(54) Title: ROR2 ANTIBODY COMPOSITIONS AND RELATED METHODS

FIG. 1

(57) Abstract: The disclosure provides antibodies, antibody fragments or antigen- binding fragments, as well as related antibody drug conjugates (ADCs) and chimeric antigen receptors (CARs), that specifically recognize human ROR2, Also provided in the disclosure are methods of using such antibodies in various diagnostic and therapeutic applications,

ROR2 Antibody Compositions and Related Methods

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001]   The subject patent application claims the benefit of priority to U.S. Provisional Patent Application Number 62/280,834 (filed January 20, 2016). The full disclosure of the priority application is incorporated herein by reference in its entirety and for all purposes.

BACKGROUND OF THE INVENTION

[0002]   Cancer is one of the leading causes of death. It is a class of diseases which is caused by malignant transformation of healthy cells, caused by genetic alterations, like chromosomal translocations, mutations in tumor suppressor genes, transcription factors or growth-factor receptors, leading to the immortalization of the cells. If the immortalization is combined with excessive proliferation the immortalized cells generate tumors with or without metastasis (in case of solid tumors) or leukemias and lymphomas (cancers of the blood). Defective apoptosis, or programmed cell death, can further contribute to malignant transformation of cells leading to cancer.

[0003]   A family of membrane associated receptor tyrosine kinases, consisting of the receptor tyrosine kinase orphan receptors-1 and -2 (ROR1 and ROR2) have been described as being specifically associated with particular cancers (Rebagay et al. (2012) *Front Oncol.* 2(34)), while being largely absent in expression on healthy tissue with, at least in case of ROR1, few exceptions (Balakrishnan et al. (2016) *Clin Cancer Res.* doi: 10.1158/1078-0432). Whether or not ROR expression is functionally associated with tumorigenesis remains unclear. However, due to the very tumor-selective expression of the ROR family members, they represent relevant targets for targeted cancer therapies. Importantly, ROR2 is expressed on the tumor cell surface in neuroblastoma, osteosarcoma, renal cell carcinoma, breast cancer, gastric cancer, prostate cancer, melanoma, squamous cell carcinoma, multiple myeloma and other cancers.

[0004]   In normal physiology, ROR2 is responsible for aspects of bone and cartilage growth during embryonic development. After birth expression of ROR2 is downregulated and ROR2 is normally undetectable or expressed at very low levels in adult tissues. Weak

expression of ROR2 has only been reported in stomach and thyroid issue (Morioka et al., Cancer Sci. 100: 1227-1233, 2009). ROR2 has previously been recognized as a target for the development of ROR2 specific antibodies (WO2013103637). However, there are no antibodies against the hROR2 target with known sequences described in the literature.

[0005] Therefore, there is a need for high-quality anti-ROR2 binding antibodies that can be used as a basis for the development of antibody-based targeted therapies of ROR2 expressing cancers. There is also a need for additional diagnostic tools for detecting ROR2 expressions in ROR2-related disease conditions, e.g. such as Western-blotting and or immunohistochemistry (IHC). The instant invention is directed to addressing these and other needs.

SUMMARY OF THE INVENTION

[0006] In one aspect, the invention provides novel, high-affinity binding domains of rabbit antibodies that specifically bind to the extracellular domain of human receptor tyrosine kinase-like orphan receptor 2 (hROR2) and that have been selected from highly diverse phage-display libraries of non-immunized rabbits using human ROR2 (hROR2) extracellular domains expressed in mammalian cells as a bait. The variable regions of rabbit antibodies have been selected by screening for the binding against the ECD of hROR2 both as recombinant proteins and also based on the binding of hROR2 over-expressed on the surface of mammalian host cells. By this strategy novel antibodies for hROR2 of high quality and favorable functional properties have been identified. Furthermore, the invention provides chimeric full-length antibodies of the rabbit variable domains fused to the constant region domains of human IgG$_1$ antibodies.

[0007] In a second aspect of the invention site-specifically conjugated antibody drug conjugates (ADCs) based on the chimeric rabbit-human anti-human ROR2 (hROR2) antibodies with an ultra-potent anthracycline toxin are provided by the invention. The site-specific conjugation is achieved by enzymatic conjugation using sortase enzyme, essentially as disclosed in WO2014140317. The ultra-potent anthracycline toxin resulting in anti-hROR2 ADCs with high potency *in vitro* has been disclosed in WO2016102679, which is incorporated by reference herein.

[0008]   Lastly, the invention provides chimeric antigen receptors (CARs) and T cells engineered with these CARs, i.e. so-called CAR-T cells, employing said anti-hROR2 binding domains showing high efficacy *in vitro*.

[0009]   Therefore the invention relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments (antigen-binding fragments) thereof, antibody drug conjugates (ADCs), or CARs having the same binding specificity for hROR2 as that of hROR2 specific antibodies containing an immunoglobulin heavy chain variable region sequence and an immunoglobulin light chain variable region sequence, respectively, shown in (i) SEQ ID NO:1 and SEQ ID NO:13; (ii) SEQ ID NO:2 and SEQ ID NO:14; (iii) SEQ ID NO:3 and SEQ ID NO:15; (iv) SEQ ID NO:4 and SEQ ID NO:16; (v) SEQ ID NO:5 and SEQ ID NO:17; (vi) SEQ ID NO:6 and SEQ ID NO:18; (vii) SEQ ID NO:7 and SEQ ID NO:19; (vii) SEQ ID NO:8 and SEQ ID NO:20; (ix) SEQ ID NO:9 and SEQ ID NO:21; (x) SEQ ID NO:10 and SEQ ID NO:22; (xi) SEQ ID NO:11 and SEQ ID NO:23; or (xii) SEQ ID NO:12 and SEQ ID NO:24.

[0010]   The invention further relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs comprising a heavy chain variable region sequence or a light chain variable region sequence, one or both of which are at least 90%, or at least 95% or greater than 95%, but less than 100% identical,to a heavy chain variable region sequence or a light chain variable region sequence respectively shown in (i) SEQ ID NO:1 and SEQ ID NO:13; (ii) SEQ ID NO:2 and SEQ ID NO:14; (iii) SEQ ID NO:3 and SEQ ID NO:15; (iv) SEQ ID NO:4 and SEQ ID NO:16; (v) SEQ ID NO:5 and SEQ ID NO:17; (vi) SEQ ID NO:6 and SEQ ID NO:18; (vii) SEQ ID NO:7 and SEQ ID NO:19; (vii) SEQ ID NO:8 and SEQ ID NO:20; (ix) SEQ ID NO:9 and SEQ ID NO:21; (x) SEQ ID NO:10 and SEQ ID NO:22; (xi) SEQ ID NO:11 and SEQ ID NO:23; or (xii) SEQ ID NO:12 and SEQ ID NO:24.

[0011]   The invention further relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs comprising a heavy chain variable region sequence or a light chain variable region sequence, one or both of which are identical to a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (i) SEQ ID NO:1 and SEQ ID NO:13; (ii) SEQ ID NO:2 and SEQ ID NO:14; (iii) SEQ ID NO:3 and SEQ ID NO:15; (iv) SEQ ID NO:4 and SEQ ID NO:16; (v) SEQ ID NO:5 and SEQ ID NO:17; (vi) SEQ ID NO:6 and

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SEQ ID NO:18; (vii) SEQ ID NO:7 and SEQ ID NO:19; (vii) SEQ ID NO:8 and SEQ ID NO:20; (ix) SEQ ID NO:9 and SEQ ID NO:21; (x) SEQ ID NO:10 and SEQ ID NO:22; (xi) SEQ ID NO:11 and SEQ ID NO:23; or (xii) SEQ ID NO:12 and SEQ ID NO:24.

[0012]   The invention further relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs comprising immunoglobulin heavy chain CDR sequences and immunoglobulin light chain CDR sequences that are at least 90%, or at least 95% or greater than 95%, but less than 100% identical, respectively, to (i) SEQ ID NOs:25-27 and SEQ ID NOs:61-63, (ii) SEQ ID NOs:28-30 and SEQ ID NOs:64-66, (iii) SEQ ID NOs:31-33 and SEQ ID NOs:67-69, (iv) SEQ ID NOs:34-36 and SEQ ID NOs:70-72, (v) SEQ ID NOs:37-39 and SEQ ID NOs:73-75, (vi) SEQ ID NOs:40-42 and SEQ ID NOs:76-78, (vii) SEQ ID NOs:43-45 and SEQ ID NOs:79-81, (viii) SEQ ID NOs:46-48 and SEQ ID NOs:82-84, (ix) SEQ ID NOs:49-51 and SEQ ID NOs:85-87, (x) SEQ ID NOs:52-54 and SEQ ID NOs:88-90, (xi) SEQ ID NOs:55-57 and SEQ ID NOs:91-93, or (xii) SEQ ID NOs:58-60 and SEQ ID NOs:94-96.

[0013]   The invention further relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs of the invention that contain heavy chain CDR1-3 sequences or light chain CDR1-3 sequences that are identical, respectively, to (i) SEQ ID NOs:25-27 and SEQ ID NOs:61-63, (ii) SEQ ID NOs:28-30 and SEQ ID NOs:64-66, (iii) SEQ ID NOs:31-33 and SEQ ID NOs:67-69, (iv) SEQ ID NOs:34-36 and SEQ ID NOs:70-72, (v) SEQ ID NOs:37-39 and SEQ ID NOs:73-75, (vi) SEQ ID NOs:40-42 and SEQ ID NOs:76-78, (vii) SEQ ID NOs:43-45 and SEQ ID NOs:79-81, (viii) SEQ ID NOs:46-48 and SEQ ID NOs:82-84, (ix) SEQ ID NOs:49-51 and SEQ ID NOs:85-87, (x) SEQ ID NOs:52-54 and SEQ ID NOs:88-90, (xi) SEQ ID NOs:55-57 and SEQ ID NOs:91-93, or (xii) SEQ ID NOs:58-60 and SEQ ID NOs:94-96.

[0014]   The invention further relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs of the invention that contain a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (i) SEQ ID NO:1 and SEQ ID NO:13; (ii) SEQ ID NO:2 and SEQ ID NO:14; (iii) SEQ ID NO:3 and SEQ ID NO:15; (iv) SEQ ID NO:4 and SEQ ID NO:16; (v) SEQ ID NO:5 and SEQ ID NO:17; (vi) SEQ ID NO:6 and SEQ ID NO:18; (vii) SEQ ID NO:7 and SEQ ID NO:19; (vii) SEQ ID NO:8 and SEQ ID NO:20; (ix)

SEQ ID NO:9 and SEQ ID NO:21; (x) SEQ ID NO:10 and SEQ ID NO:22; (xi) SEQ ID NO:11 and SEQ ID NO:23; or (xii) SEQ ID NO:12 and SEQ ID NO:24.

[0015]   The invention further relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs of the invention that contain at least one heavy chain CDR sequence selected from the group consisting of SEQ ID NOs:25-60. Some of these molecules further contain a light chain CDR sequence selected from the group consisting of SEQ ID NOs:61-96.

[0016]   The invention further relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs that contain heavy chain CDR1, CDR2, and CDR3 sequences that are respectively identical to SEQ ID NOs:25-27, SEQ ID NOs:28-30, SEQ ID NOs:31-33, SEQ ID NO:34-36, SEQ ID NOs:37-39, SEQ ID NOs:40-42, SEQ ID NOs:43-45, SEQ ID NOs:46-48, SEQ ID NOs:49-51, SEQ ID NOs:52-54, SEQ ID NOs:55-57, or SEQ ID NOs:58-60.

[0017]   The invention further relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs of the invention that contain at least one light chain CDR sequence selected from the group consisting of SEQ ID NOs:61-96. Some of these molecules additionally contain a heavy chain CDR sequence selected from the group consisting of SEQ ID NOs:25-60.

[0018]   The invention further relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs that contain light chain CDR1, CDR2, and CDR3 sequences that are respectively identical to SEQ ID NOs:61-63, SEQ ID NOs:64-66, SEQ ID NOs:67-69, SEQ ID NOs:70-72, SEQ ID NOs:73-75, SEQ ID NOs:76-78, SEQ ID NOs:79-81, SEQ ID NOs:82-84, SEQ ID NOs:85-87, SEQ ID NOs:88-90, SEQ ID NOs:91-93, or SEQ ID NOs:94-96.

[0019]   The invention further relates to anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs that contain heavy chain CDR1, CDR2 and CDR3 sequences and light chain CDR1, CDR2 and CDR3 sequences respectively shown in (i) SEQ ID NOs:25-27 and SEQ ID NOs:61-63, (ii) SEQ ID NOs:28-30 and SEQ ID NOs:64-66, (iii) SEQ ID NOs:31-33 and SEQ ID NOs:67-69, (iv) SEQ ID NOs:34-36 and SEQ ID NOs:70-72, (v) SEQ ID NOs:37-39 and SEQ ID NOs:73-75, (vi) SEQ ID NOs:40-42 and SEQ ID NOs:76-78, (vii) SEQ ID NOs:43-45 and SEQ ID NOs:79-81, (vii) SEQ ID NOs:46-48 and SEQ ID NOs:82-84, (ix) SEQ ID NOs:49-

51 and SEQ ID NOs:85-87, (x) SEQ ID NOs:52-54 and SEQ ID NOs:88-90, (xi) SEQ ID NOs:55-57 and SEQ ID NOs:91-93, or (xii) SEQ ID NOs:58-60 and SEQ ID NOs:94-96.

[0020]   In various embodiments, the anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof of the invention can be either of IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4 or IgM isotypes, or F(ab)2, Fv, scFv, IgGACH2, F(ab')2, scFv2CH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)2 fragments thereof, or non-depleting IgG, a diabody, or a bivalent antibody. In some embodiments, the anti-hROR2 antibodies or antibody-based binding proteins is an IgG selected from the group consisting of naturally occurring IgG1, IgG2, IgG3, IgG4 isotypes, or synthetic IgGs. In some other embodiments, the anti-hROR2 antibody fragment is a Fab, a scFv, or a dsFv. In some embodiments, the anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof are conjugated to a synthetic molecule. In some of these embodiments, the anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof are conjugated to a transmembrane region and an intracellular T-cell receptor (TCR) signaling domain to form a chimeric antigen receptor (CAR).

[0021]   The invention further relates to antibody drug conjugates (ADCs) comprising a hROR2-specific antibody, antibody-based binding protein or antibody fragment of the invention with a toxin payload that effects killing of hROR2 specific cells. In said ADCs the toxin payload can be conjugated non-site-specifically to the antibody, antibody-based binding protein or antibody fragment via lysine or cysteine amino acid side chains employing classical chemical linkers with maleimide functionality, or other chemical known in the art that can mediate conjugation to lysine or cysteine amino acid side chains. In said ADCs the small molecular weight payload can also be conjugated site-specifically either by chemical, chemo-enzymatic, or enzymatic conjugations known in the art, like e.g. with bifunctional linkers, linkers allowing Pictet-Spengler chemistry on formyl-glycine forming enzyme modified antibodies, by glycan-remodeled antibodies, or by bacterial transglutaminase or sortase enzymes

[0022]   In a related aspect, the invention provides pharmaceutical compositions that contain a therapeutically effective amount of an anti-hROR2 antibody, antibody-based binding protein, antibody fragment thereof, antibody drug conjugate (ADC) of the invention and a pharmaceutically acceptable carrier. Also provided in the invention are pharmaceutical combinations or kits that contain at least one of the antibody, antibody-based

binding proteins, antibody fragments thereof or antibody drug conjugates (ADCs) disclosed herein. Further provided in the invention are isolated or substantially purified polynucleotides that encode the variable region of the immunoglobulin heavy chain or immunoglobulin light chain of the anti-hROR2 antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs disclosed herein, as well as expression vectors harboring such a polynucleotide sequence.

[0023] In another aspect, the invention provides methods of killing or inhibiting the growth of a cell expressing hROR2 in a subject. The methods entail administering a therapeutically effective amount of an anti-hROR2 antibody, antibody-based binding protein, antibody fragment thereof, antibody drug conjugate (ADC), or CAR disclosed herein to a subject in need of treatment. This enables killing or inhibiting the growth of the cell expressing hROR2 in the subject. In various embodiments, the cell expressing hROR2 is a tumor cell. In a related aspect, the invention provides methods of treating a disease or condition associated with elevated expression of hROR2 in a subject. These methods involve administering a therapeutically effective amount of an anti-hROR2 antibody, antibody-based binding protein, antibody fragment thereof, antibody drug conjugate (ADC), or CAR of the invention to a subject that is afflicted with a disease or condition associated with elevated expression of hROR2. This enables treatment of the disease or condition in the subject. Some of these methods are directed to treating a cancer in the subject. Cancers that are amendable to treatment with methods of the invention include, e.g., neuroblastoma, osteosarcoma, renal cell carcinoma, breast cancer, gastric cancer, prostate cancer, melanoma, squamous cell carcinoma and multiple myeloma. In various embodiments, the administered anti-hROR2 antibody, antibody-based binding protein, antibody fragment thereof, or the antibody drug conjugates (ADC) or CARs based thereon, are F(ab)2, Fv, scFv, IgGACH2, F(ab')2, scFv2CH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, (scFv)2, or a synthetic IgG. In some methods, the administered anti-hROR2 antibody, antibody-based binding protein or antibody fragment thereof is conjugated to a synthetic molecule. In some of these embodiments, the anti-hROR2 antibody, antibody-based binding protein, antibody fragment thereof is conjugated to a transmembrane region and an intracellular T-cell receptor (TCR) signaling domain to form a chimeric antigen receptor (CAR). In some embodiments, the chimeric antigen receptor (CAR) is present on a T cell to be administered to the subject. In

some other embodiments, the antibody can be conjugated to a cytotoxic agent, a radioisotope, or a liposome.

[0024]   In another aspect, the invention provides methods for detecting an altered ROR2 level in a subject. The methods involve (a) obtaining a biological sample from the subject, (b) contacting the sample with an anti-hROR2 antibody, antibody-based binding protein, antibody fragment thereof disclosed herein, (c) determining the level of ROR2 in the biological sample, and (d) comparing the level of ROR2 in the biological sample to a control level of ROR2. This allows determination of whether the ROR2 level in the biological sample is altered relative to the control level of ROR2. In these methods, an increased ROR2 level in the subject relative to the control level is indicative of a disease or condition associated with elevated expression of ROR2 in the subject. For example, detection of elevated ROR2 expression can be indicative of the presence of neuroblastoma, osteosarcoma, renal cell carcinoma, breast cancer, gastric cancer, prostate cancer, melanoma, squamous cell carcinoma, or multiple myeloma in the subject.

[0025]   In still another aspect, the invention provides methods for detecting an ROR2-expressing tumor in a subject. These methods entail (a) administering an anti-hROR2 antibody, antibody-based binding protein, antibody fragment thereof of the invention to a subject that has, is suspected to have, or is at risk of developing an ROR2-expressing tumor, and (b) imaging the subject for a region of altered conjugated label density or concentration, wherein the density or concentration is relative to (i) background in proximal tissue or (ii) the density or concentration previously detected in the same region of the subject. The existence of a region of altered conjugated label density or concentration is an indication of the presence of an ROR2-expressing tumor in the subject.

[0026]   A further understanding of the nature and advantages of the present invention may be realized by reference to the remaining portions of the specification and claims.

DESCRIPTION OF THE DRAWINGS

[0027]   Figure 1 shows amino acid sequences of variable immunoglobulin heavy and light chains of novel rabbit anti-hROR2 mAbs as indicated. The amino acid sequence alignment of the rabbit variable domains (V_k, V_λ, and V_H) clones is shown with framework regions (FR) and complementarity determining regions (CDR) using Kabat numbering. Shown in the figure are the heavy chain variable domain sequences (SEQ ID NOs:1-12,

respectively) and the light chain variable domain sequences (SEQ ID NOs:13-24, respectively) of 12 antibodies designated XBR2-401, XBR2-416, XBR2-433, XBR2-327, XBR2-TOP9, XBR2-TOP72, ERR2-302, ERR2-308, ERR2-316, ERR2-317, ERR2-TOP2, and ERR2-TOP35. As indicated in the figure, clones XBR2-401, XBR2-416, XBR2-327, XBR2-TOP9, ERR2-308, ERR2-316, ERR2-317, and ERR2-TOP2 comprise variable domains of immunoglobulin κ light chains, while antibodies XBR2-433, XBR2-TOP72, ERR2-302, and ERR2-TOP35 comprise variable domains of immunoglobulin λ light chains.

[0028]   Figure 2 shows binding activity of chimeric rabbit/human Fabs to ROR2. (A) The binding of each chimeric rabbit/human Fab to immobilized human ROR2 (hFc-hROR2). As controls for the hROR2 specificity, ELISAs with the sister-molecule of ROR2, hROR1 (hFc-hROR1) expressed in mammalian cells and with BSA as an unrelated control were performed with the different novel antibodies identified in the phage-display selection against hFc-hROR2. (B) The panel displays the binding of chimeric rabbit/human Fab to ectopically expressed hROR2 on mouse pre-B cell (63-12) surface as analyzed by flow cytometry.

[0029]   Figure 3 is a schematic representation of the structure of various hROR2 domain proteins and control proteins displayed on HEK 293F cells for epitope mapping studies of the novel identified antibodies. Eight constructs with different isolated domains of the extracellular domains (ECD) of human ROR2 or control domains, were cloned and stably transfected into HEK 293F cells.

[0030]   Figure 4 shows binding of various antibodies to recombinantly expressed human ROR2 (hROR2). (A) The ectopic expression of hROR2 with different SNP at amino acid position 245 and mouse ROR2 (mROR2) in HEK 293F cells was detected by flow cytometry using a commercially available biotinylated rat anti-hemagglutinin (HA) monoclonal antibody (Roche) followed by phycoerythrin (PE)-conjugated streptavidin (BioLegend). (B) The binding of each novel chimeric rabbit/human Fab to hROR2-T245, hROR2-A245 and mROR2 displayed on the HEK 293F cell surface was analyzed by flow cytometry.

[0031]   Figure 5 shows epitope mapping studies for mAb XBR2-401 by flow cytometry. The epitope of chimeric rabbit/human IgG1 XBR2-401 was determined by flow cytometry using HEK 293F cells displaying different compositions of the three extracellular domains (Immunoglobulin, Frizzled and Kringle domain, abbreviated Ig, Fr and Ki) of hROR2-T245.

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Binding to hROR2 Ig, Fr, Ki, Ig+Fr, Fr+Ki and complete ECD (extracellular domain) expressed on HEK 293F cells was detected by flow cytometry.

[0032]   Figure 6 shows epitope mapping studies for XBR2-433 by flow cytometry. The epitope of chimeric rabbit/human IgG1 XBR2-433 was determined by flow cytometry using HEK 293F cells displaying different compositions of the three extracellular domains (Immunoglobulin, Frizzled and Kringle domain, abbreviated Ig, Fr and Ki) of ROR2-T245. Binding to hROR2 Ig, Fr, Ki, Ig+Fr, Fr+Ki and complete ECD (extracellular domain) expressed on HEK 293F cells was detected by flow cytometry.

[0033]   Figure 7 shows epitope mapping studies for XBR2-416 by flow cytometry. The epitope of chimeric rabbit/human IgG1 XBR2-416 was determined by flow cytometry using HEK 293F cells displaying different compositions of the three extracellular domains (Immunoglobulin, Frizzled and Kringle domain, abbreviated Ig, Fr and Ki) of hROR2-T245. Binding to hROR2 Ig, Fr, Ki, Ig+Fr, Fr+Ki and complete ECD (extracellular domain) expressed on HEK 293F cells was detected by flow cytometry.

[0034]   Figure 8 shows epitope mapping studies for ten chimeric rabbit/human Fabs by flow cytometry. The epitopes of each chimeric rabbit/human Fab were determined by flow cytometry using HEK 293F cells displaying different compositions of the three extracellular domains (Immunoglobulin, Frizzled and Kringle domain, abbreviated Ig, Fr and Ki) of hROR2-T245. Binding to hROR2 Ig, Fr, Ki, Ig+Fr, Fr+Ki and complete ECD (extracellular domain) expressed on HEK 293F cells was detected by flow cytometry.

[0035]   Figure 9 shows epitope binding studies performed by surface plasmon resonance (SPR). Shown are SPR sensorgrams obtained for the binding of different Fabs to hFc-hROR2 captured by anti-human Fc-γ antibody immobilized on a CM5 chip. Fabs were injected in different orders and mixtures to identify independent and overlapping epitopes. Resonance unit (RU, y axis) increases that exceeded the values found for previously existed Fabs indicated independent epitopes because they allow simultaneous binding. For example, the RUs found for the combination of Fab XBR2-TOP72, XBR2-327, ERR2-TOP2, XBR2-416, and XBR2-401 (right graph) exceeded the values found for XBR2-401 alone or the combination of XBR2-327, ERR2-TOP2, XBR2-416, and XBR2-401, indicating that all those five Fabs can bind simultaneously to human ROR2 and therefore to different regions of the protein. The x axis depicts the time in seconds (s).

[0036]   Figure 10 shows affinity measurements of anti-hROR2 specific Fabs to hROR2 ECD by surface plasmon resonance. (A) Shown are Biacore X100 sensorgrams obtained for the binding of each Fab to Fc-hROR2 captured by anti-human Fc-γ antibody immobilized on a CM5 chip after instantaneous background depletion. Fabs were injected at five different concentrations with the highest concentration indicated in table (B), one of the five concentrations was tested in duplicates. (B) Monovalent affinities of each Fab are shown in the table. The equilibrium dissociation constant (K_D) was calculated from k_{off}/k_{on} (k_{on}, association rate constant; k_{off}, dissociation rate constant).

[0037]   Figure 11 shows the binding activity of selected chimeric rabbit/human IgG1 to endogenous hROR2 expressed on breast cancer cells measured by flow cytometry. Human breast cancer cell line T47D is known to express hROR2, human breast cancer cell line MDA-MB-231 is known to be negative for hROR2. In contrast, T47D is known to be ROR1 negative, whereas MDA-MB-231 is known to be positive for ROR1 expression. (A) The expression of endogenous hROR2 on breast cancer cells was detected by flow cytometry using commercially available goat anti-human ROR2 polyclonal antibodies (R&D Systems) followed by Alexa Fluor 647-conjugated AffiniPure F(ab')₂ donkey anti-goat IgG (H+L) polyclonal antibodies (Jackson ImmunoResearch Laboratories). (B) Binding of chimeric rabbit/human IgG1 to hROR2 was detected by flow cytometry on the cell surface of human T47D breast cancer cells. Human MDA-MB-231 breast cancer cells were used as a negative control cell line.

[0038]   Figure 12 shows the binding activity of chimeric rabbit/human IgG1 XBR2-401 to denatured hROR2 in Western-blotting experiments using lysates from non-transfected 63-12 cells (lane 2), as well as 63-12 cells ectopically expressing hROR1 (lane 1) or hROR2 (lane 3). In addition, Western-blotting was used to determine the binding to the purified extracellular domain of hROR2 (lane 4) or hROR1 (lane 5).

[0039]   Figure 13 shows schematically how site-specifically conjugated ADCs disclosed in this invention have been generated. (A) shows schematically the mechanism of sortase-enzyme mediated antibody conjugation (SMAC-technology) as disclosed in WO2014140317. In order to generate site-specifically conjugated ADCs, recombinant antibodies were expressed with the C-terminal pentapeptide motif LPXTG (SEQ ID NO:139), which serve as recognition sites for the sortase enzyme A from *Staphylococcus aureus* (SrtA). When a glycine modified toxin substrate is incubated with pentapeptide motif

LPXTG containing antibody and sortase A enzyme, the sortase A enzyme catalyzes a transpeptidation reaction by which the glycine-modified toxin replaces the C-terminal glycine of the LPXTG motif and is covalently coupled to the threonine of the remaining LPXT (SEQ ID NO:142) sequence. This way site-specifically and C-terminally toxin-conjugated ADCs can be generated with high efficiency. (B) shows the structure of the preferred toxin, a PNU-159682 derivative comprising a glycine(5x)-ethylene-diamino (Gly(5)-EDA) linker connecting a 5x glycine stretch to the carbonyl group at C13 of the anthracycline structure, as disclosed in WO2016102697.

[0040]   Figure 14 shows binding of full length IgG1 antibodies to recombinant ECD of human and mouse ROR2. Panels (A) and (B) show the binding specificity of selected clones of the invention expressed as full-length chimeric rabbit-human IgG1 antibodies to purified, recombinant ECD of human and mouse ROR2 analyzed by ELISA. Panels (C) and (D) show the binding specificity of selected clones of the invention expressed as full-length chimeric rabbit-human IgG1 antibodies to purified, recombinant ECD of human and cynomolgus ROR2 analyzed by ELISA.

[0041]   Figure 15 shows *in vitro* cell killing activity of site-specifically conjugated PNU-ADCs generated with selected clones of the invention expressed as full-length chimeric rabbit-human IgG1 on (B) mouse breast cancer cell line EMT6, or (A) human ROR2 transfected EMT6 cells (clone 14). An unrelated, PNU-ADC with irrelevant specificity was used as an isotype-matched control ADC. Panel (C) shows the relative expression of hROR2 in hROR2 transfected EMT6-clone 14 versus untransfected EMT6 cells as detected by flow cytometry with a hROR2-specific antibody.

[0042]   Figure 16 shows a comparison of the *in vitro* activities of ROR2-targeting XBR2-401 CAR-T cells on ROR2 negative human breast cancer cells MDA-MB-231, and on ROR2 positive human breast cancer cells T47D with short and long spacer. The upper panels show the cellular proliferation of T cells with or without CARs (long and short spacer) as indicated upon co-culture with ROR2 negative MDA-MB-231 and ROR2 positive T47D cells and as analyzed by CFSE staining. Only ROR2 positive T47D cells induce very strong proliferation of T cells engineered with a ROR2 CAR with long spacer. The lower panels show the corresponding cell killing activity at different effector-target ratios of CAR T cells to breast cancer target cells as indicated. Also this data set shows strongest activity of the T cells with a ROR2 CAR having a long spacer.

[0043]   Figure 17 provides an overview of the method for specificity analysis of chimeric rabbit/human anti-human ROR2 IgG1 XBR2-401 and, as a control, chimeric rabbit/human anti-human ROR1 IgG1 XBR1-402, with the Retrogenix Cell Microarray Platform.

[0044]   Figure 18 shows a specificity analysis of chimeric rabbit/human anti-human ROR2 IgG1 XBR2-401 and, as a control, chimeric rabbit/human anti-human ROR1 IgG1 XBR1-402, with the Retrogenix Cell Microarray Platform. Primary binding hits from the large screen involving 4,336 human plasma membrane proteins (see Figure 17) were combined on a single slide and stained with chimeric rabbit/human anti-human ROR2 IgG1 XBR2-401 and, as controls, chimeric rabbit/human anti-human ROR1 IgG1 XBR1-402 and a rituximab biosimilar. ZsGreen1 signals on the left indicate the expression levels of the various human membrane proteins. In addition to their respective cognate antigens (ROR2, ROR1, and CD20), the tested antibodies for ROR1 and ROR2 in IgG1 format also bind to Fcγ receptors FCGR3B (CD16B), FCGR1A (CD64A), and FCGR2A (CD32A) as expected. Staining with the secondary antibody alone detects the human IgG3 heavy chain (IGHG3) as expected.

DETAILED DESCRIPTION

I.   Overview

[0045]   Receptor tyrosine kinase orphan receptors-1 and -2, ROR1 and ROR2, are the only two family members defining a new receptor tyrosine kinase family, based on the overall structural design and some functional similarities. Both ROR1 and ROR2 proteins are type I-single pass trans-membrane receptors with an extracellular domain (ECD) consisting of an immunoglobulin domain, a cysteine rich frizzled domain and a Kringle domain. These three extracellular domains are followed by a trans-membrane domain connecting the ECD to an intracellular portion of the protein comprising kinase domains (Rabagay et al. (2012) Frontiers Oncol. 2: 1-8).

[0046]   The human ROR1 and ROR2 proteins are 58% homologous between each other, but each of the ROR proteins is highly conserved between species. This represents a challenge for the development of human ROR2 specific monoclonal antibodies and very few antibodies are known, and no sequences of anti-ROR2 antibodies have been described in the literature.

[0047]   In order to develop anti-hROR2 antibodies, the present inventors have generated a very high-complexity naïve rabbit antibody Fab library displayed by phage and selected this library for binders to the mammalian cell expressed human ECD of hROR2 and to cell-surface expressed human ROR2, in order to select most functional and diverse antibody clones reactive with native human ROR2 protein.

[0048]   This strategy was chosen, because the antibody repertoire to be mined is still derived from natural rabbit B lymphocytes and thus selected for immune-system pre-selected antibody heavy and light chains. However, due to the applied screening strategy involving native recombinant and cell-expressed human ROR2 it was the hope that hROR2 specific antibodies would be identified with high functional qualities that are potentially useful for the therapy of human diseases associated with ROR2 expression, like in particular ROR2-positive cancer.

[0049]   As a result of the chosen strategy a number of novel rabbit high-affinity anti-human ROR2 antibodies have been identified with diverse CDR1, 2 and 3 sequences (Figure 1) and with high binding selectivity for human ROR2, but not for its most related "sister molecule", human ROR1 (Figures 2 and 3). Some of the hROR2-specific antibodies showed high affinity (single-digit nM affinities) to the hROR2 target (Figure 10). The invention is predicated in part on the generation by the present inventors of a large naïve chimeric rabbit/human Fab library and selection for binders to human ROR2. As detailed herein, several monoclonal chimeric rabbit/human Fab antibodies (mAbs) were obtained by the inventors (see Figure 1). These mAbs all bind to purified human ROR2 as analyzed by ELISA and to cell surface human ROR2 as analyzed by flow cytometry. Neither binds to ROR1, which is the closest relative of ROR2 and shares 58% amino acid sequence identity with ROR2. In addition, the affinity of the mAbs and the location of their epitopes (which are different) have been determined. Further, several mAbs (antibody clones XBR2-401, XBR2-416, XBR2-433, ERR2-302, ERR2-308, ERR2-317, XBR2-327, and XBR2-TOP72) were also converted to the chimeric rabbit/human IgG1 format, expressed in mammalian cells, and purified by Protein A affinity chromatography. Binding activities of these IgG1 antibodies were also determined.

[0050]   In addition to this, several, mAbs were expressed as chimeric rabbit/human IgG1 with C-terminal sortase-recognition tags, allowing site-specific conjugation of payloads to the antibody C-termini by sortase-enzyme mediated antibody conjugation technology

(SMAC-technology™) essentially as described in WO2014140317. These anti-hROR2 antibodies have then been site-specifically conjugated to a highly potent anthracycline-based PNU-159682 toxin derivative, Gly_5-EDA-PNU (Figure 13), in order to generate antibody drug conjugates (ADCs), essentially as disclosed in WO2016102679 (which is incorporated by reference herein). These ADCs have functionally been evaluated *in vitro*, and all have been found to effectively kill mouse breast cancer cells ectopically expressing human ROR2.

[0051]   To further investigate the therapeutic utility of ROR2-targeting mAbs, CAR-T cells based on XBR2-401 were engineered using previously described methods (*Hudecek, M., Lupo-Stanghellini, M. T., Kosasih, P. L., Sommermeyer, D., Jensen, M. C., Rader, C., and Riddell, S. R. (2013) Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells. Clin. Cancer Res. 19, 3153-3164*). In brief, *ex vivo* expanded healthy donor CD8+ CD62L+ T cells were lentivirally transduced with an EF1α promoter-driven expression cassette containing XBR2-401 in scFv format, followed by a short or long spacer, the transmembrane domain of human CD28, the signaling domain of 4-1BB, the signaling domain of CD3ζ, and a T2A-separated transmembrane EGFR fragment with truncated ligand binding and tyrosine kinase domains. FACS isolation of EGFR+ transduced T cells, revealed robust anti-ROR2 recognition in >90% of CAR-T cells. The activity of the ROR2-targeting XBR2-401 CAR-T with short and long spacer was tested against breast cancer cell lines T47D (ROR2+ ROR1−) and MDA-MB-231 (ROR2− ROR1+). Since XBR2-401 binds to a membrane-proximal epitope in the Kr domain of ROR2, the inventors hypothesized predicted that XBR2-401 CAR-T cells are more active with a long rather than a short spacer. This was confirmed with respect to proliferation, IFN-γ and IL-2 secretion, and cytotoxicity in the presence of ROR2+ ROR1− target cells (Figure 16).

[0052]   In accordance with these studies, the present invention provides monoclonal antibodies and related antibody-based binding proteins and antibody fragments (antigen-binding fragments) thereof that specifically recognize hROR2. The invention also provides antibody drug conjugates (ADCs) and chimeric antigen receptors (CARs) derived from the hROR2 antibodies described herein. Further provided in the invention are methods of using these antibody agents and related compositions in therapeutic and diagnostic applications for diseases and conditions with associated abnormal or elevated ROR2 expression, e.g., cancer.

II.   Definitions

[0053]   Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by those of ordinary skill in the art to which this invention pertains. The following references provide one of skill with a general definition of many of the terms used in this invention: *Academic Press Dictionary of Science and Technology*, Morris (Ed.), Academic Press (1ˢᵗ ed., 1992); *Oxford Dictionary of Biochemistry and Molecular Biology*, Smith et al. (Eds.), Oxford University Press (revised ed., 2000); *Encyclopaedic Dictionary of Chemistry*, Kumar (Ed.), Anmol Publications Pvt. Ltd. (2002); *Dictionary of Microbiology and Molecular Biology*, Singleton et al. (Eds.), John Wiley & Sons (3ʳᵈ ed., 2002); *Dictionary of Chemistry*, Hunt (Ed.), Routledge (1ˢᵗ ed., 1999); *Dictionary of Pharmaceutical Medicine*, Nahler (Ed.), Springer-Verlag Telos (1994); *Dictionary of Organic Chemistry*, Kumar and Anandand (Eds.), Anmol Publications Pvt. Ltd. (2002); and *A Dictionary of Biology (Oxford Paperback Reference)*, Martin and Hine (Eds.), Oxford University Press (4ᵗʰ ed., 2000). In addition, the following definitions are provided to assist the reader in the practice of the invention.

[0054]   The term "antibody" also synonymously called "immunoglobulins" (Ig), or "antigen-binding fragment" refers to polypeptide chain(s) which exhibit a strong monovalent, bivalent or polyvalent binding to a given antigen, epitope or epitopes. Unless otherwise noted, antibodies or antigen-binding fragments used in the invention can have sequences derived from any vertebrate species. They can be generated using any suitable technology, e.g., hybridoma technology, ribosome display, phage display, gene shuffling libraries, semi-synthetic or fully synthetic libraries or combinations thereof. Unless otherwise noted, the term "antibody" as used in the present invention includes intact antibodies, antigen-binding polypeptide fragments and other designer antibodies that are described below or well known in the art (see, e.g., Serafini, J Nucl. Med. 34:533-6, 1993).

[0055]   An intact "antibody" typically comprises at least two heavy (H) chains (about 50-70 kD) and two light (L) chains (about 25 kD) inter-connected by disulfide bonds. The recognized immunoglobulin genes encoding antibody chains include the kappa, lambda, alpha, gamma, delta, epsilon, and mu constant region genes, as well as the myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively.

[0056]   Each heavy chain of an antibody is comprised of a heavy chain variable region (V_H) and a heavy chain constant region. The heavy chain constant region of most IgG isotypes (subclasses) is comprised of three domains, C_{H1}, C_{H2} and C_{H3}, some IgG isotypes, like IgM or IgE comprise a fourth constant region domain, C_{H4}. Each light chain is comprised of a light chain variable region (V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system and the first component (Clq) of the classical complement system.

[0057]   The V_H and V_L regions of an antibody can be further subdivided into regions of hypervariability, also termed complementarity determining regions (CDRs), which are interspersed with the more conserved framework regions (FRs). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The locations of CDR and FR regions and a numbering system have been defined by, e.g., Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, U.S. Government Printing Office (1987 and 1991).

[0058]   An "antibody-based binding protein", as used herein, may represent any protein that contains at least one antibody-derived V_H, V_L, or C_H immunoglobulin domain in the context of other non-immunoglobulin, or non-antibody derived components. Such antibody-based proteins include, but are not limited to (i) F_c-fusion proteins of binding proteins, including receptors or receptor components with all or parts of the immunoglobulin C_H domains, (ii) binding proteins, in which V_H and or V_L domains are coupled to alternative molecular scaffolds, or (iii) molecules, in which immunoglobulin V_H, and/or V_L, and/or C_H domains are combined and/or assembled in a fashion not normally found in naturally occurring antibodies or antibody fragments (antigen-binding fragments).

[0059]   "Binding affinity" is generally expressed in terms of equilibrium association or dissociation constants (K_A or K_D, respectively), which are in turn reciprocal ratios of dissociation and association rate constants (k_{off} and k_{on}, respectively). Thus, equivalent affinities may correspond to different rate constants, so long as the ratio of the rate constants remains the same. The binding affinity of an antibody is usually be expressed as the K_D of a

monovalent fragment (e.g. a F_{ab} fragment) of the antibody, with K_D values in the single-digit nanomolar range or below (subnanomolar or picomolar) being considered as very high and of therapeutic and diagnostic relevance.

[0060]   As used herein, the term "binding specificity" refers to the selective affinity of one molecule for another such as the binding of antibodies to antigens (or an epitope or antigenic determinant thereof), receptors to ligands, and enzymes to substrates. Thus, all monoclonal antibodies that bind to a particular antigenic determinant of an entity (e.g., a specific epitope of ROR1 or ROR2) are deemed to have the same binding specificity for that entity.

[0061]   The term "Antibody Drug Conjugate", or "ADC" refers to an antibody to which a therapeutically active substance or an active pharmaceutical ingredient (API) has been covalently coupled, such that the therapeutically active substance or an active pharmaceutical ingredient (API) can be targeted to the binding target of the antibody to exhibit its pharmacologic function. The therapeutically active substance or an active pharmaceutical ingredient can be a cellular toxin that is able to effect killing of the cells targeted by the ADCs, preferably malignant or cancer cells. The covalent attachment of a therapeutically active substance, an active pharmaceutical ingredient or a cellular toxin can be performed in a non-site specific manner using standard chemical linkers that couple payloads to lysine or cysteine residues, or, preferably the conjugation is performed in a site-specific manner, that allows full control of conjugation site and drug to antibody ratio (DAR) of the ADC to be generated.

[0062]   The term "conservatively modified variant" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes

every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[0063] For polypeptide sequences, "conservatively modified variants" refer to a variant which has conservative amino acid substitutions, amino acid residues replaced with other amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0064] The term "contacting" has its normal meaning and refers to combining two or more agents (e.g., polypeptides or phage), combining agents and cells, or combining two populations of different cells. Contacting can occur in vitro, e.g., mixing an antibody and a cell or mixing a population of antibodies with a population of cells in a test tube or growth medium. Contacting can also occur in a cell or in situ, e.g., contacting two polypeptides in a cell by coexpression in the cell of recombinant polynucleotides encoding the two polypeptides, or in a cell lysate. Contacting can also occur in vivo inside a subject, e.g., by administering an agent to a subject for delivery the agent to a target cell.

[0065] The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same. Two sequences are "substantially identical" if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (i.e., 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 50 nucleotides

(or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length.

[0066]   Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482c, 1970; by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443, 1970; by the search for similarity method of Pearson and Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444, 1988; by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, Madison, WI); or by manual alignment and visual inspection (see, e.g., Brent et al., *Current Protocols in Molecular Biology*, John Wiley & Sons, Inc. (ringbou ed., 2003)). Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., Nuc. Acids Res. 25:3389-3402, 1977; and Altschul et al., J. Mol. Biol. 215:403-410, 1990, respectively.

[0067]   The term "subject" refers to human and non-human animals (especially non-human mammals). The term "subject" is used herein, for example, in connection with therapeutic and diagnostic methods, to refer to human or animal subjects. Animal subjects include, but are not limited to, animal models, such as, mammalian models of conditions or disorders associated with elevated ROR2 expression such as neuroblastoma, osteosarcoma, renal cell carcinoma, breast cancer, gastric cancer, prostate cancer, melanoma, squamous cell carcinoma, multiple myeloma and other cancers. Other specific examples of non-human subjects include, e.g., cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, monkeys.

[0068]   Artificial T cell receptors (also known as chimeric T cell receptors, chimeric immunoreceptors, chimeric antigen receptors (CARs) or T-bodies) are engineered receptors, which graft an arbitrary specificity onto an immune effector cell. Typically, these receptors are used to graft the specificity of a monoclonal antibody onto a T cell; with transfer of their coding sequence facilitated by retroviral or lentiviral vectors or by transposons. CAR-engineered T cells (also abbreviated CAR-T cells) are genetically engineered T cells armed with chimeric receptors whose extracellular recognition unit is comprised of an antibody-derived recognition domain and whose intracellular region is derived from lymphocyte

stimulating moiety(ies). The structure of the prototypic CAR is modular, designed to accommodate various functional domains and thereby to enable choice of specificity and controlled activation of T cells. The preferred antibody-derived recognition unit is a single chain variable fragment (scFv) that combines the specificity and binding residues of both the heavy and light chain variable regions of a monoclonal antibody. The most common lymphocyte activation moieties include a T-cell costimulatory (e.g. CD28) domain in tandem with a T-cell triggering (e.g. CD3zeta) moiety. By arming effector lymphocytes (such as T cells and natural killer cells) with such chimeric receptors, the engineered cell is redirected with a predefined specificity to any desired target antigen, in a non-HLA restricted manner. CAR constructs are introduced *ex vivo* into T cells from peripheral lymphocytes of a given patient using retroviral or lentiviral vectors or transposons. Following infusion of the resulting CAR-engineered T cells back into the patient, they traffic, reach their target site, and upon interaction with their target cell or tissue, they undergo activation and perform their predefined effector function. Therapeutic targets for the CAR approach include cancer and HIV-infected cells, or autoimmune effector cells.

[0069]   The terms "treat," "treating," "treatment," and "therapeutically effective" used herein do not necessarily imply 100% or complete treatment. Rather, there are varying degrees of treatment recognized by one of ordinary skill in the art as having a potential benefit or therapeutic effect. In this respect, the inventive method can provide any amount of any level of treatment. Furthermore, the treatment provided by the inventive method can include the treatment of one or more conditions or symptoms of the disease being treated.

[0070]   A "vector" is a replicon, such as plasmid, phage or cosmid, to which another polynucleotide segment may be attached so as to bring about the replication of the attached segment. Vectors capable of directing the expression of genes encoding for one or more polypeptides are referred to as "expression vectors".

III.   Antibodies, antibody-based binding proteins, antibody fragments thereof, antibody drug conjugates (ADCs), or CARs specifically binding to ROR2 and related derivative compounds

[0071]   In one aspect, the invention provides novel antibodies, antibody-based binding proteins, antibody fragments (also termed "antigen-binding fragments") thereof, ADCs or CARs that specifically bind to human ROR2 with the same binding specificity as that of

anti-ROR2 antibody exemplified herein (Figure 1). Antibodies of the invention include intact antibodies (e.g., IgG1 antibodies exemplified herein), antibody fragments (e.g., Fab antibodies exemplified herein), antibody-based binding proteins, antibody fragments thereof, ADCs or CARs which contain the antigen-binding portions of an intact antibody that retain capacity to bind the cognate antigen, ROR2. Examples of such antibody fragments include (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and C_{H1} domains; (ii) a F(ab')$_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and C_{H1} domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an intact antibody; (v) disulfide stabilized Fvs (dsFvs) which have an interchain disulfide bond engineered between structurally conserved framework regions; (vi) a single domain antibody (dAb) which consists of a V_H or V_L domain (see, e.g., Ward et al., Nature 341:544-546, 1989); and (vii) an isolated complementarity determining region (CDR) as a linear or cyclic peptide. Examples of antibody-based binding proteins are polypeptides in which the binding domains of the antibodies are combined with other polypeptides or polypeptide domains, e.g. alternative molecular scaffolds, Fc-regions, other functional or binding domains of other polypeptides or antibodies resulting in molecules with addition binding properties, e.g. bi- or multispecific proteins or antibodies. Such polypeptides can create an arrangement of binding or functional domains normally not found in naturally occurring antibodies or antibody fragments.

[0072]   Antibodies of the invention also encompass antibody fragments, like single chain antibodies. The term "single chain antibody" refers to a polypeptide comprising a V_H domain and a V_L domain in polypeptide linkage, generally linked via a spacer peptide, and which may comprise additional domains or amino acid sequences at the amino- and/or carboxyl-termini. For example, a single-chain antibody may comprise a tether segment for linking to the encoding polynucleotide. As an example, a single chain variable region fragment (scFv) is a single-chain antibody. Compared to the V_L and V_H domains of the Fv fragment which are coded for by separate genes, a scFv has the two domains joined (e.g., via recombinant methods) by a synthetic linker. This enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules.

[0073]   Antibodies of the present invention also encompass single domain antigen-binding units, which have a camelid scaffold. Animals in the camelid family include camels,

llamas, and alpacas. Camelids produce functional antibodies devoid of light chains. The heavy chain variable (V_H) domain folds autonomously and functions independently as an antigen-binding unit. Its binding surface involves only three CDRs as compared to the six CDRs in classical antigen-binding molecules (Fabs) or single chain variable fragments (scFvs). Camelid antibodies are capable of attaining binding affinities comparable to those of conventional antibodies.

[0074]   The various antibodies, antibody-based binding proteins, and antibody fragments thereof described herein can be produced by enzymatic or chemical modification of the intact antibodies, or synthesized de novo using recombinant DNA methodologies, or identified using phage display libraries. Methods for generating these antibodies, antibody-based binding proteins, and antibody fragments thereof are all well known in the art. For example, single chain antibodies can be identified using phage display libraries or ribosome display libraries, gene shuffled libraries (see, e.g., McCafferty et al., Nature 348:552-554, 1990; and U.S. Pat. No. 4,946,778). In particular, scFv antibodies can be obtained using methods described in, e.g., Bird et al., Science 242:423-426, 1988; and Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883, 1988. Fv antibody fragments can be generated as described in Skerra and Plückthun, Science 240:1038-41, 1988. Disulfide-stabilized Fv fragments (dsFvs) can be made using methods described in, e.g., Reiter et al., Int. J. Cancer 67:113-23, 1996. Similarly, single domain antibodies (dAbs) can be produced by a variety of methods described in, e.g., Ward et al., Nature 341:544-546, 1989; and Cai and Garen, Proc. Natl. Acad. Sci. USA 93:6280-85, 1996. Camelid single domain antibodies can be produced using methods well known in the art, e.g., Dumoulin et al., Nat. Struct. Biol. 11:500–515, 2002; Ghahroudi et al., FEBS Letters 414:521–526, 1997; and Bond et al., J. Mol. Biol. 332:643-55, 2003. Other types of antigen-binding fragments (e.g., Fab, F(ab')$_2$ or Fd fragments) can also be readily produced with routinely practiced immunology methods. See, e.g., Harlow & Lane, *Using Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1998.

[0075]   In some embodiments, the antibodies, antibody fragments, antibody-based binding proteins, ADCs or CARs of the invention have their heavy chain CDR1, CDR2 and CDR3 sequences and their light chain CDR1, CDR2 and CDR3 sequences that are substantially identical to that of the antibodies shown in Figure 1. The light chain and heavy chain CDR sequences of the exemplified antibodies are all indicated in the figure. In some

of these embodiments, the antibodies, antibody fragments, antibody-based binding proteins, ADCs or CARs have (1) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:25-27, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:61-63, respectively; (2) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:28-30, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:64-66, respectively; (3) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:31-33, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:67-69, respectively; (4) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:34-36, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:70-72, respectively; (5) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:37-39, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:73-75, respectively; (6) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:40-42, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:76-78, respectively; (7) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:43-45, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:79-81, respectively; (8) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:46-48, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:82-84, respectively; (9) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:49-51, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:85-87, respectively; (10) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:52-54, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:88-90, respectively; (11) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:55-57, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:91-93, respectively; or (12) heavy chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:58-60, respectively; and light chain CDR1-3 sequences that are substantially identical to SEQ ID NOs:94-96, respectively.

[0076]　　　In some embodiments, the antibodies, antibody fragments, antibody-based binding proteins, ADCs or CARs of the invention comprise the heavy chain CDR1-CDR3

and light chain CDR1-CDR3 sequences that are respectively identical to the sequences shown in (1) SEQ ID NOs:25-27 and SEQ ID NOs:61-63 (antibody XBR2-401), (2) SEQ ID NOs:28-30 and SEQ ID NOs:64-66 (antibody XBR2-416), (3) SEQ ID NOs:31-33 and SEQ ID NOs:67-69 (antibody XBR2-433), (4) SEQ ID NOs:34-36 and SEQ ID NOs:70-72 (antibody XBR2-327), (5) SEQ ID NOs:37-39 and SEQ ID NOs:73-75 (antibody XBR2-TOP9), (6) SEQ ID NOs:40-42 and SEQ ID NOs:76-78 (antibody XBR2-TOP72), (7) SEQ ID NOs:43-45 and SEQ ID NOs:79-81 (antibody ERR2-302), (8) SEQ ID NOs:46-48 and SEQ ID NOs:82-84 (antibody ERR2-308), (9) SEQ ID NOs:49-51 and SEQ ID NOs:85-87 (antibody ERR2-316), (10) SEQ ID NOs:52-54 and SEQ ID NOs:88-90 (antibody ERR2-317), (11) SEQ ID NOs:55-57 and SEQ ID NOs:91-93 (antibody ERR2-TOP2), or (12) SEQ ID NOs:58-60 and SEQ ID NOs:94-96 (antibody ERR2-TOP35).

[0077] In other embodiments, the antibodies, antibody fragments, antibody-based binding proteins, ADCs or CARs of the invention that specifically bind to human ROR2 contain (a) a light chain variable domain having a sequence that is substantially identical to any one of SEQ ID NOs:13-24, (b) a heavy chain variable domain having a sequence that is substantially identical to any one of SEQ ID NOs:1-12, or (c) both a light chain of (a) and a heavy chain of (b). In some embodiments, the antibody, antibody fragment, antibody-based binding protein, ADC or CAR of the invention comprises both a light chain of (a) and a heavy chain of (b). In some embodiments, the antibody, antibody fragment, antibody-based binding protein, ADC or CAR of the invention contains (a) a light chain variable domain having at least 90% identity to any one of SEQ ID NOs:13-24, (b) a heavy chain variable domain having at least 90% sequence identity to any one of SEQ ID NOs:1-12, or (c) both a light chain of (a) and a heavy chain of (b). In some embodiments, the percentage identity can be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or even 100%. In some embodiments, the light chain variable domain has at least 95% identity to any one of SEQ ID NOs:13-24. In some embodiments, the light chain variable domain has 100% identity to any one of SEQ ID NOs:13-24. In some embodiments, the antibody, antibody fragment, antibody-based binding protein, ADC or CAR of the invention contains a heavy chain variable domain having at least 90% identity to any one of SEQ ID NOs:1-12. In other embodiments, the percentage identity can be at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99%, or even 100%. In some embodiments, the

heavy chain variable domain has at least 95% identity to any one of SEQ ID NOs:1-12. In some embodiments, the heavy chain variable domain has 100% identity to any one of SEQ ID NOs:1-12.

[0078]   In some embodiments, the antibody, antibody fragment, antibody-based binding protein, ADC or CAR of the invention of the invention can comprise any heavy chain as described herein (e.g., heavy chains shown in Fig. 1) in combination with any suitable light chain, such as those exemplified herein. Likewise, the antibody can comprise any of the light chains as described above (e.g., light chains shown in Fig. 1) in combination with any suitable heavy chain, such as those exemplified herein. For example, in preferred embodiments, the antibody, antibody fragment, antibody-based binding protein, ADC or CAR of the invention comprises a light chain having at least 90% identity to SEQ ID NO:13 and a heavy chain having at least 90% identity to SEQ ID NO:1, or a light chain having at least 90% identity to SEQ ID NO:14 and a heavy chain having at least 90% identity to SEQ ID NO:2, or a light chain having at least 90% identity to SEQ ID NO:15 and a heavy chain having at least 90% identity to SEQ ID NO:3. In some embodiments, the antibody, antibody fragment, antibody-based binding protein, ADC or CAR of the invention can comprise the light chain and heavy chain sequences respectively shown in (1) SEQ ID NO:13 and SEQ ID NO:1, (2) SEQ ID NO:14 and SEQ ID NO:2, or (3) SEQ ID NO:15 and SEQ ID NO:3. In the various embodiments, percent (%) identity of peptide sequences can be calculated, for example, as 100 x [(identical positions)/min(TGA, TGB)], where TGA and TGB are the sum of the number of residues and internal gap positions in peptide sequences A and B in the alignment that minimizes TGA and TGB. See, e.g., Russell et al, J. Mol. Biol., 244: 332-350 (1994).

[0079]   The antibody, antibody fragment, antibody-based binding protein, ADC or CAR of the invention of the invention can be any antibody including a full length antibody or an antibody fragment that specifically recognizes or binds to the extracellular domain of human ROR2. For example, the antibody, antibody fragment or antibody-based binding protein can be polyclonal, monoclonal, recombinant, chimeric, or humanized. Furthermore, the antibody can be of any isotype including without limitation IgA, IgD, IgE, IgG, or IgM. Thus, for example, the antibody can be any IgA such as IgA1 or IgA2, or any IgG such as IgG1, IgG2, IgG3, IgG4, or synthetic IgG. The antibody can also be any antibody fragment or antibody-based binding protein having specificity for the extracellular domain of human

ROR2, such as F(ab)2, Fv, scFv, IgGACH2, F(ab')2, scFv2CH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)2, a diabody, and a bivalent antibody. The antibody can be any modified or synthetic antibody, including, but not limited to, non-depleting IgG antibodies, CARs, or other Fc or Fab variants of antibodies.

[0080]   In addition to a heavy chain as described above, the antibody, antibody-based binding proteins or antibody fragments thereof of the invention can further comprise a light chain selected from a Fab library using sequential naive chain shuffling. Likewise, in addition to a light chain as described above, the antibody of the invention can further comprise a heavy chain selected from a Fab library using sequential naive chain shuffling.

[0081]   In some embodiments, the invention provides antibodies, antibody-based binding proteins or antibody fragments thereof that are conservatively modified variants of the anti-ROR2 antibodies exemplified herein. Typically, the variable regions of these variants have an amino acid sequence that is identical to one of these exemplified sequences except for conservative substitutions at one or more amino acid residues. In some embodiments, the antibody, antibody fragment, antibody-based binding protein, ADC or CAR of the invention specifically binds to human ROR2 and contains at least one CDR having a sequence selected from the group consisting of SEQ ID NOs:25-96. The invention also provides an isolated antibody, antibody fragment, antibody-based binding protein, ADC or CAR with specificity for ROR2 containing one or more variants of the foregoing CDR sequences or substantially identically CDR sequences. The variant CDR sequences in these antibodies can include 1, 2, or 3 substitutions, insertions, deletions, or combinations thereof in a sequence selected from the group consisting of SEQ ID NOs:25-96. For example, a recombinant chimeric or humanized antibody (or fragment thereof) can include one, two, three, four, five, or six of the foregoing CDR sequences. In some embodiments, however, the recombinant chimeric or humanized antibody (or fragment thereof) includes three CDR sequences of the same light or heavy chain, e.g., light chain CDRS shown in SEQ ID NOs:61-63, SEQ ID NOs:64-66, or SEQ ID NOs:67-69; and heavy chain CDRs shown in SEQ ID NOs:25-27, SEQ ID NOs:28-30; or SEQ ID NOs:31-33. In some embodiments, the recombinant chimeric or humanized antibody (or fragment thereof) includes six CDR sequences of the same antibody, e.g., (a) SEQ ID NOs:61-63 and SEQ ID NOs:25-27; (b) SEQ ID NOs:64-66 and SEQ ID NOs:28-30; or (c) SEQ ID NOs:67-69 and SEQ ID NOs:31-33.

[0082]   In some embodiments, the invention provides antibodies, antibody-based binding proteins or antibody fragments thereof with avidity for ROR2 of about 10 μM or less, 5 μM or less, 2 μM or less, 1 μM or less, 500 nM or less, 400 nM or less, 300 nM or less, or 200 nM or less. In some embodiments, the antibodies, antibody-based binding proteins or antibody fragments thereof bind to ROR2 with an avidity of about 100 nM or less, about 75 nM or less, about 50 nM or less, about 25 nM or less, about 10 nM or less, or about 5 nM or less. In some embodiments, the antibodies, antibody-based binding proteins or antibody fragments thereof bind to ROR2 with an avidity of about 1 nM or less, about 800 pM or less, about 700 pM or less, about 600 pM or less, about 500 pM or less, about 400 pM or less, about 300 pM or less, about 200 pM or less, or about 100 pM or less. Avidity can be measured using art-known techniques, such as ELISA, biolayer inferometry, or surface plasmon resonance.

[0083]   The antibody, antibody-based binding protein or antibody fragment thereof of the invention can be produced by any suitable technique, for example, using any suitable eukaryotic or non-eukaryotic expression system. In certain embodiments, the antibody, antibody-based binding protein or antibody fragment thereof is produced using a mammalian expression system. Some specific techniques for generating the antibodies or antigen-binding fragments of the invention are exemplified herein. In some embodiments, the antibodies or antigen-binding fragments of the invention can be produced using a suitable non-eukaryotic expression system such as a bacterial expression system. Bacterial expression systems can be used to produce fragments such as a F(ab)2, Fv, scFv, IgGACH2, F(ab')2, scFv2CH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)2, and diabodies. Techniques for altering DNA coding sequences to produce such fragments are known in the art.

[0084]   The antibodies, antibody-based binding proteins or antibody fragments thereof of the invention can be conjugated to a synthetic molecule using any type of suitable conjugation. Recombinant engineering and incorporated selenocysteine (e.g., as described in U.S. Patent 8,916,159 issued on December 23, 2014) can be used to conjugate a synthetic molecule. Other methods of conjugation can include covalent coupling to native or engineered lysine side-chain amines or cysteine side-chain thiols. See, e.g., Wu et al., Nat. Biotechnol, 23: 1 137-1 146 (2005).

[0085]   In a preferred embodiment, the antibodies, antibody-based binding proteins or antibody fragments thereof of the invention conjugated to a synthetic molecule (called "ADC" for antibody drug conjugate with the synthetic molecule being a toxin) are obtained by means of site-specific sortase-enzyme mediated antibody conjugation. As disclosed in WO2014140317, sortases (also called sortase transpeptidases) form a group of prokaryotic enzymes that modify surface proteins by recognizing and cleaving a specific peptide motif called "sortase recognition tag" or "sortase tag". Usually, a given sortase enzyme recognizes one or more sortase recognition tags. Sortase enzymes can be naturally occurring, or may have undergone genetic engineering (Dorr et al., *PNAS* 2014; 111, 13343-8).

[0086]   In a preferred embodiment, the conjugate is obtained by means of site-specific sortase-enzyme mediated conjugation of (a) an antibody, antibody-based binding protein or antibody fragment thereof as described herein carrying one or more sortase recognition tags, and (b) one or more synthetic molecules carrying a glycine or oligoglycine tag, $Gly_{(n)}$. Preferably, the sortase recognition tag is fused or conjugated to the C-terminus of at least one subdomain of the antibody. Said sortase recognition tag is preferably selected from the group consisting of LPXSG (SEQ ID NO:137), LPXAG (SEQ ID NO:138), LPXTG (SEQ ID NO:139), LAXTG (SEQ ID NO:140), and NPQTG (SEQ ID NO:141), with X being any amino acid residue. Preferably, the oligoglycine tag, $Gly_{(n)}$, has a length of 1 to 21 glycine residues, preferably with a length of 3 to 5 amino acids, i.e., $Gly_{(3)}$, $Gly_{(4)}$, or $Gly_{(5)}$.

[0087]   The synthetic molecule can be any molecule such as one targeting a tumor. In some embodiments, the synthetic molecule for conjugation to the antibody is a protein (e.g., an antibody) or an RNA or DNA aptamer. In one embodiment, the antibodies, antibody-based binding proteins or antibody fragments thereof of the invention conjugated to a synthetic molecule have the general formula $A - (L - P)_n$, in which: A is an antibody, antibody-based binding protein or antibody fragment thereof as described herein, L is one or more linkers, P is one or more payloads selected from the group consisting of a label and a cytotoxic or cytostatic agent, and in which n is an integer between ≥ 1 and ≤ 10. In this embodiment, the linker preferably comprises, or consists of, at least one selected from the group consisting of: an oligopeptide linker (including cleavable and non-cleavable oligopeptide linkers), a hydrazine linker, a thiourea linker, a self-immolative linker, a succinimidyl trans-4-(maleimidylmethyl)cyclohexane-1-carboxylate (SMCC) linker, a maleimide linker, a disulfide linker, a thioether linker, and/or a maleimide linker.

[0088]   The skilled person understands that further linkers may be suitable. Such linkers may be non-cleavable or may be cleaved by changes in pH, redox potential or specific intracellular enzymes. Cleavable oligopeptide linkers include protease- or matrix metalloprotease-cleavable linkers. It is understood that the linker may comprise combinations of the above. For example, the linker may be a valine-citruline PAB linker. In a preferred embodiment, the linker comprises an oligopeptide with a sequence comprising the penta-peptide motif LPXSG (SEQ ID NO:137), LPXAG (SEQ ID NO:138), LPXTG (SEQ ID NO:139), LAXTG (SEQ ID NO:140), or NPQTG (SEQ ID NO:141) with X being any amino acid, followed by an oligo-glycine stretch, $Gly_{(n)}$, with n being an integer between ≥ 1 and ≤ 21. In a preferred embodiment, the linker is conjugated to the C-terminus of at least one subdomain of the antibody, antibody-based binding proteins or antibody fragments thereof.

[0089]   In various embodiments, suitable synthetic molecules ("payloads") for conjugation to the antibody include, e.g., therapeutic agents such as cytotoxic, cytostatic, or antiangiogenic agents, radioisotopes, and liposomes. A cytotoxic agent can be a plant, fungal, or bacterial molecule. In some embodiments, the cytotoxic agent for conjugation to the antibody of the invention is a small molecular weight toxin (MW< 2'000 Dalton, preferably MW < 1'000 Dalton), a peptide toxin, or a protein toxin. Many specific examples of these toxins are well known in the art. See, e.g., Dyba et al., Curr. Pharm. Des. 10:2311-34, 2004; Kuyucak et al., Future Med. Chem. 6:1645-58, 2014; Beraud et al., Inflamm. Allergy Drug Targets. 10:322-42, 2011; and Middlebrook et al., Microbiol. Rev. 48:199-221, 1984. In some embodiments, a therapeutic agent is conjugated to the antibody. For example, the therapeutic agent can be a maytansinoid (e.g., maytansinol or DM1 maytansinoid), a taxane, a calicheamicin, a cemadotin, a monomethylauristatin (e.g., monomethylauristatin E or monomethylauristatin F), a pyrrolobenzodiazepine (PBD), preferably an anthracycline, more preferably a derivative of the highly potent anthracycline PNU-159682. Particularly preferred derivatives of the highly potent anthracycline PNU-159682 are disclosed in WO2016102679 (which is incorporated by reference herein). Therapeutic agents also include vincristine and prednisone. In various embodiments, the therapeutic agent that may be employed in the invention can be an antimetabolite (e.g., an antifolate such as methotrexate, a fluoropyrimidine such as 5- fluorouracil, cytosine arabinoside, or an analogue of purine or adenosine); an intercalating agent an intercalating agent (for example,

an anthracycline such as doxorubicin, nemorubicine, or preferably a derivative of PNU-159682), daunomycin, epirabicin, idarubicin, mitomycin-C, dactinomycin, or mithramycin, or other intercalating agents such as pyrrolobenzodiazepine); a DNA-reactive agent such as calicheamicins, tiancimycins, and other enediynes; a platinum derivative (e.g., cisplatin or carboplatin); an alkylating agent (e.g., nitrogen mustard, melphalan, chlorambucil, busulphan, cyclophosphamide, ifosfamide nitrosoureas or thiotepa); an RNA polymerase inhibitor such as α-amanitin; an antimitotic agent (e.g., a vinca alkaloid such as vincristine, or a taxoid such as paclitaxel or docetaxel); a topoisomerase inhibitor (for example, etoposide, teniposide, amsacrine, topotecan); a cell cycle inhibitor (for example, a flavopyridol); or a microbtubule agent (e.g., an epothilone, a tubulysine, a pre-tubulysine, a discodermolide analog, or an eleutherobin analog). A therapeutic agent can be a proteosome inhibitor or a topoisomerase inhibitor such as bortezomib, amsacrine, etoposide, etoposide phosphate, teniposide, or doxorubicin. Therapeutic radioisotopes include iodine (^{131}I), yttrium (^{90}Y), lutetium (^{177}Lu), actinium (^{225}Ac), praseodymium, astatine (At), rhenium (Re), bismuth (Bi or Bi), and rhodium (Rh). Antiangiogenic agents include linomide, bevacuzimab, angiostatin, and razoxane.

[0090]   In a preferred embodiment, the synthetic toxin molecule is selected from PNU-159682 as described in Quintieri et al. (2005) and derivatives thereof (see formula (i) below), maytansine, monomethyl auristatin MMAE, and monomethyl auristatin MMAF. In a preferred embodiment, the toxin, joined to the linker at its wavy line, is of formula (i), as described in WO 2016102679 (which is incorporated by reference herein):

formula (i)

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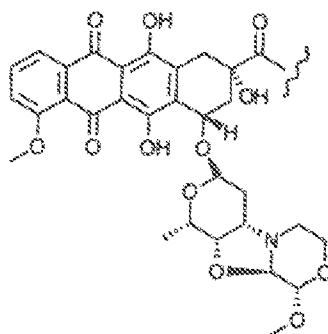
[0091]   In the embodiment where the synthetic molecule is of formula (i), it is preferred that the linker comprise an alkyldiamino group of the form $NH_2-(CH_2)_m-NH_2$, where $m \geq 1$ and ≤ 11, preferably m=2, such that one amino group is directly linked at the wavy line of formula (i) to form an amide bond. It is moreover preferred that the second amino group is linked to an oligopeptide linker, which is more preferably an oligoglycine, $Gly_{(n)}$, with n being ≥ 1 and ≤ 21. The most preferred payload is shown in Fig. 13 (B).

[0092]   In some embodiments, the synthetic molecule can be conjugated to any antibody, antibody-based binding protein, or antibody-fragment. In some embodiments, the synthetic molecule can be a label. Labels can be useful in diagnostic applications and can include, for example, contrast agents. A contrast agent can be a radioisotope label such as iodine (^{131}I or ^{125}I), indium (^{111}In), technetium (^{99}Tc), phosphorus (^{32}P), carbon (^{14}C), tritium (^{3}H), other radioisotope (e.g., a radioactive ion), or a therapeutic radioisotope such as one of the therapeutic radioisotopes listed above. Additionally, contrast agents can include radiopaque materials, magnetic resonance imaging (MRI) agents, ultrasound imaging agents, and any other contrast agents suitable for detection by a device that images an animal body. A synthetic molecule can also be a fluorescent label, a biologically active enzyme label, a luminescent label, or a chromophore label.

[0093]   In some other embodiments, the synthetic molecule can be a liposome, as described in Bendas, BioDrugs, 15: 215-224, 2001. In such embodiments, the antibody can be conjugated to a colloidal particle, e.g., a liposome, and used for controlled delivery of an agent to diseased cells. In preparing an antibody conjugated to a liposome, e.g., an immunoliposome, an agent such as a chemotherapeutic or other drug can be entrapped in the liposome for delivery to a target cell. In some embodiments, the antibodies, antibody-based binding proteins or antibody fragments thereof of the invention can also have specificity for one or more antigens in addition to ROR2. For example, the antibody of the invention can be engineered (e.g., as a bivalent diabody or a conjugated Fab dimer or trimer) to have specificity for ROR2 and another tumor antigen, e.g., an antigen associated with neuroblastoma, renal cell carcinoma, breast cancer, gastric cancer, prostate cancer, colon cancer (e.g., colon adenocarcinoma), or breast cancer (e.g., breast adenocarcinoma). The antibody can be engineered to have specificity for ROR2 and an antigen that promotes activation or targeting of cytotoxic effector cells.

[0094]   To further investigate the therapeutic utility of ROR2-targeting mAbs, chimeric

antigen receptors (CARs) and CAR-T cells based on the mAbs were generated. Typically, the chimeric antigen receptor of the invention contains a hROR2 antibody or antibody fragment described herein that is fused to a transmembrane region and an intracellular T-cell receptor (TCR) signaling domain. CAR-T cells based on XBR2-401 were engineered using previously described methods (*Hudecek, M., Lupo-Stanghellini, M. T., Kosasih, P. L., Sommermeyer, D., Jensen, M. C., Rader, C., and Riddell, S. R. (2013) Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells. Clin. Cancer Res. 19, 3153-3164*). In brief, *ex vivo* expanded healthy donor CD8+ CD62L+ T cells were lentivirally transduced with an EF1α promoter-driven expression cassette containing XBR2-401 in scFv format, followed by a short or long spacer, the transmembrane domain of human CD28, the signaling domain of 4-1BB, the signaling domain of CD3ζ, and a T2A-separated transmembrane EGFR fragment with truncated ligand binding and tyrosine kinase domains. FACS isolation of EGFR+ transduced T cells, revealed robust anti-ROR2 recognition in >90% of CAR-T cells. The activity of the ROR2-targeting XBR2-401 CAR-T with short and long spacer against breast cancer cell lines T47D (ROR2+ ROR1−) and MDA-MB-231 (ROR2− ROR1+). Since XBR2-401 binds to a membrane-proximal epitope in the Kr domain of ROR2, it is likely that XBR2-401 CAR-T cells are more active with a long rather than a short spacer. This was confirmed with respect to proliferation, IFN-γ and IL-2 secretion, and cytotoxicity in the presence of ROR2+ ROR1− target cells (Figure 16).

IV.   Polynucleotides, vectors and host cells for producing ROR2 antibodies

[0095]   The invention provides substantially purified polynucleotides (DNA or RNA) that are identical or complementary to sequences encoding polypeptides comprising segments or domains of the antibody, antibody-based binding protein or antibody fragment thereof chains described herein. In some embodiments, the polynucleotides of the invention encode the heavy chain or light chain domains sequences shown in Fig. 1. As exemplifications, a pair of polynucleotides encoding the heavy chain variable domain and light chain variable domain sequences of antibodies XBR2-401, XBR2-416 and XBR2-433 are shown in SEQ ID NOs:129 and 132, SEQ ID NOs:130 and 133, and SEQ ID NOs:131 and 134, respectively. Some of the polynucleotides of the invention comprise the nucleotide sequence as shown in SEQ ID NO:129, 130, or 131 and/or the light chain variable region

sequence as shown in SEQ ID NO:132, 133, or 134. Some other polynucleotides of the invention comprise nucleotide sequences that are substantially identical (e.g., at least 65, 80%, 95%, or 99%) to a sequence selected from SEQ ID NOs:129-134. When expressed from appropriate expression vectors, polypeptides encoded by these polynucleotides are capable of exhibiting ROR2 antigen binding capacity.

[0096]   Also provided in the invention are polynucleotides which encode at least one CDR region and usually all three CDR regions from the heavy or light chain of the antibodies described herein. Some other polynucleotides encode all or substantially all of the variable region sequence of the heavy chain and/or the light chain of the exemplified antibodies. For example, some of these polynucleotides encode the amino acid sequence of the heavy chain variable region shown in any one SEQ ID NOs:1-12, and/or the amino acid sequence of the light chain variable region shown in any one SEQ ID NOs:13-24. Because of the degeneracy of the code, a variety of nucleic acid sequences will encode each of the immunoglobulin amino acid sequences.

[0097]   The polynucleotides of the invention can encode only the variable region sequences of the exemplified antibodies. They can also encode both a variable region and a constant region of the antibody. Some of polynucleotide sequences of the invention nucleic acids encode a mature heavy chain variable region sequence that is substantially identical (e.g., at least 80%, 90%, 95% or 99%) to the mature heavy chain variable region sequence shown in any one SEQ ID NOs:1-12. Some other polynucleotide sequences encode a mature light chain variable region sequence that is substantially identical to the mature light chain variable region sequence shown in any one SEQ ID NOs:13-24. Some of the polynucleotide sequences encode a polypeptide that comprises variable regions of both the heavy chain and the light chain of one of the exemplified antibodies. Some other polynucleotides encode two polypeptide segments that respectively are substantially identical to the variable regions of the heavy chain or the light chain of one of the exemplified antibodies.

[0098]   The polynucleotide sequences can be produced by *de novo* solid-phase DNA synthesis or by PCR mutagenesis of an existing sequence (e.g., sequences as described in the Examples below) encoding an exemplified functional antibody. Direct chemical synthesis of nucleic acids can be accomplished by methods known in the art, such as the phosphotriester method of Narang et al., Meth. Enzymol. 68:90, 1979; the phosphodiester method of Brown et al., Meth. Enzymol. 68:109, 1979; the diethylphosphoramidite method of Beaucage et al.,

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Tetra. Lett., 22:1859, 1981; and the solid support method of U.S. Patent No. 4,458,066.
Introducing mutations to a polynucleotide sequence by PCR can be performed as described
in, e.g., *PCR Technology: Principles and Applications for DNA Amplification*, H.A. Erlich
(Ed.), Freeman Press, NY, NY, 1992; *PCR Protocols: A Guide to Methods and Applications*,
Innis *et al.* (Ed.), Academic Press, San Diego, CA, 1990; Mattila et al., Nucleic Acids Res.
19:967, 1991; and Eckert *et al.*, PCR Methods and Applications 1:17, 1991.

[0099]   Also provided in the invention are expression vectors and host cells for producing
the functional antibodies described herein. Specific examples of plasmid and transposon
based vectors for expressing the antibodies are described in the Examples below. Various
other expression vectors can also be employed to express the polynucleotides encoding the
functional antibody chains or binding fragments. Both viral-based and nonviral expression
vectors can be used to produce the antibodies in a mammalian host cell. Nonviral vectors
and systems include plasmids, episomal vectors, typically with an expression cassette for
expressing a protein or RNA, and human artificial chromosomes (see, e.g., Harrington et al.,
Nat. Genet. 15:345, 1997). For example, nonviral vectors useful for expression of the
antibody polynucleotides and polypeptides in mammalian (e.g., human) cells include
pCEP4, pREP4, pThioHis A, B & C, pcDNA3.1/His, pEBVHis A, B & C (Invitrogen, San
Diego, CA), MPSV vectors, and numerous other vectors known in the art for expressing
other proteins. Other useful nonviral vectors comprise expression cassettes that can be
mobilized with Sleeping Beauty, PiggyBack and other transposon systems. Useful viral
vectors include vectors based on lentiviruses or other retroviruses, adenoviruses, adeno-
associated viruses, herpes viruses, vectors based on SV40, papilloma virus, HBP Epstein
Barr virus, vaccinia virus vectors and Semliki Forest virus (SFV). See, Brent et al., *supra*;
Smith, Annu. Rev. Microbiol. 49:807, 1995; and Rosenfeld et al., Cell 68:143, 1992.

[00100]   The choice of expression vector depends on the intended host cells in which the
vector is to be expressed. Typically, the expression vectors contain a promoter and other
regulatory sequences (e.g., enhancers) that are operably linked to the polynucleotides
encoding a functional antibody chain or fragment. In some embodiments, an inducible
promoter is employed to prevent expression of inserted sequences except under inducing
conditions. Inducible promoters include, e.g., arabinose, lacZ, metallothionein promoter or a
heat shock promoter. Cultures of transformed organisms can be expanded under
noninducing conditions without biasing the population for coding sequences whose

expression products are better tolerated by the host cells. In addition to promoters, other regulatory elements may also be required or desired for efficient expression of a functional antibody chain or fragment. These elements typically include an ATG initiation codon and adjacent ribosome binding site (Kozak consensus sequence) or other sequences. In addition, the efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (see, e.g., Scharf et al., Results Probl. Cell Differ. 20:125, 1994; and Bittner et al., Meth. Enzymol., 153:516, 1987). For example, the SV40 enhancer or CMV enhancer may be used to increase expression in mammalian host cells.

[00101]   The expression vectors may also provide a secretion signal sequence position to form a fusion protein with polypeptides encoded by inserted functional antibody sequences. More often, the inserted functional antibody sequences are linked to a signal sequences before inclusion in the vector. Vectors to be used to receive sequences encoding the functional antibody light and heavy chain variable domains sometimes also encode constant regions or parts thereof. Such vectors allow expression of the variable regions as fusion proteins with the constant regions thereby leading to production of intact antibodies or fragments thereof. Typically, such constant regions are human, and preferably of human IgG1 antibodies.

[00102]   The host cells for harboring and expressing the functional antibody chains can be either prokaryotic or eukaryotic. In some preferred embodiments, mammalian host cells are used to express and to produce the antibody polypeptides of the present invention. For example, they can be either a hybridoma cell line expressing endogenous immunoglobulin genes or a mammalian cell line harboring an exogenous expression vector. These include any normal mortal or normal or abnormal immortal animal or human cell. In addition to the cell lines exemplified herein, a number of other suitable host cell lines capable of secreting intact immunoglobulins are also known in the art. These include, e.g., the CHO cell lines, various HEK 293 cell lines, various Cos cell lines, HeLa cells, myeloma cell lines, transformed B-cells and hybridomas. The use of mammalian tissue cell culture to express polypeptides is discussed generally in, e.g., Winnacker, *From Genes to Clones*, VCH Publishers, N.Y., N.Y., 1987. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. These expression vectors

usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters may be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, but are not limited to, EF1α and human UbC promoters exemplified herein, the metallothionein promoter, the constitutive adenovirus major late promoter, the dexamethasone-inducible MMTV promoter, the SV40 promoter, the MRP pol III promoter, the constitutive MPSV promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, and promoter-enhancer combinations known in the art.

[00103]   Methods for introducing expression vectors containing the polynucleotide sequences of interest vary depending on the type of cellular host. For example, calcium chloride transformation is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts (see generally Brent et al., *supra*). Other methods include, e.g., electroporation, calcium phosphate treatment, liposome-mediated transformation, injection and microinjection, ballistic methods, virosomes, immunoliposomes, polycation:nucleic acid conjugates, naked DNA, artificial virions, fusion to the herpes virus structural protein VP22 (Elliot and O'Hare, Cell 88:223, 1997), agent-enhanced uptake of DNA, and *ex vivo* transduction. For long-term, high-yield production of recombinant proteins, stable expression will often be desired. For example, cell lines which stably express the antibody chains or binding fragments can be prepared using expression vectors of the invention which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth of cells which successfully express the introduced sequences in selective media. Resistant, stably transfected cells can be proliferated using tissue culture techniques appropriate for the cell type.

[00104]   The invention further provides eukaryotic or non-eukaryotic cells (e.g., T lymphocytes) that have been recombinantly engineered to produce the antibodies, antibody-based binding proteins or antibody fragments thereof of the invention. The eukaryotic or non- eukaryotic cells can be used as an expression system to produce the antibody of the invention. In some embodiments, the invention provides ROR2 targeted immune cells that are engineered to recombinantly express an ROR2 specific antibody of the invention. For

example, the invention provides a T cell engineered to express an antibody of the invention (e.g., an scFv, scFv-Fc, or (scFv)2), which is linked to a synthetic molecule containing one or more of the following domains: a spacer or hinge region (e.g., a CD28 sequence or a IgG4 hinge-Fc sequence), a transmembrane region (e.g., a transmembrane canonical domain), and an intracellular T-cell receptor (TCR) signaling domain, thereby forming a chimeric antigen receptor (CAR) or T-body. Intracellular TCR signaling domains that can be included in a CAR (or T-body) include, but are not limited to, CD3ζ, FcR-γ, and Syk-PT signaling domains as well as the CD28, 4-1BB, and CD134 co-signaling domains. Methods for constructing T-cells expressing a CAR (or T-body) are known in the art. See, e.g., Marcu-Malina et al., Expert Opinion on Biological Therapy, Vol. 9, No. 5 (posted online on April 16, 2009).

V.   Therapeutic and diagnostic applications

[00105]   In one aspect, the invention provides methods for inhibiting cells that express ROR2 (ROR2 cells) by contacting the cells with an antibody, antibody-based binding protein or antibody fragment thereof of the invention, or an antibody drug conjugate (ADC) or an engineered cell harboring a chimeric antigen receptor (CAR) described herein. The antibody, antibody-based binding protein or antibody fragment thereof can be a naked (unconjugated) molecule or an antibody molecule conjugated to a synthetic molecule, e.g., a cytotoxic, cytostatic, or antiangiogenic agent, a radioisotope, or even to a liposome. The method can be used to inhibit ROR2 cells *in vitro* or in a subject (i.e., in vivo). The contacted ROR2 cells can be in, for example, a cell culture or animal model of a disorder associated with elevated levels of ROR2. The methods are useful, for example, to measure and/or rank (relative to another antibody) the antibody's inhibitory activity for a specific ROR2 cell type. Inhibiting ROR2 cells can include blocking or reducing the activity or growth of ROR2 cells. Inhibiting can also include the killing of ROR2 cells. While the methods are not bound by or limited to any particular mechanism of action, inhibitory activity can be mediated by blocking ROR2- mediated signaling or by blocking the signaling of an ROR2 associated receptor. Inhibitory activity can also be mediated by recruitment of immune system effectors that attack ROR2 cells, e.g., by activating constituents of the antibody-dependent cell-mediated cytotoxicity (ADCC) or complement systems.

[00106]   In some related embodiments, the invention provides methods for treating a subject that has, is suspected to have, or is at risk of developing a disorder associated with elevated levels of ROR2. Generally, the methods include administering a pharmaceutical composition that contains a therapeutically effective amount of an isolated antibody, antibody-based binding protein, antibody fragment thereof, ADC or CAR of the invention to the subject. The antibody can be any anti-ROR2 antibody of the invention as described herein. Thus, the antibody can be chimeric, humanized, synthetic, F(ab)2, Fv, scFv, IgGACH2, F(ab')2, scFv2CH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, or (scFv)2. In some embodiments, the method includes administering an IgG, an scFv, a dsFv, a F(ab')2, a diabody, or a bivalent antibody. The administered antibody or antigen-binding fragment can be conjugated to a synthetic molecule described above, e.g., a cytotoxic, cytostatic, or antiangiogenic agent, a therapeutic radioisotope, or a liposome. An exemplary cytotoxic agent is Pseudomonas exotoxin A (PE38). Disorders that can be treated include neuroblastoma, osteosarcoma, renal cell carcinoma, breast cancer, gastric cancer, prostate cancer, melanoma, squamous cell carcinoma, multiple myeloma and other disorders with elevated ROR2 expression..

[00107]   In some embodiments, the invention provides methods for treating a subject that has, is suspected to have, or is at risk of developing a disorder associated with expression of ROR2 by adoptive transfer of the genetically engineered T-cells described herein, which express an antibody or antigen-binding fragment of the invention as a chimeric antigen receptor (CAR) that selectively binds ROR2. Recombinant technology can be used to introduce CAR-encoding genetic material into any suitable T-cells, e.g., central memory T-cells from the subject to be treated. The T-cells carrying the genetic material can be expanded (e.g., in the presence of cytokines). The genetically engineered T- cells are transferred, typically by infusion, to the patient. The transferred T-cells of the invention can then mount an immune response against ROR2 expressing cells in the subject. The adoptive transfer method can be used, for example, to treat subjects that have or are suspected to have any of the cancers associated with ROR2, including neuroblastoma, osteosarcoma, renal cell carcinoma, breast cancer, gastric cancer, prostate cancer, melanoma, squamous cell carcinoma, multiple myeloma and other cancers.

[00108]   In some embodiments, the foregoing methods of treatment can further include coadministering a second therapeutic agent for treating the disorder associated with elevated

ROR2. For example, when the disorder to be treated involves an ROR2 -expressing cancer, the method can further include co-administration of a cytotoxic, cystostatic, or antiangiogenic or immune-stimulatory agent (e.g. immune-checkpoint inhibitor antibodies, for instance, but not limited to, those binding to PD1, PDL1, CTLA4, OX40, TIM3, GITR, LAG3 and the like) suitable for treating the cancer. If the cancer is a B-cell malignancy, the method can further include, for example, co-administration of rituximab, alemtuzumab, ofatumumab, ocrelizumab, or a CHOP chemotherapeutic regimen.

[00109]   In some other embodiments, the invention provides method for detecting in a biological sample an altered level of ROR2 (e.g., cell surface ROR2), for example, relative to a control, either by FACS, immunohistochemistry (IHC) or Western Blotting. Generally, the method includes contacting a biological sample with an antibody, antibody-based binding protein, antibody fragment thereof of the invention and determining the amount of antibody that selectively binds to material (e.g., cells) in the sample to thereby determine the level of ROR2 in the biological sample. A biological sample can be from a cell culture or from a test subject, e.g., a plasma or a tissue sample from a subject that has, is suspected to have, or is at risk of developing a disease or condition associated with elevated ROR2 in a subject. A control level desirably corresponds to the ROR2 level detected using the same antibody in a corresponding sample(s) from one or more control cultures or disease-free subjects. Methods of using the antibody of the invention to determine ROR2 levels can include any immunoassay such as immuno- (Western) blotting, enzyme-linked immunosorbent assay (ELISA), Immunohistochemistry (IHC) and flow cytometry, e.g., fluorescence-activated cell sorting (FACS) analysis.

[00110]   The methods of detection can be used to screen for the presence of a disorder associated with elevated ROR2. The methods include obtaining a sample from a test subject in need of screening, e.g., a subject that has, is suspected to have, or is at risk of developing a disorder associated with elevated ROR2. The level of ROR2 (e.g., the amount or concentration) in the sample is measured using an antibody, antibody-based binding protein, antibody fragment thereof of the invention, and the level in the sample is compared to a control level of ROR2. The control level represents, for example, the mean level (e.g., the amount or concentration) in sample(s) from one or, preferably, multiple control group subjects that do not have a disorder associated with elevated ROR2. Alternatively, the control level can correspond to the level or mean level of ROR2 in one or more samples

taken from the test subject at one or more prior times, such as when the test subject did not have or did not exhibit, a condition associated with elevated ROR2. A significantly higher level of ROR2 in the biological sample relative to the control level is indicative of a disorder associated with elevated ROR2 in the subject. In subjects such as humans, where cell surface ROR2 expression is largely restricted to embryonic development, a control level of ROR2 can be zero or none. Thus, in some embodiments of the method of the detection provided by the invention, any significant and detectable amount of ROR2 in a biological sample can be indicative of a disorder associated with elevated ROR2 in the subject.

[00111]   Additionally, the methods of detection can be used to monitor the progress of a disorder associated with elevated ROR2. The method includes obtaining a sample from a subject in need of screening, e.g., a subject having been diagnosed or suspected to have a disorder associated with elevated ROR2. The level of ROR2 in the sample is measured using an antibody, antibody-based binding protein, antibody fragment thereof of the invention, and the level in the sample is compared to a control level corresponding to the level or mean level of ROR2 in one or more samples taken from the test subject at one or more prior times. Levels of ROR2 that are significantly elevated or decreased relative to control indicate that the subject's disorder is deteriorating or improving, respectively. The foregoing methods of detection can be used to screen for the presence or to monitor the progress of disorders including, for example, neuroblastoma, osteosarcoma, renal cell carcinoma, breast cancer, gastric cancer, prostate cancer, melanoma, squamous cell carcinoma, multiple myeloma and other cancers.

[00112]   In some embodiments, the invention provides methods for screening a subject for an altered level of ROR2. Generally, the methods entail administering to the subject an antibody, antibody-based binding protein, antibody fragment thereof of the invention that is conjugated to a label (e.g., a contrast agent), imaging the subject in a manner suitable for detecting the label, and determining whether a region in the subject has an altered density or concentration of label as compared to the background level of label in proximal tissue. Alternatively, the methods include determining whether there is an altered density or concentration of label in a region as compared to the density or concentration of label previously detected in the same region of the subject. Methods of imaging a subject can include x-ray imaging, x-ray computed tomography (CT) imaging (e.g., CT angiography (CTA) imaging), magnetic resonance (MR) imaging, magnetic resonance angiography

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(MRA), nuclear medicine, ultrasound (US) imaging, optical imaging, elastography, infrared imaging, microwave imaging, and the like, as appropriate for detecting the label conjugated to the antibody. In a preferred embodiment, the subject has, is suspected to have, or is at risk of developing an ROR2 -expressing tumor, such as neuroblastoma, osteosarcoma, renal cell carcinoma, breast cancer, gastric cancer, prostate cancer, melanoma, squamous cell carcinoma, multiple myeloma and other cancers., and the method is used to screen for or detect the presence of the tumor. In another embodiment, the method can be used to monitor the size or density of a ROR2- expressing tumor over time, e.g., during a course of treatment.

VI.   Pharmaceutical compositions and combinations

[00113]   In another aspect, the invention provides pharmaceutical compositions that contain an antibody, an antibody fragment, an antibody-based binding protein, or an ADC as described herein and a pharmaceutically acceptable carrier. Pharmaceutical compositions can be prepared from any of the antibodies or related compounds described herein. Exemplary compositions include one or more of a chimeric antibody having SEQ ID NO:13 (light chain) and/or SEQ ID NO:1 (heavy chain), a chimeric antibody having SEQ ID NO:14 (light chain) and/or SEQ ID NO:2 (heavy chain), and a chimeric antibody having SEQ ID NO:15 (light chain) and/or SEQ ID NO:3 (heavy chain). Other antibodies, antibody fragments, antibody-based binding proteins, or ADCs suitable for the pharmaceutical compositions of the invention include those having a light chain sequence as shown in SEQ ID NOs:16-24 and/or a heavy chain sequence as shown in SEQ ID NOs:4-12. Other exemplary compositions of the invention can contain a humanized antibody having one, two, three, four, five, or six CDRs selected from the group consisting of SEQ ID NOs:25-96. In some embodiments, however, the antibody includes three CDR sequences of the same exemplified light or heavy chains shown in Figure 1. These include the heavy chain CDR1, CDR2 and CDR3 sequences and light chain CDR1, CDR2 and CDR3 sequences respectively shown in (i) SEQ ID NOs:25-27 and SEQ ID NOs:61-63 (antibody XBR2-401), (ii) SEQ ID NOs:28-30 and SEQ ID NOs:64-66 (antibody XBR2-416), (iii) SEQ ID NOs:31-33 and SEQ ID NOs:67-69 (antibody XBR2-433), (iv) SEQ ID NOs:34-36 and SEQ ID NOs:70-72 (antibody XBR2-327), (v) SEQ ID NOs:37-39 and SEQ ID NOs:73-75 (antibody XBR2-TOP9), (vi) SEQ ID NOs:40-42 and SEQ ID NOs:76-78 (antibody XBR2-TOP72), (vii)

SEQ ID NOs:43-45 and SEQ ID NOs:79-81 (antibody ERR2-302), (vii) SEQ ID NOs:46-48 and SEQ ID NOs:82-84 (antibody ERR2-308), (ix) SEQ ID NOs:49-51 and SEQ ID NOs:85-87 (antibody ERR2-316), (x) SEQ ID NOs:52-54 and SEQ ID NOs:88-90 (antibody ERR2-317), (xi) SEQ ID NOs:55-57 and SEQ ID NOs:91-93 (antibody ERR2-TOP2), and (xii) SEQ ID NOs:58-60 and SEQ ID NOs:94-96 (antibody ERR2-TOP35). In some embodiments, the pharmaceutical composition includes an antibody having six CDR sequences of the same antibody exemplified in Figure 1, e.g., (a) SEQ ID NOs:25-27 and SEQ ID NOs:61-63 (antibody XBR2-401); (b) SEQ ID NOs:28-30 and SEQ ID NOs:64-66 (antibody XBR2-416); or (c) SEQ ID NOs:31-33 and SEQ ID NOs:67-69 (antibody XBR2-433). Still another exemplary pharmaceutical composition includes a dsFv fragment, which can include one or more modifications to the amino acid sequence as appropriate and understood by one of ordinary skill in the art.

[00114] In some embodiments, the compositions of the invention contain a carrier for the antibody, the antibody fragment, the antibody-based binding protein or the ADC, desirably a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier can be any suitable pharmaceutically acceptable carrier. It can be one or more compatible solid or liquid fillers, diluents, other excipients, or encapsulating substances which are suitable for administration into a human or veterinary patient (e.g., a physiologically acceptable carrier or a pharmacologically acceptable carrier). The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the use of the active ingredient, e.g., the administration of the active ingredient to a subject. The pharmaceutically acceptable carrier can be co-mingled with one or more of the active components, e.g., a hybrid molecule, and with each other, when more than one pharmaceutically acceptable carrier is present in the composition, in a manner so as not to substantially impair the desired pharmaceutical efficacy. Pharmaceutically acceptable materials typically are capable of administration to a subject, e.g., a patient, without the production of significant undesirable physiological effects such as nausea, dizziness, rash, or gastric upset. It is, for example, desirable for a composition comprising a pharmaceutically acceptable carrier not to be immunogenic when administered to a human patient for therapeutic purposes.

[00115] Pharmaceutical compositions of the invention can additionally contain suitable buffering agents, including, for example, acetic acid in a salt, citric acid in a salt, boric acid

in a salt, and phosphoric acid in a salt. The compositions can also optionally contain suitable preservatives, such as benzalkonium chloride, chlorobutanol, parabens, and thimerosal. Pharmaceutical compositions of the invention can be presented in unit dosage form and can be prepared by any suitable method, many of which are well known in the art of pharmacy. Such methods include the step of bringing the antibody of the invention into association with a carrier that constitutes one or more accessory ingredients. In general, the composition is prepared by uniformly and intimately bringing the active agent into association with a liquid carrier, a finely divided solid carrier, or both, and then, if necessary, shaping the product.

[00116] A composition suitable for parenteral administration conveniently comprises a sterile aqueous preparation of the inventive composition, which preferably is isotonic with the blood of the recipient. This aqueous preparation can be formulated according to known methods using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation also can be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution, and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can be employed, such as synthetic mono-or di-glycerides. In addition, fatty acids such as oleic acid can be used in the preparation of injectables. Carrier formulations suitable for oral, subcutaneous, intravenous, intramuscular, etc. administrations can be found in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, PA.

[00117] Preparation of pharmaceutical compositions of the invention and their various routes of administration can be carried out in accordance with methods well known in the art. See, e.g., Remington: *The Science and Practice of Pharmacy*, Mack Publishing Co., 20th ed., 2000; and *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978. The delivery systems useful in the context of the invention include time-released, delayed release, and sustained release delivery systems such that the delivery of the inventive composition occurs prior to, and with sufficient time to cause, sensitization of the site to be treated. The inventive composition can be used in conjunction with other therapeutic agents or therapies. Such systems can avoid repeated administrations of the inventive composition, thereby increasing convenience to the subject and the physician, and may be particularly suitable for certain compositions of the invention.

[00118]   Many types of release delivery systems are available and known to those of ordinary skill in the art. Suitable release delivery systems include polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109. Delivery systems also include non-polymer systems that are lipids including sterols such as cholesterol, cholesterol esters, and fatty acids or neutral fats such as mono-di-and triglycerides; hydrogel release systems; sylastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples include, but are not limited to: (a) erosional systems in which the active composition is contained in a form within a matrix such as those described in U.S. Patents 4,452,775, 4,667,014, 4,748,034, and 5,239,660 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patents 3,832,253 and 3,854,480. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

[00119]   The invention also provides kits suitable for carrying out the methods of the invention. Typically, the kits contain two or more components required for performing the therapeutic or diagnostic methods of the invention. Kit components include, but are not limited to, one or more antibodies, antibody-based binding proteins, antibody fragments thereof, or ADCs of the invention, appropriate reagents, and/or equipment. In some embodiments, the kits can contain an antibody, antibody-based binding protein, antibody fragment thereof, or ADC of the invention and an immunoassay buffer suitable for detecting ROR2 (e.g. by ELISA, flow cytometry, magnetic sorting, or FACS). The kit may also contain one or more microtiter plates, standards, assay diluents, wash buffers, adhesive plate covers, magnetic beads, magnets, and/or instructions for carrying out a method of the invention using the kit. The kit scan include an antibody, antibody-based binding protein, antibody fragment thereof of the invention bound to a substrate (e.g., a multi-well plate or a chip), which is suitably packaged and useful to detect ROR2. In some embodiments, the kits include an antibody, antibody-based binding protein, antibody fragment thereof of the invention that is conjugated to a label, such as, a fluorescent label, a biologically active enzyme label, a luminescent label, or a chromophore label. The kits can further include reagents for visualizing the conjugated antibody, antibody-based binding protein, antibody

fragment thereof, e.g., a substrate for the enzyme. In some embodiments, the kits include an antibody or antigen-binding fragment of the invention that is conjugated to a contrast agent and, optionally, one or more reagents or pieces of equipment useful for imaging the antibody, antibody-based binding protein, antibody fragment thereof in a subject.

[00120] Generally the antibody, antibody-based binding protein, antibody fragment thereof or ADC of the invention in a kit is suitably packaged, e.g., in a vial, pouch, ampoule, and/or any container appropriate for a therapeutic or detection method. Kit components can be provided as concentrates (including lyophilized compositions), which may be further diluted prior to use, or they can be provided at the concentration of use. For use of the antibody of the invention in vivo, single dosages may be provided in sterilized containers having the desired amount and concentration of components.

EXAMPLES

[00121] The following examples are provided to further illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims.

Example 1.   Generation of human ROR2-specific monoclonal antibodies

[00122]   *Cell lines:* MDA-MB-231 and T47D from ATCC were cultured in DMEM (Invitrogen; Carlsbad, CA) supplemented with 10% (v/v) heat inactivated FBS (Thermo Scientific; Logan, UT), 100 U/mL penicillin, and 100 µg/mL streptomycin (Invitrogen). Mouse pre-B cells 63-12 (Shinkai et al., Cell 68, 855-867, 1992), as well as the 63-12 cells ectopically expressing hROR1 or hROR2, were cultured in IMEM (Invitrogen) supplemented with 10% (v/v) heat inactivated FBS (Thermo Scientific), 0.1% (v/v) beta-ME, 100 U/mL penicillin, and 100 µg/mL streptomycin (Invitrogen). HEK 293F cells were purchased from Invitrogen and maintained in FreeStyle Medium supplemented with 1% (v/v) heat inactivated FBS (Thermo Scientific), 100 U/mL penicillin, and 100 µg/mL streptomycin (Invitrogen).

[00123]   *Cloning of full-length hROR2 mammalian expression vectors:* Transposable vector backbones (pPB-Puro) were assembled from modular parts with flanking restriction sites that were synthesized or derived from sequence-verified commercially available vectors, and are described in detail in Patent WO2014013026A1. These original transposable

vector backbones were modified by exchanging IRES-driven expression of the Puromycin resistance gene in the original vector with separate, phosphoglycerate kinase promoter (PGK) driven expression. This was done by replacing the IRES sequence with an SV40-pA sequence located 3' of the multiple cloning site, followed by introduction of the PGK-promoter sequence 5' of the Puromycin resistance gene. Full-length ROR2 open reading frames were synthesized by total gene synthesis (Genscript, Piscataway) with flanking restriction sites (5'NotI/3'BstBI) and were then cloned into the multiple cloning site of the transposable vectors using the respective restriction enzymes.

[00124]   *Cell line engineering for ectopic expression of hROR2 in the EMT-6 murine breast cancer cell line:* Murine EMT-6 breast cancer cells (kind gift from Prof. Dr. med. Alfred Zippelius, University Hospital of Basel, Switzerland) were cultured in DMEM complete (Dulbecco's Modified Eagle Medium (DMEM) High Glucose (4.5 g/l) with L-Glutamine with 10% (v/v) Fetal Calf Serum (FCS), 100 IU/mL of Pen-Strep-Fungizone and 2 mM L-glutamine (all Bioconcept, Allschwil, Switzerland)) at 37°C and 5% CO_2. Cells were engineered to overexpress human ROR2 by transposition as follows: cells were centrifuged (6 min, 1200 rpm, 4°C) and resuspended in RPMI-1640 media (5x10^6 cells/mL). 400 µL of this cell suspension was then added to 400 µL of RPMI containing 13.3 µg of transposable vector pPB-PGK-Puro-ROR2, directing co-expression of full-length human ROR2 (NP_004551.2) and the puromycin-resistance gene, and 6.6 µg of transposase-containing vector pCDNA3.1_hy_mPB. DNA/EMT-6 cell mixture was transferred to electroporation cuvettes (0.4 cm-gap, 165-2088, BioRad, Cressier, Switzerland) and electroporated using the Biorad Gene Pulser II with capacitance extender at 300V and 950 µF. Then, cells were incubated for 5-10 min at room-temperature. Following the incubation, cells were centrifuged at 1200 rpm for 6 min, washed once and subsequently resuspended in DMEM complete prior to incubation at 37°C in a humidified incubator at 5% CO_2 atmosphere. One day after electroporation, cell pools stably expressing human ROR2 were selected by adding 3 µg/mL puromycin (Sigma-Aldrich, P8833).

[00125]   ROR2 expression on selected EMT-6-ROR2 cells was confirmed by flow cytometry. Briefly, following trypsinization, 10^6 cells were centrifuged in FACS tubes; obtained pellets were resuspended in buffer (PBS with 2% (v/v) FCS). Cells were then incubated with XBR2-401 (mAb003); 30 min, 4°C, final concentration 2 µg/mL), followed by centrifugation and washing. Cells were then resuspended as previously and incubated

with anti-human IgG antibody (Fc gamma-specific) PE (eBioscience, Vienna, Austria, 12-4998-82) with a 1:250 dilution in the dark (30 min, 4°C), washed once in buffer and kept on ice until FACS sorting.

[00126]   Using a FACS Aria II, cells were single cell sorted into a 96-well flat-bottom plate containing 200 µL of DMEM complete per well. This plate was incubated at 37°C and clones were expanded to 6-well plates before analysis of ROR2-expression by flow cytometry as outlined above, using a FACSCalibur instrument (BD Biosciences) and FlowJo analytical software (Tree Star, Ashland, OR) for analysis.

Figure 15C shows the FACS analysis data of clone 14 (high ROR2-expressing) and WT (ROR2 negative) EMT-6, detected with anti-ROR2 antibody XBR2-401 (mAb003).

Example 2.   Generation of high-complexity rabbit Fab library and reagents for screening

[00127]   *Construction, expression, purification, and biotinylation of recombinant human ROR2 protein hFc-hROR2-T[245]*: A human ROR2 cDNA from Thermo Scientific (Clone ID: 40/46553) was used as template. ROR2 has a single nucleotide polymorphism (SNP) at amino acid position 245. As part of the cloning, we mutated the less common alanine at position 245 of clone 40/46553 to the more frequent threonine. Briefly, two cDNA fragments encoding N-terminal and C-terminal portions of the extracellular domain (ECD) of ROR2 were PCR-amplified with (i) primers hROR2ECD_F (gcctaagcttgtctccgggtgccgaagtggaggttctggatccgaacg) (SEQ ID NO:97) and hROR2_A245T_R (gctcacgcggcttgggtgtccgggagcgcgcgtcgc) (SEQ ID NO:98) and (ii) primers hROR2_A245T_F (gcgacgcgcgctcccggacacccaagccgcgtgagc) (SEQ ID NO:99) and hROR2ECD_R (agctctcgagtcacccatcttgctgctgtctcggggactacacgagg) (SEQ ID NO:100). Subsequently, the whole ROR2 ECD (amino acids 55-394) was assembled by overlap extension PCR using the flanking primers hROR2ECD_F and hROR2ECD_R and cloned into pCEP4-hFc (Hofer et al., 2008) via *Hind*III/*Xho*I. The resulting pCEP4-hFc-hROR2 construct was then transiently transfected into HEK 293F cells (Invitrogen) using 293fectin (Invitrogen) and conditions detailed in the manufacturer's protocol. Transfected cells were cultured in FreeStyle protein-free medium (Invitrogen) and the hFc-hROR2 fusion protein was purified from supernatants by Protein A affinity chromatography. Purified hFc-hROR2 was biotinylated using the Biotin-Tag Micro Biotinylation kit (Sigma-Aldrich). Briefly, 300 µg hFc-hROR2 in 50 µL of 0.1 M sodium phosphate buffer (pH 7.2) was incubated with 1

μL of 5 mg/mL biotinamidohexanoic acid 3-sulfo-N-hydroxysuccinide ester for 30 min at room temperature with gentle stirring. Biotinylated hFc-hROR2 was isolated using MicroSpin G-50 Columns provided by the kit.

[00128] *Construction, expression, and purification of recombinant human ROR1 (hROR1-His) and human ROR2 (hROR2-His) proteins*: hROR1-His was PCR-amplified with primers SP-hROR1_F (5'

gctgggtaccggcgcgccaccatggactggacttggagaatcctgtttctcgtagctgctgcaactggagcacactccgcccggggg

cgccgccgcccag 3') (SEQ ID NO:101) and hROR1-His_R (5'

cggcctcgagtcagtgatggtgatggtggtgctccatcttgttcttctcctt 3') (SEQ ID NO:102) using pCEP4-hFc-hROR1 (Yang et al., 2011) as template, while hROR2-His was PCR-amplified with primers SP-hROR2_F

(gctgggtaccggcgcgccaccatggactggacttggagaatcctgtttctcgtagctgctgcaactggagcacactccgaagtgga

ggttctggatccg) (SEQ ID NO:103) and hROR2-His_R

(cggcctcgagtcagtgatggtgatggtggtgccccatcttgctgctgtctcg) (SEQ ID NO:104) using pCEP4-hFc-hROR2 as template. Following cloning into pCEP4 (Invitrogen) separately via *KpnI/XhoI*, the constructs were transiently transfected into HEK 293F cells (Invitrogen) using 293fectin (Invitrogen), and the corresponding recombinant protein products were purified by Immobilized Metal Ion Affinity Chromatography using a 1-mL HisTrap column (GE Healthcare) as described (Kwong and Rader, 2009). The quality and quantity of purified hROR1-His and hROR2-His were analyzed by SDS-PAGE and A_{280} absorbance, respectively.

[00129] *Generation and selection of naïve chimeric rabbit/human Fab libraries*: All rabbit handling was carried out by veterinary personnel at Pocono Rabbit Farm & Laboratory (Canadensis, PA) or R & R Research (Stanwood, WA). A total of nine rabbits (ages 3-4 months) were used. Five of these rabbits were of the New Zealand White (NZW) strain, with three obtained from Pocono Rabbit Farm & Laboratory (Canadensis, PA) and two obtained from R & R Research (Stanwood, WA). Four b9 wild-type rabbits were derived from a separate R & R Research colony that originated from a pedigreed colony developed and characterized at the National Institute of Allergy and Infectious Diseases (NIAID) (McCartney-Francis et al., 1984; Popkov et al., 2003). Spleen and bone marrow from each rabbit were collected and processed for total RNA preparation and RT-PCR amplification of rabbit V_κ, V_λ, and V_H encoding sequences using established protocols (Rader, 2009). Rabbit

(rb) V_κ/human (hu) C_κ/rbV_H and rbV_λ/huC_λ/rbV_H segments, respectively, were assembled in one fusion step based on 3-fragment overlap extension PCR. Note that the V_L derived from b9 rabbits were also assembled with V_H from NZW rabbits. The Fab-encoding fragments were digested with *SfiI* and ligated with *SfiI*-treated phage display vector pC3C (Hofer et al., 2007) at 16°C for 24 h. Subsequently, 15 µg purified pC3C-rbV_κ/hcC_κ/rbV_H ligated products were transformed into *E. coli* strain SR320 (a kind gift from Dr. Sachdev S. Sidhu, University of Toronto, Toronto, Ontario, Canada) by 30 separate electroporations (each using 0.5 µg DNA in 50 µl electrocompetent cells) and yielded 7.5×10^9 independent transformants for library κ. For library λ, 4.8×10^9 independent transformants were obtained using the same procedure. Using VCSM13 helper phage (Stratagene; La Jolla, CA), the phagemid libraries were converted to phage libraries and stored at -80°C. Phage library κ and library λ were re-amplified using XL1-Blue (Stratagene) or ER2738 (Lucigen) and mixed equally before four rounds of panning against biotinylated hFc-hROR2. During the panning, 5 µg/mL antigen was pre-incubated with streptavidin coated magnetic beads (Dynabeads MyOne Streptavidin C1; Invitrogen) at 37°C for 30 min and then binders from the phage library were captured in the presence of 1 mg/mL unspecific polyclonal human IgG (Thermo Scientific). Starting from the third round of panning, the input phage was negatively depleted by incubation with empty beads before selection against antigen-loaded beads. Following selection, supernatants of IPTG-induced bacterial clones were analyzed by ELISA and by flow cytometry. Repeated clones were identified by DNA fingerprinting with *AluI*, and the V_L and V_H sequences of unique clones were determined by DNA sequencing (Figure 1).

Example 3.   Expression and purification of chimeric rabbit/human Fab and full-length IgG1 antibodies

[00130]   *Construction, expression, and purification of chimeric rabbit/human Fab and IgG1:* MAbs XBR2-401, XBR2-416, and XBR2-433 in chimeric rabbit/human Fab format were cloned into *E. coli* expression plasmid pC3C-His and expressed and purified as described (Kwong and Rader, 2009). For the expression of mAbs XBR2-401, XBR2-416, and XBR2-433 in chimeric rabbit/human IgG1 format, the previously described vector PIGG-R11 was used (Yang et al., 2011). The various primer sequences used in the expression are shown in Table 1. The V_H encoding sequences of Fab XBR2-401, XBR2-

416, and XBR2-433 were PCR amplified using primers 4-1_VH_F and 4-1_VH_R, 4-16_VH_F and 4-16_VH_R, and 4-33_VH_F and 4-33_VH_R, respectively, and cloned via *ApaI/SacI* into PIGG-R11. The light chain encoding sequences of Fab 4-1, 4-16, and 4-33 were PCR amplified using primers 4-1_κ_F, 4-16_κ_F, and 4-33_λ_F, respectively, in combination with LEAD-B, and cloned via *HindIII/XbaI* into PIGG-R11 with the corresponding heavy chain encoding sequences. Note that an internal *ApaI* site in FR4 of V_H encoding sequences of Fab XBR2-401 was removed by silent mutation in primer 4-1_VH_R. In addition, for , XBR2-416, and XBR2-433, we changed a TAG stop codon, which was suppressed during selection in *E. coli* strain XL1-Blue, to CAG (glutamine) encoding the first amino acid of native V_H of both , XBR2-416, and XBR2-433 (Figure 1) with primers 4-16_VH_F and 4-33_VH_F. The resulting PIGG-XBR2-401, PIGG-XBR2-416, and PIGG-XBR2-433 plasmids were transiently transfected into HEK 293F cells (Invitrogen) using 293fectin (Invitrogen), and the corresponding protein products were purified with a 1-mL recombinant Protein A HiTrap column (GE Healthcare, Piscataway, NJ) as described (Yang et al., 2011; Yang and Rader, 2012). The quality and quantity of purified IgG1 were analyzed by SDS-PAGE and A_{280} absorbance, respectively.

[00131] All the other mAbs in chimeric rabbit/human Fab format were cloned into *E. coli* expression plasmid pET11a and expressed and purified as described (Kwong and Rader, 2009) For the expression of mAbs ERR2-302, ERR2-308, ERR2-317, XBR2-327 and XBR2-TOP72 in chimeric rabbit/human IgG1 format, pCEP4 (Invitrogen) was used to clone the heavy chain and light chain separately. For heavy chain, a gBlock containing a heavy-chain signal peptide encoding sequence, V_H of ERR2-302 and C_H1 (1-49) of human IgG1 was synthesized by IDT (San Jose, CA) and amplified with primers KpnI/AscI-Signal and CH1-internal/overlap-R, and fused to C_H1 (50-88)- C_H2- C_H3 amplified from PIGG with primers CH1-internal/overlap-F and HC-CH3-R-XhoI by overlap extension PCR with primers KpnI/AscI-Signal and HC-CH3-R-XhoI, and then cloned into pCEP4 by *AscI/XhoI*. Note that a *NheI* site was introduced into C_H1 at Ala^{12} by synonymous mutation when the gBLOCK was synthesized. This construct served as vector to clone other mAb heavy chain by replacing the V_H using *AscI/NheI*: V_H of ERR2-308, ERR2-317, XBR2-327 and XBR2-TOP72 were amplified with forward primer ERR2-308 HC-F, ERR2-317 HC-F, XBR2-327 and ERR2-308 HC-F and reverse primer VH-CH1-R-EheI separately, followed by extension PCR to add the signal peptide with primer KpnI/AscI-Signal and VH-CH1-R-EheI. Then

each V$_H$ was inserted into the vector by *AscI*/*Nhe*I. For light chain cloning, while lambda light chains of ERR2-302 and XBR2-TOP72 were amplified with primers ERR2-302 LC-F and XBR2-TOP72 LC-F separately combined with primer LC-R-XhoI, kappa light chains of ERR2-308, ERR2-317 and XBR2-327 were amplified with primers ERR2-308 KC-F, ERR2-317 KC-F and ERR2-317 KC-F separately combined with primer KC-R-XhoI. Then signal peptide was added by extension PCR with forward primer KpnI/AscI-Signal and reverse primer LC-R-XhoI or KC-R-XhoI. Subsequently, each light chain PCR products was cloned into pCEP4 by *AscI*/*Xho*I. The resulting constructs containing heavy chain or light chain for each IgG were co-transfected transiently into HEK 293F cells (Invitrogen) using 293fectin (Invitrogen), and the corresponding recombinant protein products were purified with a 1-mL recombinant Protein A HiTrap column (GE Healthcare, Piscataway, NJ) as described (Yang et al., 2011; Yang and Rader, 2012). The quality and quantity of purified IgG1 were analyzed by SDS-PAGE and A$_{280}$ absorbance, respectively.

Table 1. Primer sequences for cloning antibody sequences

Primer	Sequence	SEQ ID NO:
4-1_VH_F	GAGGAGGAGCTCACTCTCAGTCAGTGAAGGAGTCCGA GGGAG	105
4-1_VH_R	CGATGGGCCCTTGGTGGAGGCTGAAGAGACGGTGACG AGGGTCCCTGGTCCCCAGATGTT	106
4-16_VH_F	GAGGAGGAGCTCACTCTCAGGAGCAGCTGGAGGAGTC CGGG	107
4-16_VH_R	CGATGGGCCCTTGGTGGAGGCTGAGGAGATGGTGACC AGGGTGCCTGGCCCCCACAAGTC	108
4-33_VH_F	GAGGAGGAGCTCACTCTCAGTCAGTGAAGGAGTCCGA GGGA	109
4-33_VH_R	CGATGGGCCCTTGGTGGAGGCTGAGGAGATGGTGACC AGGGTGCCTGGCCCCCAGATGTT	110
4-1_κ_F	GAGAAGCTTGTTGCTCTGGATCTCTGGTGCCTACGGGG ACCCTATGCTGACCCAGACTCC	111

4-16_x_F	GAGAAGCTTGTTGCTCTGGATCTCTGGTGCCTACGGGCAAGTGCTGACCCAGACTCCATC	112
4-33_λ_F	GAGAAGCTTGTTGCTCTGGATCTCTGGTGCCTACGGGTCCTTCGTGCTGACTCAGCCAGC	113
LEAD-B	GGCCATGGCTGGTTGGGCAGC	114
Kpnl/Ascl-Signal	GGTACCGGCGCGCCACCATGGACTGGACTTGGAGAATCCTGTTTCTCGTAGCTGCTGCAA	115
CH1-internal/overlap-R	GCCGCTGGTCAGGGCTCCTG	116
CH1-internal/overlap-F	CAGGAGCCCTGACCAGCGGC	117
HC-CH3-R-Xhol	GGCCTCGAGTCATTTACCCGGAGACAGGGA	118
ERR2-308 HC-F	TTTCTCGTAGCTGCTGCAACTGGAGCACACTCCCAGGAGCAGCTGGAGGAGTCC	119
ERR2-317 HC-F	TTTCTCGTAGCTGCTGCAACTGGAGCACACTCCCAGTCGTTGGAGGAGTCCGGG	120
XBR2-327 HC-F	TTTCTCGTAGCTGCTGCAACTGGAGCACACTCCCAGGAGCAGCTGAAGGAGTCC	121
VH-CH1-R-Ehel	GGAGGGCGCCAGGGGGAAGACCGATGGGCCCTTGGT	122
ERR2-302 LC-F	TTTCTCGTAGCTGCTGCAACTGGAGCACACTCCTCCTATGAGCTGACACAGCTG	123
XBR2-TOP72 LC-F	TTTCTCGTAGCTGCTGCAACTGGAGCACACTCCCAGTTTGTGCTGACTCAGTCG	124
ERR2-308 KC-F	TTTCTCGTAGCTGCTGCAACTGGAGCACACTCCGACCCTATGCTGACCCAGACT	125
ERR2-317 KC-F	TTTCTCGTAGCTGCTGCAACTGGAGCACACTCCGACCCTGTGCTGACCCAGACT	126
LC-R-Xhol	GGCCTCGAGTTATGAACATTCTGTAGGGGC	127

| KC-R-XhoI. | GGCCTCGAGTTAACACTCTCCCCTGTTGAA | 128 |

Example 4. Examination of antibody binding activities

[00132]   *ELISA:* Binding properties of the ROR2 antibodies were examined via ELISA. For coating, each well of a 96-well Costar 3690 plate (Corning, Corning, NY) was incubated with 100 ng antigen in 25 µL coating buffer (0.1 M Na_2CO_3, 0.1 M $NaHCO_3$, pH 9.6) for 1 h at 37°C. After blocking with 150 µL 3% (w/v) BSA/TBS for 1 h at 37°C, 100 ng / 50 µL of Fab was added, incubated for 2 h at 37°C, washed with 150 µL PBS ten times and incubated with 50 µL of a 1:1000 dilution of a mouse anti-His tag mAb conjugated to horse radish peroxidase (HRP) (R&D Systems, Minneapolis, MN) in 1% (w/v) BSA/TBS for 1 h at 37°C. Washing with PBS was repeated and colorimetric detection was performed using 2,2'-azino-bis (3-ethylbenzthiazoline)-6-sulfonic acid (Roche) as substrate according to the manufacturer's directions. The absorbance was measured at 405 nm using a SpectraMax M5 microplate reader (Molecular Devices; Sunnyvale, CA) and SoftMax Pro software (Molecular Devices), as shown in Figure 2A.

[00133]   *Flow cytometry:* Antigen binding characteristics of the antibodies were also examined via flow cytometry analysis. Specifically, cells were stained using standard flow cytometry methodology. Briefly, for purified anti-ROR2 Fab, $0.1\sim1 \times 10^6$ cells were stained with 1 µg / 100 µL of Fab on ice for 1 h. After washing twice with ice-cold flow cytometry buffer (PBS containing 1% (v/v) BSA, 0.1% sodium azide and 1 mM EDTA), the cells were incubated with a 1:1000 dilution of a mouse anti-His tag mAb conjugated to Alexa Fluor 488 (Qiagen) in 100 µL flow cytometry buffer on ice for 30 min. For purified anti-ROR2 IgG1, $0.1\sim1 \times 10^6$ cells were incubated with 100 ng / 100 µL of IgG on ice for 1 h. After washing twice with ice-cold flow cytometry buffer, cells were stained with a 1:500 dilution of goat anti-human IgG, Fcγ pAbs conjugated to APC (Jackson ImmunoResearch) in 100 µL flow cytometry buffer on ice for 30 min. Finally, 4',6-diamidino-2-phenylindole (DAPI) was added to a final concentration of 100 ng / mL to exclude dead cells from analysis. Cells were analyzed using a FACSCalibur instrument (BD Biosciences) and FlowJo analytical software (Tree Star, Ashland, OR). Results of the study are shown in Figure 2B, Figures 4-8, and Figure 11.

[00134]   Additional studies were performed to examine cross-reactivity and epitope mapping of the isolated antibodies. In these studies, extracellular domains of human ROR2

with Thr at 145 (hROR2-T^{245}) (55-394) and human ROR2 with less frequent SNP hROR2-
A^{245} (55-394), as well as mouse ROR2 (34-403), were separately fused to a (G$_4$S)$_3$ linker
followed by an HA tag (YPYDVPDYAS) and a PDGFRB segment (amino acids 513-561)
that included the transmembrane domain (amino acids 533-553), and stably displayed on
HEK 293F cells (Figure 3). Then, the cross reactivity of all the mAbs in chimeric rabbit /
human Fab format to hROR2-T^{245}, hROR2-A^{245} and mROR2 was tested by flow using these
cells (Figure 4). Similarly, different compositions of the three extracellular domains of
hROR2-T^{245}, Ig (amino acids 55-145), Fr (169-303), Ki (316-394), Ig+Fr (55-303), Fr+Ki
(169-394) were also stably displayed on HEK 293F cells separately (Figure 3). Using these
ROR2 domain displaying cell lines, the epitopes of mAbs XBR2-401 (Figure 5), XBR2-433
(Figure 6), and XBR2-416 (Figure 7) were determined with chimeric rabbit/human IgG1 by
flow cytometry. Briefly, 0.1~1 x 10^6 cells were incubated with purified (1 µg/mL) chimeric
rabbit/human IgG1 on ice for 1 h. After washing twice with ice-cold flow cytometry buffer
(PBS containing 1% (v/v) BSA, 0.1% sodium azide and 1 mM EDTA), the cells were
incubated with a 1:500 dilution of goat anti-human IgG, Fcγ pAbs conjugated to APC
(Jackson ImmunoResearch) in 100 µL flow cytometry buffer on ice for 30 min. After
washing twice with ice-cold flow cytometry buffer, the cells were incubated with a 1:500
dilution of biotinylated rat anti-HA mAb 3F10 (Roche) in 100 µL flow cytometry buffer on
ice for 1 h, washed as before, and stained with 2 µg/mL PE-conjugated streptavidin (BD
Biosciences) on ice for 30 min. DAPI was added to a final concentration of 100 ng/mL to
exclude dead cells from analysis. All the epitopes of other mAbs were determined with
chimeric rabbit/human Fab by flow cytometry as the same procedure described above. Cells
were analyzed using a FACSCalibur instrument (BD Biosciences) and FlowJo analytical
software (Tree Star, Ashland, OR).

[00135]   *Surface plasmon resonance:* Furthermore, a surface plasmon resonance study was
performed to measure the affinities of all Fabs to hFc-hROR2 and epitope mapping studies
were performed on a Biacore X100 instrument using Biacore reagents and software (GE
Healthcare, Piscataway, NJ). Anti-Human IgG (Fc) antibody was immobilized on a CM5
sensor chip following the instruction of Human Antibody Capture Kit (GE Healthcare,
Piscataway, NJ). Then hFc-hROR2 fusion proteins were captured at certain density
(indicated in Figure 9 and Figure 10B). Each sensor chip included an empty flow cell for
instantaneous background depletion. All binding assays used 1x HBS-EP+ running buffer

(10 mM HEPES, 150 mM NaCl, 3 mM EDTA (pH 7.4), and 0.05% (v/v) Surfactant P20) and a flow rate of 30 mL/min. For affinity measurements, all Fabs were injected at five different concentrations (dilution factor was 2), with one of which was tested in duplicates (the highest concentrations for each Fab were indicated in Figure 10B). The sensor chips were regenerated with 3 M $MgCl_2$ from the Human Antibody Capture Kit without any loss of binding capacity. Calculation of association (k_{on}) and dissociation (k_{off}) rate constants was based on a 1:1 Langmuir binding model. The equilibrium dissociation constant (K_D) was calculated from k_{off} / k_{on}. For epitope mapping studies, each Fab was prepared at 500 nM alone or in a mixture in 1x HBS-EP+ running buffer and then injected in order as indicated in Figure 9.

[00136]   *Western blotting:* A Western blotting assay was performed to examine the binding activity of the isolated antibodies for denatured ROR2 polypeptide. Cells or proteins were lysed by 1x sample buffer (containing 1% β-mercaptoethanol) and boiled before running on NuPAGE Novex 4-12% Bis-Tris gels (Invitrogen). After membrane transferring and blocking by 5% milk, 2 μg/mL chimeric rabbit/human IgG1 XBR2-401 was applied to detect the denatured proteins, followed by incubation with 1/1000 anti-human Fc conjugated to HRP before developing using ECL Prime Western Blotting Detection Reagent (GE Healthcare). As shown in Figure 12, the results indicate the antibody indeed recognizes denatured hROR2.

Example 5.   Expression of purified, recombinant twin strep-tagged human, murine and cynomolgus ROR2.

[00137]   Twin strep-tagged human, mouse and cynomolgus ROR2-extracellular domain was produced as follows: the nucleotide sequence encoding the extracellular domain of human ROR2 (NP_004551.2), murine ROR2 (NP_038874.3) and cynomolgus ROR2 (XP_005582291.1) were each N-terminally fused to a signal sequence (MNFGLRLIFLVLTLKGVQC) and C-terminally fused with a sequence encoding a twin strep-tag (SAWSHPQFEKGGGSGGGSGGSAWSHPQFEKG). The entire nucleotide sequences with flanking 5'NotI and 3'HindIII sites were produced by total gene synthesis (GenScript, Piscataway, USA), assembled in the mammalian expression vector pCB14 and verified by DNA sequencing. This vector, a derivative of the episomal mammalian expression vector pCEP4 (Invitrogen), carries the EBV replication origin, encodes the EBV

nuclear antigen (EBNA-1) to permit extrachromosomal replication, and contains a puromycin selection marker in place of the original hygromycin B resistance gene.

[00138]   Recombinant human twin strep-tagged ROR2 (NP_004551.2), murine twin strep-tagged ROR2 (NP_038874.3) and cynomolgus twin strep-tagged ROR2 (XP_005582291.1) were expressed and purified in-house according to the following protocol: the EBNA expression vector pCB14b-ROR2-ECD-TwinStrep (human ROR2), pCB14g-mouseROR2-ECD-TwinStrep (mouse ROR2) or pCB14b-cynomolgusROR2-ECD-TwinStrep (cynomolgus monkey ROR2), directing expression of ROR2 extracellular domain (ECD), C-terminally tagged with a TwinStrep tag, was transfected into HEK293T using Lipofectamine® LTX with PLUS™ Reagent (Thermo Fisher Scientific, 15388100). Following a 1-day incubation (37°C, 5% CO_2, growth media: Dulbecco's Modified Eagle Medium (DMEM) High Glucose (4.5 g/l) with L-Glutamine with 10% (v/v) Fetal Calf Serum (FCS), 100 IU/mL of Pen-Strep-Fungizone and 2 mM L-glutamine (all Bioconcept)), cells were expanded under selection conditions (2 μg/mL of puromycin (Sigma-Aldrich, P8833-25 mg stock at 2 mg/mL)). Cells were split and further expanded (37°C, 5% CO_2); once confluency was reached, tissue culture dishes were coated with 20 μg/ml poly-L-Lysine (Sigma-Aldrich, P1524) for 2 hrs at 37°C and washed twice with PBS. Then, cells were trypsinized, washed with PBS and split 1:3 onto poly-L-lysine-coated plates. Again after reaching confluency, cells were washed with PBS followed by with media replacement using production media (DMEM/F-12, Gibco/Thermo Fisher Scientific, 31330-03) supplemented with 1 μg/mL puromycin (Sigma-Aldrich, P8833), 100 IU/mL of Pen-Strep-Fungizone (Bioconcept, 4-02F00-H), 161 μg/mL of N-acetyl-L-cysteine (Sigma-Aldrich, A8199) and 10 μg/mL of L-glutathione reduced (Sigma-Aldrich, G6529). Supernatant, harvested bi-weekly and filtered (0.22 μm) to remove cells, was stored at 4°C until purification. For purification, filtered supernatant was loaded onto a Streptactin® Superflow® high capacity cartridge (IBA, Göttingen, Germany, 2-1238-001) column; purification and elution was performed according to the manufacturer's protocol on an AEKTA pure (GE Healthcare). Fractions were analyzed for protein purity and integrity by SDS-PAGE. Protein-containing fractions were mixed and subjected to buffer exchange using Amicon filtration units (Millipore, Schaffhausen, Switzerland) to reach a dilution of ≥1:100 in PBS, and then sterile filtered using a low retention filter (0.20 μm, Carl Roth, Karlsruhe, Germany, PA49.1).

Example 6.   Expression of purified, recombinant anti-human ROR2 and isotype control antibodies

[00139]   *Expression vectors:* Antibody variable region coding regions were produced by total gene synthesis (GenScript) using MNFGLRLIFLVLTLKGVQC as leader sequence, and were assembled with human IgH-γ 1 and IgL-κ (ERR2-308, ERR2-316, ERR2-317, XBR2-327, Huluc63) or IgL-λ (ERR2-302, ERR2-Top35) constant regions in the expression vector pCB14.

[00140]   Expression vectors encoding each of the full-length heavy and light chains were assembled in a proprietary mammalian expression vector, Switzerland. Antibodies were transiently expressed in CHO cells by methods known in the art and recombinant antibodies were purified by standard protein A purification from CHO cell supernatants, as known in the art. In short, the CHO cell supernatants were harvested by centrifugation and sterile filtered (0.2 μm) before FPLC-based affinity purification using Amsphere protein A columns (JSR Life Sciences) performed as described below.

[00141]   *In-house expression and purification:* pCB14-based expression vectors were transfected into HEK293T cells using Lipofectamine® LTX Reagent with PLUS™ Reagent (Thermo Fisher Scientific, Reinach, Switzerland, 15388100); following a 1-day incubation (37°C, 5% CO₂, growth media: Dulbecco's Modified Eagle Medium (DMEM) High Glucose (4.5 g/l) with L-Glutamine with 10% (v/v) Fetal Calf Serum (FCS), 100 IU/mL of Pen-Strep-Fungizone and 2 mM L-glutamine (all Bioconcept, Allschwil, Switzerland)), cells were expanded under selection conditions (2 μg/mL of puromycin (Sigma-Aldrich, Buchs SG, Switzerland, P8833-25 mg stock at 2 mg/mL)). Cells were split and further expanded (37°C, 5% CO₂); once confluency was reached, tissue culture dishes were coated with 20 μg/ml poly-L-Lysine (Sigma-Aldrich, P1524) for 2h at 37°C and washed twice with PBS. Then, cells were trypsinized and split 1:3 onto poly-L-lysine-coated plates. Again after reaching confluency, cells were washed with PBS followed by media replacement to production media (DMEM/F-12, Gibco/Thermo Fisher Scientific, 31330-03) supplemented with 1 μg/mL puromycin (Sigma, P8833), 100 IU/mL of Pen-Strep-Fungizone (Bioconcept), 161 μg/mL of N-acetyl-L-cysteine (Sigma-Aldrich, A8199) and 10 μg/mL of L-glutathione reduced (Sigma-Aldrich, G6529). Supernatant, harvested bi-weekly and filtered (0.22 μm) to remove cells, was stored at 4°C until purification.

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[00142] For purification, filtered supernatant was loaded onto a PBS-equilibrated Protein A HiTrap column (GE Healthcare, Frankfurt am Main, Germany, 17-0405-01) or a JSR Amsphere™ Protein A column (JSR Life Sciences, Leuven, Belgium, JWT203CE) and washed with PBS; elution was performed using 0.1 M glycine (pH 2.5) on an AEKTA pure (GE Healthcare). Fractions were immediately neutralized with 1 M Tris-HCl buffer (pH 8.0), and analyzed for protein purity and integrity by SDS-PAGE. Protein-containing fractions were mixed and subjected to buffer exchange using Amicon filtration units (Millipore, Schaffhausen, Switzerland, UFC901008) to reach a dilution of 1:100 in the buffer listed in Table 2, and then sterile filtered using a low retention filter (0.20 µm, Carl Roth, Karlsruhe, Germany, PA49.1).

[00143] Table 2 lists the antibodies used in subsequent examples, along with their final concentration and buffer.

Table 2. List of antibodies used in the Examples

Antibody (ref.)	Antibody SEQ ID HC/LC	C-Terminal Tags (HC: Heavy Chain, LC: Light Chain)	Buffer	Final conc. (mg/mL)
ERR2-302 (mAb004)	HC: SEQ ID NO. 7 LC: SEQ ID NO. 19	HC: LPETG-Strep LC: G₅SLPETG-Strep	PBS	2.7
ERR2-308 (mAb037)	HC: SEQ ID NO. 8 LC: SEQ ID NO. 20	HC: LPETG-Strep LC: G₅SLPETG-Strep	PBS	4.5
ERR2-316 (mAb005)	HC: SEQ ID NO. 9 LC: SEQ ID NO. 21	HC: LPETG-Strep LC: G₅SLPETG-Strep	PBS	3.2
ERR2-317 (mAb006)	HC: SEQ ID NO. 10 LC: SEQ ID NO. 22	HC: LPETG-Strep LC: G₅SLPETG-Strep	PBS	2.5
XBR2-327 (mAb007)	HC: SEQ ID NO. 4 LC: SEQ ID NO. 16	HC: LPETG-Strep LC: G₅SLPETG-Strep	PBS	3.7
ERR2-top35 (mAb138)	HC: SEQ ID NO. 12 LC: SEQ ID NO. 24	HC: LPETG-Strep LC: G₅SLPETG-Strep	PBS	3.3
XBR2-	HC: SEQ ID NO. 11	HC: LPETG-Strep	PBS	6.0

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| 401 (mAb003) | LC: SEQ ID NO. 13 | LC: G₅SLPETG-Strep | | |
| Huluc63 (mAb047) | HC: SEQ ID NO. 135 LC: SEQ ID NO. 136 | HC: LPETG-Strep LC: G₅SLPETG-Strep | PBS | 8.0 |

[00144]   Huluc63 corresponds to an unrelated control antibody; the heavy and light chain sequences are given below.

SEQ ID NO. Name	Amino Acid Sequence (with constant domain underlined)
SEQ ID NO. 135 Huluc63 HC amino acid sequence	EVQLVESGGGLVQPGGSLRLSCAASGFDFSRYWMSWVRQAPGKGLEW IGEINPDSSTINYAPSLKDKFIISRDNAKNSLYLQMNSLRAEDTAVYYCA RPDGNYWYFDVWGQGTLVTVSS̲A̲S̲T̲K̲G̲P̲S̲V̲F̲P̲L̲A̲P̲S̲S̲K̲S̲T̲S̲G̲G̲T̲A̲A̲L̲G̲ C̲L̲V̲K̲D̲Y̲F̲P̲E̲P̲V̲T̲V̲S̲W̲N̲S̲G̲A̲L̲T̲S̲G̲V̲H̲T̲F̲P̲A̲V̲L̲Q̲S̲S̲G̲L̲Y̲S̲L̲S̲S̲V̲V̲T̲V̲P̲S̲S̲S̲L̲ G̲T̲Q̲T̲Y̲I̲C̲N̲V̲N̲H̲K̲P̲S̲N̲T̲K̲V̲D̲K̲K̲V̲E̲P̲K̲S̲C̲D̲K̲T̲H̲T̲C̲P̲P̲C̲P̲A̲P̲E̲L̲L̲G̲G̲P̲S̲V̲F̲L̲ F̲P̲P̲K̲P̲K̲D̲T̲L̲M̲I̲S̲R̲T̲P̲E̲V̲T̲C̲V̲V̲V̲D̲V̲S̲H̲E̲D̲P̲E̲V̲K̲F̲N̲W̲Y̲V̲D̲G̲V̲E̲V̲H̲N̲A̲K̲T̲K̲ P̲R̲E̲E̲Q̲Y̲N̲S̲T̲Y̲R̲V̲V̲S̲V̲L̲T̲V̲L̲H̲Q̲D̲W̲L̲N̲G̲K̲E̲Y̲K̲C̲K̲V̲S̲N̲K̲A̲L̲P̲A̲P̲I̲E̲K̲T̲I̲S̲K̲ A̲K̲G̲Q̲P̲R̲E̲P̲Q̲V̲Y̲T̲L̲P̲P̲S̲R̲D̲E̲L̲T̲K̲N̲Q̲V̲S̲L̲T̲C̲L̲V̲K̲G̲F̲Y̲P̲S̲D̲I̲A̲V̲E̲W̲E̲S̲N̲G̲Q̲P̲ E̲N̲N̲Y̲K̲T̲T̲P̲P̲V̲L̲D̲S̲D̲G̲S̲F̲F̲L̲Y̲S̲K̲L̲T̲V̲D̲K̲S̲R̲W̲Q̲Q̲G̲N̲V̲F̲S̲C̲S̲V̲M̲H̲E̲A̲L̲H̲N̲H̲ Y̲T̲Q̲K̲S̲L̲S̲L̲S̲P̲G̲K̲
SEQ ID NO. 136 Huluc63 LC amino acid sequence	DIQMTQSPSSLSASVGDRVTITCKASQDVGIAVAWYQQKPGKVPKLLIY WASTRHTGVPDRFSGSGSGTDFTLTISSLQPEDVATYYCQQYSSYPYTF GQGTKVEIK̲R̲T̲V̲A̲A̲P̲S̲V̲F̲I̲F̲P̲P̲S̲D̲E̲Q̲L̲K̲S̲G̲T̲A̲S̲V̲V̲C̲L̲L̲N̲N̲F̲Y̲P̲R̲E̲A̲K̲V̲Q̲ W̲K̲V̲D̲N̲A̲L̲Q̲S̲G̲N̲S̲Q̲E̲S̲V̲T̲E̲Q̲D̲S̲K̲D̲S̲T̲Y̲S̲L̲S̲S̲T̲L̲T̲L̲S̲K̲A̲D̲Y̲E̲K̲H̲K̲V̲Y̲A̲C̲E̲ V̲T̲H̲Q̲G̲L̲S̲S̲P̲V̲T̲K̲S̲F̲N̲R̲G̲E̲C̲

Example 7.   Cross-reactivity of anti-human ROR2 antibodies on murine and cynomolgus ROR2

[00145]   Each well of a 96-well plate was coated with 50 µL of 2 µg/mL twinstrep-tagged human, murine or cynomolgus ROR2 (from Example 4) in 0.1 M bicarbonate coating buffer (pH 9.6), and incubated for 12h at 4°C. An additional 96-well plate was likewise prepared but with anti-human Fc (Jackson ImmunoResearch, 109-006-008).

After blocking with 150 µL of 3% (w/v) bovine serum albumin (BSA)/TBS for 1h at 37°C, the following antibodies were added to a well within each plate at a concentration of 0.5

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µg/mL, and serially diluted (dilution factor 4) with 1% (w/v) BSA/TBS, before incubation for 1h at 37°C: ERR2-302 (mAb004), ERR2-308 (mAb037), ERR2-316 (mAb0005), ERR2-317 (mAb006), XBR2-327 (mAb007), ERR2-Top35 (mab130), and XBR2-401 (mab003). After washing with PBS with 0.05% Tween 20, HRP-conjugated F(ab')₂ anti-human FC-gamma (Jackson Immunoresearch, 109-036-008) was then added at a 1:10'000 dilution, 50 µl per well, and incubated for 1h at 37°C prior to detection using an Spark 10M plate reader (Tecan). Curves of OD 490 nm versus antibody concentration (ng/mL) were fitted with Graphpad Prism Software (Graphpad Software, La Jolla, CA, U.S.A.). The IC_{50} values, determined using the built-in "log(inhibitor) vs. response -- Variable slope (four parameters)" IC_{50} determination function of Prism Software, are reported in Table 3 for human and mouse ROR2, and in Table 4 for human and cynomolgus ROR2. As shown in Figure 14, the anti-human ROR2 antibodies ERR2-302 and ERR2-Top35 bind human ROR2 (panel A, C) and mouse ROR2 (panel B); the remaining are not cross-reactive with mouse ROR2. All of the inventive anti-human ROR2 antibodies are, however, cross-reactive with cynomolgus ROR2 (panel D).

Table 3. Human and mouse ROR2 binding by anti-ROR2 antibodies.

| Antibody | IC50 ng/mL | | | ratio IC50 | |
	anti-human Fc	human ROR2	mouse ROR2	human ROR2/IgG	mouse ROR2/IgG
ERR2-302	32.2	102.3	60.6	0.31	0.53
ERR2-308	33.6	466.8	150.7	0.07	0.22
ERR2-316	40	29.9	994.6	1.34	0.04
ERR2-317	39.1	1354.1	167.2	0.03	0.23
XBR2-327	43.3	167901.4	443.7	0	0.1
XBR2-401	85.6	50.5	361.1	1.69	0.24
ERR2-top35	41.9	24.4	17.3	1.72	2.41

Table 4. Human and cynomolgus ROR2 binding by anti-ROR2 antibodies

| Antibody | IC50 ng/mL | | | ratio IC50 | |
	anti-human Fc	human ROR2	cynomolgus ROR2	human ROR2/IgG	cynomolgus ROR2/IgG
ERR2-302	13.4	5.5	7.1	0.41	0.53
ERR2-308	16.7	18.6	4.6	1.11	0.28
ERR2-316	11.2	40.7	44	3.63	3.93
ERR2-317	8.4	16.8	19	2	2.26
XBR2-327	10.2	0.57	0	0.06	0

| XBR2-401 | 6.8 | 41.9 | 43.3 | 6.16 | 6.37 |
| ERR2-top35 | 14.3 | 46.2 | 53.1 | 3.23 | 3.71 |

Example 8.   Binding affinities of anti-human ROR2 antibodies on human and cynomolgus ROR2

Surface plasmon resonance for the measurement of the affinities of anti-ROR2 antibodies to human ROR2 and cynomolgus ROR2 was performed on a Biacore T200 instrument (GE Healthcare). Goat α-human Fcγ-specific IgG (Jackson ImmunoResearch, 109-005-098) was covalently immobilized on a CM5 sensor chip (GE Healthcare, BR-1005-30. For affinity measurements, purified anti-human ROR2 antibodies were used. In all cases, anti-ROR2 antibodies were diluted to 7.5 µg/ml in 1x HBS-EP+ running buffer (10 mM HEPES, 150 mM NaCl, 3 mM EDTA (pH 7.4), and 0.05% (v/v) Tween P20) and captured for 60 s with a flow of 10 µl/min. Human ROR2-TwinStrep or cynomolgus ROR2-TwinStrep was diluted in running buffer using 2-fold serial dilutions ranging from 200nM to 12.5 nM. Association and dissociation were measured at a flow of 30µl/min for 120s and 200s, respectively. Calculation of association (k_{on}) and dissociation (k_{off}) rate constants was based on a 1:1 Langmuir binding model. The equilibrium dissociation constant (K_d) was calculated from k_{off}/k_{on}. Values are reported in Table 5.

Table 5: Binding characteristics of anti-ROR2 antibodies against human and cynomolgus ROR2 as determined by SPR

Antigen	Antibody	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_d (nM)
Human ROR2	XBR2-401 (mab003)	9.78E+02	≤ 1.00E-05	≤ 10.2
Cynomolgus ROR2	XBR2-401 (mab003)	8.71E+03	≤ 1.00E-05	≤ 1.1
Human ROR2	ERR2-302 (mab004)	1.07E+05	5.60E-03	52.4
Cynomolgus ROR2	ERR2-302 (mab004)	1.08E+05	5.27E-03	48.6
Human ROR2	ERR2-308 (mab037)	1.37E+04	1.53E-04	11.2
Cynomolgus ROR2	ERR2-308 (mab037)	2.38E+04	1.27E-04	5.4

Example 9.   Generation of site-specifically conjugated ADCs using SMAC-technology

[00146]   *Sortase A enzyme.* Recombinant and affinity purified Sortase A enzyme from *Staphylococcus aureus* was produced in *E. coli* as disclosed in WO2014140317A1.

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[00147]   *Generation of glycine-modified toxins.* In order to generate SMAC-technology[TM] conjugated ADCs with pentaglycine-modified PNU-159682 derivative Gly₅-EDA-PNU (Figure 13B) was manufactured as disclosed in WO2016102697. The identity and the purity of the pentaglycine-modified PNU toxin was confirmed by mass-spectrometry and HPLC. The Gly₅-modified toxin exhibited > 95% purity, as determined by the single peak in the HPLC chromatogram.

[00148]   *Sortase-mediated antibody conjugation.* The above-mentioned toxin was conjugated to anti-ROR2 antibodies as per Table 3 by incubating LPETG-tagged mAbs [10µM] with glycine modified toxin [200µM] and 3 µM Sortase A in the listed conjugation buffer for 3.5h at 25°C. The reaction was stopped by passing it through an rProtein A GraviTrap column (BioRad). Bound conjugate was eluted with 5 column volumes of elution buffer (0.1 M glycine pH 2.5, 50 nM NaCl), with 1 column volume fractions collected into tubes containing 25% v/v 1M HEPES pH 8 to neutralise the acid. Protein containing fractions were pooled and formulated in the formulation buffer of Table 7 using a ZebaSpin desalting column.

[00149]   *ADC analytics.* DAR was assessed by Reverse Phase Chromatography performed on a Polymer Labs PLRP 2.1mm x 5cm, 5µm column run at 1mL/min/80°C with a 25 minute linear gradient between 0.05 to 0.1% TFA/H₂O and 0.04 to 0.1% TFA/CH₃CN. Samples were first reduced by incubation with DTT at pH 8.0 at 37°C for 15 minutes. The DAR determined by Reverse Phase Chromatography is summarized in Table 6 below.

Table 6: Manufacturing conditions and analytical summary of ADCs manufactured in this study. DAR, drug-to-antibody ratio. ND, not determined.

ADC (ref.)	mAb (ref.)	Toxin	Conjugation Buffer	Formulation Buffer	DAR
ERR2-302-G5-PNU (adc142)	ERR2-302 (mAb004)	G5-PNU	50mM HEPES (pH 7.5), 15mM NaCl, 5mM CaCl₂	10mM sodium succinate (pH 5.0), 175 mM sucrose, 0.02% (w/v) Tween 20	3.5
ERR2-308-G5-PNU (adc143)	ERR2-308 (mAb037)	G5-PNU	50mM HEPES (pH 7.5), 15mM NaCl, 5mM CaCl₂	10mM sodium succinate (pH 5.0), 175 mM sucrose, 0.02% (w/v) Tween 20	3.5
ERR2-317-G5-PNU	ERR2-317 (mAb006)	G5-PNU	50mM HEPES (pH 7.5), 15mM NaCl, 5mM CaCl₂	10mM sodium succinate (pH 5.0), 175	3.5

(adc145)				mM sucrose, 0.02% (w/v) Tween 20	
XBR2-327-GS-PNU (adc146)	XBR2-327 (mAb007)	GS-PNU	50mM HEPES (pH 7.5), 15mM NaCl, 5mM CaCl2	10mM sodium succinate (pH 5.0), 175 mM sucrose, 0.02% (w/v) Tween 20	3.8
XBR2-401-GS-PNU (adc096)	XBR2-401 (mAb003)	GS-PNU	50mM HEPES (pH 7.5), 15mM NaCl, 5mM CaCl2	10mM sodium succinate (pH 5.0), 175 mM sucrose, 0.02% (w/v) Tween 20	3.6
ERR2-top35-GS-PNU (adc207)	ERR2-top35 (mAb130)	GS-PNU	50mM HEPES (pH 7.5), 150mM NaCl, 1mM CaCl2	25mM HEPES (pH 6.8), 150mM NaCl	ND
Huluc63-GS-PNU (adc101)	Huluc63 (mAb047)	GS-PNU	50mM HEPES (pH 7.5), 15mM NaCl, 5mM CaCl2	10mM sodium succinate (pH 5.0), 175 mM sucrose, 0.02% (w/v) Tween 20	3.6

Example 10. *In vitro cytotoxicity assays of anti-ROR2 antibody-based ADCs on wild type EMT-6 and ROR2-overexpressing EMT-6 breast cancer cells*

[00150]   Cytotoxicity of the anti-ROR2 ADCs of Table 5 was investigated using wild type (WT) EMT-6 and EMT-6 cells engineered to overexpress human ROR2 (from Example 1). Huluc-G5-PNU (adc101) was included as isotype control.

[00151]   For this, 9.75×10^3 WT EMT-6 and hROR2 overexpressing EMT-6 cells per well were plated on 96-well plates (excluding edge wells, which contained water) in 75 µL DMEM supplemented with 10% by vol. FCS, 100 IU/ml Pen-Strep-Fungizone and 2 mM L-Glutamine and were grown at 37°C in a humidified incubator at 7.5% CO_2 atmosphere. After 1-day incubation, each ADC was added to respective wells in an amount of 25µL of 3.5-fold serial dilutions in growth medium (resulting in final ADC concentrations from 20 µg/mL to 0.88 ng/ml). After 4 additional days, plates were removed from the incubator and equilibrated to room temperature. After approximately 30 min, 50 µL was removed from each well, and then 50 µL of CellTiter-Glo® 2.0 Luminescent Solution (Promega, G9243) was added to each well. After shaking the plates at 750 rpm for 5 min followed by 20 min incubation without shaking, luminescence was measured on a Tecan iControl plate reader.

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Curves of luminescence versus ADC concentration (ng/mL) were fitted with Graphpad Prism Software. The IC50 values, determined using the built-in "log(inhibitor) vs. response - - Variable slope (four parameters)" IC_{50} determination function of Prism Software, are reported in Table 7.

Table 7: *In vitro* cell killing of EMT-6 cells and EMT-6/hROR2 expressing cells by anti-ROR2 or isotype control ADCs (IC_{50}, ng/mL)

ADC	WT EMT-6	hROR2-overexpressing EMT-6
ERR2-302-G5-PNU (adc142)	2.1×10^{4}	3.2
ERR2-308-G5-PNU (adc143)	8'983	10.3
ERR2-317-G5-PNU (adc145)	25'205	6.2
XBR2-401-G5-PNU (adc096)	7'452	10.2
XBR2-327-G5-PNU (adc146)	11'568	10.2
ERR2-top35-G5-PNU (adc207)	364	7.2
Hulnc63-G5-PNU (adc101)	3398	1'207

[00152]   Figure 15 shows the dose-repose curves of the *in vitro* cell killing assays on WT and hROR2-overexpressing EMT6 cells with the ADCs of Table 7. As per the above Table and Figure 15, the ADCs of the invention provide specific killing dependent on ROR2 expression status.

Example 11.   Functional evaluation of CAR-T cells expressing anti-hROR2 CAR based on XBR2-401

[00153]   CAR-T cells based on XBR2-401 were engineered using previously described methods (Hudecek, M., Lupo-Stanghellini, M. T., Kosasih, P. L., Sommermeyer, D., Jensen, M. C., Rader, C., and Riddell, S. R. (2013) Receptor affinity and extracellular domain modifications affect tumor recognition by ROR1-specific chimeric antigen receptor T cells. *Clin. Cancer Res.* 19, 3153-3164). *Ex vivo* expanded primary human $CD8^{+}$ $CD62L^{+}$ T cells were lentivirally transduced with XBR2-401 CARs containing CD3ζ and 4-1BB signaling domains and a short or long spacer. Transduced T cells were purified via tEGFR by FACS and their phenotype was assesed the day before functional assays. CD8+ purity varied between 97% and 99%, tEGFR expression varied between 95% and 99%. Following 72 h co-culture with ROR2-positive or ROR2-negative human breast cancer cells, CFSE-stained

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CD8+ CD62L+ cells were analyzed by flow cytometry, revealing target-dependent proliferation of XBR2-401 (Figure 16; upper panel). Selective cytotoxicity was measured with a luciferase-based cytotoxity assay following 11 h of co-culture with ROR2-positive and ROR2-negative cells (Figure 16; lower panel).

Example 12.   Specificity analysis of XBR2-401

[00154]   Figure 18 provides an overview of the Retrogenix Cell Microarray Platform. *Primary screen:* Purified chimeric rabbit/human IgG1 XBR2-401 targeting ROR2 and purified chimeric rabbit/human IgG1 XBR1-402 targeting ROR1 were pooled to a concentration of 2 μg/mL each. The pool was screened for binding against fixed HEK293 cells/slides expressing 4,336 human plasma membrane proteins individually (13 slide sets; n=2 slides per slide set). All transfection efficiencies exceeded the minimum threshold. An AlexaFluor647 anti-human IgG Fc detection antibody was used. Primary hits (duplicate spots) were identified by analyzing fluorescence (AlexaFluor647 and ZsGreen1) on ImageQuant. Vectors encoding all hits were sequenced to confirm their correct identities. Confirmation screen: Vectors encoding all hits, plus control vectors, were spotted in duplicate on new slides, and used to reverse transfect human HEK293 cells as before. All transfection efficiencies exceeded the minimum threshold. Identical fixed slides were treated with each of the two test antibodies (XBR2-401 and XBR1-402) individually, plus positive and negative controls (n=2 slides per treatment). Slides were analyzed as before (Figure 18).

[00155]   Some additional references cited here are listed below.

Hofer, T., W. Tangkeangsirisin, M.G. Kennedy, R.G. Mage, S.J. Raiker, K. Venkatesh, H. Lee, R.J. Giger, and C. Rader. 2007. Chimeric rabbit/human Fab and IgG specific for members of the Nogo-66 receptor family selected for species cross-reactivity with an improved phage display vector. *J Immunol Meth* 318:75-87.

Hofer, T., J.D. Thomas, T.R. Burke, Jr., and C. Rader. 2008. An engineered selenocysteine defines a unique class of antibody derivatives. *Proc Natl Acad Sci U S A* 105:12451-12456.

Kwong, K.Y., S. Baskar, H. Zhang, C.L. Mackall, and C. Rader. 2008. Generation, affinity maturation, and characterization of a human anti-human NKG2D monoclonal antibody with dual antagonistic and agonistic activity. *J Mol Biol* 384:1143-1156.

Kwong, K.Y., and C. Rader. 2009. E. coli expression and purification of Fab antibody fragments. *Curr Protoc Protein Sci* Chapter 6:Unit 6 10.

McCartney-Francis, N., R.M. Skurla, Jr., R.G. Mage, and K.E. Bernstein. 1984. Kappa-chain allotypes and isotypes in the rabbit: cDNA sequences of clones encoding b9 suggest an evolutionary pathway and possible role of the interdomain disulfide bond in quantitative allotype expression. *Proc Natl Acad Sci U S A* 81:1794-1798.

Popkov, M., R.G. Mage, C.B. Alexander, S. Thundivalappil, C.F. Barbas, 3rd, and C. Rader.

2003. Rabbit immune repertoires as sources for therapeutic monoclonal antibodies: the impact of kappa allotype-correlated variation in cysteine content on antibody libraries selected by phage display. *J Mol Biol* 325:325-335.

Rader, C. 2009. Generation and selection of rabbit antibody libraries by phage display. *Methods Mol Biol* 525:101-128, xiv.

Yang, J., S. Baskar, K.Y. Kwong, M.G. Kennedy, A. Wiestner, and C. Rader. 2011. Therapeutic potential and challenges of targeting receptor tyrosine kinase ROR1 with monoclonal antibodies in B-cell malignancies. *PloS One* 6:e21018.

Yang, J., and C. Rader. 2012. Cloning, expression, and purification of monoclonal antibodies in scFv-Fc format. *Methods Mol Biol* 901:209-232.

[00156]   Some additional nucleotide sequences described herein are listed below.

SEQ ID NO:129.   5'-
CAGTCAGTGAAGGAGTCCGAGGGAGGTCTCTTCAAGCCAACGGATACCCTGACA
CTCACCTGCACAGTCTCTGGATTCTCCCTCAGTAGCTATGGAGTGACCTGGGTCC
GCCAGGCTCCAGGGAGCGGGCTGGAATGGATCGGATACATTAATACTGCTGGTA
ACACATACTACGCGAGCTGGGCGAAAAGCCGGTCCACCATCACCAGGAACACC
AACGAGAACACGGTGACTCTGAAAATGACCAGTCTGACAGCCGCGGACACGGC
CACCTATTTCTGTGCGAGAGATTGGACATCCCTTAACATCTGGGGACCAGGGAC
CCTCGTCACCGTCTCTTCA-3'

SEQ ID NO:130.   5'-
CAGGAGCAGCTGGAGGAGTCCGGGGGGTCGCCTGGTCACGCCTGGGACACCCCTG
ACACTCACCTGCACAGTCTCTGGATTCTCCCTCAGTAGCTACTGGATGAGCTGGG
TCCGCCAGGCTCCAGGGAAGGGGCTGGAATGGATCGGGACCATTACGCCTGGTG
GTAACACGGACTACGCGACCTGGGCGAAAGCCCGATTCACCGTCTCCAAAACCT
CGACCACGGTGGATCTAAAAATCACCAGTCCGACAACCGAGGACACGGCCACCT
ATTTCTGTGCCAGAGGGATAGGTGGTGCTGCTGACTTGTGGGGGCCAGGCACCC
TGGTCACCATCTCCTCA-3'

SEQ ID NO:131.   5'-
CAGTCAGTGAAGGAGTCCGAGGGAGGTCTCTTCAAGCCAACGGATAGCCTGACA
CTCACCTGCACAGTCTCTGGATTCTCCCTCAGTACCTATGGAGTGAGCTGGGTCC
GCCAGGCTCCAGGGAACGGGCTGGAATGGATCGGGGCCATTGGTAGTAGTGGT
AGCGCAAACTACGCGAGCTGGGCGAAAGACCGATCCACCATCACCAGAAACAC
CAACCTGAACACGGTGACTCTGAAAATGACCAGTCTGACAGCCGCGGACACGGC
CACCTATTTCTGTGCGAGAGATGGTTACTATAGTAGTGGCTGGGGTCCCTACTTT
AACATCTGGGGCCAGGCACCCTGGTCACCATCTCCTCA-3'

SEQ ID NO:132.   5'-
GACCCTATGCTGACCCAGACTCCATCCTCCACGTCTACCGCTGTGGGAGACACA
GTCACCATCAAGTGCCAGGCTAGTCAGAGCATTAGTAGTGACTTATCCTGGTAT
CAGCAGAAACCAGGGCAGCGTCCCAAGCTCCTGATCTACCAGGCATCCACTCTG
GCATCTGGGGTCCCATCGCGGTTCAAAGGCAGTGGATATGGGACAGAGTACACT
CTCACCATCAGCGGCGTGCAGCGTGAGGATGCTGCCATCTACTACTGTCTAGGT
GGTTATGCTGATGCTTCTTATCGAACTGCTTTCGGCGGAGGAACCAAGCTGGAG
ATCAAA-3'

SEQ ID NO:133.   5'-
CAAGTGCTGACCCAGACTCCATCCTCCACGTCTGCCGCTGTGGGAGGCACAGTC
ACCATCAAGTGCCAGGCCAGTCAGAGCATTAGTAGTAGCGACTTATCCTGGTAT
CAGCAGAAACCAGGGCAGCCTCCCAAGCTCCTGATCTATGCTACATCCTATCTG
GCATCTGGAGTCCCATCGCGGTTCAAAGGCAGTGGATCTGGGACAGAGTACACT
CTCACCATCAGCGGCGTGCAGCGTGAGGATGCTGCCACCTACTACTGTCTAGGT
GGTTATCCTAATACTTCTTACCGGTCTGCTTTCGGCGGAGGGACCAAGGTGGAG
ATCAAA-3'

SEQ ID NO:134.   5'-
TCCTTCGTGCTGACTCAGCCAGCCTCAGTGCAGGTGAACTTGGGACAGACGGTC
TCCCTCACATGCACTGCAGATACACTGAGCAGAAGTTATGCTTCCTGGTACCAG
CAGAAGCCAGGCCAGGCCCCTGTGCTGCTCATCTACAGGGATACCAGTCGGCCC
TCAGGGGTCCCTGACCGCTTCTCTGGCTCCAGCTCAGGGAACACGGCCACCCTG
ACCATCAGTGGGGCCCAGGCTGGGGACGAGGCTGACTACTATTGTGCTACAAGC
GGTGGCAGTGGCAGCAACCCTCAGTATGTGTTCGGCGGAGGGACCCAGCTGACC
GTCACAGGC-3'

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[00157]   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 one 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 appended claims.

[00158]   All publications, databases, GenBank sequences, patents, and patent applications
cited in this specification are herein incorporated by reference as if each was specifically and
individually indicated to be incorporated by reference.

WE CLAIM:

1.　　　　An antibody, an antibody-based binding protein or an antibody fragment that specifically binds to the extracellular domain of human receptor tyrosine kinase-like orphan receptor 2 (ROR2) with the same binding specificity as that of a second antibody, wherein the second antibody comprises a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (i) SEQ ID NO:1 and SEQ ID NO:13; (ii) SEQ ID NO:2 and SEQ ID NO:14; (iii) SEQ ID NO:3 and SEQ ID NO:15; (iv) SEQ ID NO:4 and SEQ ID NO:16; (v) SEQ ID NO:5 and SEQ ID NO:17; (vi) SEQ ID NO:6 and SEQ ID NO:18; (vii) SEQ ID NO:7 and SEQ ID NO:19; (vii) SEQ ID NO:8 and SEQ ID NO:20; (ix) SEQ ID NO:9 and SEQ ID NO:21; (x) SEQ ID NO:10 and SEQ ID NO:22; (xi) SEQ ID NO:11 and SEQ ID NO:23; or (xii) SEQ ID NO:12 and SEQ ID NO:24.

2.　　　　The antibody, antibody-based binding protein or antibody fragment of claim 1, comprising a heavy chain variable region sequence and a light chain variable region sequence, one or both of which are at least 90% or at least 95% identical to a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (i) SEQ ID NO:1 and SEQ ID NO:13; (ii) SEQ ID NO:2 and SEQ ID NO:14; (iii) SEQ ID NO:3 and SEQ ID NO:15; (iv) SEQ ID NO:4 and SEQ ID NO:16; (v) SEQ ID NO:5 and SEQ ID NO:17; (vi) SEQ ID NO:6 and SEQ ID NO:18; (vii) SEQ ID NO:7 and SEQ ID NO:19; (vii) SEQ ID NO:8 and SEQ ID NO:20; (ix) SEQ ID NO:9 and SEQ ID NO:21; (x) SEQ ID NO:10 and SEQ ID NO:22; (xi) SEQ ID NO:11 and SEQ ID NO:23; or (xii) SEQ ID NO:12 and SEQ ID NO:24.

3.　　　　The antibody, antibody-based binding protein or antibody fragment of claim 1, comprising a heavy chain variable region sequence and a light chain variable region sequence, one or both of which are identical to a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (i) SEQ ID NO:1 and SEQ ID NO:13; (ii) SEQ ID NO:2 and SEQ ID NO:14; (iii) SEQ ID NO:3 and SEQ ID NO:15; (iv) SEQ ID NO:4 and SEQ ID NO:16; (v) SEQ ID NO:5 and SEQ ID NO:17; (vi) SEQ ID NO:6 and SEQ ID NO:18; (vii) SEQ ID NO:7 and SEQ ID NO:19; (vii) SEQ ID NO:8 and SEQ ID NO:20; (ix) SEQ ID NO:9 and SEQ ID NO:21; (x) SEQ ID NO:10 and SEQ ID

NO:22; (xi) SEQ ID NO:11 and SEQ ID NO:23; or (xii) SEQ ID NO:12 and SEQ ID NO:24.

4.   The antibody, antibody-based binding protein or antibody fragment of claim 1, comprising a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (i) SEQ ID NO:1 and SEQ ID NO:13; (ii) SEQ ID NO:2 and SEQ ID NO:14; (iii) SEQ ID NO:3 and SEQ ID NO:15; (iv) SEQ ID NO:4 and SEQ ID NO:16; (v) SEQ ID NO:5 and SEQ ID NO:17; (vi) SEQ ID NO:6 and SEQ ID NO:18; (vii) SEQ ID NO:7 and SEQ ID NO:19; (vii) SEQ ID NO:8 and SEQ ID NO:20; (ix) SEQ ID NO:9 and SEQ ID NO:21; (x) SEQ ID NO:10 and SEQ ID NO:22; (xi) SEQ ID NO:11 and SEQ ID NO:23; or (xii) SEQ ID NO:12 and SEQ ID NO:24.

5.   The antibody, antibody-based binding protein or antibody fragment of claim 1, comprising heavy chain CDR1-3 sequences and light chain CDR1-3 sequences that are at least 90% or at least 95% identical, respectively, to (i) SEQ ID NOs:25-27 and SEQ ID NOs:61-63, (ii) SEQ ID NOs:28-30 and SEQ ID NOs:64-66, (iii) SEQ ID NOs:31-33 and SEQ ID NOs:67-69, (iv) SEQ ID NOs:34-36 and SEQ ID NOs:70-72, (v) SEQ ID NOs:37-39 and SEQ ID NOs:73-75, (vi) SEQ ID NOs:40-42 and SEQ ID NOs:76-78, (vii) SEQ ID NOs:43-45 and SEQ ID NOs:79-81, (viii) SEQ ID NOs:46-48 and SEQ ID NOs:82-84, (ix) SEQ ID NOs:49-51 and SEQ ID NOs:85-87, (x) SEQ ID NOs:52-54 and SEQ ID NOs:88-90, (xi) SEQ ID NOs:55-57 and SEQ ID NOs:91-93, or (xii) SEQ ID NOs:58-60 and SEQ ID NOs:94-96.

6.   The antibody, antibody-based binding protein or antibody fragment of claim 1, comprising a heavy chain CDR sequence selected from the group consisting of SEQ ID NOs:25-60.

7.   The antibody, antibody-based binding protein or antibody fragment of claim 6, further comprising a light chain CDR sequence selected from the group consisting of SEQ ID NOs:61-96.

8.   The antibody, antibody-based binding protein or antibody fragment of claim 6, comprising heavy chain CDR1, CDR2, and CDR3 sequences that are respectively

identical to SEQ ID NOs:25-27, SEQ ID NOs:28-30, SEQ ID NOs:31-33, SEQ ID NO:34-36, SEQ ID NOs:37-39, SEQ ID NOs:40-42, SEQ ID NOs:43-45, SEQ ID NOs:46-48, SEQ ID NOs:49-51, SEQ ID NOs:52-54, SEQ ID NOs:55-57, or SEQ ID NOs:58-60.

9.   The antibody, antibody-based binding protein or antibody fragment of claim 8, comprising heavy chain CDR1, CDR2 and CDR3 sequences and light chain CDR1, CDR2 and CDR3 sequences respectively shown in (i) SEQ ID NOs:25-27 and SEQ ID NOs:61-63, (ii) SEQ ID NOs:28-30 and SEQ ID NOs:64-66, (iii) SEQ ID NOs:31-33 and SEQ ID NOs:67-69, (iv) SEQ ID NOs:34-36 and SEQ ID NOs:70-72, (v) SEQ ID NOs:37-39 and SEQ ID NOs:73-75, (vi) SEQ ID NOs:40-42 and SEQ ID NOs:76-78, (vii) SEQ ID NOs:43-45 and SEQ ID NOs:79-81, (vii) SEQ ID NOs:46-48 and SEQ ID NOs:82-84, (ix) SEQ ID NOs:49-51 and SEQ ID NOs:85-87, (x) SEQ ID NOs:52-54 and SEQ ID NOs:88-90, (xi) SEQ ID NOs:55-57 and SEQ ID NOs:91-93, or (xii) SEQ ID NOs:58-60 and SEQ ID NOs:94-96.

10.   The antibody, antibody-based binding protein or antibody fragment of claim 1, comprising a light chain CDR sequence selected from the group consisting of SEQ ID NOs:61-96.

11.   The antibody, antibody-based binding protein or antibody fragment of claim 10, further comprising a heavy chain CDR sequence selected from the group consisting of SEQ ID NOs:25-60.

12.   The antibody, antibody-based binding protein or antibody fragment of claim 10, comprising light chain CDR1, CDR2, and CDR3 sequences that are respectively identical to SEQ ID NOs:61-63, SEQ ID NOs:64-66, SEQ ID NOs:67-69, SEQ ID NOs:70-72, SEQ ID NOs:73-75, SEQ ID NOs:76-78, SEQ ID NOs:79-81, SEQ ID NOs:82-84, SEQ ID NOs:85-87, SEQ ID NOs:88-90, SEQ ID NOs:91-93, or SEQ ID NOs:94-96.

13.   The antibody, antibody-based binding protein or antibody fragment of claim 1, wherein the antibody or antibody fragment is chimeric or humanized.

14.   The antibody, antibody-based binding protein or antibody fragment of claim 1, wherein the antibody or antibody fragment is IgA1, IgA2, IgD, IgE, IgG1, IgG2,

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IgG3, IgG4, synthetic IgG, IgM, F(ab)2, Fv, scFv, IgGACH2, F(ab')2, scFv2CH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)2, a non-depleting IgG, a diabody, or a bivalent antibody.

15.   The antibody, antibody-based binding protein or antibody fragment of claim 1, which is conjugated to a synthetic molecule.

16.   The antibody, antibody-based binding protein or antibody fragment of claim 15, wherein the synthetic molecule is a cytotoxic agent, a label, a therapeutic radioisotope, or a liposome.

17.   The antibody or antigen-binding fragment of claim 16, wherein the cytotoxic agent is selected from a small molecule weight toxin, or a peptide toxin, or a protein toxin.

18.   An antibody drug conjugate (ADC), comprising the antibody or antibody fragment of claim 1 and at least one cytotoxic agent.

19.   The antibody drug conjugate of claim 18, wherein the cytotoxic agent is a small molecular weight toxin, a peptide toxin, or a protein toxin.

20.   The antibody drug conjugate of claim 18, wherein the antibody or antibody fragment is conjugated to the cytotoxic agent via sortase enzyme mediated antibody conjugation (SMAC).

21.   The antibody drug conjugate of claim 20, wherein the cytotoxic agent is anthracycline (PNU) toxin derivative $Gly_{(n)}$-EDA-PNU, where n is any number from 1 to 21.

22.   The antibody drug conjugate of claim 18, wherein the antibody or antibody fragment is chimeric or humanized.

23.   The antibody drug conjugate of claim 18, wherein the antibody or antibody fragment comprises heavy chain CDR1, CDR2 and CDR3 sequences and light chain CDR1, CDR2 and CDR3 sequences respectively shown in (1) SEQ ID NOs:25-27 and SEQ ID NOs:61-63, (2) SEQ ID NOs:43-45 and SEQ ID NOs:79-81, (3) SEQ ID NOs:46-48 and

SEQ ID NOs:82-84, (4) SEQ ID NOs:52-54 and SEQ ID NOs:88-90, (5) SEQ ID NOs:34-36 and SEQ ID NOs:70-72, or (6) SEQ ID NOs:58-60 and SEQ ID NOs:94-96.

24.   The antibody drug conjugate of claim 18, wherein the antibody or antibody fragment comprises a heavy chain variable region sequence and a light chain variable region sequence respectively shown in (1) SEQ ID NO:1 and SEQ ID NO:13; (2) SEQ ID NO:7 and SEQ ID NO:19; (3) SEQ ID NO:8 and SEQ ID NO:20; (4) SEQ ID NO:10 and SEQ ID NO:22; (5) SEQ ID NO:4 and SEQ ID NO:16; or (6) SEQ ID NO:12 and SEQ ID NO:24.

25.   A chimeric antigen receptor (CAR), comprising the antibody or antibody fragment of claim 1 that is fused to a transmembrane region and an intracellular T-cell receptor (TCR) signaling domain.

26.   The chimeric antigen receptor of claim 25, wherein the antibody or antibody fragment is chimeric or humanized.

27.   The chimeric antigen receptor of claim 25, wherein the antibody or antibody fragment comprises heavy chain CDR1, CDR2 and CDR3 sequences and light chain CDR1, CDR2 and CDR3 sequences respectively shown in SEQ ID NOs:25-27 and SEQ ID NOs:61-63.

28.   The chimeric antigen receptor of claim 25, wherein the antibody or antibody fragment comprises a heavy chain variable region sequence and a light chain variable region sequence respectively shown SEQ ID NO:1 and SEQ ID NO:13.

29.   A pharmaceutical composition comprising (1) a therapeutically effective amount of (a) an antibody, antibody-based binding protein or antibody fragment of claim 1 or (b) an antibody drug conjugate (ADC) of claim 18, and (2) a pharmaceutically acceptable carrier.

30.   A kit comprising (1) the antibody or antigen-binding fragment of claim 1 or (2) the antibody drug conjugate (ADC) of claim 18.

31.   A polynucleotide encoding the variable region of the heavy chain or light chain of the antibody, antibody-based binding protein or antibody fragment of claim 1.

32.   A vector harboring the polynucleotide of claim 31.

33.   A method of killing or inhibiting the growth of a cell expressing ROR2 in a subject, comprising administering a pharmaceutical composition of claim 29 to a subject in need thereof, thereby killing or inhibiting the growth of the cell expressing ROR2 in the subject.

34.   The method of claim 33, wherein the cell is a tumor cell.

35.   The method of claim 33, wherein the pharmaceutical composition comprises an antibody drug conjugate (ADC) comprising (1) the antibody, antibody-based binding protein or antibody fragment of claim 1 and (2) a cytotoxic agent.

36.   The method of claim 35, wherein the cytotoxic agent is a small molecular weight toxin, a peptide toxin, or a protein toxin.

37.   A method of treating a disease or condition associated with elevated expression of ROR2 in a subject, comprising administering a pharmaceutical composition of claim 29 to a subject having a disease or condition associated with elevated expression of ROR2, thereby treating the disease or condition associated with elevated expression of ROR2 in the subject.

38.   The method of claim 37, wherein the disease or condition is a cancer.

39.   The method of claim 37, wherein the disease or condition is neuroblastoma, osteosarcoma, renal cell carcinoma, breast cancer, gastric cancer, prostate cancer, melanoma, squamous cell carcinoma, or multiple myeloma.

40.   The method of claim 37, wherein the pharmaceutical composition comprises an antibody drug conjugate (ADC) comprising (1) the antibody or antibody fragment of claim 1 and (2) a cytotoxic agent.

41.   The method of claim 40, wherein the cytotoxic agent is a small molecular weight toxin, a peptide toxin, or a protein toxin.

42.   The method of claim 37, wherein the antibody or antibody fragment is fused to a transmembrane region and an intracellular T-cell receptor (TCR) signaling domain to form a chimeric antigen receptor (CAR).

ROR2 mAbs

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 6

FIG. 6

FIG. 7

FIG. 8

FIG. 9

FIG. 10

clone	XBR2-401	XBR2-416	XBR2-433	XBR2-327	ERR2-302	ERR2-308	ERR2-316	ERR2-317	XBR2-TOP9	XBR2-TOP72*	ERR2-TOP2	ERR2-TOP35
Immobilization RU	5000	10000	5000*	5000*	5000	5000	5000	5000	10000	10000	5000	5000
hFc-ROR2 RU	386.6	832.4	505.3	312.5	582.1	318.1	325.1	383.3	863.8	867.9	470.9	366.9
Fab Con.^{H} (nM)	200	500	2000	200	200	200	200	200	500	500	1000	200
K$_{on}$ (10^4 M^{-1} s^{-1})	2.02	4.98	4.73	9.56	10.4	2.71	334	8.35	32.8	3.70	2.98	19.7
K$_{off}$ (10^{-3} s^{-1})	0.0782	17.34	108	5.463	5.44	0.286	17.81	13.23	46.7	153.6	2.419	8.51
K$_d$ (nM)	3.87	348	2280	57.2	52.5	10.6	5.34	159	142	4150	81.1	43.3
Chi^2	0.167	20.9	7.05	4.07	8.01	1.41	22.7	0.826	153	0.237	94.4	4.06

FIG. 11

FIG. 12

1. 63-12 / hROR1
2. 63-12
3. 63-12 / hROR2
4. hROR2-ECD-HIS
5. hROR1-ECD-HIS
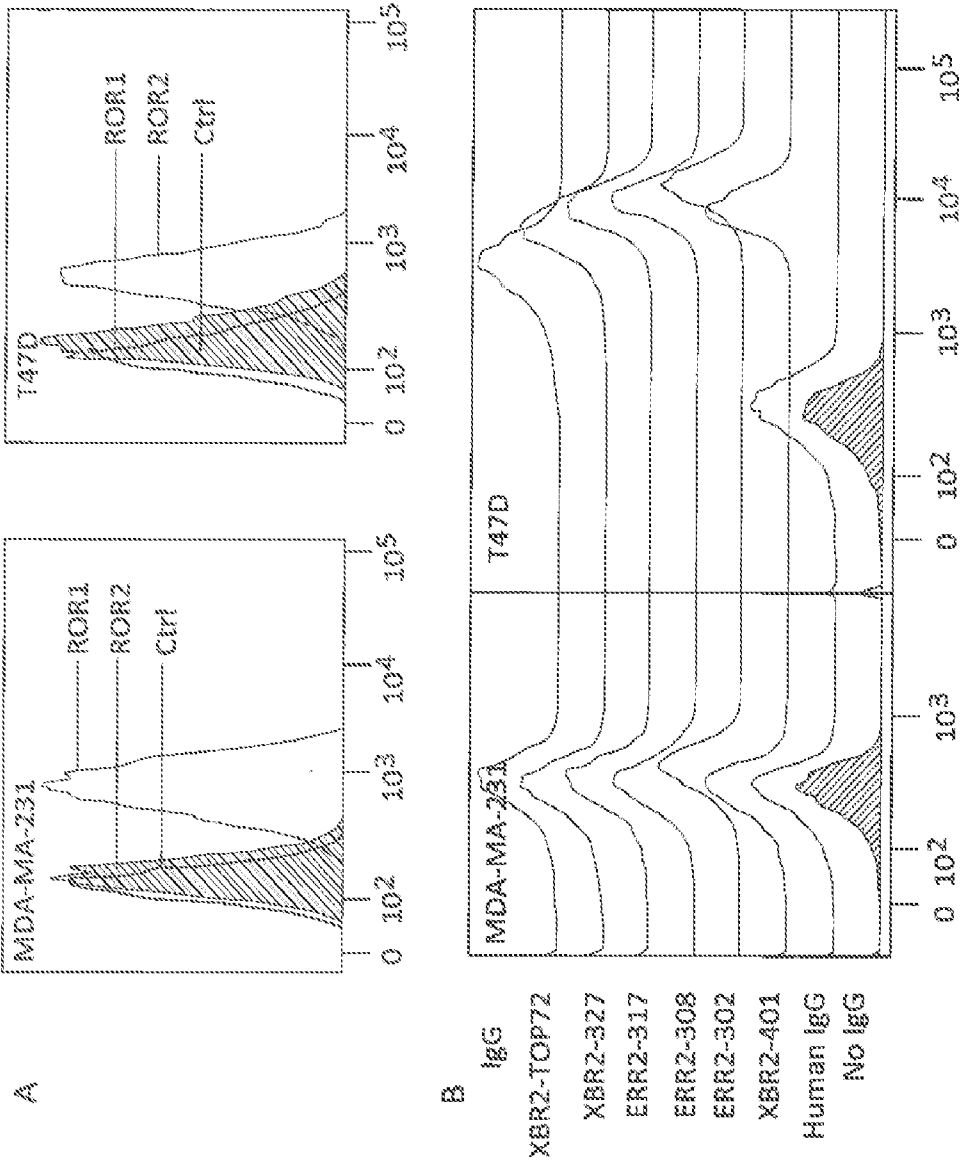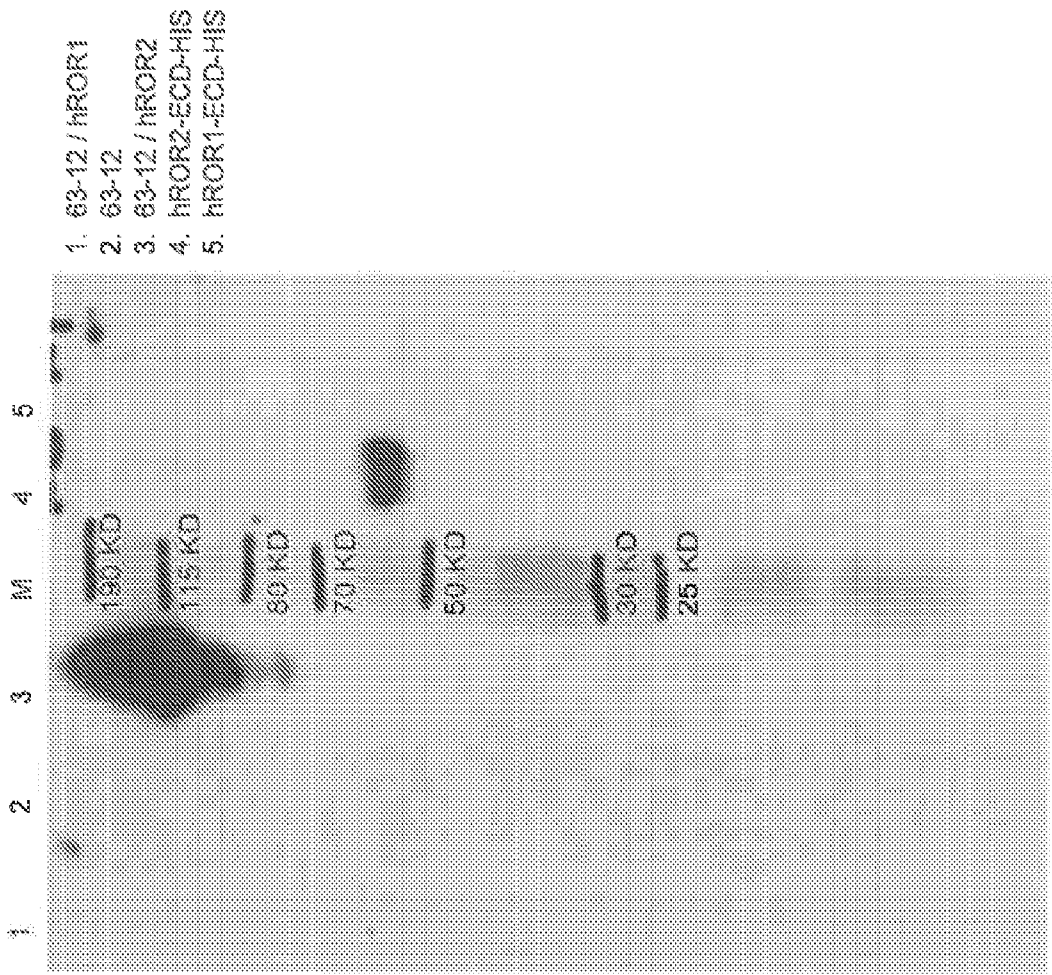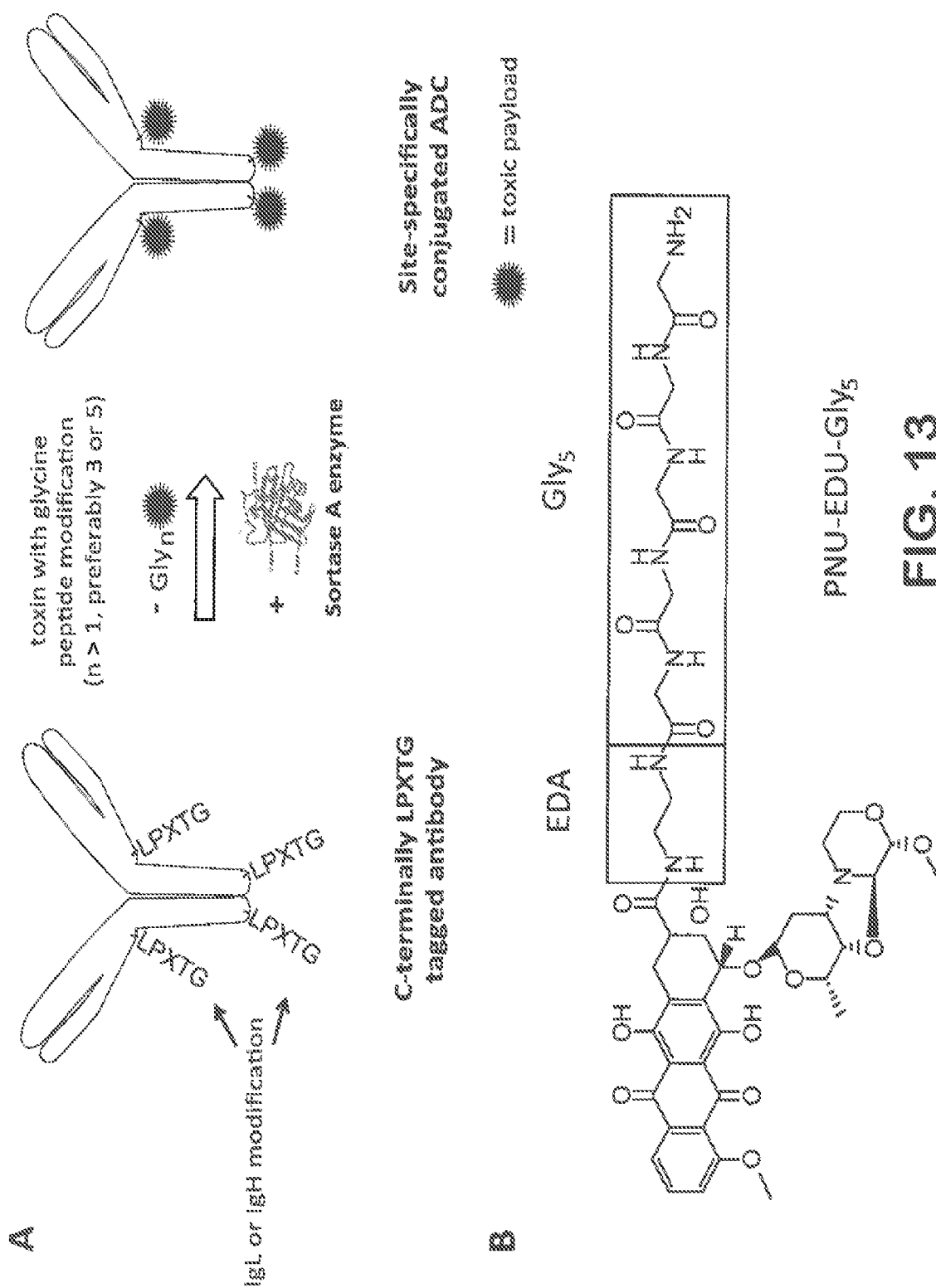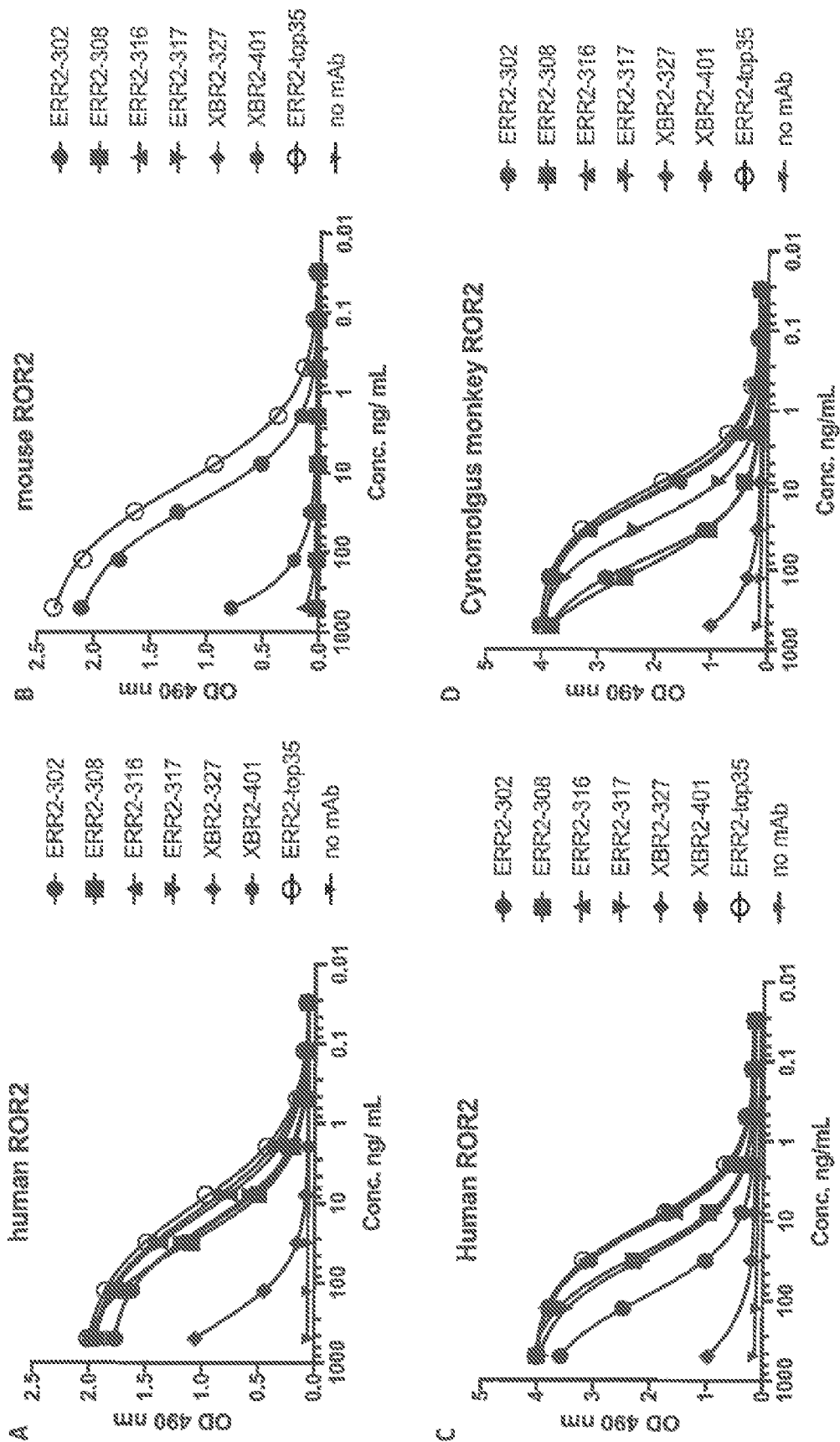
FIG. 13

FIG. 14
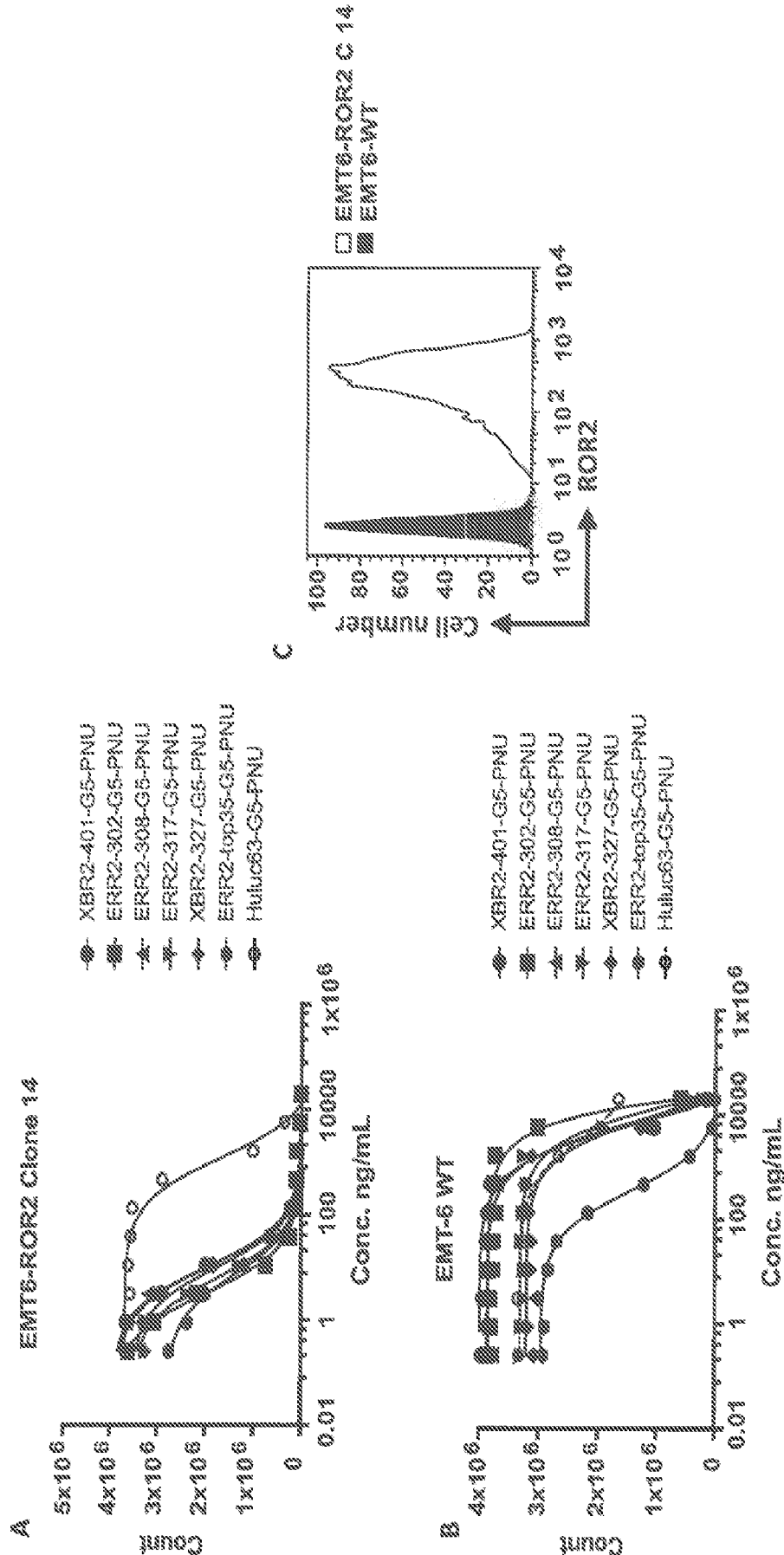
FIG. 15
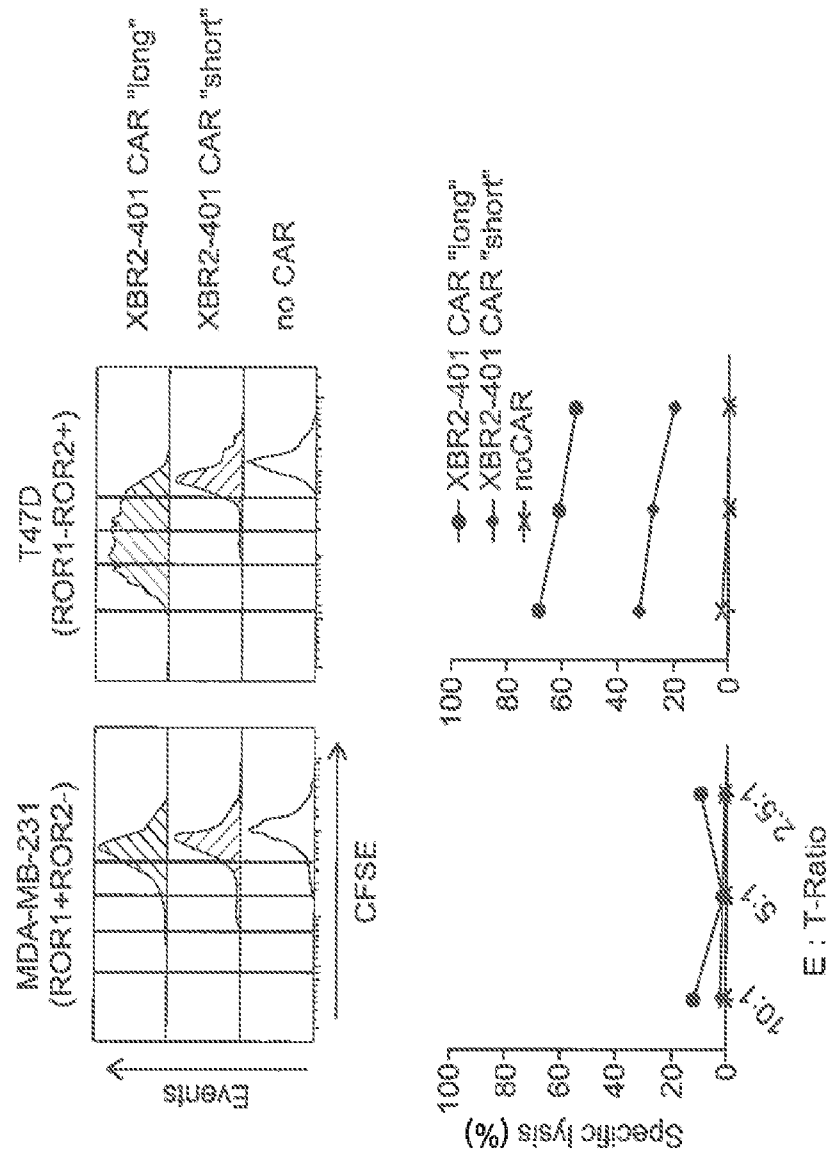
FIG. 16
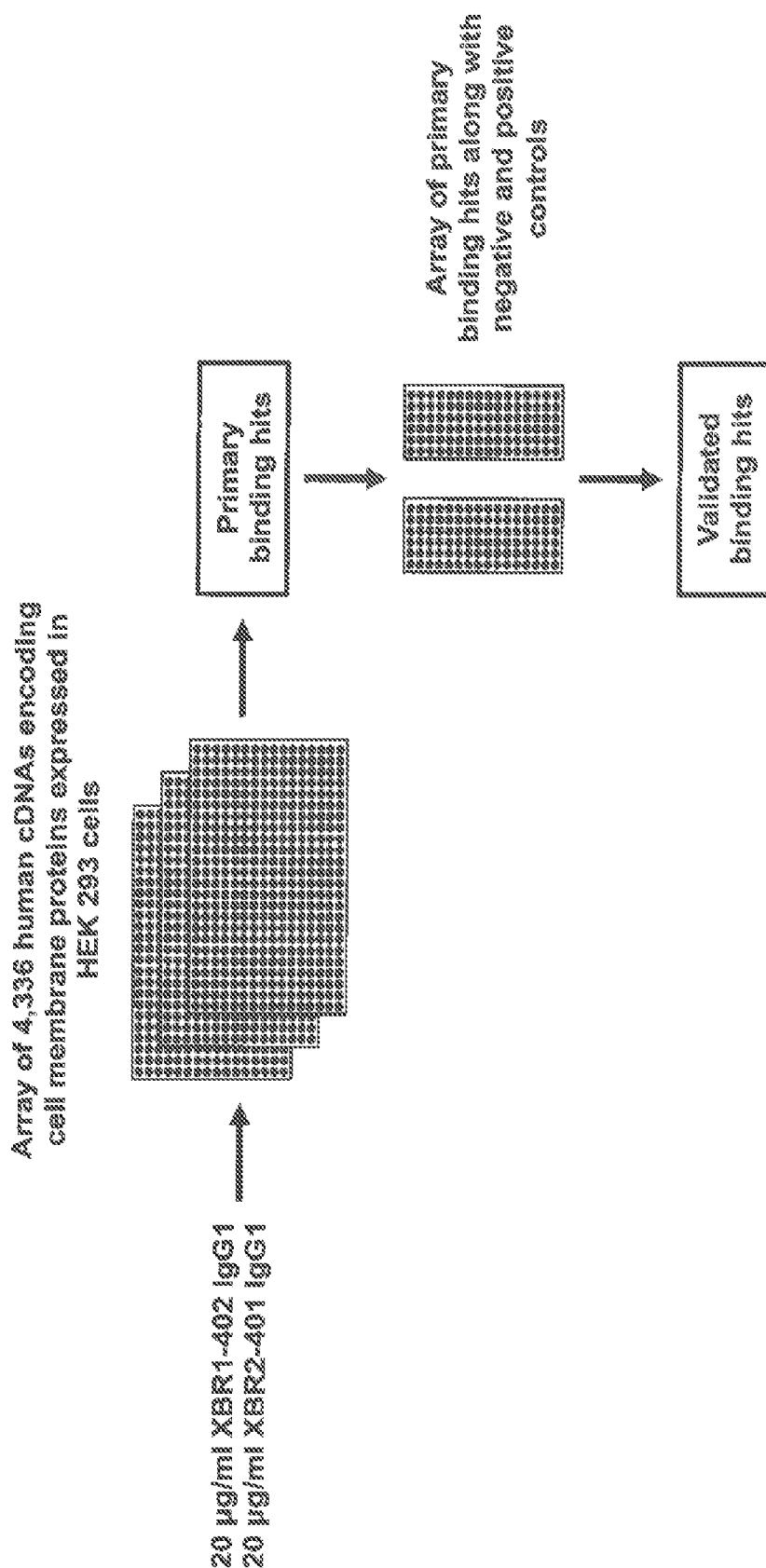
FIG. 17

FIG. 18

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/28   C07K14/725
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 A61K

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/103637 A1 (UNIV LELAND STANFORD JUNIOR [US]) 11 July 2013 (2013-07-11) cited in the application claims 1-24; examples 2-4,5 -----	1-42
X	WO 2015/171938 A1 (NOVODIAX INC [US]) 12 November 2015 (2015-11-12) example 8 ----- -/--	1-42

[X] Further documents are listed in the continuation of Box C.   [X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 March 2017	03/04/2017

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

INTERNATIONAL SEARCH REPORT

C(Continuation).	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	R&d: "Human ROR2 Antibody, catalog number MAB2064", R&DCtalog, 13 October 2015 (2015-10-13), page 1, XP055358072, Retrieved from the Internet: URL:https://resources.rndsystems.com/pdfs/datasheets/mab2064.pdf [retrieved on 2017-03-23] page 1 -----	1-42
X	Abcam: "Anti-ROR2 antibody [6F2D10] ab201962", abcam catalogue, 1 March 2015 (2015-03-01), pages 1-3, XP055358061, Retrieved from the Internet: URL:http://www.abcam.com/ROR2-antibody-6F2D10-ab201962.pdf [retrieved on 2017-03-23] page 1 -----	1-42
X	ZUFAN DEBEBE ET AL: "Ror2 as a Therapeutic Target in Cancer", PHARMACOLOGY AND THERAPEUTICS., vol. 150, 1 June 2015 (2015-06-01), pages 143-148, XP055358520, GB ISSN: 0163-7258, DOI: 10.1016/j.pharmthera.2015.01.010 paragraph [0008] page 147, left-hand column -----	1-42

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

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			WO	2013103637 A1	11-07-2013
WO 2015171938	A1	12-11-2015	AU	2015255883 A1	27-10-2016
			CN	106415269 A	15-02-2017
			EP	3140653 A1	15-03-2017
			US	2017074867 A1	16-03-2017
			WO	2015171938 A1	12-11-2015