNES
38	VK_aa A10	MKYLLPTAAAGLLLLAAQPAMANIVMTQSPSS MYASLGERVTITCKASQDIYSYLSWFQQKPGKS PKTLIYRANRLVDGVPSRFSGSGSGQDYSLTISS LDYEDVGIYYCLQYDEFPYTFGSGTKLEIERAD AAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDI NVKWKIDGSERQNGVLNSWTDQDSKDSTYSMS STLTLTKDEYERHNSYTCEATHKTSTSPIVKSFN RNES
39	VK_aa A11	MKYLLPTAAAGLLLLAAQPAMAETTVTQSPVS LSMAIGEKVTIRCMTSTDIDDALNWYQQKPGE PPKLLISEGNSLRPGVPSRFSSSGNGTDFVFTIEN MLSEDVADYYCLQSDNLPLTFGSGTKLEIKRAD AAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDI NVKWKIDGSERQNGVLNSWTDQDSKDSTYSMS STLTLTKDEYERHNSYTCEATHKTSTSPIVKSFN RNES

SEQ ID No.	Description	Sequence
40	VK_aa A12	MKYLLPTAAAGLLLLAAQPAMADIKMTQSPSS MYASLGERVTITCKASQDINSYLSWFQQKPGKS PMTLTHRANRLVDGVPSRFSGSGSGQDYSLTIS SLENEDMGIYYCLQYDEFPYTFGGGTKLEIKRA DAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKD INVKWKIDGSERQNGVLNSWTDQDSKDSTYSM SSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSF NRNES
41	VK_aa A13	MKYLLPTAAAGLLLLAAQPAMANIVMTQSPSS MYASLGERVTIICKSSQDINSYLSWFQQKPGKS PKTLIFRANRLVDGVPSRFSGSGSGQDYSLTISS LEYEDMGIYYCLQYDEFPYTFGGGTKLEVKRA DAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKD INVKWKIDGSERQNGVLNSWTDQDSKDSTYSM SSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSF NRNES
42	VK_aa A14	MKYLLPTAAAGLLLLAAQPAMADVVMSQSPSS LAVSTGEKVTLSCKSSQSLLNSRTRKNYLAWY QQKPGQSPKLLIYWTSTRESGVPNRFTGSGSGT DFTLTISSVQAEDLAVYYCKQSYDLPWTFGGGT KLEIKRADAAPTVSIFPPSSEQLTSGGASVVCFL NNFYPKDINVKWKIDGSERQNGVLNSWTDQDS KDSTYSMSSTLTLTKDEYERHNSYTCEATHKTS TSPIVKSFNRNES
43	VK_nt A1	TATCGCAACTCTCTACTGTTTCTCCATACCCG TTTTTTTGGATGGAGTGAAACGATGAAATAC CTATTGCCTACGGCAGCCGCTGGATTGTTAT TACTCGCTGCCCAACCAGCCATGGCCGACAT CGTTATGTCTCAGTCTCCATCTTCCATGTATG CATCTCTAGGAGAGAGAGTCACTATCACTTG CAAGGCGAGTCAGGACATTAATAGCTATTTA AACTGGTTCCAGCAGAAACCAGGCAAATCTC CTAAGACCCTGATCTATCGTGCAAACAGATT GGTAGATGGGGTCCCATCAAGGTTCAGTGGC AGTGGATCTGGGCACGATTATTTTCTTACCAT TCGCAGCCTGGAATATGAAGATATGGGAATT TATTATTGTCTACAGTATGATGAGTTTCCGTA CACGTTCGGAGGGGGGACCAAGCTGGAAATA AAACGGGCTGATGCTGCACCAACTGTATCCA TCTTCCCACCATCCAGTGAGCAGTTAACATCT GGAGGTGCCTCAGTCGTGTGCTTCTTGAACA ACTTCTACCCCAAAGACATCAATGTCAAGTG GAAGATTGATGGCAGTGAACGACAAAATGGC GTCCTGAACAGTTGGACTGATCAGGACAGCA AAGACAGCACCTACAGCATGAGCAGCACCCT CACGTTGACCAAGGACGAGTATGAACGACAT AACAGCTATACCTGTGAGGCCACTCACAAGA CATCAACTTCACCCATTGTCAAGAGCTTCAAC

SEQ ID No.	Description	Sequence
		AGGAATGAGTCTTAAGTGATTAGCTAATTCTA GAACGCGTCACTTGGCACTGGCCGTCG
44	VK_nt A2	TTATCGCAACTCTCTACTGTTTCTCCATACCC GTTTTTTTGGATGGAGTGAAACGATGAAATA CCTATTGCCTACGGCAGCCGCTGGATTGTTA TTACTCGCTGCCCAACCAGCCATGGCCGACA TCAAAATGACCCAGTCTCCATCTTCCATGTAT GCATCTCTAGGAGAGAGAGTCACTATCACTT GCAAGGCGAGTCAGGACATTAATAGCTATTT AAGCTGGTTCCAGCAGAAACCAGGGAAATCT CCTAAGACCCTGATCTATCGTGCAAACAGAT TGGTAGATGGGGTCCCATCAAGGTTCAGTGG CAGTGGATCTGGGCAAGATTATTCTCTCACC ATCAGCAGCCTGGAGTATGAAGATATGGGAA TTTATTATTGTCTACAGTATGATGAATTTCCG TACACGTTCGGAGGGGGGACCAAGCTGGAAA TAAAACGGGCTGATGCTGCACCAACTGTATC CATCTTCCCACCATCCAGTGAGCAGTTAACAT CTGGAGGTGCCTCAGTCGTGTGCTTCTTGAA CAACTTCTACCCCAAAGACATCAATGTCAAGT GGAAGATTGATGGCAGTGAACGACAAAATGG CGTCCTGAACAGTTGGACTGATCAGGACAGC AAAGACAGCACCTACAGCATGAGCAGCACCC TCACGTTGACCAAGGACGAGTATGAACGACA TAACAGCTATACCTGTGAGGCCACTCACAAG ACATCAACTTCACCCATTGTCAAGAGCTTCAA CAGGAATGAGTCTTAAGTGATTAGCTAATTCT AGAACGCGTCACTTGGCACTGGCCGTCGTTT TACAACGTCGTGACTGGGAAAA
45	VK_nt A3	TATCGCAACTCTCTACTGTTTCTCCATACCCG TTTTTTTGGATGGAGTGAAACGATGAAATAC CTATTGCCTACGGCAGCCGCTGGATTGTTAT TACTCGCTGCCCAACCAGCCATGGCCGACAT TCAGCTGACCCAGTCTCCATCTTCCATGTATG CATCTCTAGGAGAGAGAGTCACTATCGCTTG CAAGGCGAGTCAGGACATTAATAGCTATTTA AGCTGGTTCCAGCAGAAACCAGGGAAATCTC CTAAGACCCTGATCCATCGTGCAAACAGATT GGTAGATGGGGTCCCATCAAGGTTCAGTGGC AGTGGATCTGGGCAAGATTATTCTCTCACCA TCAGCAGCCTGGAGTATGAAGATATCGGAAT TTATTATTGTCTACAGTATGATGAGTTTCCGT ACACGTTCGGAGGGGGGACCAAGCTGGAAAT AAAACGGGCTGATGCTGCACCAACTGTATCC ATCTTCCCACCATCCAGTGAGCAGTTAACATC TGGAGGTGCCTCAGTCGTGTGCTTCTTGAAC AACTTCTACCCCAAAGACATCAATGTCAAGT GGAAGATTGATGGCAGTGAACGACAAAATGG

SEQ ID No.	Description	Sequence
		CGTCCTGAACAGTTGGACTGATCAGGACAGC AAAGACAGCACCTACAGCATGAGCAGCACCC TCACGTTGACCAAGGACGAGTATGAACGACA TAACAGCTATACCTGTGAGGCCACTCACAAG ACATCAACTTCACCCATTGTCAAGAGCTTCAA CAGGAATGAGTCTTAAGTGATTAGCTAATTCT AGAACGCGTCACTTGGCACTGGCCGTCGTTT TACAACGTCG
46	VK_nt A4	TCGCAACTCTCTACTGTTTCTCCATACCCGTT TTTTTGGATGGAGTGAAACGATGAAATACCT ATTGCCTACGGCAGCCGCTGGATTGTTATTA CTCGCTGCCCAACCAGCCATGGCCGACATCT TGCTGACTCAGTCTCCATCTTCCATGTATACA TCTCTAGGAGAGAGAGTCACTATCACTTGCA AGGCGAGTCAGGACATTAATAGCTATTTAAG CTGGTTCCAGCAGAAACCAGGAAAATCTCCT AAGACCCTGATCTATCGTGCAAACAAATTGG TAGATGGGGTCCCATCAAGATTCAGTGGCAG TGGATCTGGGCAAGATTATTCTCTCACCATCA GCAGCCTGGAGTCTGAAGATATGGGAATTTA TTATTGTCTACAGTATGATGAGTTTCCGTACA CGTTCGGAGGGGGGACCAAGCTGGAAATCAA ACGGGCTGATGCTGCACCAACTGTATCCATC TTCCCACCATCCAGTGAGCAGTTAACATCTG GAGGTGCCTCAGTCGTGTGCTTCTTGAACAA CTTCTACCCCAAAGACATCAATGTCAAGTGG AAGATTGATGGCAGTGAACGACAAAATGGCG TCCTGAACAGTTGGACTGATCAGGACAGCAA AGACAGCACCTACAGCATGAGCAGCACCCTC ACGTTGACCAAGGACGAGTATGAACGACATA ACAGCTATACCTGTGAGGCCACTCACAAGAC ATCAACTTCACCCATTGTCAAGAGCTTCAACA GGAATGAGTCTTAAGTGATTAGCTAATTCTA GAACGCGTCACTTGG
47	VK_nt A5	TCGCAACTCTCTACTGTTTCTCCATACCCGTT TTTTTGGATGGAGTGAAACGATGAAATACCT ATTGCCTACGGCAGCCGCTGGATTGTTATTA CTCGCTGCCCAACCAGCCATGGCCGACATCA AAATGACCCAGTCTCCATCTTCCATGTATGCA TCTCTAGGAGAGAGAGTCACTATCACTTGCA AGGCGAGTCAGGACATTAATAGCTATTTAAG CTGGTTCCAGCAGAAACCAGGGAAATCTCCT AAGACCCTGATCTATCGTGCAAAGAGATTGA TAGATGGGGTCCCATCAAGGTTCAGTGGCAG TGGATCTGGGCAAGATTATTCTCTCACCATCA GCAGCCTGGAGTATGAAGATATGGGAATTTA TTATTGTCTACAGTATGATGAGTTTCCTTACA CGTTCGGAGGGGGGACAAAGTTGGAAATAAA

SEQ ID No.	Description	Sequence
		ACGGGCTGATGCTGCACCAACTGTATCCATC TTCCCACCATCCAGTGAGCAGTTAACATCTG GAGGTGCCTCAGTCGTGTGCTTCTTGAACAA CTTCTACCCCAAAGACATCAATGTCAAGTGG AAGATTGATGGCAGTGAACGACAAAATGGCG TCCTGAACAGTTGGACTGATCAGGACAGCAA AGACAGCACCTACAGCATGAGCAGCACCCTC ACGTTGACCAAGGACGAGTATGAACGACATA ACAGCTATACCTGTGAGGCCACTCACAAGAC ATCAACTTCACCCATTGTCAAGAGCTTCAACA GGAATGAGTCTTAAGTGATTAGCTAATTCTA GAACGCGTCACTTGGCACTGGCCGTCGTTTT ACAACGTCG
48	VK_nt A6	TTTTATCGCAACTCTCTACTGTTTCTCCATAC CCGTTTTTTTGGATGGAGTGAAACGATGAAA TACCTATTGCCTACGGCAGCCGCTGGATTGT TATTACTCGCTGCCCAACCAGCCATGGCCGA CATCGTTATGTCTCAGTCTCCATCTTCCATGT ATGCATCTCTAGGAGAGAGAGTCACTATCAC TTGCAAGGCGAGTCAAGACATTAATAGCTAT TTAAGCTGGTTCCAGCAGAAACCAGGGAAAT CTCCTAAGACCCTGACCTATCGTGCAAACAG ATTGGTAGAAGGGGTCCCATCAAGGTTCAGT GGCAGTGGATCTGGGCAAGATTATTCTCTCA CCATCAGCAGCCTGGAATATGAAGATATGGG AATTTATTATTGTCTACAGTATGATGAGTTTC CGTACACGTTCGGAGGGGGGACCAAGCTGGA AATAAAACGGGCTGATGCTGCACCAACTGTA TCCATCTTCCCACCATCCAGTGAGCAGTTAAC ATCTGGAGGTGCCTCAGTCGTGTGCTTCTTG AACAACTTCTACCCCAAAGACATCAATGTCAA GTGGAAGATTGATGGCAGTGAACGACAAAAT GGCGTCCTGAACAGTTGGACTGATCAGGACA GCAAAGACAGCACCTACAGCATGAGCAGCAC CCTCACGTTGACCAAGGACGAGTATGAACGA CATAACAGCTATACCTGTGAGGCCACTCACA AGACATCAACTTCACCCATTGTCAAGAGCTTC AACAGGAATGAGTCTTAAGTGATTAGCTAAT TCTAGAACGCGTCACTTGGCACTGGCCGTCG TTTTACAACGT
49	VK_nt A7	GCAACTCTCTACTGTTTCTCCATACCCGTTTT TTTGGATGGAGTGAAACGATGAAATACCTAT TGCCTACGGCAGCCGCTGGATTGTTATTACT CGCTGCCCAACCAGCCATGGCCGACATTGTG ATGACCCAGTCTCCATCTTCCATGTATACATC TCTAGGAGAGAGAGTCACTATCACTTGCAAG GCGAGTCAGGACATTAATAGCTATTTAAGCT GGTTCCAGCAGAAACCAGGGAAATCTCCTAA

SEQ ID No.	Description	Sequence
		GACCCTGATCTATCGTGCAAACAGATTGATA GATGGGGTCCCATCAAGGTTCAGTGGCAGTG GATCTGGGCAAGATTATTCTCTCACCATCAG CAGCCTGGAGTATGAAGATATGGGAATTTAT TATTGTCTACAGTATGATGAGTTTCCATTCAC GTTCGGCTCGGGGACAAAGTTGGAAATAAAA CGGGCTGATGCTGCACCAACTGTATCCATCT TCCCACCATCCAGTGAGCAGTTAACATCTGG AGGTGCCTCAGTCGTGTGCTTCTTGAACAAC TTCTACCCCAAAGACATCAATGTCAAGTGGA AGATTGATGGCAGTGAACGACAAAATGGCGT CCTGAACAGTTGGACTGATCAGGACAGCAAA GACAGCACCTACAGCATGAGCAGCACCCTCA CGTTGACCAAGGACGAGTATGAACGACATAA CAGCTATACCTGTGAGGCCACTCACAAGACA TCAACTTCACCCATTGTCAAGAGCTTCAACAG GAATGAGTCTTAAGTGATTAGCTAATTCTAGA ACGCGTCACTTGGCACTGGCCGTCGTTTTAC AACGTCGTGACTGGGAAAACCCTGGC
50	VK_nt A8	GAGTGAAACGATGAAATACCTATTGCCTACG GCAGCCGCTGGATTGTTATTACTCGCTGCCC AACCAGCCATGGCCAACATCGTTATGACCCA GTCTCCAGTATCCCTGTCCATGGCTATAGGA GAAAAAGTCACCATCAGATGCATAACCAACA CTGATATTGATGATGCTATGAACTGGTACCA GCAAAAGCCAGGGGAACCTCCTAAGCTCCTT ATTTCAGAAGGCAATACTCTTCGTCCTGGAG TCCCATCCCGATTCTCCAGCAGTGGCTATGG TACAGATTTTGTTTTTACAATTGAAAACATGC TCTCAGAAGATGTTGCAGATTACTACTGTTTG CAAACTGATAACTTGCCTCTCACGTTCGGCTC GGGGACAAAGTTGGCAATAAAACGGGCTGAT GCTGCACCAACTGTATCCATCTTCCCACCATC CAGTGAGCAGTTAACATCTGGAGGTGCCTCA GTCGTGTGCTTCTTGAACAACTTCTACCCCAA AGACATCAATGTCAAGTGGAAGATTGATGGC AGTGAACGACAAAATGGCGTCATGAACAGTT GGACTGATCAGGACAGCAAAGACAGCACCTA CAGCATGAGCAGCACCCTCACGTTGACCAAG GACGAGTATGAACGACATAACAGCTATACCT GTGAGGCCACTCACAAGACATCAACTTCACC CATTGTCAAGAGCTTCAACAGGAATGAGTCT TAAGTGATTAG
51	VK_nt A9	TCGCAACTCTCTACTGTTTCTCCATACCCGTT TTTTTGGATGGAGTGAAACGATGAAATACCT ATTGCCTACGGCAGCCGCTGGATTGTTATTA CTCGCTGCCCAACCAGCCATGGCCAACATCG TTATGACCCAGTCTCCATCTTCCATGTATGCA

SEQ ID No.	Description	Sequence
		TCTCTAGGAGAGAGAGTCACTATCACTTGCA AGGCGAGTCAGGACATTAATAGCTATTTAAG CTGGTTCCAGCAGAAACCAGGGAAATCTCCT AAGACCCTGATCTATCGTGCAAACAGATTGG TAGATGGGGTCCCATCAAGGTTCAGTGGCAG TGGATCTGGGCAAGATTATTCTCTCACCATCA GCAGCCTGGAGTATGAAGATATGGGAATTTA TTATTGTCTACAGTATGATGAGTTTCCGTACA CGTTCGGAGGGGGGACCAAACTGGAAATAAA ACGGGCTGATGCTGCACCAACTGTATCCATC TTCCCACCATCCAGTGAGCAGTTAACATCTG GAGGTGCCTCAGTCGTGTGCTTCTTGAACAA CTTCTACCCCAAAGACATCAATGTCAAGTGG AAGATTGATGGCAGTGAACGACAAAATGGCG TCCTGAACAGTTGGACTGATCAGGACAGCAA AGACAGCACCTACAGCATGAGCAGCACCCTC ACGTTGACCAAGGACGAGTATGAACGACATA ACAGCTATACCTGTGAGGCCACTCACAAGAC ATCAACTTCACCCATTGTCAAGAGCTTCAACA GGAATGAGTCTTAAGTGATTAGCTAATTCTA GAACGCGTCACTTGGCACTGGCCGTCGTTTT ACA
52	VK_nt A10	GCAACTCTCTACTGTTTCTCCATACCCGTTTT TTTGGATGGAGTGAAACGATGAAATACCTAT TGCCTACGGCAGCCGCTGGATTGTTATTACT CGCTGCCCAACCAGCCATGGCCAACATCGTT ATGACCCAGTCTCCATCTTCCATGTATGCATC TCTAGGAGAGAGGGTCACTATCACTTGCAAG GCGAGTCAGGACATTTATAGCTATTTAAGCT GGTTCCAGCAGAAACCAGGCAAATCTCCTAA GACCCTGATCTATCGTGCAAACAGATTGGTA GATGGGGTCCCATCAAGGTTCAGTGGCAGTG GATCTGGGCAAGATTATTCTCTCACCATCAG CAGCCTGGACTATGAAGATGTGGGAATTTAT TATTGTCTACAGTATGATGAGTTTCCGTACAC GTTCGGCTCGGGGACAAAGTTGGAAATAGAA CGGGCTGATGCTGCACCAACTGTATCCATCT TCCCACCATCCAGTGAGCAGTTAACATCTGG AGGTGCCTCAGTCGTGTGCTTCTTGAACAAC TTCTACCCCAAAGACATCAATGTCAAGTGGA AGATTGATGGCAGTGAACGACAAAATGGCGT CCTGAACAGTTGGACTGATCAGGACAGCAAA GACAGCACCTACAGCATGAGCAGCACCCTCA CGTTGACCAAGGACGAGTATGAACGACATAA CAGCTATACCTGTGAGGCCACTCACAAGACA TCAACTTCACCCATTGTCAAGAGCTTCAACAG GAATGAGTCTTAAGTGATTAGCTAATTCTAGA ACGCGTCACTTGGCACTGGCCGTCGTTTTA

SEQ ID No.	Description	Sequence
53	VK_nt A11	CGCAACTCTCTACTGTTTCTCCATACCCGTTT TTTTGGATGGAGTGAAACGATGAAATACCTA TTGCCTACGGCAGCCGCTGGATTGTTATTAC TCGCTGCCCAACCAGCCATGGCCGAAACAAC TGTGACCCAGTCTCCAGTATCCCTGTCCATG GCTATAGGAGAAAAAGTCACCATCAGATGCA TGACCAGCACTGATATTGATGATGCTCTGAA CTGGTACCAGCAAAAGCCAGGGGAACCTCCT AAACTCCTTATTTCAGAAGGCAATAGTCTTCG TCCTGGAGTCCCATCCCGATTCTCCAGCAGT GGCAATGGTACAGATTTTGTTTTTACAATTGA AAACATGCTCTCAGAAGATGTTGCAGATTAC TACTGTTTGCAAAGTGATAACTTGCCTCTCAC GTTCGGCTCGGGGACAAAGTTGGAAATAAAA CGGGCTGATGCTGCACCAACTGTATCCATCT TCCCACCATCCAGTGAGCAGTTAACATCTGG AGGTGCCTCAGTCGTGTGCTTCTTGAACAAC TTCTACCCCAAAGACATCAATGTCAAGTGGA AGATTGATGGCAGTGAACGACAAAATGGCGT CCTGAACAGTTGGACTGATCAGGACAGCAAA GACAGCACCTACAGCATGAGCAGCACCCTCA CGTTGACCAAGGACGAGTATGAACGACATAA CAGCTATACCTGTGAGGCCACTCACAAGACA TCAACTTCACCCATTGTCAAGAGCTTCAACAG GAATGAGTCTTAAGTGATTAGCTAATTCTAGA ATGCGTCACTTGGCACTGGCCGTCGTTTTAC AACGTCGTGAC
54	VK_nt A12	TATCGCAACTCTCTACTGTTTCTCCATACCCG TTTTTTTGGATGGAGTGAAACGATGAAATAC CTATTGCCTACGGCAGCCGCTGGATTGTTAT TACTCGCTGCCCAACCAGCCATGGCCGACAT CAAAATGACCCAGTCTCCATCTTCCATGTATG CATCTCTAGGAGAGAGAGTCACTATCACTTG CAAGGCGAGTCAGGACATTAATAGCTATTTA AGCTGGTTCCAGCAGAAACCAGGGAAATCTC CTATGACCCTGACCCATCGTGCAAACAGATT GGTAGATGGGGTCCCATCAAGGTTCAGTGGC AGTGGATCTGGGCAAGATTATTCTCTCACCA TCAGCAGCCTGGAGAATGAAGATATGGGAAT TTATTATTGTCTACAGTATGATGAGTTTCCGT ACACGTTCGGAGGGGGGACCAAGCTGGAAAT AAAACGGGCTGATGCTGCACCAACTGTATCC ATCTTCCCACCATCCAGTGAGCAGTTAACATC TGGAGGTGCCTCAGTCGTGTGCTTCTTGAAC AACTTCTACCCCAAAGACATCAATGTCAAGT GGAAGATTGATGGCAGTGAACGACAAAATGG CGTCCTGAACAGTTGGACTGATCAGGACAGC AAAGACAGCACCTACAGCATGAGCAGCACCC

SEQ ID No.	Description	Sequence
		TCACGTTGACCAAGGACGAGTATGAACGACA TAACAGCTATACCTGTGAGGCCACTCACAAG ACATCAACTTCACCCATTGTCAAGAGCTTCAA CAGGAATGAGTCTTAAGTGATTAGCTAATTCT AGAACGCGTCACTTGGCACTGGCCGTCGTTT TACAACGTCGT
55	VK_nt A13	CGCAACTCTCTACTGTTTCTCCATACCCGTTT TTTTGGATGGAGTGAAACGATGAAATACCTA TTGCCTACGGCAGCCGCTGGATTGTTATTAC TCGCTGCCCAACCAGCCATGGCCAACATCGT TATGACCCAGTCTCCATCTTCCATGTATGCAT CTCTAGGAGAGAGAGTCACTATCATTTGCAA GTCGAGTCAGGACATTAATAGCTATTTAAGTT GGTTCCAGCAGAAACCAGGGAAGTCTCCTAA GACCCTGATCTTTCGTGCAAACAGATTGGTA GATGGGGTCCCATCAAGGTTCAGTGGCAGTG GATCTGGGCAAGATTATTCTCTCACCATCAG CAGCCTGGAGTATGAAGATATGGGAATTTAT TATTGTCTACAGTATGATGAGTTTCCGTACAC GTTCGGAGGGGGGACCAAGCTGGAAGTAAAA CGGGCTGATGCTGCACCAACCGTATCCATCT TCCCACCATCCAGTGAGCAGTTAACATCTGG AGGTGCCTCAGTCGTGTGCTTCTTGAACAAC TTCTACCCCAAAGACATCAATGTCAAGTGGA AGATTGATGGCAGTGAACGACAAAATGGCGT CCTGAACAGTTGGACTGATCAGGACAGCAAA GACAGCACCTACAGCATGAGCAGCACCCTCA CGTTGACCAAGGACGAGTATGAACGACATAA CAGCTATACCTGTGAGGCCACTCACAAGACA TCAACTTCACCCATTGTCAAGAGCTTCAACAG GAATGAGTCTTAAGTGATTAGCTAATTCTAGA ACGCGTCACTTGGCACTGGCCGTCGTTTTAC AACGTCGTGA
56	VK_nt A14	TCGCAACTCTCTACTGTTTCTCCATACCCGTT TTTTTGGATGGAGTGAAACGATGAAATACCT ATTGCCTACGGCAGCCGCTGGATTGTTATTA CTCGCTGCCCAACCAGCCATGGCCGACGTTG TGATGTCACAGTCTCCATCCTCCCTGGCTGT GTCAACAGGAGAGAAGGTCACTTTGAGCTGC AAATCCAGTCAGAGTCTGCTCAACAGTAGAA CCCGAAAGAACTACTTGGCTTGGTACCAGCA GAAACCAGGGCAGTCTCCTAAACTGCTGATC TACTGGACATCCACTAGGGAATCTGGGGTCC CTAATCGCTTCACAGGCAGTGGATCTGGGAC AGATTTCACTCTCACCATCAGCAGTGTGCAG GCTGAAGACCTGGCAGTTTATTACTGCAAGC AATCTTATGATCTTCCGTGGACGTTCGGTGG GGGCACCAAACTGGAAATCAAACGGGCTGAT

SEQ ID No.	Description	Sequence
		GCTGCACCAACTGTATCCATCTTCCCACCATC CAGTGAGCAGTTAACATCTGGAGGTGCCTCA GTCGTGTGCTTCTTGAACAACTTCTACCCCAA AGACATCAATGTCAAGTGGAAGATTGATGGC AGTGAACGACAAAATGGCGTCCTGAACAGTT GGACTGATCAGGACAGCAAAGACAGCACCTA CAGCATGAGCAGCACCCTCACGTTGACCAAG GACGAGTATGAACGACATAACAGCTATACCT GTGAGGCCACTCACAAGACATCAACTTCACC CATTGTCAAGAGCTTCAACAGGAATGAGTCT TAAGTGATTAGCTAATTCTAGAACGCGTCACT TGGCACTGGCCGTCGT
57	VH_CDR1_aa A1	VSGFTFSSYAMS
58	VH_CDR1_aa A1, A3, A8, A12 and A13	GFTFSSYAMS
59	VH_CDR1_aa A2	ASGFTFSTYAMS
60	VH_CDR1_aa A2	GFTFSTYAMS
61	VH_CDR1_aa A3, A8, A12 and A13	ASGFTFSSYAMS
62	VH_CDR1_aa A4	ASGFTFSNYGMS
63	VH_CDR1_aa A4	GFTFSNYGMS
64	VH_CDR1_aa A5	ASGFTFSNYDMS
65	VH_CDR1_aa A5	GFTFSNYDMS
66	VH_CDR1_aa A6	ASGFTFSPYAMS
67	VH_CDR1_aa A6	GFTFSPYAMS
68	VH_CDR1_aa A7	ASGFSFSSYAMS
69	VH_CDR1_aa A7	GFSFSSYAMS
70	VH_CDR1_aa A9	ASGFTFSSNAMS
71	VH_CDR1_aa A9	GFTFSSNAMS
72	VH_CDR1_aa A10	ASGFAFSSYAMS
73	VH_CDR1_aa A10	GFAFSSYAMS
74	VH_CDR1_aa A11	ASGFTFSRYGMS
75	VH_CDR1_aa A11	GFTFSRYGMS
76	VH_CDR1_aa A14	ATGYTFSSYWIE
77	VH_CDR1_aa A14	GYTFSSYWIE
78	VH_CDR1_nt A1	GTCTCTGGATTCACTTTCAGTAGCTATGCCAT GTCT
79	VH_CDR1_nt A1, A3, A8, A12 and A13	GGATTCACTTTCAGTAGCTATGCCATGTCT
80	VH_CDR1_nt A2	GCCTCTGGATTCACTTTCAGTACCTATGCCAT GTCT
81	VH_CDR1_nt A2	GGATTCACTTTCAGTACCTATGCCATGTCT
82	VH_CDR1_nt A3, A8, A12 and A13	GCCTCTGGATTCACTTTCAGTAGCTATGCCAT GTCT
83	VH_CDR1_nt A4	GCCTCTGGATTCACTTTCAGTAACTATGGCAT GTCT
84	VH_CDR1_nt A4	GGATTCACTTTCAGTAACTATGGCATGTCT

SEQ ID No.	Description	Sequence
85	VH_CDR1_nt A5	GCCTCTGGATTCACTTTCAGTAACTATGACATGTCT
86	VH_CDR1_nt A5	GGATTCACTTTCAGTAACTATGACATGTCT
87	VH_CDR1_nt A6	GCCTCTGGATTCACTTTCAGTCCCTATGCCATGTCT
88	VH_CDR1_nt A6	GGATTCACTTTCAGTCCCTATGCCATGTCT
89	VH_CDR1_nt A7	GCCTCTGGATTCTCTTTCAGTAGCTATGCCATGTCT
90	VH_CDR1_nt A7	GGATTCTCTTTCAGTAGCTATGCCATGTCT
91	VH_CDR1_nt A9	GCCTCTGGATTCACTTTCAGTAGCAATGCCATGTCC
92	VH_CDR1_nt A9	GGATTCACTTTCAGTAGCAATGCCATGTCC
93	VH_CDR1_nt A10	GCCTCTGGATTCGCTTTCAGTAGCTATGCCATGTCT
94	VH_CDR1_nt A10	GGATTCGCTTTCAGTAGCTATGCCATGTCT
95	VH_CDR1_nt A11	GCCTCTGGATTCACTTTCAGTAGATATGGCATGTCT
96	VH_CDR1_nt A11	GGATTCACTTTCAGTAGATATGGCATGTCT
97	VH_CDR1_nt A14	GCTACTGGCTACACATTCAGTAGTTACTGGATAGAG
98	VH_CDR1_nt A14	GGCTACACATTCAGTAGTTACTGGATAGAG
99	VH_CDR2_aa A1	AINFNRGTTY
100	VH_CDR2_aa A1	AINFNRGTTYYSDTVKG
101	VH_CDR2_aa A2 and A13	GINSNRGTTY
102	VH_CDR2_aa A2 and A13	GINSNRGTTYYPDTVKG
103	VH_CDR2_aa A3	AININRGTTY
104	VH_CDR2_aa A3	AININRGTTYYSDTVKG
105	VH_CDR2_aa A4	AMNNNGASTY
106	VH_CDR2_aa A4	AMNNNGASTYYPDTVKG
107	VH_CDR2_aa A5	AINRKGHSTY
108	VH_CDR2_aa A5	AINRKGHSTYYPDTVQG
109	VH_CDR2_aa A6	AINSNRGTTYYPDTVKG
110	VH_CDR2_aa A6 and A12	AINSNRGTTY
111	VH_CDR2_aa A7	AININRGTPY
112	VH_CDR2_aa A7	AININRGTPYYPDTVKG
113	VH_CDR2_aa A8	AINPNGGSTY
114	VH_CDR2_aa A8	AINPNGGSTYYPDTVKG
115	VH_CDR2_aa A9	AINSKGGGTY
116	VH_CDR2_aa A9	AINSKGGGTYYPDTVRG
117	VH_CDR2_aa A10	AINNRGGGTY
118	VH_CDR2_aa A10	AINNRGGGTYYPDTVRG
119	VH_CDR2_aa A11	AINPNGGTTY
120	VH_CDR2_aa A11	AINPNGGTTYYPDTVKG

SEQ ID No.	Description	Sequence
121		AINSNRGTTYSDTVKG
122	VH_CDR2_aa A14	EILPGIGNTN
123	VH_CDR2_aa A14	EILPGIGNTNYNEKFKG
124	VH_CDR2_nt A1	GCCATTAATTTTAATCGTGGTACCACCTAC
125	VH_CDR2_nt A1	GCCATTAATTTTAATCGTGGTACCACCTACTA TTCAGACACTGTGAAGGGC
126	VH_CDR2_nt A2 and A13	GGCATTAATAGTAATCGTGGTACCACCTAC
127	VH_CDR2_nt A2 and A13	GGCATTAATAGTAATCGTGGTACCACCTACT ATCCAGACACTGTGAAGGGC
128	VH_CDR2_nt A3	GCCATTAATATTAATCGTGGTACCACCTAC
129	VH_CDR2_nt A3	GCCATTAATATTAATCGTGGTACCACCTACTA TTCAGACACTGTGAAGGGC
130	VH_CDR2_nt A4	GCCATGAATAATAATGGTGCTAGCACCTAC
131	VH_CDR2_nt A4	GCCATGAATAATAATGGTGCTAGCACCTACT ATCCAGACACTGTGAAGGGC
132	VH_CDR2_nt A5	GCCATTAATCGTAAAGGTCATAGTACCTAC
133	VH_CDR2_nt A5	GCCATTAATCGTAAAGGTCATAGTACCTACTA TCCAGACACTGTGCAGGGC
134	VH_CDR2_nt A6	GCCATTAATAGTAATCGTGGTACCACCTACTA TCCAGACACTGTGAAGGGC
135	VH_CDR2_nt A6 and A12	GCCATTAATAGTAATCGTGGTACCACCTAC
136	VH_CDR2_nt A7	GCCATTAATATTAATCGTGGTACCCCCTAT
137	VH_CDR2_nt A7	GCCATTAATATTAATCGTGGTACCCCCTATTA TCCAGACACTGTGAAGGGC
138	VH_CDR2_nt A8	GCCATTAATCCTAATGGTGGTAGTACCTAC
139	VH_CDR2_nt A8	GCCATTAATCCTAATGGTGGTAGTACCTACTA TCCAGACACTGTGAAGGGC
140	VH_CDR2_nt A9	GCCATTAATAGTAAAGGTGGTGGCACCTAC
141	VH_CDR2_nt A9	GCCATTAATAGTAAAGGTGGTGGCACCTACT ATCCAGACACTGTGAGGGGC
142	VH_CDR2_nt A10	GCCATTAATAATAGAGGTGGTGGCACCTAC
143	VH_CDR2_nt A10	GCCATTAATAATAGAGGTGGTGGCACCTACT ATCCAGACACTGTGAGGGGC
144	VH_CDR2_nt A11	GCCATTAATCCTAATGGTGGTACTACCTAC
145		GCCATTAATCCTAATGGTGGTACTACCTACTA CAATGAGAAATTCAAGGGC
146	VH_CDR2_nt A12	GCCATTAATAGTAATCGTGGTACCACCTACTA TTCAGACACTGTGAAGGGC
147	VH_CDR2_nt A14	GAGATTTTACCTGGAATTGGTAATACTAAC
148	VH_CDR2_nt A14	GAGATTTTACCTGGAATTGGTAATACTAACTA CAATGAGAAATTCAAGGGC
149	VH_CDR3_aa A1 and A3	SRHRYSDYDYAMDY
150	VH_CDR3_aa A2	VRHRYTNYDYAMDY
151	VH_CDR3_aa A4	VRHNNYVDYAMDY

SEQ ID No.	Description	Sequence
152	VH_CDR3_aa A5	VRLDDNYYFFDY
153	VH_CDR3_aa A6	VRHRYNNYDYAMDY
154	VH_CDR3_aa A7	VRHRNSNNDYAMDY
155	VH_CDR3_aa A8 and A11	ARLPWSPYTLDY
156	VH_CDR3_aa A9	VSHGDNKYFYAMDY
157	VH_CDR3_aa A10	VRHDNLNYDYAMDS
158	VH_CDR3_aa A12	TRHRYSDYDYAMDY
159	VH_CDR3_aa A13	VRHRYIDYDYAMDY
160	VH_CDR3_aa A14	SGGYSTVYWYFDV
161	VH_CDR3_nt A1 and A3	TCAAGACACCGCTATAGTGACTACGACTATG CTATGGACTAC
162	VH_CDR3_nt A2	GTAAGACACCGCTATACTAACTACGACTATG CTATGGACTAC
163	VH_CDR3_nt A4	GTAAGACATAATAACTACGTTGACTATGCTAT GGACTAT
164	VH_CDR3_nt A5	GTAAGACTTGACGATAACTACTACTTCTTTGA CTAC
165	VH_CDR3_nt A6	GTAAGACACCGCTATAATAACTACGACTATG CTATGGACTAC
166	VH_CDR3_nt A7	GTAAGACACCGCAATAGTAACAACGACTATG CTATGGACTAC
167	VH_CDR3_nt A8 and A11	GCAAGACTCCCATGGTCCCCCTATACTTTGG ACTAC
168	VH_CDR3_nt A9	GTAAGCCATGGGGATAATAAGTACTTTTATG CTATGGACTAC
169	VH_CDR3_nt A10	GTGAGACATGACAATCTTAACTATGACTATG CTATGGACTCC
170	VH_CDR3_nt A12	ACAAGACACCGCTATAGTGACTACGACTATG CTATGGACTAC
171	VH_CDR3_nt A13	GTAAGACACCGCTATATTGACTACGACTATG CTATGGACTAC
172	VH_CDR3_nt A14	GCAAGTGGGGGGTATAGTACCGTCTATTGGT ATTTTGATGTC
173	VK_CDR1_aa A1	KASQDINSYLNW
174	VK_CDR1_aa A10	KASQDIYSYLSW
175	VK_CDR1_aa A11	MTSTDIDDALNW
176	VK_CDR1_aa A13	KSSQDINSYLSW
177	VK_CDR1_aa A14	QSLLNSRTRKNY
178	VK_CDR1_aa A14	KSSQSLLNSRTRKNYLA
179	VK_CDR1_aa A2, A3, A4, A5, A6, A7, A9 and A12	KASQDINSYLSW
180	VK_CDR1_aa A8	ITNTDIDDAMNW
181	VK_CDR1_nt A1	AAGGCGAGTCAGGACATTAATAGCTATTTAA ACTGG
182	VK_CDR1_nt A2, A3,	AAGGCGAGTCAGGACATTAATAGCTATTTAA

SEQ ID No.	Description	Sequence
	A4, A5, A7, A9 and A12	GCTGG
183	VK_CDR1_nt A6	AAGGCGAGTCAAGACATTAATAGCTATTTAA GCTGG
184	VK_CDR1_nt A8	ATAACCAACACTGATATTGATGATGCTATGAA CTGG
185	VK_CDR1_nt A10	AAGGCGAGTCAGGACATTTATAGCTATTTAA GCTGG
186	VK_CDR1_nt A11	ATGACCAGCACTGATATTGATGATGCTCTGA ACTGG
187	VK_CDR1_nt A13	AAGTCGAGTCAGGACATTAATAGCTATTTAA GTTGG
188	VK_CDR1_nt A14	CAGAGTCTGCTCAACAGTAGAACCCGAAAGA ACTAC
189	VK_CDR1_nt A14	AAATCCAGTCAGAGTCTGCTCAACAGTAGAA CCCGAAAGAACTACTTGGCT
190	VK_CDR2_aa A1, A2, A3, A9, A10, A12 and A13	RANRLVD
191	VK_CDR2_aa A1, A2, A9 and A10	TLIYRANRLV
192	VK_CDR2_aa A3	TLIHRANRLV
193	VK_CDR2_aa A4	TLIYRANKLV
194	VK_CDR2_aa A4	RANKLVD
195	VK_CDR2_aa A5	TLIYRAKRLI
196	VK_CDR2_aa A5	RAKRLID
197	VK_CDR2_aa A6	TLTYRANRLV
198	VK_CDR2_aa A6	RANRLVE
199	VK_CDR2_aa A7	TLIYRANRLI
200	VK_CDR2_aa A7	RANRLID
201	VK_CDR2_aa A8	LLISEGNTLR
202	VK_CDR2_aa A8	EGNTLRP
203	VK_CDR2_aa A11	LLISEGNSLR
204	VK_CDR2_aa A11	EGNSLRP
205	VK_CDR2_aa A12	TLTHRANRLV
206	VK_CDR2_aa A13	TLIFRANRLV
207	VK_CDR2_aa A14	KLLIYWTSTRE
208	VK_CDR2_aa A14	WTSTRES
209	VK_CDR2_nt A1, A2, A3, A9, A10, A12 and A13	CGTGCAAACAGATTGGTAGAT
210	VK_CDR2_nt A1, A2, A9 and A10	ACCCTGATCTATCGTGCAAACAGATTGGTA
211		CCCTCATCTATCCTCCAAACACATTCCTACA
212	VK_CDR2_nt A3	ACCCTGATCCATCGTGCAAACAGATTGGTA
213	VK_CDR2_nt A4	ACCCTGATCTATCGTGCAAACAAATTGGTA
214	VK_CDR2_nt A4	CGTGCAAACAAATTGGTAGAT

SEQ ID No.	Description	Sequence
215	VK_CDR2_nt A5	ACCCTGATCTATCGTGCAAAGAGATTGATA
216	VK_CDR2_nt A5	CGTGCAAAGAGATTGATAGAT
217	VK_CDR2_nt A6	ACCCTGACCTATCGTGCAAACAGATTGGTA
218	VK_CDR2_nt A6	CGTGCAAACAGATTGGTAGAA
219	VK_CDR2_nt A7	ACCCTGATCTATCGTGCAAACAGATTGATA
220	VK_CDR2_nt A7	CGTGCAAACAGATTGATAGAT
221	VK_CDR2_nt A8	CTCCTTATTTCAGAAGGCAATACTCTTCGT
222	VK_CDR2_nt A8	GAAGGCAATACTCTTCGTCCT
223	VK_CDR2_nt A11	CTCCTTATTTCAGAAGGCAATAGTCTTCGT
224	VK_CDR2_nt A11	GAAGGCAATAGTCTTCGTCCT
225	VK_CDR2_nt A12	ACCCTGACCCATCGTGCAAACAGATTGGTA
226	VK_CDR2_nt A13	ACCCTGATCTTTCGTGCAAACAGATTGGTA
227	VK_CDR2_nt A14	AAACTGCTGATCTACTGGACATCCACTAGGG AA
228	VK_CDR2_nt A14	TGGACATCCACTAGGGAATCT
229	VK_CDR3_aa A1, A2, A3, A4, A5, A6, A9, A10, A12 and A13	LQYDEFPYT
230	VK_CDR3_aa A7	LQYDEFPFT
231	VK_CDR3_aa A8	LQTDNLPLT
232	VK_CDR3_aa A11	LQSDNLPLT
233	VK_CDR3_aa A14	KQSYDLPWT
234	VK_CDR3_nt A1, A3, A4, A6, A9, A10, A12 and A13	CTACAGTATGATGAGTTTCCGTACACG
235	VK_CDR3_nt A2	CTACAGTATGATGAATTTCCGTACACG
236	VK_CDR3_nt A5	CTACAGTATGATGAGTTTCCTTACACG
237	VK_CDR3_nt A7	CTACAGTATGATGAGTTTCCATTCACG
238	VK_CDR3_nt A8	TTGCAAACTGATAACTTGCCTCTCACG
239	VK_CDR3_nt A11	TTGCAAAGTGATAACTTGCCTCTCACG
240	VK_CDR3_nt A14	AAGCAATCTTATGATCTTCCGTGGACG
241	VH_CDR2_aa A12	AINSNRGTTYYSDTVKG
242	VH_CDR2_nt A11	GCCATTAATCCTAATGGTGGTACTACCTACTA TCCAGACACTGTGAAGGGC
243	VK14-111 (GenBank V01563) n.t. 813-848	AAGGCGAGTCAGGACATTAATAGCTATTTAA GCTGG
244	VK14-111 (GenBank V01563) n.t. 879-908	ACCCTGATCTATCGTGCAAACAGATTGGTAG AT
245	VK14-111 (GenBank V01563) n.t. 879-908	ACCCTGATCTATCGTGCAAACAGATTGGTA
246	VK14-111 (GenBank V01563) n.t. 1008-1034	CTACAGTATGATGAGTTTCCTCCCACA
247	VH5-12 (GenBank AJ851868) n.t. 926028-926063	GCCTCTGGATTCGCTTTCAGTAGCTATGACAT GTCT
248	VH5-12 (GenBank AJ851868) n.t. 926022-	GGATTCGCTTTCAGTAGCTATGACATGTCT

SEQ ID No.	Description	Sequence
	926063	
249	VH5-12 (GenBank AJ851868) n.t. 926106-926135	TACATTAGTAGTGGTGGTGGTAGCACCTAC
250	VH5-12 (GenBank AJ851868) n.t. 926106-926156	TACATTAGTAGTGGTGGTGGTAGCACCTACTATCCAGACACTGTGAAGGGC
251	VH5-6 (GenBank AJ851868) n.t. 983326-983361	GCCTCTGGATTCACTTTCAGTAGCTATTACATGTCT
252	VH5-6 (GenBank AJ851868) n.t. 983320-983361	GGATTCACTTTCAGTAGCTATTACATGTCT
253	VH5-6 (GenBank AJ851868) n.t. 983404-983433	GCCATTAATAGTAATGGTGGTAGCACCTAC
254	VH5-6 (GenBank AJ851868) n.t. 983404-983454	GCCATTAATAGTAATGGTGGTAGCACCTACTATCCAGACACTGTGAAGGGC
255	VK17-121 (GenBank AJ231258) n.t. 594-629	ATAACCAGCACTGATATTGATGATGATATGAACTGG
256	VK17-121 (GenBank AJ231258) n.t. 588-629	GGCTACACATTCACTGGCTACTGGATAGAG
257	VK17-121 (GenBank AJ231258) n.t. 660-689	CTCCTTATTTCAGAAGGCAATACTCTTCGT
258	VK17-121 (GenBank AJ231258) n.t. 660-692	CTCCTTATTTCAGAAGGCAATACTCTTCGTCCT
259	VK17-121 (GenBank AJ231258) n.t. 789-815	TTGCAAAGTGATAACTTGCCTCTCACA
260	VK8-21 (GenBank Y15982) n.t. 430-465	CAGAGTCTGCTCAACAGTAGAACCCGAAAGAACTAC
261	VK8-21 (GenBank Y15982) n.t. 421-471	AAATCCAGTCAGAGTCTGCTCAACAGTAGAACCCGAAAGAACTACTTGGCT
262	VK8-21 (GenBank Y15982) n.t. 502-534	AAACTGCTGATCTACTGGGCATCCACTAGGGAA
263	VK8-21 (GenBank Y15982) n.t. 514-537	TGGGCATCCACTAGGGAATCT
264	VK8-21 (GenBank Y15982) n.t. 634-658	AAGCAATCTTATAATCTTCCCACAGTG
265	VH1-9 (GenBank AC090843) n.t. 86263-86299	GCTACTGGCTACACATTCACTGGCTACTGGATAGAG
266	VH1-9 (GenBank AC090843) n.t. 86341-86370	GAGATTTTACCTGGAAGTGGTAGTACTAAC
267	VH1-9 (GenBank AC090843) n.t. 86341-	GAGATTTTACCTGGAAGTGGTAGTACTAACTACAATGAGAAGTTCAAGGGC

SEQ ID No.	Description	Sequence
	86391	
268	A1_HuVH_aa	METDTLLLWVLLLWVPGSTGEVQLVESGGGL VQPGGSLRLSCAASGFTFSSYAMSWVRQAPGK GLEWVAAINFNRGTTYYSDTVKGRFTISRDNAK NSLYLQMNSLRAEDTAVYYCSRHRYSDYDYAM DYWGQGTMVTVSSASTKGPSVFPLAPSSKSTSG GTAALGCLVKDYFPEPVTVSWNSGALTSGVHT FPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVN HKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
269	A1_HuVH_nt	ATGGAAACCGACACCCTGCTGCTGTGGGTCC TGCTGCTCTGGGTGCCAGGCTCTACCGGCGA GGTGCAGCTGGTGGAATCCGGCGGAGGCCT GGTCCAGCCTGGCGGATCCCTGAGACTGTCC TGTGCCGCCTCCGGCTTCACCTTCTCCAGCT ACGCCATGTCCTGGGTCCGACAGGCTCCAGG CAAGGGCCTGGAATGGGTGGCCGCCATCAAC TTCAACCGGGGCACCACCTACTACTCCGACA CCGTGAAGGGCCGGTTTACCATCTCCCGGGA CAACGCCAAGAACTCCCTGTACCTGCAGATG AACTCCCTGCGGGCCGAGGACACCGCCGTGT ACTACTGCTCCCGGCACCGGTACTCCGACTA CGACTACGCCATGGACTACTGGGGCCAGGGC ACCATGGTCACCGTGTCCTCCGCCTCCACCA AGGGCCCCTCCGTGTTTCCTCTGGCCCCCTC CAGCAAGTCTACCTCTGGCGGCACCGCCGCA CTGGGCTGCCTGGTCAAGGACTATTTCCCCG AGCCCGTGACCGTGTCCTGGAACTCTGGCGC CCTGACCTCCGGCGTGCACACCTTTCCAGCC GTGCTGCAGTCCTCCGGCCTGTACTCCCTGT CCTCCGTCGTGACCGTGCCCTCCAGCTCTCT GGGCACCCAGACCTACATCTGCAACGTGAAC CACAAGCCCTCCAACACCAAGGTGGACAAGC GGGTGGAACCCAAGTCCTGCGACAAGACCCA CACCTGTCCCCCCTGCCCTGCCCCTGAACTG CTGGGAGGACCTTCCGTGTTCCTGTTCCCTC CAAAGCCCAAGGACACCCTGATGATCTCCCG GACCCCCGAAGTGACCTGCGTGGTGGTGGAC GTGTCCCACGAGGACCCTGAAGTGAAGTTCA ATTGGTACGTGGACGGCGTGGAAGTGCACAA TGCCAAGACCAAGCCCAGAGAGGAACAGTAC AACTCCACCTACCGGGTGGTGTCCGTGCTGA

SEQ ID No.	Description	Sequence
		CCGTGCTGCACCAGGACTGGCTGAACGGCAA AGAGTACAAGTGCAAGGTCTCCAACAAGGCT CTGCCTGCCCCCATCGAAAAGACCATCTCCA AGGCCAAGGGGCAGCCTCGCGAGCCTCAGGT GTACACACTGCCCCCTAGCCGGGAAGAGATG ACCAAGAACCAGGTGTCCCTGACCTGTCTGG TCAAAGGCTTCTACCCCTCCGATATCGCCGT GGAATGGGAGTCCAACGGCCAGCCCGAGAAC AACTACAAGACCACCCCCCCTGTGCTGGACT CCGACGGCTCATTCTTCCTGTACTCCAAGCT GACCGTGGACAAGTCCCGGTGGCAGCAGGG CAACGTGTTCTCCTGCTCCGTGATGCACGAG GCCCTGCACAACCACTACACCCAGAAGTCCC TGTCCCTGAGCCCCGGCAAGTGATAGTCTAG A
270	A1_HuVK_aa	METDTLLLWVLLLWVPGSTGDIQMTQSPSSLS ASVGDRVTITCKASQDINSYLNWFQQKPGKAP KSLIYRANRLVDGVPSKFSGSGSGHDYTLTISSL QPEDFATYYCLQYDEFPYTFGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSLSST LTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGECSARQSTPFVCEYQGQSSDLP
271	A1_HuVK_nt	ATGGAAACCGACACCCTGCTGCTGTGGGTCC TGCTGCTCTGGGTGCCAGGCTCCACCGGCGA CATCCAGATGACCCAGTCCCCCTCCAGCCTG TCCGCCTCTGTGGGCGACAGAGTGACCATCA CATGCAAGGCCTCCCAGGACATCAACTCCTA CCTGAACTGGTTCCAGCAGAAGCCCGGCAAG GCCCCCAAGTCCCTGATCTACCGGGCCAACC GGCTGGTGGACGGCGTGCCCTCCAAGTTCTC CGGCTCTGGCTCCGGCCACGACTATACCCTG ACCATCTCCAGCCTGCAGCCCGAGGACTTCG CCACCTACTACTGCCTGCAGTACGACGAGTT CCCCTACACCTTCGGCCAGGGCACCAAGCTG GAAATCAAGCGGACCGTGGCCGCTCCCTCCG TGTTCATCTTCCCACCCTCCGACGAGCAGCT GAAGTCCGGCACCGCCTCCGTCGTGTGCCTG CTGAACAACTTCTACCCCCGCGAGGCCAAGG TGCAGTGGAAGGTGGACAACGCCCTGCAGTC CGGCAACTCCCAGGAATCCGTCACCGAGCAG GACTCCAAGGACAGCACCTACTCCCTGTCCT CCACCCTGACCCTGTCCAAGGCCGACTACGA GAAGCACAAGGTGTACGCCTGCGAAGTGACC CACCAGGGCCTGTCCAGCCCCGTGACCAAGT CCTTCAACCGGGGCGAGTGCTCTGCCCGGCA GTCCACCCCTTTCGTGTGCGAGTACCAGGGC CAGTCCTCCGACCTGCCCTGATAGTCTAGAG

SEQ ID No.	Description	Sequence
		GGCCCTATTCTATAGTGTCACCTAAATG
272	Tyrosine-protein kinase transmembrane receptor ROR1 (ECD a.a 30-407)	QETELSVSAELVPTSSWNISSELNKDSYLTLDEP MNNITTSLGQTAELHCKVSGNPPPTIRWFKNDA PVVQEPRRLSFRSTIYGSRLRIRNLDTTDTGYFQ CVATNGKEVVSSTGVLFVKFGPPPTASPGYSDE YEEDGFCQPYRGIACARFIGNRTVYMESLHMQ GEIENQITAAFTMIGTSSHLSDKCSQFAIPSLCH YAFPYCDETSSVPKPRDLCRDECEILENVLCQT EYIFARSNPMILMRLKLPNCEDLPQPESPEAAN CIRIGIPMADPINKNHKCYNSTGVDYRGTVSVT KSGRQCQPWNSQYPHTHTFTALRFPELNGGHS YCRNPGNQKEAPWCFTLDENFKSDLCDIPACDS KDSKEKNKMEILYI
273		CTACACTATCATCAATTTCCCTACACC
274		AACCCCACTCACCACATTAATACCTATTTAAC CTCC
275	Amino acid consensus sequence heavy chain CDR1	G-(F/Y)-(T/S/A)-F-S-(S/T/N/P/R)-(Y/N)-(A/G/D/W)-(M/I)-(S/E)
276	Amino acid consensus sequence heavy chain CDR1 without A14	G-(F/Y)-(T/S/A)-F-S-(S/T/N/P/R)-(Y/N)-(A/G/D)-M-S
277	Amino acid consensus sequence heavy chain CDR2	(A/G/E)-(I/M)-(N/L)-(F/S/I/N/R/P)-(N/K/R/G)-(R/G/I)-(G/H/A)-(T/S/G/N)-(T/P)-(Y/N)-Y-(S/P/N)-(D/E)-(T/K)-(V/F)-(K/Q/R)-G
278	Amino acid consensus sequence heavy chain CDR2 without A14	(A/G)-(I/M)-(N)-(F/S/I/N/R/P)-(N/K/R)-(R/G)-(G/H/A)-(T/S/G)-(T/P)-Y-Y-(S/P)-D-T-V/-(K/Q/R)-G
279	Amino acid consensus sequence heavy chain CDR3	(S/V/T/A)-(R/S)-(H/L/G)-(R/N/D/P/G)-(Y/N/D/W)-(S/T/Y/N/L)-(D/N/V/Y/P/K/T)-(Y/D/N)-(D/Y/F/T)-(Y/F/L/*)-(A/*)-(M/*)-D-(Y/S)
280	Amino acid consensus sequence heavy chain CDR3 without A14	(S/V/T/A)-(R/S)-(H/L)-(R/N/D/P/G)-(Y/N/D/W)-(S/T/Y/N/L)-(D/N/V/Y/P/K)-(Y/D)-(D/Y/F/T)-(Y/F/L)-A-M-D-Y
281	Amino acid consensus sequence light chain CDR1	(K/I/M)-(A/T/S)-(S/N)-(Q/T)-(D/S)-(I/L)-(N/D/Y/L)-(S/D/N)-(Y/A/S)-(L/M/R)-(N/S/T)-(W/R)
282	Amino acid consensus sequence light chain CDR1 without A14	(K/I/M)-(A/T)-(S/N)-(Q/T)-D-I-(N/D/Y)-(S/D)-(Y/A)-(L/M)-(N/S)-W
283	Amino acid consensus sequence light chain CDR2	(R/E/P)-(A/G/K)-(N/K/L)-(R/K/T/S/L)-(L/I)-(V/I/R/Y)-(D/E/P/W)-(G/T)
284	Amino acid consensus sequence light chain CDR2 without A14	(R/E)-(A/G)-(N/K)-(R/K/T/S)-I-(V/I/R)-(D/E/P)-G
285	Amino acid consensus	(L/K)-Q-(Y/T/S)-D-(E/N/L)-(F/L)-P-(Y/L/F/W)-T

SEQ ID No.	Description	Sequence
	sequence light chain CDR3	
286	Amino acid consensus sequence light chain CDR3 without A14	L-Q-(Y/T/S)-D-(E/N)-(F/L)-P-(Y/L/F)-T
287	Full length isoform 1 human ROR1 identifier Q01973-1	MHRPRRRGTR PPLLALLAAL LLAARGAAAQ ETELSVSAEL VPTSSWNISS ELNKDSYLTL DEPMNNITTS LGQTAELHCK VSGNPPPTIR WFKNDAPVVQ EPRRLSFRST IYGSRLRIRN LDTTDTGYFQ CVATNGKEVV SSTGVLFVKF GPPPTASPGY SDEYEEDGFC QPYRGIACAR FIGNRTVYME SLHMQGEIEN QITAAFTMIG TSSHLSDKCS QFAIPSLCHY AFPYCDETSS VPKPRDLCRD ECEILENVLC QTEYIFARSN PMILMRLKLP NCEDLPQPES PEAANCIRIG IPMADPINKN HKCYNSTGVD YRGTVSVTKS GRQCQPWNSQ YPHTHTFTAL RFPELNGGHS YCRNPGNQKE APWCFTLDEN FKSDLCDIPA CDSKDSKEKN KMEILYILVP SVAIPLAIAL LFFFICVCRN NQKSSSAPVQ RQPKHVRGQN VEMSMLNAYK PKSKAKELPL SAVRFMEELG ECAFGKIYKG HLYLPGMDHA QLVAIKTLKD YNNPQQWTEF QQEASLMAEL HHPNIVCLLG AVTQEQPVCM LFEYINQGDL HEFLIMRSPH SDVGCSSDED GTVKSSLDHG DFLHIAIQIA AGMEYLSSHF FVHKDLAARN ILIGEQLHVK ISDLGLSREI YSADYYRVQS KSLLPIRWMP PEAIMYGKFS SDSDIWSFGV VLWEIFSFGL QPYYGFSNQE VIEMVRKRQL LPCSEDCPPR MYSLMTECWN EIPSRRPRFK DIHVRLRSWE GLSSHTSSTT PSGGNATTQT TSLSASPVSN LSNPRYPNYM FPSQGITPQG QIAGFIGPPI PQNQRFIPIN GYPIPPGYAA FPAAHYQPTG PPRVIQHCPP PKSRSPSSAS GSTSTGHVTS LPSSGSNQEA NIPLLPHMSI PNHPGGMGIT VFGNKSQKPY KIDSKQASLL GDANIHGHTE SMISAEL
287	Human ROR1 isoform 2 identifier Q01973-2	M LFEYINQGDL HEFLIMRSPH SDVGCSSDED GTVKSSLDHG DFLHIAIQIAAGMEYLSSHF FVHKDLAARN ILIGEQLHVK ISDLGLSREI YSADYYRVQS KSLLPIRWMPPEAIMYGKFS SDSDIWSFGV VLWEIFSFGL QPYYGFSNQE VIEMVRKRQL LPCSEDCPPR MYSLMTECWN EIPSRRPRFK DIHVRLRSWE GLSSHTSSTT PSGGNATTQT TSLSASPVSN LSNPRYPNYM FPSQGITPQG QIAGFIGPPI PQNQRFIPIN GYPIPPGYAA FPAAHYQPTG PPRVIQHCPP PKSRSPSSAS GSTSTGHVTS LPSSGSNQEA

SEQ ID No.	Description	Sequence
		NIPLLPHMSI PNHPGGMGIT VFGNKSQKPY KIDSKQASLL GDANIHGHTE SMISAEL

DB2/ 22702142.1

CDR Sequence List Summary												
	VH						VK					
Antibody	aa CDR1	aa CDR2	aa CDR3	nt CDR1	nt CDR2	nt CDR3	aa CDR1	aa CDR2	aa CDR3	nt CDR1	nt CDR2	nt CDR3
A1	57, 58	99, 100	149	78, 79	124, 125	161	173	190, 191	229	181	209, 210	234
A2	59, 60	101, 102	150	80, 81	126, 127	162	179	190, 191	229	182	209, 210	235
A3	58, 61	103, 104	149	79, 82	128, 129	161	179	190, 192	229	182	209, 212	234
A4	62, 63	105, 106	151	83, 84	130, 131	163	179	193, 194	229	182	213, 214	234
A5	64, 65	107, 108	152	85, 86	132, 133	164	179	195, 196	229	182	215, 216	236
A6	66, 67	109, 110	153	87, 88	134, 135	165	179	197, 198	229	183	217, 218	234
A7	68, 69	111, 112	154	89, 90	136, 137	166	179	199, 200	230	182	219, 220	237
A8	58, 61	113, 114	155	79, 82	138, 139	167	180	201, 202	231	184	221, 222	238
A9	70, 71	115, 116	156	91, 92	140, 141	168	179	190, 191	229	182	209, 210	234
A10	72, 73	117, 118	157	93, 94	142, 143	169	174	190, 191	229	185	209, 210	234
A11	74, 75	119, 120	155	95, 96	144, 267	167	175	203, 204	232	186	223, 224	239
A12	58, 61	110, 266	158	79, 82	135, 146	170	179	190, 205	229	182	209, 225	234
A13	58, 61	101, 102	159	79, 82	126, 127	171	176	190, 206	229	187	209, 226	234
A14	76, 77	122, 123	160	97, 98	147, 148	172	177, 178	207, 208	233	188, 189	227, 228	240

Figure 15

46/46

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2011/054645

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 C07K16/30 A61K39/395 A61P35/02
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, EMBASE, FSTA, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 2007/051077 A2 (UNIV CALIFORNIA [US]; KIPPS THOMAS J [US]; FUKUDA TETSUYA [JP]; ENDO T) 3 May 2007 (2007-05-03) the whole document	1-34
X	WO 2007/146957 A2 (IRM LLC [BM]; WARMUTH MARKUS [US]; FOSTER KARIN [US] IRM LLC; WARMUTH) 21 December 2007 (2007-12-21) the whole document	1-34

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 February 2012	02/03/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sirim, Pinar

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Information on patent family members

International application No
PCT/US2011/054645

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