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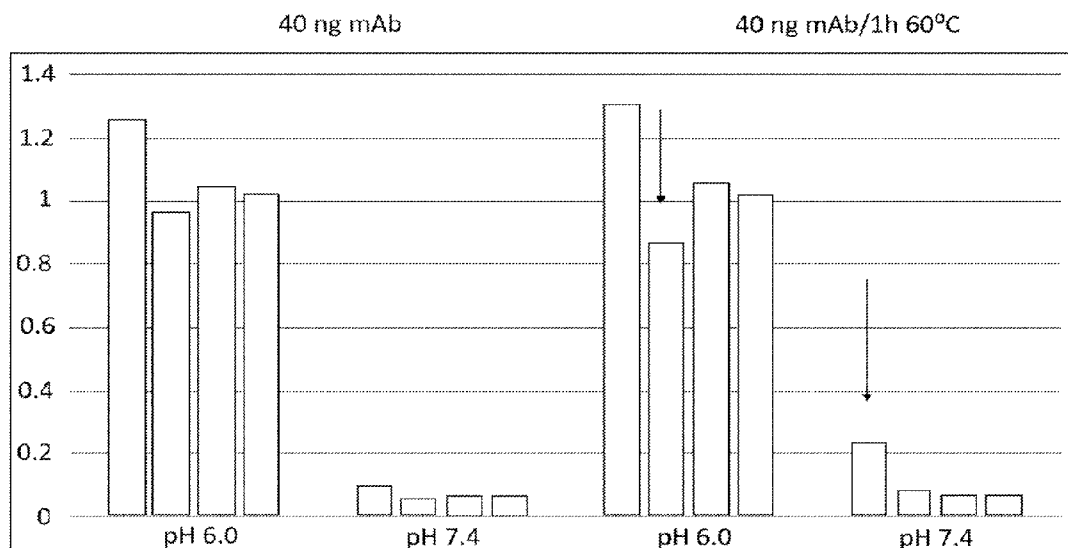
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There are 4 antibodies tested under each condition (i.e., 11 bars in each set of bars). The 11 bars under each condition from left to right represent respectively clones BAP048.6-2-11-Chimera-CAB, (#3949), BAP048.7-humC02D09-CAB (#3947), BAP048.7-humA03C05-CAB (#3950), and BAP048.7-humA03F01-CAB (#3948).

FIG. 5

(57) Abstract: A polypeptide having a heavy chain variable region and/or light chain variable region that specifically binds to ROR2 protein as well as antibodies and antibody fragments containing the heavy chain variable region and/or the light chain variable region that bind to ROR2 protein. Pharmaceutical compositions and kits comprising the polypeptide or antibodies and antibody fragments containing the polypeptide are also provided, as well as use of such polypeptide or antibodies or antibody fragments in methods of treating ROR2-expressing cancers.



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**CANCER TREATMENT WITH A CONDITIONALLY ACTIVE ANTI-ROR2
ANTIBODY-DRUG CONJUGATE**

REFERENCE TO SEQUENCE LISTING

[0001] This application includes a sequence listing submitted herewith as an XML file named “BIAT-1035WOSequence Listing” created on February 10, 2023, and containing 174,000 bytes. The material contained in this text file is incorporated herein by reference.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims priority to U.S. provisional application no. 63/313,943 filed on February 25, 2022, the entire disclosure of which is hereby incorporated by reference as if fully set forth herein.

FIELD OF THE DISCLOSURE

[0003] This disclosure relates anti-ROR2 antibodies, antibody fragments and immunoconjugates of such antibodies and antibody fragments and uses of the antibodies, antibody fragments and immunoconjugates in diagnostic and therapeutic methods.

BACKGROUND OF THE DISCLOSURE

[0004] Receptor tyrosine kinases (RTKs) are a family of cell surface receptors that regulate a range of normal cellular processes through ligand-controlled tyrosine kinase activity. Over the past 20 years, deregulation of RTKs has been shown to play a critical role in cancer development and progression. RTKs are now recognized as prognostic molecular biomarkers and as targets of oncology therapeutics.

[0005] ROR2, also called receptor tyrosine kinase-like orphan receptor 2, is a membrane-bound RTK that is activated by non-canonical Wnt signaling through its association with the Wnt5A glycoprotein during normal bone and cartilage development. ROR2 has only one transmembrane domain, which separates its extracellular and intracellular domains (FIG. 1). ROR2 is known to play crucial roles in the normal development of various organs and tissues. In mammals, ROR2- and Wnt5A-deficient mice exhibit similar abnormalities during developmental morphogenesis, reflecting their defects in convergent extension movements and planar cell polarity. Furthermore, mutations of the human ROR2 gene are responsible for the genetic skeletal disorders dominant brachydactyly type B and recessive Robinow syndrome. ROR2 has been found to mediate polarized cell migration and malfunction of ROR2 results in heritable skeletal disorders and tumor invasion (Minami et al., “Ror-family

receptor tyrosine kinases in noncanonical Wnt signaling: their implications in developmental morphogenesis and human diseases,” *Dev Dyn.*, vol. 239, pp.1-15, 2010).

[0006] ROR2 has also been reported to have pro-tumorigenic effects. US 2014/0322234 discloses that the expression and activity of ROR2 in various cancers is different from normal tissues. Thus, it is suggested that dysregulation of ROR2 plays a role in the pathogenesis of a variety of human cancers. US 2014/0322234 also contemplates that antibodies against ROR2 may be used in diagnosis of cancers and inhibition of cancer cell growth. For example, such antibodies may be conjugated to a cytotoxic agent that has a high degree of cytotoxicity for cancer cells expressing ROR2, such that the cytotoxic agent can effectively kill the cancer cells. The ROR2 gene may also be used in classification of cancers according to the ROR2 expression pattern in the cancers.

[0007] Ford et al. (“The dual role of the novel Wnt receptor tyrosine kinase, ROR2, in human carcinogenesis,” *International Journal of Cancer*, vol. 133, pp. 779–787, 2013) further explores the mechanism of ROR2 in carcinogenesis. This reference discloses that ROR2 is involved in the development and progression of cancers. Specifically, ROR2 has been found to play a pivotal role in carcinogenesis of numerous cancers including colon cancer, hepatocellular carcinoma, metastatic melanoma and renal cell carcinoma. For example, ROR2 is over-expressed in osteosarcoma, melanoma, renal cell carcinoma, prostate carcinoma, squamous cell carcinomas of the head and neck and stromal tumors. ROR2 thus has the potential of being a drug target for cancer treatments by inhibition of the Wnt signaling pathway.

[0008] Further, Debebe et al., (“ROR2 as a therapeutic target in cancer,” *Pharmacol. Ther.*, vol. 50, pp. 143-148, 2015) discloses that ROR2 mediates both canonical and non-canonical signaling pathways. ROR2 is highly expressed in osteosarcoma and renal cell carcinomas, as well as in melanoma, colon cancer, squamous cell carcinoma of the head and neck, and breast cancer. In the majority of these cancer types, ROR2 expression is associated with more aggressive cancer states. Thus, this reference also suggests that ROR2 is a potential target for cancer treatment.

[0009] WO 2013/103637 discloses ROR2 as a therapeutic target and prognostic marker for cancers, and the use of conjugates comprising an antibody that recognizes and binds ROR2 and a cytotoxic agent (see Abstract).

[0010] WO 2017/197234 discloses a polypeptide having a heavy chain variable region and/or light chain variable region that specifically binds to ROR2 protein as well as antibodies and antibody fragments containing the heavy chain variable region and/or the light chain variable region that bind to ROR2 protein. Pharmaceutical compositions and kits

comprising the polypeptide or antibodies and antibody fragments containing the polypeptide are also provided.

[0011] Chang C. et al., “Novel conditionally active biologic anti-Axl antibody-drug conjugate demonstrates anti-tumor efficacy and improved safety profile,” AACR Annual Meeting, April 2016, describes conditionally active biologics (CAB)-Axl antibodies conjugated to a model toxin payload to generate CAB-Axl-ADCs (antibody drug conjugates) (Abstract).

[0012] WO2016/138071 describes methods of generating conditionally active biologic proteins, which are more active at an aberrant condition than a normal physiological condition (see Abstract).

[0013] Though monoclonal antibodies against ROR2 are commercially available, anti-ROR2 antibodies suitable for cancer therapy have not been reported. The present invention provides anti-ROR2 antibodies or antibody fragments that are suitable for therapeutic and diagnostic use, especially for diagnosis and treatment of cancers. Some of the anti-ROR2 antibodies or antibody fragments have a higher binding affinity to ROR2 in a tumor in comparison with ROR2 present in normal tissue. These anti-ROR2 antibodies or antibody fragments of the present invention have at least comparable efficacy as well as a longer half-life, but reduced side-effects, in comparison with monoclonal anti-ROR2 antibodies known in the art. This may permit use of higher dosages of these anti-ROR2 antibodies or antibody fragments, thus providing a more effective therapeutic option without a corresponding significant increase in side effects.

SUMMARY OF THE DISCLOSURE

[0014] In one aspect, the present invention provides an isolated polypeptide that specifically binds to the ROR2 protein and uses of the polypeptide in methods of treating a ROR2-expressing tumor that involves administering the polypeptide to a human in need of such treatment.

[0015] The polypeptide includes a heavy chain variable region having three complementarity determining regions (CDRs) H1, H2, and H3 sequences, wherein:

the H1 sequence is GYTX₁TEX₂X₃X₄H (SEQ ID NO:1) or GYSITTGX₂₉YWN (SEQ ID NO:4);

the H2 sequence is X₅X₆X₇X₈NNGGTGYNQKFKG (SEQ ID NO:2) or YITYDGSX₃₀NYNPSLKN (SEQ ID NO:5); and

the H3 sequence is X₉X₁₀X₁₁SX₁₂YX₁₃YX₁₄X₁₅SYFX₁₆X₁₇X₁₈ (SEQ ID NO:3) or CSX₃₁X₃₂X₃₃X₃₄VX₃₅X₃₆X₃₇LDX₃₈ (SEQ ID NO:6);

wherein

X₁ is F or E,

X₂ is Y or D,

X₃ is T or C,

X₄ is M or D or E or Y,

X₅ is G or S,

X₆ is I or E,

X₇ is N or C or L or V,

X₈ is T or D or E,

X₉ is A or M or T,

X₁₀ is R or H,

X₁₁ is G or E,

X₁₂ is L or F,

X₁₃ is S or G,

X₁₄ is G or D,

X₁₅ is N or E,

X₁₆ is D or L,

X₁₇ is Y or C or T,

X₁₈ is W or L,

X₂₉ is Y or E or R or T,

X₃₀ is K or N,

X₃₁ is R or G or H or W or Y,

X₃₂ is F or C or N or Q,

X₃₃ is E or S,

X₃₄ is G or E or F or H or M or Q or S,

X₃₅ is W or A or I or P or Q or T or V,

X₃₆ is Y or G or N or Q,

X₃₇ is G or S or T, and

X₃₈ is Y or I; and

a light chain variable region having three complementarity determining regions (CDRs) L1, L2, and L3 sequences, wherein:

the L1 sequence is SATSSX₁₉X₂₀X₂₁MX₂₂ (SEQ ID NO:7) or RASESVDRYGNSX₃₉IH (SEQ ID NO:10);

the L2 sequence is X₂₃TSNLAS (SEQ ID NO:8) or X₄₀TYX₄₁LES (SEQ ID NO:11); and

the L3 sequence is QX₂₄X₂₅SX₂₆YPFX₂₇X₂₈ (SEQ ID NO:9) or QQX₄₂NX₄₃DPX₄₄TX₄₅ (SEQ ID NO:12);

wherein

X₁₉ is V or E,

X₂₀ is S or D,

X₂₁ is Y or C or D,

X₂₂ is H or G or L,

X₂₃ is G or C or H or P,

X₂₄ is Q or E,

X₂₅ is R or H,

X₂₆ is S or D or G or I or Q or V,

X₂₇ is T or D,

X₂₈ is F or D or E,

X₃₉ is F or S or T,

X₄₀ is R or C or D or E or W,

X₄₁ is N or D,

X₄₂ is T or I or P,

X₄₃ is E or V,

X₄₄ is W or T, and

X₄₅ is F or T.

[0016] In one embodiment of this aspect, the heavy chain variable region polypeptide includes three complementarity determining regions H1, H2, and H3 having an amino acid sequence selected from SEQ ID NOS: 18-26.

[0017] In one embodiment of this aspect, the light chain variable region polypeptide includes three complementarity determining regions L1, L2, and L3 having an amino acid sequence selected from SEQ ID NOS: 13-17 and 27.

[0018] In another embodiment of this aspect, any one of the the heavy chain variable region polypeptides as described in the embodiments above, may be combined with any one of the light chain variable region polypeptides as described in the embodiments above.

[0019] In another aspect, the present invention provides an isolated polypeptide as described above which has up to one substitution in CDRs H1, H2 and H3, relative to the parent polypeptide and/or up to one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide. This includes (1) isolated polypeptides with one substitution in CDRs H1, H2 and H3, relative to the parent polypeptide, (2) isolated polypeptides with one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide, and (3) isolated polypeptides with

one substitution in CDRs H1, H2 and H3, and one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide.

[0020] In one embodiment of this aspect, the possible single point mutations in CDRs H1, H2 and H3 are shown in FIGS. 2A-1 and 2A-2 and the possible single point mutations in CDRs L1, L2 and L3 are shown in FIGS. 3A-1 and 3A-2.

[0021] In another embodiment of this aspect, the possible single point mutations of CDRs H1, H2 and H3 are shown in FIGS. 2B-1 and 2B-2 and the possible single point mutations of CDRs L1, L2 and L3 are shown in FIGS. 3B-1 and 3B-2.

[0022] In another aspect, each of the heavy chain variable region polypeptides and each of the light chain variable region polypeptides may each have 1, 2, 3, 4 or 5 independently selected substitutions in the CDRs selected from the point mutations shown in Figures 2B-1-2 and Figures 2A-1-2, respectively.

[0023] In yet another aspect, the present invention provides an anti-ROR2 antibody or antibody fragment that includes any one of the polypeptides of the invention as described above.

[0024] In an embodiment of this aspect, the present invention provides an anti-ROR2 antibody or antibody fragment that includes any one of the heavy chain variable region polypeptides as described above combined with any one of the light chain variable region polypeptides as described above.

[0025] In one embodiment of this aspect, the present invention provides an anti-ROR2 antibody or antibody fragment that includes a heavy chain variable region having an amino acid sequence selected from SEQ ID NOS: 18-26, combined with a light chain variable region having an amino acid sequence selected from SEQ ID NOS: 13-17 and 27.

[0026] In a further embodiment of this aspect, the present invention provides an anti-ROR2 antibody or antibody fragment that includes a heavy chain variable region having an amino acid sequence selected from SEQ ID NO: 18, SEQ ID NO: 21, and SEQ ID NO: 22, combined with a light chain variable region having an amino acid sequence selected from SEQ ID NOS: 13-16.

[0027] In a further embodiment of this aspect, the present invention provides an anti-ROR2 antibody or antibody fragment that specifically binds with a higher binding affinity to the ROR2 protein at a value of a condition in a tumor microenvironment in comparison with the binding affinity to the ROR2 protein at a different value of the same condition that occurs in a non-tumor microenvironment.

[0028] In yet a further embodiment of this aspect, the condition in the tumor microenvironment and the condition in the non-tumor microenvironment is pH.

[0029] In yet a further embodiment of this aspect, the pH in the tumor microenvironment is in a range of from 5.8 to 6.8 and the pH in the non-tumor microenvironment is in a range of from 7.0 to 7.6.

[0030] In a further embodiment of this aspect, the antibody or antibody fragment has a ratio of binding affinity to the ROR2 protein at a value of a condition in a tumor microenvironment to a binding affinity to the ROR2 protein at a different value of the same condition in a non-tumor microenvironment of at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.

[0031] In yet a further embodiment of this aspect, the antibody or antibody fragment of any one of the above embodiments is a chimeric antibody, a multispecific antibody, or a humanized antibody.

[0032] In yet another aspect, the present invention provides an immunoconjugate that includes the antibody or antibody fragment of the invention as described above, conjugated to at least one agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.

[0033] In one embodiment of this aspect, the immunoconjugate is an antibody-drug conjugate (ADC) in which a conditionally active biologic (CAB) anti-ROR2 antibody or antibody fragment is conjugated to one or more heterologous molecule(s) via a cleavable linker (CAB-ROR2-ADC). The CAB anti-ROR2 antibody or antibody fragment may be BA3021, where BA3021 is an antibody or antibody fragment having a heavy chain variable region having an amino acid sequence of SEQ ID NO. 16 and a light chain variable region having an amino acid sequence of SEQ ID NO. 21. The CAB-ROR2-ADC may be BA3021-cleavable linker-MMAE_(n), in which the heterologous molecule is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive.

[0034] In yet another aspect, the present invention provides a pharmaceutical composition that includes the polypeptide, the antibody or antibody fragment, or the immunoconjugate of the invention as described above, together with a pharmaceutically acceptable carrier.

[0035] In yet another aspect, the present invention provides a kit for diagnosis or treatment including the polypeptide, the antibody or antibody fragment, or the immunoconjugate of the present invention as described above.

[0036] In another aspect, the present invention provides a method of treating a ROR2-expressing tumor using the above-described polypeptide, antibody or antibody fragment, or immunoconjugate of the present invention as described above.

[0037] In one embodiment of this aspect, the method of treating a ROR2-expressing tumor of the present invention includes administering to a human subject in need of such treatment, a conditionally active ROR2 polypeptide, antibody or antibody fragment, or immunoconjugate as described above, to a human subject in need of such treatment.

[0038] In a further embodiment of this aspect, the method of treating a ROR2-expressing tumor includes administering to a human subject in need of such treatment, a CAB-ROR2-ADC as described above.

[0039] In a further embodiment of this aspect, the human subject in need of such treatment is a human subject with cancer or a ROR2-expressing tumor.

[0040] In a particular embodiment of this aspect, the cancer may be selected from sarcoma, ovarian cancer, melanoma, non-small cell lung cancer (NSCLC), breast carcinoma, and head and neck cancer.

[0041] In another embodiment of this aspect, the method of treating a ROR2-expressing tumor includes administering to a human subject in need of such treatment as described in any of the embodiments above, a pharmaceutical composition containing a CAB-ROR2-ADC which is BA3021-cleavable linker-MMAE_(n), as described above.

[0042] In yet a further embodiment of this aspect, the pharmaceutical composition as described in any of the embodiments above is administered at a dose up to 3.3 mg/kg of the human subject weight every 21 days or 3 weeks.

[0043] In yet a further embodiment of this aspect, the pharmaceutical composition as described in any of the embodiments above is administered at a dose of 1.8 mg/kg of the human subject weight every 14 days or 2 weeks.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] FIG. 1 is a schematic diagram of the structure of human ROR2 protein. The protein contains an Ig-like domain (Ig), a frizzled or cysteine-rich (CRD) domain, and a kringle (Kr) domain in the extracellular domain. The extracellular and intracellular domains are separated by a transmembrane (TM) domain. The intracellular domain contains a tyrosine kinase (TK) domain and a proline-rich domain (PR) flanked by serine/threonine (ST) rich domains.

[0045] FIG. 2A shows an index for FIGS. 2A-1 and 2A-2. FIG. 2B shows an index for FIGS. 2B-1 and 2B-2. FIGS. 2A-1, 2A-2, 2B-1, and 2B-2 show sequence alignments of exemplary heavy chain variable regions of anti-ROR2 antibodies of the present invention.

[0046] FIG. 3A shows an index for FIGS. 3A-1 and 3A-2. FIG. 3B shows an index for FIGS. 3B-1 and 3B-2. FIGS. 3A-1, 3A-2, 3B-1, and 3B-2 show sequence alignments of exemplary light chain variable regions of anti-ROR2 antibodies of the present invention.

[0047] FIG. 4 shows a size exclusion chromatograph indicating that the anti-ROR2 antibodies of the invention do not aggregate, as described in Example 1.

[0048] FIG. 5 shows pH-dependent binding profiles of anti-ROR2 antibodies of the present invention for binding to ROR2, as described in Example 1.

[0049] FIGS. 6A-6B show on and off rates of conditionally active antibodies of the invention as measured by surface plasmon resonance (SPR) assay, as described in Example 1.

[0050] FIGS. 7A-7C show effects on tumor volume of treatment of xenografted mice with a paclitaxel-conjugated anti-ROR2 antibody of the present invention, as described in Example 2.

[0051] FIG. 8 shows the pH dependent binding affinity of an exemplary conditionally active antibody BAP048 measured by pH titration.

[0052] FIG. 9 shows the binding affinity of the conditionally active antibody BAP048 to ROR2 proteins of human, cynomolgus, and mouse.

[0053] FIG. 10 shows cell killing of the conditionally active antibody BAP048 conjugated to Monomethyl auristatin E (MMAE) on HEK293 cells expressing human ROR2.

[0054] FIGS. 11A-11C show cell killing of the conditionally active antibody BAP048 conjugated to MMAE on LCLC103H cells.

[0055] FIGS. 12A-12C show cell killing of the conditionally active antibody BAP048 conjugated to MMAE on HT1080 cells.

[0056] FIG. 13 shows treatment of mouse tumors induced by LCLC103H using the conditionally active antibody BAP048 conjugated to MMAE.

[0057] FIG. 14 shows treatment of mouse tumors induced by LCLC103H using the conditionally active antibody BAP048 conjugated to MMAE through different linkers.

[0058] FIGS. 15A-15B show treatment of mouse tumors induced by HT1080 or MDA-MB-436 respectively, using the conditionally active antibody BAP048 conjugated to MMAE.

[0059] FIG. 16 shows the variable dosing response in NSCLC patients enrolled in a BA3021 Phase 1 trial by ROR tumor membrane percent score (TmPS), in which tumor membrane ROR2 expression was associated with antitumor response in two of the five NSCLC patients with evaluable ROR2 TmPS.

[0060] FIG. 17 shows the dosing response for two evaluable metastatic melanoma patients enrolled in a BA3021 Phase 1 trial.

[0061] FIGS. 18A-18B show pre-treatment (FIG. 18A) and post-treatment (FIG. 18B) CT scans of one of two lung lesions that were cleared in a metastatic melanoma patient who received BA3021 (ozuriftamab vedotin) and achieved a complete response.

[0062] FIGS. 19A-19D show ROR2 staining in sarcoma at 40x magnification. FIG. 19A (Q9403, % Score $\geq 1+$: 100, slide #88), FIG. 19B (QMTB313-08, % Score $\geq 1+$: 70, slide #75), and FIG. 19C (Q9453, % Score $\geq 1+$: 50, slide #93) show ROR2 H-1 in sarcoma and FIG. 19D shows Mouse IgG1 in sarcoma (negative control, slide #88).

[0063] FIGS. 20A-20D show ROR2 staining in ovarian cancer at 40x magnification. FIG. 20A (Q5488, % Score $\geq 1+$: 70, slide #120), FIG. 20B (Q7499-02, % Score $\geq 1+$: 50, slide #124), and FIG. 20C (Q6946-01, % Score $\geq 1+$: 20, slide #122) show ROR2 H-1 in ovarian cancer and FIG. 20D shows Mouse IgG1 in ovarian cancer (negative control, slide #175).

[0064] FIGS. 21A-21D show ROR2 staining in NSCLC at 40x magnification. FIG. 21A (QMTB397-09, % Score $\geq 1+$: 100, slide #239), FIG. 21B (Q4044-049, % Score $\geq 1+$: 10, slide #253), and FIG. 21C (QMTB249-02, % Score $\geq 1+$: 0, slide #238) show ROR2 H-1 in NSCLC and FIG. 21D shows Mouse IgG1 in NSCLC (QMTB397-09, negative control, slide #294).

[0065] FIGS. 22A-22D show ROR2 staining in triple-negative breast cancer (TNBC) at 40x magnification. FIG. 22A (Q933, % Score $\geq 1+$: 90, slide #369), FIG. 22B (Q9286, % Score $\geq 1+$: 60, slide #290), and FIG. 22C (Q9335, % Score $\geq 1+$: 15, slide #371) show ROR2 H-1 in TNBC and FIG. 22D shows Mouse IgG1 in TNBC (negative control, slide #384).

[0066] FIG. 23 shows the proportion of positive and negative ROR2 cases in each cancer indication, based on a ROR2 Cut-Off: $\geq 1+$ Intensity in $\geq 10\%$ Tumor Cells which indicates a positive case.

[0067] FIG. 24 shows the average ROR2 Plasma Membrane H-Scores by cancer indication.

[0068] FIGS. 25A-25F show ROR2 in normal human TMA tissues at 20x magnification. FIG. 25A shows ROR2 in normal kidney, % Score $\geq 1+$: 0 (slide #375), FIG. 25B shows ROR2 in normal liver, % Score $\geq 1+$: 0 (slide #375), FIG. 25C shows ROR2 in normal intestine, % Score $\geq 1+$: 0 (slide #375), FIG. 25D shows ROR2 in normal brain, % Score $\geq 1+$: 0 (slide #375), FIG. 25E shows ROR2 in normal thyroid, % Score $\geq 1+$: 10 (slide #375), and FIG. 25F shows ROR2 in normal tonsil, % Score $\geq 1+$: 10 (slide #375).

[0069] FIG. 26 shows precision and reproducibility of ROR2 in Sarcoma Q9403.

[0070] FIG. 27 shows precision and reproducibility of ROR2 in Sarcoma QMTB313-07.

[0071] FIG. 28 shows precision and reproducibility of ROR2 in Ovarian Cancer Q7499-02.

[0072] FIG. 29 shows precision and reproducibility of ROR2 in Ovarian Cancer QMTB400-05.

[0073] FIG. 30 shows precision and reproducibility of ROR2 in NSCLC Q6949-01.

[0074] FIG. 31 shows precision and reproducibility of ROR2 in NSCLC Q4044.

[0075] FIG. 32 shows precision and reproducibility of ROR2 in TNBC Q9333.

[0076] FIG. 33 shows precision and reproducibility of ROR2 in TNBC Q9250.

[0077] FIG. 34 shows an embodiment of the dosing schedule for use of the polypeptides of the present invention for the treatment of non-small cell lung cancer.

DEFINITIONS

[0078] In order to facilitate understanding of the examples provided herein, certain frequently occurring terms are defined herein.

[0079] In connection with a measured quantity, the term "about" as used herein refers to the normal variation in that measured quantity that would be expected by a skilled person making the measurement and exercising a level of care commensurate with the objective of the measurement and the precision of the measuring equipment used. Unless otherwise indicated, "about" refers to a variation of +/- 10% of the value provided.

[0080] The term "affinity" as used herein refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (e.g., an antibody) and its binding partner (e.g., an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (e.g., antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_d). Affinity can be measured by common methods known in the art, including those described herein. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0081] The term "affinity matured" antibody as used herein refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen. The term "amino acid" as used herein refers to any organic compound that contains an amino group (--NH₂) and a carboxyl group (--COOH); preferably either as free groups or alternatively after condensation as part of peptide bonds. The "twenty naturally encoded polypeptide-forming alpha-amino acids" are understood in the art and refer to: alanine (ala or A), arginine (arg or R), asparagine (asn or N), aspartic acid (asp or D), cysteine (cys or C), glutamic acid (glu or E), glutamine (gin or Q), glycine (gly or G), histidine (his or H), isoleucine (ile or I), leucine (leu or L), lysine (lys or K), methionine (met or M), phenylalanine (phe or F), proline (pro or P), serine (ser or S), threonine (thr or T), tryptophan (tip or W), tyrosine (tyr or Y), and valine (val or V).

[0082] The term "antibody" as used herein refers to intact immunoglobulin molecules, as well as fragments of immunoglobulin molecules, such as Fab, Fab', (Fab')₂, Fv, and SCA fragments, that are capable of binding to an epitope of an antigen. These antibody fragments,

which retain some ability to selectively bind to an antigen (e.g., a polypeptide antigen) of the antibody from which they are derived, can be made using well known methods in the art (see, e.g., Harlow and Lane, supra), and are described further, as follows. Antibodies can be used to isolate preparative quantities of the antigen by immunoaffinity chromatography. Various other uses of such antibodies are to diagnose and/or stage disease (e.g., neoplasia) and for therapeutic application to treat disease, such as for example: neoplasia, autoimmune disease, AIDS, cardiovascular disease, infections, and the like. Chimeric, human-like, humanized or fully human antibodies are particularly useful for administration to human patients.

[0083] An Fab fragment consists of a monovalent antigen-binding fragment of an antibody molecule, and can be produced by digestion of a whole antibody molecule with the enzyme papain, to yield a fragment consisting of an intact light chain and a portion of a heavy chain.

[0084] An Fab' fragment of an antibody molecule can be obtained by treating a whole antibody molecule with pepsin, followed by reduction, to yield a molecule consisting of an intact light chain and a portion of a heavy chain. Two Fab' fragments are obtained per antibody molecule treated in this manner.

[0085] An (Fab')₂ fragment of an antibody can be obtained by treating a whole antibody molecule with the enzyme pepsin, without subsequent reduction. A (Fab')₂ fragment is a dimer of two Fab' fragments, held together by two disulfide bonds.

[0086] An Fv fragment is defined as a genetically engineered fragment containing the variable region of a light chain and the variable region of a heavy chain expressed as two chains.

[0087] The term “antibody fragment” as used herein refers to a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments.

[0088] The terms “anti-ROR2 antibody,” “ROR2 antibody” and “an antibody that binds to ROR2” as used herein refer to an antibody that is capable of binding ROR2 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting ROR2. In one embodiment, the extent of binding of an anti-ROR2 antibody to an unrelated, non-ROR2 protein is less than about 10% of the binding of the antibody to ROR2 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to ROR2 has a dissociation constant (K_d) of $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g. 10^{-8}M or less, e.g. from 10^{-8}M to 10^{-13}M , e.g., from

10^{-9}M to 10^{-13}M). In certain embodiments, an anti-ROR2 antibody binds to an epitope of ROR2 that is conserved among ROR2 from different species.

[0089] The term “binding” as used herein refers to interaction of the variable region or an Fv of an antibody with an antigen with the interaction depending upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the antigen. For example, an antibody variable region or Fv recognizes and binds to a specific protein structure rather than to proteins generally. As used herein, the term “specifically binding” or “binding specifically” means that an antibody variable region or Fv binds to or associates with more frequently, more rapidly, with greater duration and/or with greater affinity with a particular antigen than with other proteins. For example, an antibody variable region or Fv specifically binds to its antigen with greater affinity, avidity, more readily, and/or with greater duration than it binds to other antigens. For another example, an antibody variable region or Fv binds to a cell surface protein (antigen) with materially greater affinity than it does to related proteins or other cell surface proteins or to antigens commonly recognized by polyreactive natural antibodies (i.e., by naturally occurring antibodies known to bind a variety of antigens naturally found in humans). However, “specifically binding” does not necessarily require exclusive binding or non-detectable binding of another antigen, this is meant by the term “selective binding”. In one example, “specific binding” of an antibody variable region or Fv (or other binding region) binds to an antigen, means that the an antibody variable region or Fv binds to the antigen with an equilibrium constant (KD) of 100 nM or less, such as 50nM or less, for example 20nM or less, such as, 15nM or less, or 10 nM or less, or 5nM or less, 2 nM or less, or 1 nM or less.

[0090] The terms “cancer” and “cancerous” as used herein refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth/proliferation. Examples of cancer include, but are not limited to, melanoma, carcinoma, lymphoma (e.g., Hodgkin's and non-Hodgkin's lymphoma), blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer (NSCLC), adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, leukemia and other lymphoproliferative disorders, fibrosarcoma, osteosarcoma and various types of head and neck cancer.

[0091] The terms “cell proliferative disorder” and “proliferative disorder” as used herein refer to disorders that are associated with some degree of abnormal cell proliferation. In one embodiment, the cell proliferative disorder is cancer.

[0092] The term “chemotherapeutic agent” as used herein refers to a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN®); alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylomelamine; acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan (HYCAMTIN®), CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scoplectin, and 9-aminocamptothecin); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin; podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, chlorophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosoureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII (see, e.g., Nicolaou et al., *Angew. Chem. Intl. Ed. Engl.*, 33: 183-186 (1994)); CDP323, an oral alpha-4 integrin inhibitor; dynemicin, including dynemicin A; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores), aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, caminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including ADRIAMYCIN®, morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, doxorubicin HCl liposome injection (DOXIL®), liposomal doxorubicin TLC D-99 (MYOCET®), pegylated liposomal doxorubicin (CAELYX®), and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate,

gemcitabine (GEMZAR®), tegafur (UFTORAL®), capecitabine (XELODA®), an epothilone, and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, methotrexate, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanane, testolactone; anti-adrenals such as aminogluthethimide, mitotane, trilostane; folic acid replenisher such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diazi quone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triazi quone; 2,2',2'-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); thiotepa; taxoid, e.g., paclitaxel (TAXOL®), albumin-engineered nanoparticle formulation of paclitaxel (ABRAXANE™), and docetaxel (TAXOTERE®); chloranbucil; 6-thioguanine; mercaptopurine; methotrexate; platinum agents such as cisplatin, oxaliplatin (e.g., ELOXATIN®), and carboplatin; vincas, which prevent tubulin polymerization from forming microtubules, including vinblastine (VELBAN®), vincristine (ONCOVIN®), vindesine (ELDISINE®, FILDESIN®), and vinorelbine (NAVELBINE®); etoposide (VP-16); ifosfamide; mitoxantrone; leucovorin; novantrone; edatrexate; daunomycin; aminopterin; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMF®); retinoids such as retinoic acid, including bexarotene (TARGRETIN®); bisphosphonates such as clodronate (for example, BONEFOS® or OSTAC®), etidronate (DIDROCAL®), NE-58095, zoledronic acid/zoledronate (ZOMETA®), alendronate (FOSAMAX®), pamidronate (AREDIA®), tiludronate (SKELID®), or risedronate (ACTONEL®); troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in aberrant cell proliferation, such as, for example, PKC-alpha, Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as THERATOPE® vaccine and gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; topoisomerase I inhibitor (e.g., LURTOTECAN®); rmRH (e.g., ABARELIX®); BAY439006 (sorafenib;

Bayer); SU-11248 (sunitinib, SUTENT®, Pfizer); perifosine, COX-2 inhibitor (e.g. celecoxib or etoricoxib), proteasome inhibitor (e.g. PS341); bortezomib (VELCADE®); CCI-779; tipifarnib (R11577); orafenib, ABT510; Bcl-2 inhibitor such as oblimersen sodium (GENASENSE®); pixantrone; EGFR inhibitors (see definition below); tyrosine kinase inhibitors (see definition below); serine-threonine kinase inhibitors such as rapamycin (sirolimus, RAPAMUNE®); farnesyltransferase inhibitors such as lonafarnib (SCH 6636, SARASAR™); and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone; and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

[0093] Chemotherapeutic agents as defined herein include “anti-hormonal agents” or “endocrine therapeutics,” which act to regulate, reduce, block, or inhibit the effects of hormones that can promote the growth of cancer. They may be hormones themselves, including, but not limited to: anti-estrogens with mixed agonist/antagonist profile, including, tamoxifen (NOLVADEX®), 4-hydroxytamoxifen, toremifene (FARESTON®), idoxifene, droloxifene, raloxifene (EVISTA®), trioxifene, keoxifene, and selective estrogen receptor modulators (SERMs) such as SERM3; pure anti-estrogens without agonist properties, such as fulvestrant (FASLODEX®), and EM800 (such agents may block estrogen receptor (ER) dimerization, inhibit DNA binding, increase ER turnover, and/or suppress ER levels); aromatase inhibitors, including steroidal aromatase inhibitors such as formestane and exemestane (AROMASIN®), and nonsteroidal aromatase inhibitors such as anastrozole (ARIMIDEX®), letrozole (FEMARA®) and aminoglutethimide, and other aromatase inhibitors include vorozole (RIVISOR®), megestrol acetate (MEGASE®), fadrozole, and 4(5)-imidazoles; lutenizing hormone-releasing hormone agonists, including leuprolide (LUPRON® and ELIGARD®), goserelin, buserelin, and triptorelin; sex steroids, including progestines such as megestrol acetate and medroxyprogesterone acetate, estrogens such as diethylstilbestrol and premarin, and androgens/retinoids such as fluoxymesterone, all transretinoic acid and fenretinide; onapristone; anti-progesterones; estrogen receptor down-regulators (ERDs); anti-androgens such as flutamide, nilutamide and bicalutamide; and pharmaceutically acceptable salts, acids or derivatives of any of the above; as well as combinations of two or more of the above.

[0094] The term “chimeric” antibody as used herein refers to an antibody in which a portion of the heavy and/or light chain is derived from a particular source or species, while the remainder of the heavy and/or light chain is derived from a different source or species.

[0095] The term “class” of an antibody as used herein refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively.

[0096] The term "conditionally active antibody" as used herein refers to an antibody which is more active under a condition in the tumor microenvironment compared to under a condition in the non-tumor microenvironment. The conditions in the tumor microenvironment include lower pH, higher concentrations of lactate and pyruvate, hypoxia, lower concentration of glucose, and slightly higher temperature in comparison with non-tumor microenvironment. For example, a conditionally active antibody is virtually inactive at normal body temperature, but is active at a higher temperature in a tumor microenvironment. In yet another aspect, the conditionally active antibody is less active in normal oxygenated blood, but more active under a less oxygenated environment exists in tumor. In yet another aspect, the conditionally active antibody is less active in normal physiological pH 7.2-7.8, but more active under an acidic pH 5.8-7.0, or 6.0-6.8 that exists in a tumor microenvironment. There are other conditions in the tumor microenvironment known to a person skilled in the field may also be used as the condition in the present invention under which the anti-ROR2 antibodies to have different binding affinity to ROR2.

[0097] The term “constitutive” as used herein, as for example applied to ROR2 activity, refers to continuous signaling activity of the receptor kinase that is not dependent on the presence of a ligand or other activating molecules. Depending on the nature of the receptor kinase, all of the activity may be constitutive or the activity of the receptor may be further activated by the binding of other molecules (e.g. ligands). Cellular events that lead to activation of receptor kinase are well known among those of ordinary skill in the art. For example, activation may include oligomerization, e.g., dimerization, trimerization, etc., into higher order receptor complexes. Complexes may comprise a single species of protein, i.e., a homomeric complex. Alternatively, complexes may comprise at least two different protein species, i.e., a heteromeric complex. Complex formation may be caused by, for example, overexpression of normal or mutant forms of receptor on the surface of a cell. Complex formation may also be caused by a specific mutation or mutations in a receptor.

[0098] The term “cytostatic agent” as used herein refers to a compound or composition which arrests growth of a cell either in vitro or in vivo. Thus, a cytostatic agent may be one which significantly reduces the percentage of cells in S phase. Further examples of cytostatic

agents include agents that block cell cycle progression by inducing G0/G1 arrest or M-phase arrest. The humanized anti-Her2 antibody trastuzumab (HERCEPTIN®) is an example of a cytostatic agent that induces G0/G1 arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxanes, and topoisomerase II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin. Certain agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in Mendelsohn and Israel, eds., *The Molecular Basis of Cancer*, Chapter 1, entitled “Cell cycle regulation, oncogenes, and antineoplastic drugs” by Murakami et al. (W.B. Saunders, Philadelphia, 1995), e.g., p. 13. The taxanes (paclitaxel and docetaxel) are anticancer drugs both derived from the yew tree. Docetaxel (TAXOTERE®, Rhone-Poulenc Rorer), derived from the European yew, is a semisynthetic analogue of paclitaxel (TAXOL®, Bristol-Myers Squibb). Paclitaxel and docetaxel promote the assembly of microtubules from tubulin dimers and stabilize microtubules by preventing depolymerization, which results in the inhibition of mitosis in cells.

[0099] The term “cytotoxic agent” as used herein refers to a substance that inhibits or prevents a cellular function and/or causes cell death or destruction. Cytotoxic agents include, but are not limited to radioactive isotopes (e.g., At^{211} , I^{131} , I^{125} , Y^{90} , Re^{186} , Re^{188} , Sm^{153} , Bi^{212} , P^{32} , Pb^{212} and radioactive isotopes of Lu); chemotherapeutic agents or drugs (e.g., methotrexate, adriamycin, vinca alkaloids (vincristine, vinblastine, etoposide), doxorubicin, melphalan, mitomycin C, chlorambucil, daunorubicin or other intercalating agents); growth inhibitory agents; enzymes and fragments thereof such as nucleolytic enzymes; antibiotics; toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including fragments and/or variants thereof; and the various antitumor or anticancer agents disclosed below.

[0100] The term “diabodies” as used herein refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H - V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

[0101] The term “detectably label” as used herein refers to any substance whose detection or measurement, either directly or indirectly, by physical or chemical means, is indicative of the presence of the CTCs in a sample. Representative examples of useful detectable labels, include, but are not limited to the following: molecules or ions directly or indirectly

detectable based on light absorbance, fluorescence, reflectance, light scatter, phosphorescence, or luminescence properties; molecules or ions detectable by their radioactive properties; molecules or ions detectable by their nuclear magnetic resonance or paramagnetic properties. Included among the group of molecules indirectly detectable based on light absorbance or fluorescence, for example, are various enzymes which cause appropriate substrates to convert, e.g., from non-light absorbing to light absorbing molecules, or from non-fluorescent to fluorescent molecules.

[0102] The term "diagnostics" as used herein refers to determination of a subject's susceptibility to a disease or disorder, determination as to whether a subject is presently affected by a disease or disorder, prognosis of a subject affected by a disease or disorder (e.g., identification of pre- metastatic or metastatic cancerous states, stages of cancer, or responsiveness of cancer to therapy), and therametrics (e.g., monitoring a subject's condition to provide information as to the effect or efficacy of therapy). In some embodiments, the diagnostic method of this invention is particularly useful in detecting early stage cancers.

[0103] The term "diagnostic agent" as used herein refers to a molecule which can be directly or indirectly detected and is used for diagnostic purposes. The diagnostic agent may be administered to a subject or a sample. The diagnostic agent can be provided per se or may be conjugated to a vehicle such as a conditionally active antibody.

[0104] The term "effector functions" as used herein refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g. B cell receptor); and B cell activation.

[0105] The term "effective amount" of an agent as used herein, e.g., a pharmaceutical formulation, refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic or prophylactic result.

[0106] The term "Fc region" as used herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as

described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

[0107] The term “framework” or “FR” as used herein refers to variable domain residues other than hypervariable region (HVR or H1-3 in the heavy chain and L1-3 in the light chain) residues. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in V_H (or V_L): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

[0108] The term “full length antibody,” “intact antibody,” or “whole antibody” refers to an antibody which comprises an antigen-binding variable region (V_H or V_L) as well as a light chain constant domain (CL) and heavy chain constant domains, CH1, CH2 and CH3. The constant domains may be native sequence constant domains (e.g. human native sequence constant domains) or amino acid sequence variants thereof. Depending on the amino acid sequence of the constant domain of their heavy chains, full length antibodies can be assigned to different “classes”. There are five major classes of full length antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into “subclasses” (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called alpha, delta, epsilon, gamma, and mu, respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

[0109] The terms “host cell,” “host cell line,” and “host cell culture” as used herein are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “transformants” and “transformed cells,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

[0110] The term “human antibody” as used herein is one which possesses an amino acid sequence which corresponds to that of an antibody produced by a human or a human cell or derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues.

[0111] The term “human consensus framework” as used herein is a framework which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin V_L or V_H framework sequences. Generally, the selection of human

immunoglobulin V_L or V_H sequences is from a subgroup of variable domain sequences.

Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, NIH Publication 91-3242, Bethesda Md. (1991), vols. 1-3. In one embodiment, for the V_L, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In one embodiment, for the V_H, the subgroup is subgroup III as in Kabat et al., *supra*.

[0112] The term “humanized” antibody as used herein refers to a chimeric antibody comprising amino acid residues from non-human HVRs and amino acid residues from human FRs. In certain embodiments, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the HVRs (e.g., CDRs) correspond to those of a non-human antibody, and all or substantially all of the FRs correspond to those of a human antibody. A humanized antibody optionally may comprise at least a portion of an antibody constant region derived from a human antibody. A “humanized form” of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

[0113] The term “hypervariable region” or “HVR” as used herein refers to each of the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops (“hypervariable loops”). Generally, native four-chain antibodies comprise six HVRs; three in the V_H (H1, H2, H3), and three in the V_L (L1, L2, L3). HVRs generally comprise amino acid residues from the hypervariable loops and/or from the “complementarity determining regions” (CDRs), the latter being of highest sequence variability and/or involved in antigen recognition. Exemplary hypervariable loops occur at amino acid residues 26-32 (L1), 50-52 (L2), 91-96 (L3), 26-32 (H1), 53-55 (H2), and 96-101 (H3). (Chothia and Lesk, *J. Mol. Biol.*, vol. 196, pp. 901-917 1987) Exemplary CDRs (CDR-L1, CDR-L2, CDR-L3, CDR-H1, CDR-H2, and CDR-H3) occur at amino acid residues 24-34 of L1, 50-56 of L2, 89-97 of L3, 31-35B of H1, 50-65 of H2, and 95-102 of H3 (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. 1991). With the exception of CDR1 in V_H, CDRs generally comprise the amino acid residues that form the hypervariable loops. CDRs also comprise “specificity determining residues,” or “SDRs,” which are residues that contact antigen. SDRs are contained within regions of the CDRs called abbreviated-CDRs, or a-CDRs. Exemplary a-CDRs (a-CDR-L1, a-CDR-L2, a-CDR-L3, a-CDR-H1, a-CDR-H2, and a-CDR-H3) occur at amino acid residues 31-34 of L1, 50-55 of L2, 89-96 of L3, 31-35B of H1, 50-58 of H2, and 95-102 of H3. (See Almagro and Fransson, *Front. Biosci.*, vol. 13, pp.1619-1633, 2008). Unless otherwise indicated, HVR residues and other residues in the variable domain (e.g., FR residues) are numbered herein according to Kabat et al., *supra*.

[0114] The term “immunoconjugate” as used herein is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a cytotoxic agent.

[0115] The term “individual” or “subject” as used herein refers to a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

[0116] The term “inhibiting cell growth or proliferation” as used herein means decreasing a cell's growth or proliferation by at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, and includes inducing cell death.

[0117] The term “isolated” antibody as used herein is one which has been separated from a component of its natural environment. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (e.g., SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (e.g., ion exchange or reverse phase High Performance Liquid Chromatography (HPLC)). For review of methods for assessment of antibody purity, see, e.g., Flatman et al., *J. Chromatogr. B*, vol. 848, pp. 79-87, 2007.

[0118] The term “isolated” nucleic acid as used herein refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

[0119] The term “isolated nucleic acid encoding an anti-ROR2 antibody” as used herein refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

[0120] The term “ligand-independent” as used herein, as for example applied to receptor signaling activity, refers to signaling activity that is not dependent on the presence of a ligand. A receptor having ligand-independent kinase activity will not necessarily preclude the binding of ligand to that receptor to produce additional activation of the kinase activity.

[0121] The term “metastasis” as used herein refers to all ROR2-involving processes that support cancer cells to disperse from a primary tumor, penetrate into lymphatic and/or blood vessels, circulate through the bloodstream, and grow in a distant focus (metastasis) in normal tissues elsewhere in the body. In particular, it refers to cellular events of tumor cells such as proliferation, migration, anchorage independence, evasion of apoptosis, or secretion of angiogenic factors, that underlie metastasis and are stimulated or mediated by non-catalytic

or catalytic activities of ROR2, preferably including ROR2 phosphorylation and/or ROR2-mediated signal transduction.

[0122] The term "microenvironment" as used herein means any portion or region of a tissue or body that has constant or temporal, physical or chemical differences from other regions of the tissue or regions of the body. For tumors, the term "tumor microenvironment" as used herein refers to the environment in which a tumor exists, which is the non-cellular area within the tumor and the area directly outside the tumorous tissue but does not pertain to the intracellular compartment of the cancer cell itself. The tumor and the tumor microenvironment are closely related and interact constantly. A tumor can change its microenvironment, and the microenvironment can affect how a tumor grows and spreads. Typically, the tumor microenvironment has a low pH in the range of 5.8 to 7.0, more commonly in the range of 6.0 to 6.8, in the range of 6.2-6.8. On the other hand, a normal physiological pH is in the range of 7.2-7.8. The tumor microenvironment is also known to have lower concentration of glucose and other nutrients, but higher concentration of lactic acid, in comparison with blood plasma. Furthermore, the tumor microenvironment can have a temperature that is 0.3 to 1 °C higher than the normal physiological temperature. The tumor microenvironment has been discussed in Gillies et al., "MRI of the Tumor Microenvironment," *Journal of Magnetic Resonance Imaging*, vol. 16, pp.430-450, 2002, hereby incorporated by reference herein its entirety. The term "non-tumor microenvironment" refers to a microenvironment at a site other than a tumor.

[0123] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical and/or bind the same epitope, except for possible variant antibodies, e.g., containing naturally occurring mutations or arising during production of a monoclonal antibody preparation, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations, which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody of a monoclonal antibody preparation is directed against a single determinant on an antigen. Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including but not limited to the hybridoma method, recombinant DNA methods, phage-display methods, and methods utilizing transgenic animals containing all or part of the human

immunoglobulin loci, such methods and other exemplary methods for making monoclonal antibodies being described herein.

[0124] The term “naked antibody” as used herein refers to an antibody that is not conjugated to a heterologous moiety (e.g., a cytotoxic moiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

[0125] The term “native antibodies” as used herein refers to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (V_H), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains ($CH1$, $CH2$, and $CH3$). Similarly, from N- to C-terminus, each light chain has a variable region (V_L), also called a variable light domain or a light chain variable domain, followed by a constant light (C_L) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

[0126] The term “package insert” as used herein is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products.

[0127] The term “percent (%) amino acid sequence identity” with respect to a reference polypeptide sequence as used herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly

available from Genentech, Inc., South San Francisco, Calif., or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

[0128] In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

$$100 \text{ times the fraction } X/Y$$

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

[0129] The term “pharmaceutical formulation” as used herein refers to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

[0130] The term “pharmaceutically acceptable carrier” as used herein refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

[0131] The terms “purified” and “isolated” used herein refer to an antibody according to the invention or to a nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term “purified” as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type are present. An “isolated” nucleic acid molecule which encodes a particular polypeptide refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the polypeptide;

however, the molecule may include some additional bases or moieties which do not deleteriously affect the basic characteristics of the composition.

[0132] The term “recombinant antibody” as used herein refers to an antibody (e.g. a chimeric, humanized, or human antibody or antigen-binding fragment thereof) that is expressed by a recombinant host cell comprising nucleic acid encoding the antibody. Examples of “host cells” for producing recombinant antibodies include: (1) mammalian cells, for example, Chinese Hamster Ovary (CHO), COS, myeloma cells (including Y0 and NS0 cells), baby hamster kidney (BHK), HeLa and Vero cells; (2) insect cells, for example, sf9, sf21 and Tn5; (3) plant cells, for example plants belonging to the genus *Nicotiana* (e.g. *Nicotiana tabacum*); (4) yeast cells, for example, those belonging to the genus *Saccharomyces* (e.g. *Saccharomyces cerevisiae*) or the genus *Aspergillus* (e.g. *Aspergillus niger*); (5) bacterial cells, for example *Escherichia coli* cells or *Bacillus subtilis* cells, etc.

[0133] The term “ROR2” as used herein, refers to receptor tyrosine kinase-like orphan receptor 2, which is a predicted 943-amino acid protein with in vitro protein kinase activity, shown in Genbank accession number AAI30523. Many lineage-restricted receptor tyrosine kinases were initially identified as ‘orphans’ homologous to known receptors, and only subsequently used to identify their unknown growth factors. DeChiara et al. (2000) identified one such orphan, encoded by ROR2 as shown in FIG. 1.

[0134] The term “therapeutically effective amount” of the antibody of the invention is meant a sufficient amount of the antibody to treat said cancer, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the antibodies and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific antibody employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific antibody employed; the duration of the treatment; drugs used in combination or coincidental with the specific antibody employed; and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

[0135] The term “single chain Fv” (“scFv”) as used herein is a covalently linked V_H:V_L heterodimer which is usually expressed from a gene fusion including V_H and V_L encoding genes linked by a peptide-encoding linker. “dsFv” is a V_H:V_L heterodimer stabilised by a

disulfide bond. Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling monovalent scFvs by a peptide linker, such as divalent sc(Fv)₂.

[0136] The term “treatment,” “treat,” or “treating” as used herein refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, antibodies of the invention are used to delay development of a disease or to slow the progression of a disease.

[0137] The term “tumor” as used herein refers to all neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues. The terms “cancer,” “cancerous,” “cell proliferative disorder,” “proliferative disorder” and “tumor” are not mutually exclusive as referred to herein.

[0138] The term “variable region” or “variable domain” as used herein refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (V_H and V_L, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, e.g., Kindt et al. Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single V_H or V_L domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a V_H or V_L domain from an antibody that binds the antigen to screen a library of complementary V_L or V_H domains, respectively. See, e.g., Portolano et al., *J. Immunol.*, vol. 150, pp. 880-887, 1993; Clarkson et al., *Nature*, vol. 352, pp. 624-628, 1991.

[0139] The term “vector” as used herein refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as “expression vectors.”

DETAILED DESCRIPTION

[0140] For illustrative purposes, the principles of the present invention are described by referencing various exemplary embodiments. Although certain embodiments of the invention are specifically described herein, one of ordinary skill in the art will readily recognize that the same principles are equally applicable to, and can be employed in, other systems and methods. Before explaining the disclosed embodiments of the present invention in detail, it is to be understood that the invention is not limited in its application to the details of any particular embodiment shown. Additionally, the terminology used herein is for the purpose of description and not for limitation. Furthermore, although certain methods are described with reference to steps that are presented herein in a certain order, in many instances, these steps can be performed in any order as may be appreciated by one skilled in the art; the novel method is therefore not limited to the particular arrangement of steps disclosed herein.

[0141] It must be noted that as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural references unless the context clearly dictates otherwise. Furthermore, the terms “a” (or “an”), “one or more”, and “at least one” can be used interchangeably herein. The terms “comprising”, “including”, “having” and “constructed from” can also be used interchangeably.

[0142] Unless otherwise indicated, all numbers expressing quantities of ingredients, properties such as molecular weight, percent, ratio, reaction conditions, and so forth used in the specification and claims are to be understood as being modified in all instances by the term “about,” whether or not the term “about” is present. Accordingly, unless indicated to the contrary, the numerical parameters set forth in the specification and claims are approximations that may vary depending upon the desired properties sought to be obtained by the present disclosure. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the disclosure are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains certain errors necessarily resulting from the standard deviation found in their respective testing measurements.

[0143] It is to be understood that each component, compound, substituent, or parameter disclosed herein is to be interpreted as being disclosed for use alone or in combination with one or more of each and every other component, compound, substituent, or parameter disclosed herein.

[0144] It is also to be understood that each amount/value or range of amounts/values for each component, compound, substituent, or parameter disclosed herein is to be interpreted as also being disclosed in combination with each amount/value or range of amounts/values disclosed for any other component(s), compounds(s), substituent(s), or parameter(s) disclosed herein and that any combination of amounts/values or ranges of amounts/values for two or more component(s), compounds(s), substituent(s), or parameters disclosed herein are thus also disclosed in combination with each other for the purposes of this description.

[0145] It is further understood that each lower limit of each range disclosed herein is to be interpreted as disclosed in combination with each upper limit of each range disclosed herein for the same component, compounds, substituent, or parameter. Thus, a disclosure of two ranges is to be interpreted as a disclosure of four ranges derived by combining each lower limit of each range with each upper limit of each range. A disclosure of three ranges is to be interpreted as a disclosure of nine ranges derived by combining each lower limit of each range with each upper limit of each range, etc. Furthermore, specific amounts/values of a component, compound, substituent, or parameter disclosed in the description or an example is to be interpreted as a disclosure of either a lower or an upper limit of a range and thus can be combined with any other lower or upper limit of a range or specific amount/value for the same component, compound, substituent, or parameter disclosed elsewhere in the application to form a range for that component, compound, substituent, or parameter.

A. Anti-ROR2 Antibodies

[0146] In one aspect, the present invention provides an isolated polypeptide that specifically binds to human ROR2 protein and uses of the polypeptide in methods of treating a ROR2-expressing tumor that involves administering the polypeptide to a human in need of such treatment.

[0147] The polypeptide comprises a heavy chain variable region having three complementarity determining regions H1, H2, and H3 sequences, wherein:

the H1 sequence is GYTX₁TEX₂X₃X₄H (SEQ ID NO:1) or GYSITTGX₂₉YWN (SEQ ID NO:4);

the H2 sequence is X₅X₆X₇X₈NNGGTGYNQKFKG (SEQ ID NO:2) or YITYDGSX₃₀NYNPSLKN (SEQ ID NO:5); and

the H3 sequence is X₉X₁₀X₁₁SX₁₂YX₁₃YX₁₄X₁₅SYFX₁₆X₁₇X₁₈ (SEQ ID NO:3) or CSX₃₁X₃₂X₃₃X₃₄VX₃₅X₃₆X₃₇LDX₃₈ (SEQ ID NO:6);

wherein

X₁ is F or E,

X₂ is Y or D,
 X₃ is T or C,
 X₄ is M or D or E or Y,
 X₅ is G or S,
 X₆ is I or E,
 X₇ is N or C or L or V,
 X₈ is T or D or E,
 X₉ is A or M or T,
 X₁₀ is R or H,
 X₁₁ is G or E,
 X₁₂ is L or F,
 X₁₃ is S or G,
 X₁₄ is G or D,
 X₁₅ is N or E,
 X₁₆ is D or L,
 X₁₇ is Y or C or T,
 X₁₈ is W or L,
 X₂₉ is Y or E or R or T,
 X₃₀ is K or N,
 X₃₁ is R or G or H or W or Y,
 X₃₂ is F or C or N or Q,
 X₃₃ is E or S,
 X₃₄ is G or E or F or H or M or Q or S,
 X₃₅ is W or A or I or P or Q or T or V,
 X₃₆ is Y or G or N or Q,
 X₃₇ is G or S or T, and
 X₃₈ is Y or I; and

a light chain variable region having three complementarity determining regions L1, L2, and L3 sequences, wherein:

the L1 sequence is SATSSX₁₉X₂₀X₂₁MX₂₂ (SEQ ID NO:7) or

RASESVDRYGNSX₃₉IH (SEQ ID NO:10);

the L2 sequence is X₂₃TSNLAS (SEQ ID NO:8) or X₄₀TYX₄₁LES (SEQ ID NO:11);

and

the L3 sequence is QX₂₄X₂₅SX₂₆YPFX₂₇X₂₈ (SEQ ID NO:9) or

QQX₄₂NX₄₃DPX₄₄TX₄₅ (SEQ ID NO:12);

wherein

X₁₉ is V or E,

X₂₀ is S or D,

X₂₁ is Y or C or D,

X₂₂ is H or G or L,

X₂₃ is G or C or H or P,

X₂₄ is Q or E,

X₂₅ is R or H,

X₂₆ is S or D or G or I or Q or V,

X₂₇ is T or D,

X₂₈ is F or D or E,

X₃₉ is F or S or T,

X₄₀ is R or C or D or E or W,

X₄₁ is N or D,

X₄₂ is T or I or P,

X₄₃ is E or V,

X₄₄ is W or T, and

X₄₅ is F or T,

with the proviso that X₁ to X₂₈ cannot simultaneously be F, Y, T, M, G, I, N, T, A, R, G, L, S, G, N, D, Y, W, V, S, Y, H, G, Q, R, S, T, and F, respectively in order to exclude the non-conditionally active parent antibody.

[0148] The alignment of the heavy chain variable regions is shown in FIGS. 2A-1, 2A-2, 2B-1, and 2B-2.

[0149] The alignment of the light chain variable regions is shown in FIGS. 3A-1, 3A-2, 3B-1 and 3B-2.

[0150] In another aspect, the present invention provides an isolated polypeptide as described above which has up to one substitution in CDRs H1, H2 and H3, relative to the parent polypeptide and up to one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide. This includes (1) isolated polypeptides with one substitution in CDRs H1, H2 and H3, relative to the parent polypeptide, (2) isolated polypeptides with one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide, and (3) isolated polypeptides with one substitution in CDRs H1, H2 and H3, and one substitution in CDRs L1, L2 and L3, relative to the parent polypeptide, relative to the parent polypeptide.

[0151] The possible single point mutations in CDRs H1, H2 and H3 are shown in FIGS. 2A-1, 2A-2 and the possible single point mutations in CDRs L1, L2 and L3 are shown in FIGS.

3A-1 and 3A-2. Other possible single point mutations in CDRs H1, H2 and H3 are shown in FIGS. 2B-1, and 2B-2 and other possible single point mutations in CDRs L1, L2 and L3 are shown in FIGS. 3B-1 and 3B-2

[0152] In another aspect, each of the heavy chain variable region polypeptides and each of the light chain variable region polypeptides may each have 0, 1, 2, 3, 4 or 5 independently selected substitutions in the CDRs selected from the point mutations shown in Figures 2B-1-2 and Figures 2A-1-2, respectively, as long as at least one of the heavy and light chain variable region polypeptides has at least one point mutation relative to the parent polypeptide. For example, the heavy chain variable region polypeptide may have three point mutations relative to the parent polypeptide and the light chain variable region may have five point mutations relative to the parent polypeptide

[0153] The present invention identified these heavy chain variable regions and light chain variable regions, respectively, from the heavy chain variable region and light chain variable region of a parent antibody through a method disclosed in U.S. Patent No. 8,709,755. This method of generating a conditionally active antibody is hereby incorporated by reference herein.

[0154] The DNAs encoding the heavy chain variable region and light chain variable region of the parent antibody were evolved to generate mutant antibody libraries using Comprehensive Positional Evolution (CPE), which each position in the heavy chain variable region and light chain variable region of the parent antibody is randomized one at a time. Each mutant heavy chain/light chain in the libraries has only one single point mutation, in comparison with the heavy chain variable region or light chain variable region of the parent antibody (FIGS. 2A-1, 2A-2, 2B-1, 2B-2, 3A-1, 3A-2, 3B-1, and 3B-2). The mutants in the libraries were screened for selective binding affinity to human ROR2 at pH 6.0 over pH 7.4 by ELISA. The mutant heavy chain/light chain variable regions that are more active at pH 6.0 than at pH 7.4 were selected as the heavy chain/light chain variable regions of conditionally active antibodies, with the single point mutations indicated in each of the heavy chain and light chain variable region (Tables 1 and 2, FIGS. 2A-1, 2A-2, 2B-1, 2B-2, 3A-1, 3A-2, 3B-1, and 3B-2).

Table 1. Conditionally active anti-ROR2 antibody light chain variable regions

Clone	Affinity ELISA Mutants		
	pH 6.0	pH 7.4	ratio , pH 6.0/7.4
LC-V029E	0.406	0.129	3.15
LC-S030D	0.733	0.252	2.91
LC-Y031C	1.341	0.700	1.92
LC-Y031D	0.911	0.405	2.25
LC-H033G	1.103	0.536	2.06
LC-H033L	0.690	0.332	2.08
LC-G049C	0.468	0.133	3.52
LC-G049H	1.873	0.118	15.87
LC-G049P	0.660	0.268	2.46
LC-Q089E	0.438	0.251	1.74
LC-R090H	0.560	0.256	2.19
LC-S092D	1.127	1.122	1.00
LC-S092V	1.226	1.182	1.04
LC-T096D	0.755	0.474	1.59
LC-F097D	0.190	0.107	1.77
LC-F097E	0.489	0.215	2.28

Table 2. Conditionally active anti-ROR2 antibody heavy chain variable regions

Clone	Affinity ELISA mutants		
	pH 6.0	pH 7.4	ratio , pH 6.0/7.4
HC-F029E	1.342	0.742	1.81
HC-Y032D	1.470	0.510	2.88
HC-T033C	0.626	0.232	2.70
HC-M034D	1.244	0.211	5.90
HC-M034E	1.165	0.185	6.30
HC-M034Y	0.903	0.391	2.31
HC-G050S	0.828	0.318	2.60
HC-I051E	1.039	0.100	10.39
HC-N052C	0.995	0.431	2.31
HC-N052L	0.911	0.433	2.10
HC-N052V	0.870	0.340	2.56
HC-T053D	1.108	0.222	4.99
HC-T053E	0.540	0.157	3.44
HC-A097I	1.892	1.730	1.892
HC-A097M	1.977	1.701	1.977
HC-A097S	1.666	1.692	1.666
HC-A097T	1.834	1.692	1.666
HC-R098H	0.928	0.353	2.63
HC-R098Q	0.799	0.276	2.89
HC-G099E	0.616	0.421	1.46
HC-L101F	1.461	1.412	1.461
HC-S103G	0.794	0.655	1.21
HC-G105D	0.509	0.379	1.34
HC-N106E	0.224	0.115	1.95
HC-D110L	1.675	1.403	1.575
HC-Y111C	1.079	0.793	1.36
HC-Y111T	1.862	1.403	1.575
HC-W112L	1.746	1.704	1.746

[0155] In another aspect, the present invention includes the heavy chain variable regions as represented in FIGS. 2A-1, 2A-2, 2B-1, and 2B-2 and the light chain variable regions as presented in FIGS. 3A-1, 3A-2, 3B-1, and 3B-2. Amino acid sequences of the heavy chain variable regions are SEQ ID NOS: 18-26. The amino acid sequences of the light chain variable regions are SEQ ID NOS: 13-17 and 27. These heavy chain variable regions and light chain variable regions can specifically bind to human ROR2. Antibodies or antibody fragments comprising one of these heavy chain variable regions and light chain variable regions have been found to have higher binding affinity to ROR2 at a pH in the tumor microenvironment than at a pH in a non-tumor microenvironment. For example, the antibodies and antibody fragments have a higher binding affinity to ROR2 at pH 6.0 than at pH 7.4.

[0156] The anti-ROR2 antibodies or antibody fragments have a higher binding affinity to ROR2 in a tumor in comparison with their binding affinity to ROR2 in a normal tissue. These anti-ROR2 antibodies or antibody fragments have a longer half-life and reduced side-effects, as well as comparable efficacy, in comparison with monoclonal anti-ROR2 antibodies known in the art. These features permit use of a higher dosage of these anti-ROR2 antibodies or antibody fragments to be delivered to a patient thus being a more effective therapeutic option.

[0157] Though the present invention includes the heavy chain variable regions and light chain variable regions presented in FIGS. 2A-1, 2A-2, 2B-1, 2B-2, 3A-1, 3A-2, 3B-1, and 3B-2, and having amino acid sequences with SEQ ID NOS: 13-24, the present invention also provides variants thereof that can specifically bind to human ROR2. In order to derive these variants, the complementarity determining regions (CDRs) of the heavy chain variable regions (H1-H3) and the complementarity determining regions of the light chain variable regions (L1-L3) should remain intact. However, the amino acid sequence of the heavy chain variable regions and light chains variable regions outside of the complementarity determining regions may be mutated in accordance with the principles of substitution, insertion and deletion as discussed in this application.

[0158] In deriving these variants, one is guided by the process as described herein. The variants of the heavy chain variable regions and light chain variable regions may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the heavy chain variable regions and light chain variable regions, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the heavy chain variable regions and light chain variable regions. Any combination of deletion, insertion, and substitution can be made to arrive at the antibodies or antibody fragments of the present invention, provided that they possess the desired characteristics, e.g., antigen-binding to human ROR2 and/or conditional activity.

Substitution, Insertion, and Deletion Variants

[0159] In certain embodiments, antibody or antibody fragment variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the CDRs and framework regions (FRs). Conservative substitutions are shown in Table 3 under the heading of “conservative substitutions.” More substantial changes are provided in Table 3 under the heading of “exemplary substitutions,” and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into

an antibody or antibody fragment of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, or decreased immunogenicity.

Table 3: Amino acid substitutions

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	Val; Leu; Ile	Val
Arg (R)	Lys; Gln; Asn	Lys
Asn (N)	Gln; His; Asp, Lys; Arg	Gln
Asp (D)	Glu; Asn	Glu
Cys (C)	Ser; Ala	Ser
Gln (Q)	Asn; Glu	Asn
Glu (E)	Asp; Gln	Asp
Gly (G)	Ala	Ala
His (H)	Asn; Gln; Lys; Arg	Arg
Ile (I)	Leu; Val; Met; Ala; Phe; Norleucine	Leu
Leu (L)	Norleucine; Ile; Val; Met; Ala; Phe	Ile
Lys (K)	Arg; Gln; Asn	Arg
Met (M)	Leu; Phe; Ile	Leu
Phe (F)	Trp; Leu; Val; Ile; Ala; Tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Val; Ser	Ser
Trp (W)	Tyr; Phe	Tyr
Tyr (Y)	Trp; Phe; Thr; Ser	Phe
Val (V)	Ile; Leu; Met; Phe; Ala; Norleucine	Leu

[0160] Amino acids may be grouped according to common side-chain properties:

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

[0161] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0162] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g. a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the

parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more CDR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0163] Alterations (e.g., substitutions) may be made in CDRs, e.g., to improve antibody affinity. Such alterations may be made in CDR “hotspots,” i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, *Methods Mol. Biol.*, vol. 207, pp. 179-196, 2008), and/or SDRs (a-CDRs), with the resulting variant V_H or V_L being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in *Methods in Molecular Biology*, vol. 178, pp. 1-37, 2001). In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g., error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves CDR-directed approaches, in which several CDR residues (e.g., 4-6 residues at a time) are randomized. CDR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0164] In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody or antibody fragment to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in CDRs. Such alterations may be outside of CDR “hotspots” or SDRs. In certain embodiments of the variant V_H and V_L sequences provided above, each CDR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0165] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells, *Science*, vol. 244, pp. 1081-1085, 1989. In this method, a residue or group of target residues (e.g., charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody or antibody fragment with antigen is affected. Further substitutions may be introduced at the amino acid locations

demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody or antibody fragment and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0166] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g. for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

[0167] Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. It is known that when a humanized antibody is produced by simply grafting only CDRs in V_H and V_L of an antibody derived from a non-human animal in FRs of the V_H and V_L of a human antibody, the antigen binding activity is reduced in comparison with that of the original antibody derived from a non-human animal. It is considered that several amino acid residues of the V_H and V_L of the non-human antibody, not only in CDRs but also in FRs, are directly or indirectly associated with the antigen binding activity. Hence, substitution of these amino acid residues with different amino acid residues derived from FRs of the V_H and V_L of the human antibody would reduce of the binding activity. In order to resolve the problem, in antibodies grafted with human CDR, attempts have to be made to identify, among amino acid sequences of the FR of the V_H and V_L of human antibodies, an amino acid residue which is directly associated with binding to the antibody, or which interacts with an amino acid residue of CDR, or which maintains the three-dimensional structure of the antibody and which is directly associated with binding to the antigen. The reduced antigen binding activity could be increased by replacing the identified amino acids with amino acid residues of the original antibody derived from a non-human animal.

[0168] Modifications and changes may be made in the structure of the antibodies of the present invention, and in the DNA sequences encoding them, and still obtain a functional molecule that encodes an antibody with desirable characteristics.

[0169] In making the changes in the amino sequences, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring

interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydrophathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydrophathic index on the basis of their hydrophobicity and charge characteristics these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (−0.4); threonine (−0.7); serine (−0.8); tryptophan (−0.9); tyrosine (−1.3); proline (−1.6); histidine (−3.2); glutamate (−3.5); glutamine (−3.5); aspartate (−3.5); asparagine (−3.5); lysine (−3.9); and arginine (−4.5).

[0170] A further object of the present invention also encompasses function-conservative variants of the antibodies of the present invention.

[0171] “Function-conservative variants” are those in which a given amino acid residue in a protein or enzyme has been changed without altering the overall conformation and function of the polypeptide, including, but not limited to, replacement of an amino acid with one having similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, hydrophobic, aromatic, and the like). Amino acids other than those indicated as conserved may differ in a protein so that the percent protein or amino acid sequence similarity between any two proteins of similar function may vary and may be, for example, from 70% to 99% as determined according to an alignment scheme such as by the Cluster Method, wherein similarity is based on the MEGALIGN algorithm. A “function-conservative variant” also includes a polypeptide which has at least 60% amino acid identity as determined by BLAST or FASTA algorithms, preferably at least 75%, more preferably at least 85%, still preferably at least 90%, and even more preferably at least 95%, and which has the same or substantially similar properties or functions as the native or parent protein to which it is compared.

[0172] Two amino acid sequences are “substantially homologous” or “substantially similar” when greater than 80%, preferably greater than 85%, preferably greater than 90% of the amino acids are identical, or greater than about 90%, preferably greater than 95%, are similar (functionally identical) over the whole length of the shorter sequence. Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wis.) pileup program, or any of sequence comparison algorithms such as BLAST, FASTA, etc.

[0173] For example, certain amino acids may be substituted by other amino acids in a protein structure without appreciable loss of activity. Since the interactive capacity and nature of a

protein define the protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and, of course, in its DNA encoding sequence, while nevertheless obtaining a protein with like properties. It is thus contemplated that various changes may be made in the sequences of the antibodies or antibody fragments of the invention, or corresponding DNA sequences which encode said antibodies or antibody fragments, without appreciable loss of their biological activity.

[0174] It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e. still obtain a biological functionally equivalent protein.

[0175] As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

Glycosylation Variants

[0176] In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0177] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. *TIBTECH*, vol. 15, pp. 26-32, 1997. The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the “stem” of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

[0178] In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to

Asn 297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to “defucosylated” or “fucose-deficient” antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. *J. Mol. Biol.*, vol. 336, pp. 1239-1249, 2004; Yamane-Ohnuki et al. *Biotech. Bioeng.*, vol. 87, pp. 614-622, 2004. Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. *Arch. Biochem. Biophys.*, vol. 249, pp. 533-545, 1986; US Pat Appl No US 2003/0157108 A; and WO 2004/056312 A1, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. *Biotech. Bioeng.*, vol. 87, pp. 614-622, 2004; Kanda, Y. et al., *Biotechnol. Bioeng.*, vol. 94, pp. 680-688, 2006; and WO2003/085107).

[0179] Antibody variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878; U.S. Pat. No. 6,602,684; and US 2005/0123546. Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087; WO 1998/58964; and WO 1999/22764.

Fc Region Variants

[0180] In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (e.g., a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (e.g. a substitution) at one or more amino acid positions.

[0181] In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody *in vivo* is important yet certain effector functions (such as ADCC) are unnecessary or deleterious. *In vitro* and/or *in vivo* cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, *Annu. Rev. Immunol.*, vol. 9, pp. 457-492, 1991. Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Pat. No. 5,500,362 (see also, e.g. Hellstrom et al. *Proc. Nat'l Acad. Sci. USA*, vol. 83, pp. 7059-7063, 1986) and Hellstrom, I et al., *Proc. Nat'l Acad. Sci. USA*, vol. 82, pp. 1499-1502, 1985; U.S. Pat. No. 5,821,337 (see also Bruggemann et al., *J. Exp. Med.*, vol. 166, pp. 1351-1361, 1987). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, Calif.; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, Wis.). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in a animal model such as that disclosed in Clynes et al. *Proc. Nat'l Acad. Sci. USA*, vol. 95, pp. 652-656, 1998. C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, e.g., C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro et al., *J. Immunol. Methods*, vol. 202, pp.163-171, 1996; Cragg, M. S. et al., *Blood*, vol. 101, pp. 1045-1052, 2003; and Cragg, M. S. and M. J. Glennie, *Blood*, vol. 103, pp. 2738-2743, 2004). FcRn binding and *in vivo* clearance/half life determinations can also be performed using methods known in the art (see, e.g., Petkova, S. B. et al., *Int'l. Immunol.*, vol. 18, pp. 1759-1769, 2006).

[0182] Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Pat. No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (U.S. Pat. No. 7,332,581).

[0183] Certain antibody variants with improved or diminished binding to FcRs are described. (See, e.g., U.S. Pat. No. 6,737,056; WO 2004/056312, and Shields et al., *J. Biol. Chem.*, vol. 9, pp. 6591-6604, 2001).

[0184] In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, e.g., substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

[0185] In some embodiments, alterations are made in the Fc region that result in altered (i.e., either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), e.g., as described in U.S. Pat. No. 6,194,551, WO 99/51642, and Idusogie et al. *J. Immunol.*, vol. 164, pp. 4178-4184, 2000.

[0186] Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer et al., *J. Immunol.*, vol. 117, pp. 587-593, 1976 and Kim et al., *J. Immunol.*, vol. 24, p. 249, 1994), are described in US2005/0014934. Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include/e those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, e.g., substitution of Fc region residue 434 (U.S. Pat. No. 7,371,826). See also Duncan & Winter, *Nature*, vol. 322, pp. 738-740, 1988; U.S. Pat. No. 5,648,260; U.S. Pat. No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

Cysteine Engineered Antibody Variants

[0187] In certain embodiments, it may be desirable to create cysteine engineered antibodies, e.g., “thioMAbs,” in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and 5400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, e.g., in U.S. Pat. No. 7,521,541.

Antibody Derivatives

[0188] In certain embodiments, an antibody or antibody fragment provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody or antibody fragment include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1,3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (e.g., glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight and may be branched or unbranched. The number of polymers attached to the antibody or antibody fragment may vary, and if more than one polymer are attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody or antibody fragment to be improved, whether the derivative will be used in a therapy under defined conditions, etc.

[0189] In another embodiment, conjugates of an antibody or antibody fragment and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam et al., *Proc. Natl. Acad. Sci. USA*, vol. 102, pp. 11600-11605, 2005). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

[0190] In another aspect, the present invention provides an anti-ROR2 antibody or antibody fragment including the heavy chain variable region polypeptides or light chain variable region polypeptides. The heavy chain variable region polypeptides comprise the H1, H2, and H3 regions with SEQ ID NOS: 1-6. The light chain variable region polypeptides comprise the L1, L2, and L3 regions with SEQ ID NOS: 7-12.

[0191] The anti-ROR2 antibody or antibody fragment of the invention has a higher binding affinity to ROR2 under a condition in tumor microenvironment than under a condition in a non-tumor microenvironment. In one embodiment, the condition in tumor microenvironment and the condition in a non-tumor microenvironment are both pH. The anti-ROR2 antibodies

or antibody fragments of the invention thus can selectively bind to ROR2 at a pH about 5.0-6.8 but will have a lower binding affinity to ROR2 at a pH about 7.2-7.8 encountered in a normal physiological environment. As shown Example 1, the anti-ROR2 antibodies or antibody fragments have higher binding affinity to ROR2 at pH 6.0 than at pH 7.4.

[0192] In certain embodiments, the anti-ROR2 antibodies or antibody fragments of the present invention have a dissociation constant (K_d) with ROR2 under a condition in tumor microenvironment of about $\leq 1 \mu\text{M}$, $\leq 100 \text{ nM}$, $\leq 10 \text{ nM}$, $\leq 1 \text{ nM}$, $\leq 0.1 \text{ nM}$, $\leq 0.01 \text{ nM}$, or $\leq 0.001 \text{ nM}$ (e.g., 10^{-8}M or less, or from 10^{-8}M to 10^{-13}M , or from 10^{-9}M to 10^{-13}M). In one embodiment, the ratio of the K_d of the antibody or antibody fragment with ROR2 at a value of the condition in tumor microenvironment to the K_d at a different value of the same condition in non-tumor microenvironment is at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.

[0193] In one embodiment, K_d is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen using the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating Fab with a minimal concentration of (^{125}I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (see, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with $5 \mu\text{g/ml}$ of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [^{125}I]-antigen are mixed with serial dilutions of a Fab of interest (e.g., consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta et al., *Cancer Res.* 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (e.g., about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (e.g., for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, $150 \mu\text{l/well}$ of scintillant (MICROSCINT-20™; Packard) is added, and the plates are counted on a TOPCOUNT™ gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

[0194] According to another embodiment, K_d is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE®-3000 (BIAcore, Inc., Piscataway, N.J.) at 25° C. with immobilized antigen CM5 chips at about 10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIAcore, Inc.) are activated with N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 µg/ml (~0.2 µM) before injection at a flow rate of 5 µl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20™) surfactant (PBST) at 25° C. at a flow rate of approximately 25 µl/min. Association rates (k_{on}) and dissociation rates (k_{off}) are calculated using a simple one-to-one Langmuir binding model (BIACORE® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (K_d) is calculated as the ratio k_{off}/k_{on} . See, e.g., Chen et al., *J. Mol. Biol.* 293:865-881 (1999). If the on-rate exceeds $10^6 \text{ M}^{-1} \text{ s}^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation=295 nm; emission=340 nm, 16 nm band-pass) at 25° C. of a 20 nM anti-antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophotometer (Aviv Instruments) or a 8000-series SLM-AMINCO™ spectrophotometer (ThermoSpectronic) with a stirred cuvette.

[0195] The anti-ROR2 antibodies of the invention may be a chimeric, humanized or human antibody. In one embodiment, an anti-ROR2 antibody fragment is employed, e.g., a Fv, Fab, Fab', Fab'-SH, scFv, a diabody, a triabody, a tetrabody or an $F(ab')_2$ fragment and multispecific antibodies formed from antibody fragments. In another embodiment, the antibody is a full length antibody, e.g., an intact IgG antibody or other antibody class or isotype as defined herein. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.*, vol. 9, pp. 129-134, 2003. For a review of scFv fragments, see, e.g., Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Pat. Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Pat. No. 5,869,046.

[0196] The diabodies of the invention may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA*, vol. 90, pp. 6444-6448, 1993 for examples of diabodies. Examples of triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.*, vol. 9, pp. 129-134, 2003.

[0197] In some embodiments, the invention comprises single-domain antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, Mass.; see, e.g., U.S. Pat. No. 6,248,516 B1).

[0198] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (e.g. *E. coli* or phage), as described herein.

[0199] In some embodiments, the anti-ROR2 antibodies of the invention may be chimeric antibodies. Certain chimeric antibodies are described, e.g., in U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, vol. 81, pp. 6851-6855, 1984). In one example, the chimeric antibody comprises a non-human variable region (e.g., a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, the chimeric antibody is a "class switched" antibody in which the class or subclass of the antibody has been changed relative to the class or subclass of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

[0200] In certain embodiments, the chimeric antibody of the invention is a humanized antibody. Typically, such a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which CDRs (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody may optionally also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (e.g., the antibody from which the CDR residues are derived), e.g., to restore or improve antibody specificity or affinity.

[0201] Humanized antibodies and methods of making them are reviewed, e.g., in Almagro and Fransson, *Front. Biosci.*, vol. 13, pp. 1619-1633, 2008, and are further described, e.g., in Riechmann et al., *Nature*, vol. 332, pp. 323-329, 1988; Queen et al., *Proc. Nat'l Acad. Sci. USA*, vol. 86, pp. 10029-10033, 1989; U.S. Pat. Nos. 5,821,337, 7,527,791, 6,982,321, and

7,087,409; Kashmiri et al., *Methods*, vol. 36, pp. 25-34, 2005 (describing SDR (a-CDR) grafting); Padlan, *Mol. Immunol.*, vol. 28, pp. 489-498, 1991 (describing “resurfacing”); Dall'Acqua et al., *Methods*, vol. 36, pp. 43-60, 2005 (describing “FR shuffling”); and Osbourn et al., *Methods*, vol. 36, pp. 61-68, 2005 and Klimka et al., *Br. J. Cancer*, vol. 83, pp. 252-260, 2000 (describing the “guided selection” approach to FR shuffling).

[0202] Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the “best-fit” method (see, e.g., Sims et al. *J. Immunol.*, vol. 151, p. 2296, 1993); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, e.g., Carter et al. *Proc. Natl. Acad. Sci. USA*, vol. 89, p. 4285, 1992; and Presta et al. *J. Immunol.*, vol. 151, p. 2623, 1993); human mature (somatically mutated) framework regions or human germline framework regions (see, e.g., Almagro and Fransson, *Front. Biosci.*, vol. 13, pp. 1619-1633, 2008); and framework regions derived from screening FR libraries (see, e.g., Baca et al., *J. Biol. Chem.*, vol. 272, pp. 10678-10684, 1997 and Rosok et al., *J. Biol. Chem.*, vol. 271, pp. 22611-22618, 1996).

[0203] In some embodiments, the anti-ROR2 antibodies of the invention are multispecific, e.g. bispecific antibodies. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for ROR2 and the other is for another antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of ROR2. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express ROR2. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

[0204] Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, *Nature*, vol. 305, pp. 537-540, 1983), WO 93/08829, and Traunecker et al., *EMBO J.* vol. 10, pp. 3655-3659, 1991), and “knob-in-hole” engineering (see, e.g., U.S. Pat. No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, e.g., U.S. Pat. No. 4,676,980, and Brennan et al., *Science*, vol. 229, pp. 81-83, 1985); using leucine zippers to produce bi-specific antibodies (see, e.g., Kostelny et al., *J. Immunol.*, vol. 148, pp. 1547-1553, 1992); using “diabody” technology for making bispecific antibody fragments (see, e.g., Hollinger et al., *Proc. Natl. Acad. Sci. USA*, vol. 90, pp. 6444-6448, 1993); and using single-chain Fv (scFv) dimers (see, e.g. Gruber et al., *J. Immunol.*, vol. 152, pp. 5368-5374, 1994);

and preparing trispecific antibodies as described, e.g., in Tutt et al. *J. Immunol.*, vol. 147, pp. 60-69, 1991.

[0205] Engineered antibodies with three or more functional antigen binding sites, including “Octopus antibodies,” are also included herein (see, e.g. US 2006/0025576A1).

[0206] The antibody or antibody fragment may also include a “Dual Acting Fab” or “DAF” comprising an antigen binding site that binds to ROR2 as well as another, different antigen (such as Ror1, see, US 2008/0069820, for example).

[0207] The anti-ROR2 antibodies or antibody fragments of the invention may be produced using recombinant methods and compositions, which are described in detail in US 2016/0017040.

[0208] The physical/chemical properties and/or biological activities of the anti-ROR2 antibodies or antibody fragments of the invention may be tested and measured by various assays known in the art. Some of these assays are described in U.S. Patent No. 8,853,369.

B. Immunoconjugates

[0209] In another aspect, the invention also provides immunoconjugates comprising an anti-ROR2 antibody or antibody fragment conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

[0210] In one embodiment, the immunoconjugate is an antibody-drug conjugate (ADC) in which an antibody or antibody fragment is conjugated to one or more drugs, including but not limited to a maytansinoid (see U.S. Pat. Nos. 5,208,020, 5,416,064 and European Patent EP 0 425 235 B1); an auristatin such as monomethylauristatin drug moieties DE and DF (MMAE and MMAF) (see U.S. Pat. Nos. 5,635,483 and 5,780,588, and 7,498,298); a dolastatin; a calicheamicin or derivative thereof (see U.S. Pat. Nos. 5,712,374, 5,714,586, 5,739,116, 5,767,285, 5,770,701, 5,770,710, 5,773,001, and 5,877,296; Hinman et al., *Cancer Res.*, vol. 53, pp. 3336-3342, 1993; and Lode et al., *Cancer Res.*, vol. 58, pp. 2925-2928, 1998); an anthracycline such as daunomycin or doxorubicin (see Kratz et al., *Current Med. Chem.*, vol. 13, pp. 477-523, 2006; Jeffrey et al., *Bioorganic & Med. Chem. Letters*, vol. 16, pp. 358-362, 2006; Torgov et al., *Bioconj. Chem.*, vol. 16, pp. 717-721, 2005; Nagy et al., *Proc. Natl. Acad. Sci. USA*, vol. 97, pp. 829-834, 2000; Dubowchik et al., *Bioorg. & Med. Chem. Letters*, vol. 12, pp. 1529-1532, 2002; King et al., *J. Med. Chem.*, vol. 45, pp. 4336-4343, 2002; and U.S. Pat. No. 6,630,579); methotrexate; vindesine; a taxane such as docetaxel, paclitaxel, larotaxel, tesetaxel, and ortataxel; a trichothecene; and CC1065.

[0211] In another embodiment, an immunoconjugate comprises an antibody or antibody fragment as described herein conjugated to an enzymatically active toxin or fragment thereof, including but not limited to diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, *Aleurites fordii* proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcumin, croton, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes.

[0212] In another embodiment, an immunoconjugate comprises an antibody or antibody fragment as described herein conjugated to a radioactive atom to form a radioconjugate. A variety of radioactive isotopes are available for the production of radioconjugates. Examples include At²¹¹, I¹³¹, I¹²⁵, Y⁹⁰, Re¹⁸⁶, Re¹⁸⁸, Sm¹⁵³, Bi²¹², P³², Pb²¹² and radioactive isotopes of Lu. When the radioconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

[0213] Conjugates of an antibody/antibody fragment and cytotoxic agent may be made using a variety of bifunctional protein coupling agents such as N-succinimidyl-3-(2-pyridyldithio) propionate (SPDP), succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis(p-azidobenzoyl)hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a ricin immunotoxin can be prepared as described in Vitetta et al., *Science*, vol. 238, pp. 1098-, 1987. Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026. The linker may be a “cleavable linker” facilitating release of a cytotoxic drug in the cell. For example, an acid-labile linker, peptidase-sensitive linker, photolabile linker, dimethyl linker or disulfide-containing linker (Chari et al., *Cancer Res.*, vol. 52, pp. 127-131, 1992; U.S. Pat. No. 5,208,020) may be used.

[0214] The immunoconjugates herein expressly contemplate, but are not limited to conjugates prepared with cross-linker reagents including, but not limited to, BMPS, EMCS,

GMBS, HBVS, LC-SMCC, MBS, MPBH, SBAP, SIA, SLAB, SMCC, SMPB, SMPH, sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and SVSB (succinimidyl-(4-vinylsulfone)benzoate) which are commercially available (e.g., from Pierce Biotechnology, Inc., Rockford, Ill., U.S.A).

[0215] An exemplary embodiment of an ADC comprises an antibody or antibody fragment (Ab) which targets a tumor cell, a drug moiety (D), and a linker moiety (L) that attaches Ab to D. In some embodiments, the antibody is attached to the linker moiety (L) through one or more amino acid residues, such as lysine and/or cysteine. In some embodiments, the drug moiety is an antineoplastic agent. For example, the CAB-ROR2-ADC may be BA3021-cleavable linker-MMAE_(n), in which the heterologous molecule is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive.

[0216] An exemplary ADC has Formula I as Ab-(L-D)_p, where p is 1 to about 20. In some embodiments, the number of drug moieties that can be conjugated to an antibody is limited by the number of free cysteine residues. In some embodiments, free cysteine residues are introduced into the antibody amino acid sequence by the methods described herein.

Exemplary ADC of Formula I include, but are not limited to, antibodies that have 1, 2, 3, or 4 engineered cysteine amino acids (Lyon et al., *Methods in Enzym.*, vol. 502, pp. 123-138, 2012). In some embodiments, one or more free cysteine residues are already present in an antibody, without the use of engineering, in which case the existing free cysteine residues may be used to conjugate the antibody to a drug. In some embodiments, an antibody is exposed to reducing conditions prior to conjugation of the antibody in order to generate one or more free cysteine residues.

a) Exemplary Linkers

[0217] A “Linker” (L) is a bifunctional or multifunctional moiety that can be used to link one or more moieties such as drug moieties (D) to an antibody or antibody fragment (Ab) to form an immunoconjugate such as an ADC of the Formula I. In some embodiments, ADCs can be prepared using a Linker having reactive functionalities for covalently attaching to the drug and to the antibody. For example, in some embodiments, a cysteine thiol of an antibody or antibody fragment (Ab) can form a bond with a reactive functional group of a linker or a drug-linker intermediate to make an ADC.

[0218] In one aspect, a linker has a functionality that is capable of reacting with a free cysteine present on an antibody to form a covalent bond. Nonlimiting exemplary such reactive functionalities include maleimide, haloacetamides, α -haloacetyl, activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl

esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates, and isothiocyanates. See, e.g., the conjugation method at page 766 of Klussman, et al, *Bioconjugate Chemistry*, vol. 15, pp. 765-773, 2004.

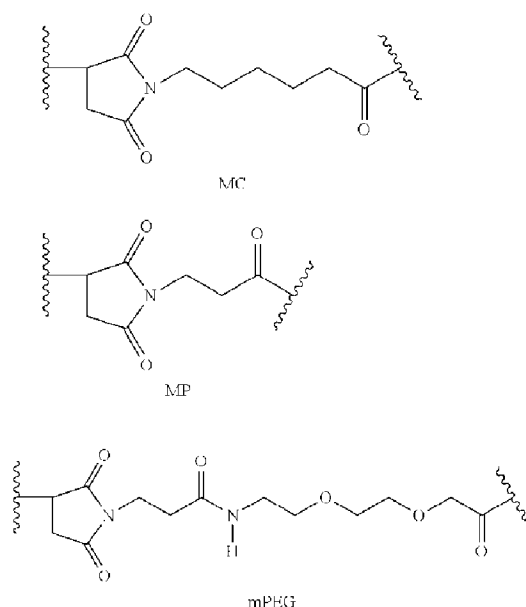
[0219] In some embodiments, a linker has a functionality that is capable of reacting with an electrophilic group present on an antibody. Exemplary such electrophilic groups include, but are not limited to, aldehyde and ketone carbonyl groups. In some embodiments, a heteroatom of the reactive functionality of the linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Nonlimiting exemplary such reactive functionalities include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide.

[0220] A linker may comprise one or more linker components. Exemplary linker components include 6-maleimidocaproyl ("MC"), maleimidopropanoyl ("MP"), valine-citrulline ("val-cit" or "vc"), alanine-phenylalanine ("ala-phe"), p-aminobenzyloxycarbonyl (a "PAB"), N-Succinimidyl 4-(2-pyridylthio) pentanoate ("SPP"), and 4-(N-maleimidomethyl)cyclohexane-1 carboxylate ("MCC"). Various linker components are known in the art, some of which are described below.

[0221] A linker may be a "cleavable linker," facilitating release of a drug. Nonlimiting exemplary cleavable linkers include acid-labile linkers (e.g., comprising hydrazone), protease-sensitive (e.g., peptidase-sensitive) linkers, photolabile linkers, or disulfide-containing linkers (Chari et al., *Cancer Research*, vol. 52, pp. 127-131, 1992; U.S. Pat. No. 5,208,020).

[0222] In certain embodiments, a linker has the following Formula II as $\text{---}A_a\text{---}W_w\text{---}Y_y\text{---}$, wherein A is a "stretcher unit", and a is an integer from 0 to 1; W is an "amino acid unit", and w is an integer from 0 to 12; Y is a "spacer unit", and y is 0, 1, or 2. An ADC comprising the linker of Formula II has the Formula I(A): $\text{Ab}-(A_a\text{---}W_w\text{---}Y_y\text{---}D)_p$, wherein Ab, D, and p are defined as above for Formula I. Exemplary embodiments of such linkers are described in U.S. Pat. No. 7,498,298.

[0223] In some embodiments, a linker component comprises a "stretcher unit" (A) that links an antibody to another linker component or to a drug moiety. Nonlimiting exemplary stretcher units are shown below (wherein the wavy line indicates sites of covalent attachment to an antibody, drug, or additional linker components):



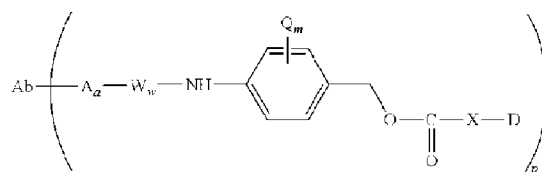
[0224] In some embodiments, a linker component comprises an “amino acid unit” (W). In some such embodiments, the amino acid unit allows for cleavage of the linker by a protease, thereby facilitating release of the drug from the immunoconjugate upon exposure to intracellular proteases, such as lysosomal enzymes (Doronina et al., *Nat. Biotechnol.*, vol. 21, pp. 778-784, 2003). Exemplary amino acid units include, but are not limited to, dipeptides, tripeptides, tetrapeptides, and pentapeptides. Exemplary dipeptides include, but are not limited to, valine-citrulline (vc or val-cit), alanine-phenylalanine (af or ala-phe); phenylalanine-lysine (fk or phe-lys); phenylalanine-homolysine (phe-homolys); and N-methyl-valine-citrulline (Me-val-cit). Exemplary tripeptides include, but are not limited to, glycine-valine-citrulline (gly-val-cit) and glycine-glycine-glycine (gly-gly-gly). An amino acid unit may comprise amino acid residues that occur naturally and/or minor amino acids and/or non-naturally occurring amino acid analogs, such as citrulline. Amino acid units can be designed and optimized for enzymatic cleavage by a particular enzyme, for example, a tumor-associated protease, cathepsin B, C and D, or a plasmin protease.

[0225] Typically, peptide-type linkers can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to a liquid phase synthesis method (e.g., E. Schroder and K. Lübke (1965) “The Peptides”, volume 1, pp 76-136, Academic Press).

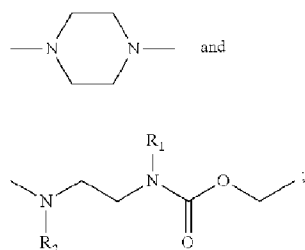
[0226] In some embodiments, a linker component comprises a “spacer unit” (Y) that links the antibody to a drug moiety, either directly or through a stretcher unit and/or an amino acid unit. A spacer unit may be “self-immolative” or a “non-self-immolative.” A “non-self-immolative” spacer unit is one in which part or all of the spacer unit remains bound to the

drug moiety upon cleavage of the ADC. Examples of non-self-immolative spacer units include, but are not limited to, a glycine spacer unit and a glycine-glycine spacer unit. In some embodiments, enzymatic cleavage of an ADC containing a glycine-glycine spacer unit by a tumor-cell associated protease results in release of a glycine-glycine-drug moiety from the remainder of the ADC. In some such embodiments, the glycine-glycine-drug moiety is subjected to a hydrolysis step in the tumor cell, thus cleaving the glycine-glycine spacer unit from the drug moiety.

[0227] A “self-immolative” spacer unit allows for release of the drug moiety. In certain embodiments, a spacer unit of a linker comprises a p-aminobenzyl unit. In some such embodiments, a p-aminobenzyl alcohol is attached to an amino acid unit via an amide bond, and a carbamate, methylcarbamate, or carbonate is made between the benzyl alcohol and the drug (Hamann et al. *Expert Opin. Ther. Patents*, vol. 15, pp. 1087-1103, 2005). In some embodiments, the spacer unit comprises p-aminobenzyloxycarbonyl (PAB). In some embodiments, an ADC comprising a self-immolative linker has the structure:



wherein Q is $\text{—C}_1\text{—C}_8$ alkyl, $\text{—O—(C}_1\text{—C}_8 \text{ alkyl)}$, -halogen, -nitro, or -cyano; m is an integer ranging from 0 to 4; X may be one or more additional spacer units or may be absent; and p ranges from 1 to about 20. In some embodiments, p ranges from 1 to 10, 1 to 7, 1 to 5, or 1 to 4. Nonlimiting exemplary X spacer units include:



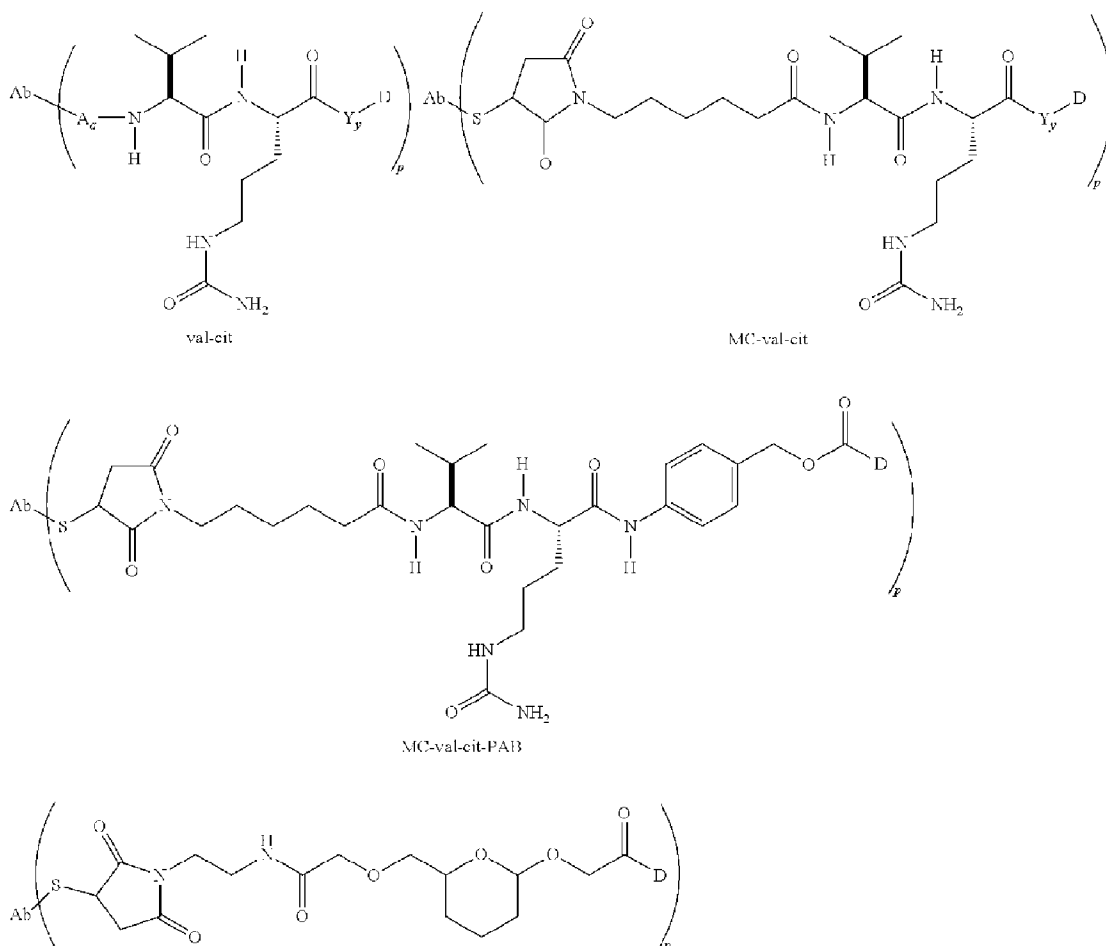
wherein R_1 and R_2 are independently selected from H and $\text{C}_1\text{—C}_6$ alkyl. In some embodiments, R_1 and R_2 are each —CH_3 .

[0228] Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group, such as 2-aminoimidazol-5-methanol derivatives (U.S. Pat. No. 7,375,078; Hay et al., *Bioorg. Med. Chem. Lett.*, vol. 9, p. 2237-, 1999) and ortho- or para-aminobenzylacetals. In some embodiments, spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al., *Chemistry Biology*, vol. 2, pp.

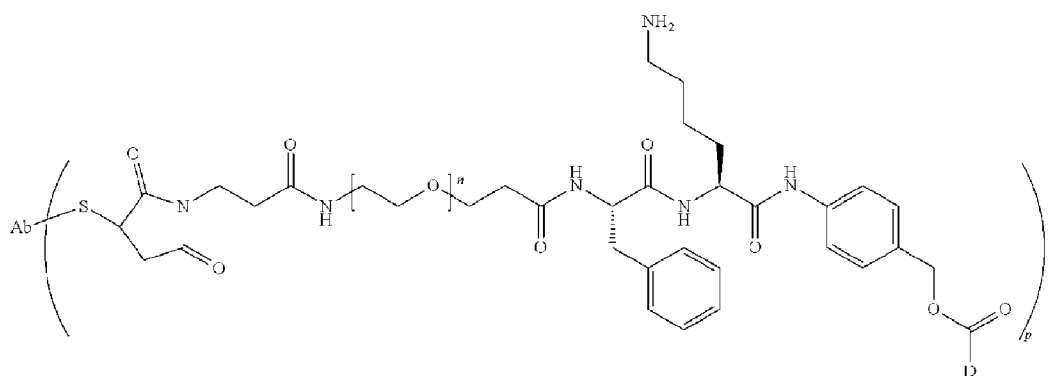
223-, 1995), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm et al., *J. Amer. Chem. Soc.*, vol. 94, p. 5815-, 1972) and 2-aminophenylpropionic acid amides (Amsberry et al., *J. Org. Chem.*, vol. 55, p. 5867, 1990). Linkage of a drug to the α -carbon of a glycine residue is another example of a self-immolative spacer that may be useful in ADCs (Kingsbury et al., *J. Med. Chem.*, vol. 27, p.1447, 1984).

[0229] In some embodiments, linker L may be a dendritic type linker for covalent attachment of more than one drug moiety to an antibody through a branching, multifunctional linker moiety (Sun et al. *Bioorganic & Medicinal Chemistry Letters*, vol. 12, pp. 2213-2215, 2002; Sun et al., *Bioorganic & Medicinal Chemistry*, vol.11, pp. 1761-1768, 2003). Dendritic linkers can increase the molar ratio of drug to antibody, i.e. loading, which is related to the potency of the ADC. Thus, where an antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

[0230] Nonlimiting exemplary linkers are shown below in the context of an ADC of Formula I:



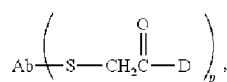
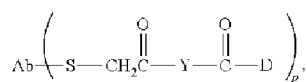
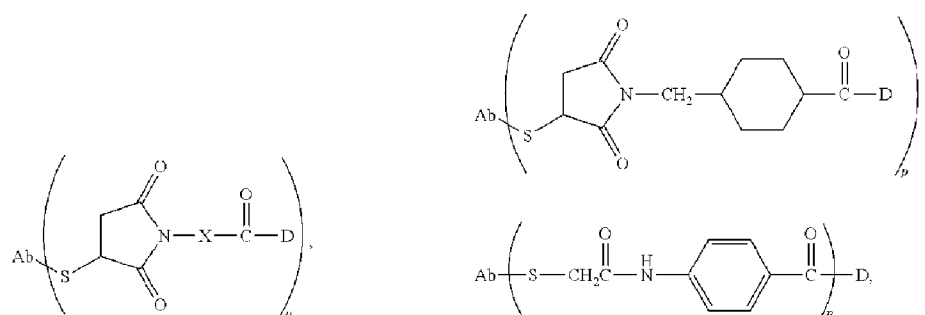
wherein R_1 and R_2 are independently selected from H and C_1 - C_6 alkyl. In some embodiments, R_1 and R_2 are each $-CH_3$.



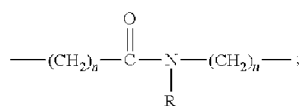
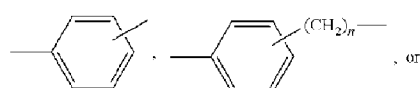
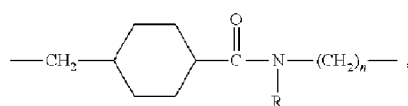
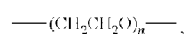
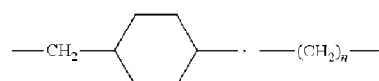
Phe-Lys-PAB-Ab

wherein n is 0 to 12. In some embodiments, n is 2 to 10. In some embodiments, n is 4 to 8.

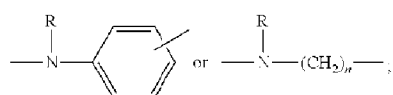
[0231] Further nonlimiting exemplary ADCs include the structures:



where X is:



Y is:



each R is independently H or C₁-C₆ alkyl; and n is 1 to 12.

[0232] In some embodiments, a linker is substituted with groups that modulate solubility and/or reactivity. As a nonlimiting example, a charged substituent such as sulfonate (SO_3^-) or ammonium may increase water solubility of the linker reagent and facilitate the coupling reaction of the linker reagent with the antibody and/or the drug moiety, or facilitate the coupling reaction of Ab-L (antibody-linker intermediate) with D, or D-L (drug-linker intermediate) with Ab, depending on the synthetic route employed to prepare the ADC. In some embodiments, a portion of the linker is coupled to the antibody and a portion of the linker is coupled to the drug, and then the Ab-(linker portion)^a is coupled to drug-(linker portion)^b to form the ADC of Formula I.

[0233] The compounds of the invention expressly contemplate, but are not limited to, ADCs prepared with the following linker reagents: bis-maleimido-trioxyethylene glycol (BMPEO), N-(β -maleimidopropoxy)-N-hydroxy succinimide ester (BMPS), N-(ϵ -maleimidocaproxy) succinimide ester (EMCS), N-[γ -maleimidobutyryloxy]succinimide ester (GMBS), 1,6-hexane-bis-vinylsulfone (HBVS), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate) (LC-SMCC), m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS), 4-(4-N-Maleimidophenyl)butyric acid hydrazide (MPBH), succinimidyl 3-(bromoacetamido)propionate (SBAP), succinimidyl iodoacetate (SIA), succinimidyl (4-iodoacetyl)aminobenzoate (SIAB), N-succinimidyl-3-(2-pyridylthio) propionate (SPDP), N-succinimidyl-4-(2-pyridylthio)pentanoate (SPP), succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB), succinimidyl 6-[(beta-maleimidopropionamido)hexanoate] (SMPH), iminothiolane (IT), sulfo-EMCS, sulfo-GMBS, sulfo-KMUS, sulfo-MBS, sulfo-SIAB, sulfo-SMCC, and sulfo-SMPB, and succinimidyl-(4-vinylsulfone)benzoate (SVSB), and including bis-maleimide reagents: dithiobismaleimidoethane (DTME), 1,4-Bismaleimidobutane (BMB), 1,4 Bismaleimidyl-2,3-dihydroxybutane (BMDB), bismaleimidoethane (BMOE), bismaleimidoethane (BMOE), BM(PEG)₂ (shown below), and BM(PEG)₃ (shown below); bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCl), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as toluene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). In some embodiments, bis-maleimide reagents allow the attachment of the thiol group of a cysteine in the antibody to a thiol-containing drug moiety, linker, or linker-drug intermediate. Other

functional groups that are reactive with thiol groups include, but are not limited to, iodoacetamide, bromoacetamide, vinyl pyridine, disulfide, pyridyl disulfide, isocyanate, and isothiocyanate.

[0234] Certain useful linker reagents can be obtained from various commercial sources, such as Pierce Biotechnology, Inc. (Rockford, Ill.), Molecular Biosciences Inc. (Boulder, Colo.), or synthesized in accordance with procedures described in the art; for example, in Toki et al., *J. Org. Chem.*, vol. 67, pp. 1866-1872, 2002; Dubowchik, et al., *Tetrahedron Letters*, vol. 38, pp. 5257-60, 1997; Walker, *J. Org. Chem.*, vol. 60, pp. 5352-5355, 1995; Frisch et al., *Bioconjugate Chem.*, vol. 7, pp. 180-186, 1995; U.S. Pat. No. 6,214,345; WO 02/088172; US2003130189; US2003096743; WO 03/026577; WO 03/043583; and WO 04/032828.

[0235] Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See, e.g., WO94/11026.

b) Exemplary Drug Moieties

1) Maytansine and Maytansinoids

[0236] In some embodiments, an immunoconjugate comprises an antibody conjugated to one or more maytansinoid molecules. Maytansinoids are derivatives of maytansine, and are mitototic inhibitors which act by inhibiting tubulin polymerization. Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S. Pat. No. 3,896,111).

Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinoids are disclosed, for example, in U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533.

[0237] Maytansinoid drug moieties are attractive drug moieties in antibody-drug conjugates because they are: (i) relatively accessible to prepare by fermentation or chemical modification or derivatization of fermentation products, (ii) amenable to derivatization with functional groups suitable for conjugation through non-disulfide linkers to antibodies, (iii) stable in plasma, and (iv) effective against a variety of tumor cell lines.

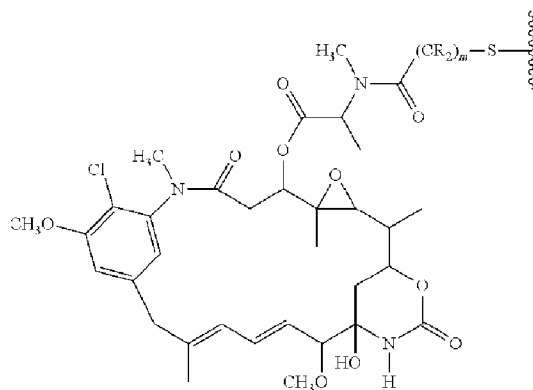
[0238] Certain maytansinoids suitable for use as maytansinoid drug moieties are known in the art and can be isolated from natural sources according to known methods or produced using genetic engineering techniques (see, e.g., Yu et al., *PNAS*, vol. 99, pp. 7968-7973, 2002). Maytansinoids may also be prepared synthetically according to known methods.

[0239] Exemplary maytansinoid drug moieties include, but are not limited to, those having a modified aromatic ring, such as: C-19-dechloro (U.S. Pat. No. 4,256,746) (prepared, for example, by lithium aluminum hydride reduction of ansamycin P2); C-20-hydroxy (or C-20-demethyl)+/-C-19-dechloro (U.S. Pat. Nos. 4,361,650 and 4,307,016) (prepared, for example, by demethylation using *Streptomyces* or *Actinomyces* or dechlorination using LAH); and C-20-demethoxy, C-20-acyloxy (—OCOR), +/-dechloro (U.S. Pat. No. 4,294,757) (prepared, for example, by acylation using acyl chlorides), and those having modifications at other positions of the aromatic ring.

[0240] Exemplary maytansinoid drug moieties also include those having modifications such as: C-9-SH (U.S. Pat. No. 4,424,219) (prepared, for example, by the reaction of maytansinol with H_2S or P_2S_5); C-14-alkoxymethyl(demethoxy/ CH_2OR)(U.S. Pat. No. 4,331,598); C-14-hydroxymethyl or acyloxymethyl (CH_2OH or CH_2OAc) (U.S. Pat. No. 4,450,254) (prepared, for example, from *Nocardia*); C-15-hydroxy/acyloxy (U.S. Pat. No. 4,364,866) (prepared, for example, by the conversion of maytansinol by *Streptomyces*); C-15-methoxy (U.S. Pat. Nos. 4,313,946 and 4,315,929) (for example, isolated from *Trewia nudiflora*); C-18-N-demethyl (U.S. Pat. Nos. 4,362,663 and 4,322,348) (prepared, for example, by the demethylation of maytansinol by *Streptomyces*); and 4,5-deoxy (U.S. Pat. No. 4,371,533) (prepared, for example, by the titanium trichloride/LAH reduction of maytansinol).

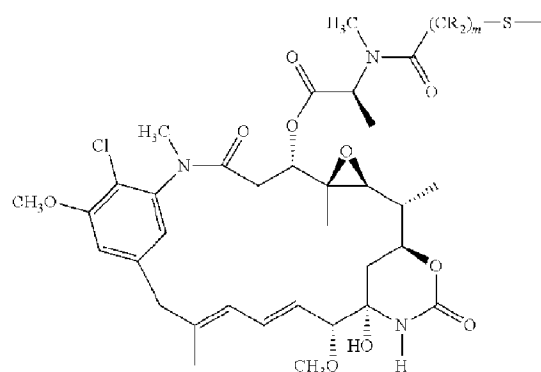
[0241] Many positions on maytansinoid compounds are useful as the linkage position. For example, an ester linkage may be formed by reaction with a hydroxyl group using conventional coupling techniques. In some embodiments, the reaction may occur at the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with a hydroxyl group, and the C-20 position having a hydroxyl group. In some embodiments, the linkage is formed at the C-3 position of maytansinol or a maytansinol analogue.

[0242] Maytansinoid drug moieties include those having the structure:

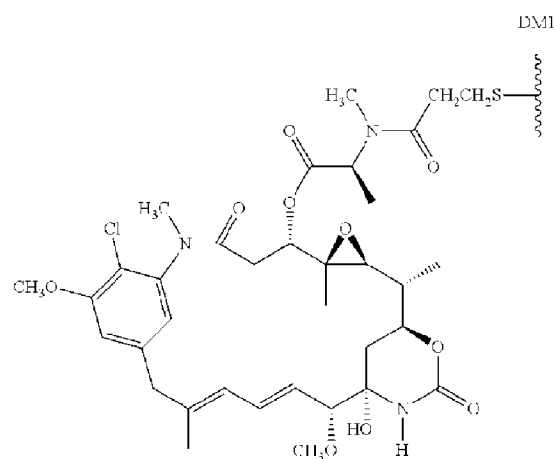


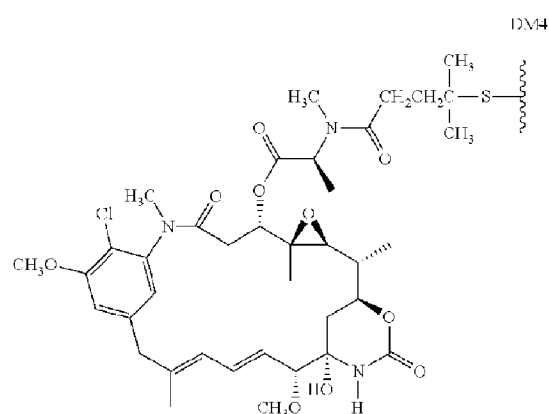
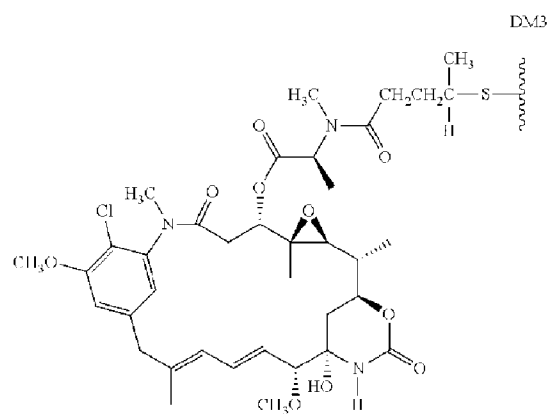
where the wavy line indicates the covalent attachment of the sulfur atom of the maytansinoid drug moiety to a linker of an ADC. Each R may independently be H or a C₁-C₆ alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propyl, i.e., m is 1, 2, or 3 (U.S. Pat. No. 633,410; U.S. Pat. No. 5,208,020; Chari et al., *Cancer Res.*, vol. 52, pp. 127-131, 1992; Liu et al., *Proc. Natl. Acad. Sci. USA*, vol. 93, pp. 8618-8623, 1996).

[0243] All stereoisomers of the maytansinoid drug moiety are contemplated for the ADC of the invention, i.e. any combination of R and S configurations at the chiral carbons (U.S. Pat. No. 7,276,497; U.S. Pat. No. 6,913,748; U.S. Pat. No. 6,441,163; U.S. Pat. No. 633,410 (RE39151); U.S. Pat. No. 5,208,020; Widdison et al (2006) *J. Med. Chem.* 49:4392-4408. In some embodiments, the maytansinoid drug moiety has the following stereochemistry:



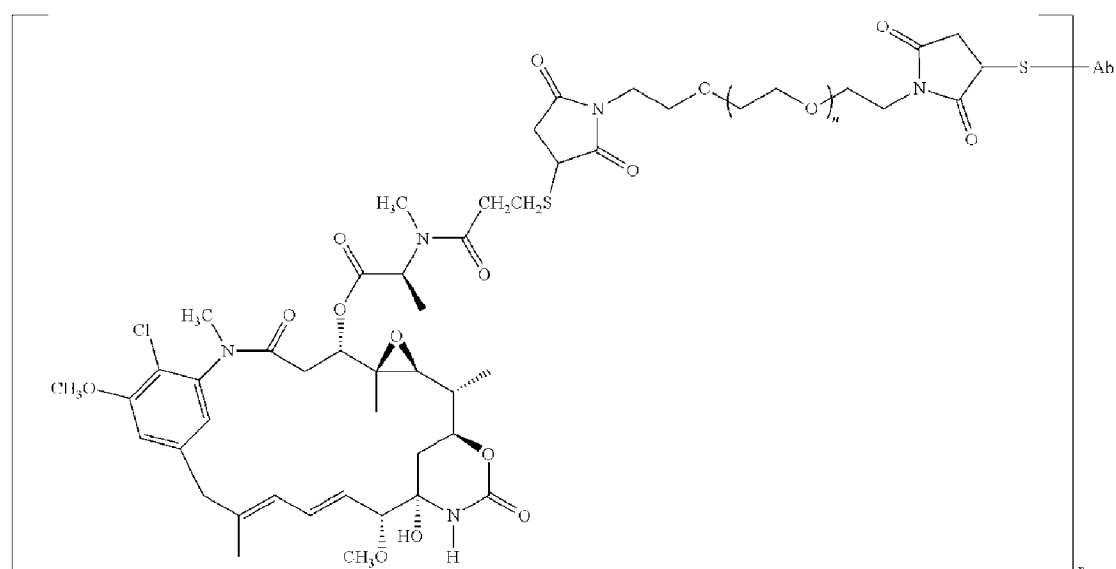
[0244] Exemplary embodiments of maytansinoid drug moieties include, but are not limited to, DM1; DM3; and DM4, having the structures:





wherein the wavy line indicates the covalent attachment of the sulfur atom of the drug to a linker (L) of an antibody-drug conjugate.

[0245] Exemplary antibody-drug conjugates where DM1 is linked through a BMPEO linker to a thiol group of the antibody have the structure and abbreviation:



where Ab is antibody; n is 0, 1, or 2; and p is 1 to about 20. In some embodiments, p is 1 to 10, p is 1 to 7, p is 1 to 5, or p is 1 to 4.

[0246] Immunoconjugates containing maytansinoids, methods of making the same, and their therapeutic use are disclosed, for example, in U.S. Pat. Nos. 5,208,020 and 5,416,064; US 2005/0276812 A1; and European Patent EP 0 425 235 B1. See also Liu et al., *Proc. Natl. Acad. Sci. USA*, vol. 93, pp. 8618-8623, 1996; and Chari et al., *Cancer Research*, vol. 52, pp. 127-131, 1992.

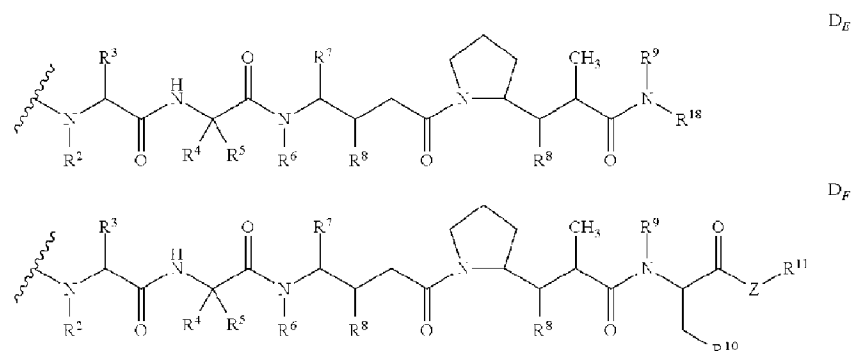
[0247] In some embodiments, antibody-maytansinoid conjugates may be prepared by chemically linking an antibody to a maytansinoid molecule without significantly diminishing the biological activity of either the antibody or the maytansinoid molecule. See, e.g., U.S. Pat. No. 5,208,020. In some embodiments, ADC with an average of 3-4 maytansinoid molecules conjugated per antibody molecule has shown efficacy in enhancing cytotoxicity of target cells without negatively affecting the function or solubility of the antibody. In some instances, even one molecule of toxin/antibody is expected to enhance cytotoxicity over the use of naked antibody.

[0248] Exemplary linking groups for making antibody-maytansinoid conjugates include, for example, those described herein and those disclosed in U.S. Pat. No. 5,208,020; EP Patent 0 425 235 B1; Chari et al., *Cancer Research*, vol. 52, pp. 127-131, 1992; US 2005/0276812 A1; and US 2005/016993 A1.

2) Auristatins and Dolastatins

[0249] Drug moieties include dolastatins, auristatins, and analogs and derivatives thereof (U.S. Pat. No. 5,635,483; U.S. Pat. No. 5,780,588; U.S. Pat. No. 5,767,237; U.S. Pat. No. 6,124,431). Auristatins are derivatives of the marine mollusk compound dolastatin-10. While not intending to be bound by any particular theory, dolastatins and auristatins have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al., *Antimicrob. Agents and Chemother.*, vol. 45, pp. 3580-3584, 2001) and have anticancer (U.S. Pat. No. 5,663,149) and antifungal activity (Pettit et al., *Antimicrob. Agents Chemother.*, vol. 42, pp. 2961-2965, 1998). The dolastatin/auristatin drug moiety may be attached to the antibody through the N (amino) terminus or the C (carboxyl) terminus of the peptidic drug moiety (WO 02/088172; Doronina et al., *Nature Biotechnology*, vol. 21, pp. 778-784, 2003; Francisco et al., *Blood*, vol. 102, pp. 1458-1465, 2003).

[0250] Exemplary auristatin embodiments include the N-terminus linked monomethylauristatin drug moieties D_E and D_F, disclosed in U.S. Pat. No. 7,498,298 and U.S. Pat. No. 7,659,241:



wherein the wavy line of D_E and D_F indicates the covalent attachment site to an antibody or antibody-linker component, and independently at each location:

R² is selected from H and C₁-C₈ alkyl;

R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula —(CR^aR^b)_n—

wherein R^a and R^b are independently selected from H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

R⁶ is selected from H and C₁-C₈ alkyl;

R⁷ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

each R⁸ is independently selected from H, OH, C₁-C₈ alkyl, C₃-C₈ carbocycle and O—(C₁-C₈ alkyl);

R⁹ is selected from H and C₁-C₈ alkyl;

R¹⁰ is selected from aryl or C₃-C₈ heterocycle;

Z is O, S, NH, or NR¹², wherein R¹² is C₁-C₈ alkyl;

R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, C₃-C₈ heterocycle, —(R¹³O)_m—R¹⁴, or —(R¹³O)_m—CH(R¹⁵)₂;

m is an integer ranging from 1-1000;

R¹³ is C₂-C₈ alkyl;

R¹⁴ is H or C₁-C₈ alkyl;

each occurrence of e is independently H, COOH, —(CH₂)_n—N(R¹⁶)₂, —(CH₂)_n—SO₃H, or —(CH₂)_n—SO₃—C₁-C₈ alkyl;

each occurrence of e is independently H, C₁-C₈ alkyl, or —(CH₂)_n—COOH;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2$ -aryl, $-C(R^8)_2-C(R^8)_2-(C_3-C_8 \text{ heterocycle})$, and $-C(R^8)_2-C(R^8)_2-(C_3-C_8 \text{ carbocycle})$; and

n is an integer ranging from 0 to 6.

[0251] In one embodiment, R^3 , R^4 and R^7 are independently isopropyl or sec-butyl and R^5 is $-H$ or methyl. In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^5 is $-H$, and R^7 is sec-butyl.

[0252] In yet another embodiment, R^2 and R^6 are each methyl, and R^9 is $-H$.

[0253] In still another embodiment, each occurrence of R^8 is $-OCH_3$.

[0254] In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is $-H$, R^7 is sec-butyl, each occurrence of R^8 is $-OCH_3$, and R^9 is $-H$.

[0255] In one embodiment, Z is $-O-$ or $-NH-$.

[0256] In one embodiment, R^{10} is aryl.

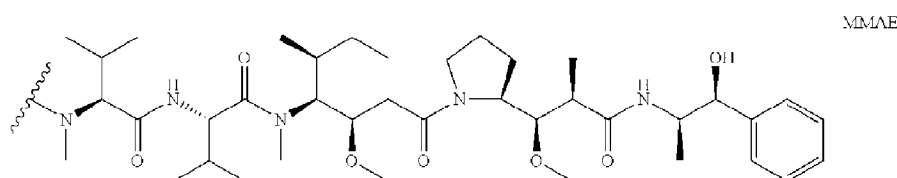
[0257] In an exemplary embodiment, R^{10} is -phenyl.

[0258] In an exemplary embodiment, when Z is $-O-$, R^{11} is $-H$, methyl or t-butyl.

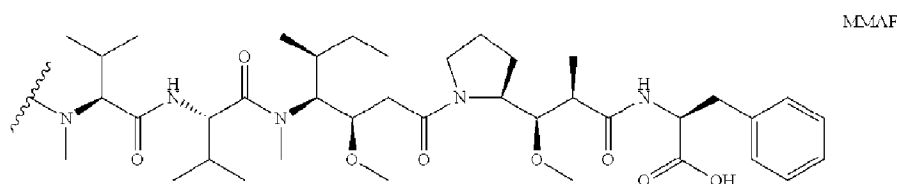
[0259] In one embodiment, when Z is $-NH$, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-N(R^{16})_2$, and R^{16} is $-C_1-C_8$ alkyl or $-(CH_2)_n-COOH$.

[0260] In another embodiment, when Z is $-NH$, R^{11} is $-CH(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$.

[0261] An exemplary auristatin embodiment of formula D_E is MMAE, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:



[0262] An exemplary auristatin embodiment of formula D_E is MMAF, wherein the wavy line indicates the covalent attachment to a linker (L) of an antibody-drug conjugate:



[0263] Other exemplary embodiments include monomethylvaline compounds having phenylalanine carboxy modifications at the C-terminus of the pentapeptide auristatin drug moiety (WO 2007/008848) and monomethylvaline compounds having phenylalanine sidechain modifications at the C-terminus of the pentapeptide auristatin drug moiety (WO 2007/008603).

[0264] Nonlimiting exemplary embodiments of ADCs of Formula I comprising MMAF and various linker components further include Ab-MC-PAB-MMAF and Ab-PAB-MMAF. Immunoconjugates comprising MMAF attached to an antibody by a linker that is not proteolytically cleavable have been shown to possess activity comparable to immunoconjugates comprising MMAF attached to an antibody by a proteolytically cleavable linker (Doronina et al., *Bioconjugate Chem.*, vol. 17, pp. 114-124, 2006). In some such embodiments, drug release is believed to be effected by antibody degradation in the cell.

[0265] Typically, peptide-based drug moieties can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to a liquid phase synthesis method (see, e.g., E. Schröder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press). Auristatin/dolastatin drug moieties may, in some embodiments, be prepared according to the methods of: U.S. Pat. No. 7,498,298; U.S. Pat. No. 5,635,483; U.S. Pat. No. 5,780,588; Pettit et al., *J. Am. Chem. Soc.*, vol. 111, pp. 5463-5465, 1998; Pettit et al., *Anti-Cancer Drug Design*, vol. 13, pp. 243-277, 1998; Pettit et al., *Synthesis*, vol. 6, pp. 719-725, 1996; Pettit et al., *J. Chem. Soc. Perkin Trans.* vol. 15, pp. 859-863, 1996; and Doronina, *Nat. Biotechnol.*, vol. 21, pp. 778-784, 2003.

[0266] In some embodiments, auristatin/dolastatin drug moieties of formulas D_E such as MMAE, and D_E, such as MMAF, and drug-linker intermediates and derivatives thereof, such as MC-MMAF, MC-MMAE, MC-vc-PAB-MMAF, and MC-vc-PAB-MMAE, may be prepared using methods described in U.S. Pat. No. 7,498,298; Doronina et al., *Bioconjugate Chem.*, vol. 17, pp. 114-124, 2006; and Doronina et al., *Nat. Biotech.*, vol. 21, pp. 778-784, 2003 and then conjugated to an antibody of interest.

3) Calicheamicin

[0267] In some embodiments, the immunoconjugate comprises an antibody or antibody fragment conjugated to one or more calicheamicin molecules. The calicheamicin family of antibiotics, and analogues thereof, are capable of producing double-stranded DNA breaks at sub-picomolar concentrations (Hinman et al., *Cancer Research*, vol. 53, pp. 3336-3342, 1993; Lode et al., *Cancer Research*, vol. 58, pp. 2925-2928, 1998). Calicheamicin has intracellular sites of action but, in certain instances, does not readily cross the plasma membrane. Therefore, cellular uptake of these agents through antibody-mediated internalization may, in some embodiments, greatly enhance their cytotoxic effects. Nonlimiting exemplary methods of preparing antibody-drug conjugates with a calicheamicin

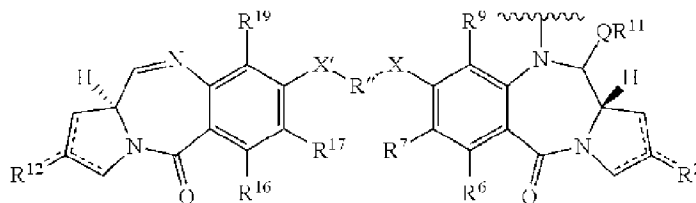
drug moiety are described, for example, in U.S. Pat. No. 5,712,374; U.S. Pat. No. 5,714,586; U.S. Pat. No. 5,739,116; and U.S. Pat. No. 5,767,285.

4) Pyrrolobenzodiazepines

[0268] In some embodiments, an ADC comprises a pyrrolobenzodiazepine (PBD). In some embodiments, PDB dimers recognize and bind to specific DNA sequences. The natural product anthramycin, a PBD, was first reported in 1965 (Leimgruber et al., *J. Am. Chem. Soc.*, vol. 87, pp. 5793-5795, 1965; Leimgruber et al., *J. Am. Chem. Soc.*, vol. 87, pp. 5791-5793, 1965). Since then, a number of PBDs, both naturally-occurring and analogues, have been reported (Thurston et al., *Chem. Rev.* vol. 1994, pp. 433-465 1994, including dimers of the tricyclic PBD scaffold (U.S. Pat. No. 6,884,799; U.S. Pat. No. 7,049,311; U.S. Pat. No. 7,067,511; U.S. Pat. No. 7,265,105; U.S. Pat. No. 7,511,032; U.S. Pat. No. 7,528,126; U.S. Pat. No. 7,557,099). Without intending to be bound by any particular theory, it is believed that the dimer structure imparts the appropriate three-dimensional shape for isohelicity with the minor groove of B-form DNA, leading to a snug fit at the binding site (Kohn, In *Antibiotics III*. Springer-Verlag, New York, pp. 3-11 (1975); Hurley and Needham-VanDevanter, *Acc. Chem. Res.*, vol. 19, pp. 230-237, 1986). Dimeric PBD compounds bearing C2 aryl substituents have been shown to be useful as cytotoxic agents (Hartley et al *Cancer Res.*, vol. 70, pp. 6849-6858, 2010; Antonow, *J. Med. Chem.* vol. 53, pp. 2927-2941, 2010; Howard et al., *Bioorganic and Med. Chem. Letters*, vol. 19, pp. 6463-6466, 2009).

[0269] PBD dimers have been conjugated to antibodies and the resulting ADC shown to have anti-cancer properties. Nonlimiting exemplary linkage sites on the PBD dimer include the five-membered pyrrolo ring, the tether between the PBD units, and the N10-C11 imine group (WO 2009/016516; US 2009/304710; US 2010/047257; US 2009/036431; US 2011/0256157; WO 2011/130598).

[0270] Nonlimiting exemplary PBD dimer components of ADCs are of:



and salts and solvates thereof, wherein:

the wavy line indicates the covalent attachment site to the linker;

the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

R^2 is independently selected from H, OH, $=O$, $=CH_2$, CN, R, OR, $=CH-R^D$, $=C(R^D)_2$, $O-SO_2-R$, CO_2R and COR, and optionally further selected from halo or dihalo, wherein R^D is independently selected from R, CO_2R , COR, CHO, CO_2H , and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH_2 , NHR, NRR' , NO_2 , Me_3Sn and halo;

R^7 is independently selected from H, R, OH, OR, SH, SR, NH_2 , NHR, NRR' , NO_2 , Me_3Sn and halo;

Q is independently selected from O, S and NH;

R^{11} is either H, or R or, where Q is O, SO_3M , where M is a metal cation;

R and R' are each independently selected from optionally substituted C_{1-8} alkyl, C_{1-12} alkyl, C_{3-8} heterocyclyl, C_{3-20} heterocycle, and C_{5-20} aryl groups, and optionally in relation to the group NRR' , R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

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

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

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

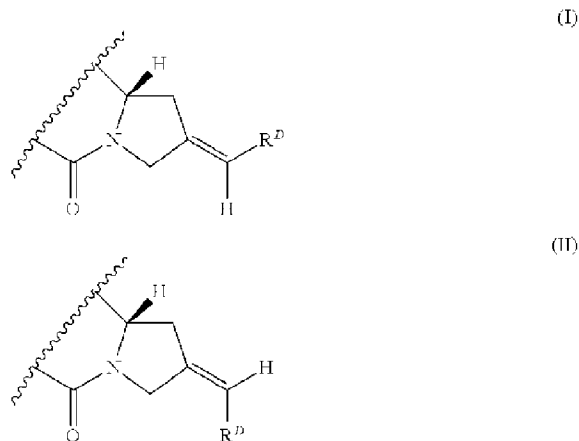
[0271] In some embodiments, R and R' are each independently selected from optionally substituted C_{1-12} alkyl, C_{3-20} heterocycle, and C_{5-20} aryl groups, and optionally in relation to the group NRR' , R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring. In some embodiments, R^9 and R^{19} are H. In some embodiments, R^6 and R^{16} are H.

[0272] In some embodiments, R^7 and R^{17} are both OR^{7A} , where R^{7A} is optionally substituted C_{1-4} alkyl. In some embodiments, R^{7A} is Me. In some embodiments, R^{7A} is CH_2Ph , where Ph is a phenyl group. In some embodiments, X is O. In some embodiments, R^{11} is H. In some embodiments, there is a double bond between C2 and C3 in each monomer unit.

[0273] In some embodiments, R^2 and R^{12} are independently selected from H and R. In some embodiments, R^2 and R^{12} are independently R. In some embodiments, R^2 and R^{12} are independently optionally substituted C_{5-20} aryl or C_{5-7} aryl or C_{8-10} aryl. In some embodiments, R^2 and R^{12} are independently optionally substituted phenyl, thienyl, naphthyl, pyridyl, quinolinyl, or isoquinolinyl. In some embodiments, R^2 and R^{12} are independently selected from $=O$, $=CH_2$, $=CH-R^D$, and $=C(R^D)_2$. In some embodiments, R^2 and R^{12} each $=CH_2$. In some embodiments, R^2 and R^{12} are each H. In some embodiments, R^2 and R^{12} are each $=O$. In

some embodiments, R^2 and R^{12} are each $=CF_2$. In some embodiments, R^2 and/or R^{12} are independently $=C(R^D)_2$. In some embodiments, R^2 and/or R^{12} are independently $=CH-R^D$.

[0274] In some embodiments, when R^2 and/or R^{12} is $=CH-R^D$, each group may independently have either configuration shown below:



In some embodiments, a $=CH-R^D$ is in configuration (I). In some embodiments, R'' is a C_3 alkylene group or a C_5 alkylene group.

[0275] The linkers of PBD dimer-val-cit-PAB-Ab and the PBD dimer-Phe-Lys-PAB-Ab are protease cleavable, while the linker of PBD dimer-maleimide-acetal is acid-labile.

[0276] PBD dimers and ADCs comprising PBD dimers may be prepared according to methods known in the art. See, e.g., WO 2009/016516; US 2009/304710; US 2010/047257; US 2009/036431; US 2011/0256157; WO 2011/130598.

5) Anthracyclines

[0277] In some embodiments, an ADC may comprise anthracycline. Anthracyclines are antibiotic compounds that exhibit cytotoxic activity. While not intending to be bound by any particular theory, studies have indicated that anthracyclines may operate to kill cells by a number of different mechanisms, including: 1) intercalation of the drug molecules into the DNA of the cell thereby inhibiting DNA-dependent nucleic acid synthesis; 2) production by the drug of free radicals which then react with cellular macromolecules to cause damage to the cells, and/or 3) interactions of the drug molecules with the cell membrane (see, e.g., C. Peterson et al., "Transport And Storage Of Anthracycline In Experimental Systems And Human Leukemia" in *Anthracycline Antibiotics In Cancer Therapy*; N. R. Bachur, "Free Radical Damage" id. at pp. 97-102). Because of their cytotoxic potential anthracyclines have been used in the treatment of numerous cancers such as leukemia, breast carcinoma, lung carcinoma, ovarian adenocarcinoma and sarcomas (see e.g., P. H-Wiernik, in *Anthracycline: Current Status And New Developments*, p. 11).

[0278] Nonlimiting exemplary anthracyclines include doxorubicin, epirubicin, idarubicin, daunomycin, nemorubicin, and derivatives thereof. Immunoconjugates and prodrugs of daunorubicin and doxorubicin have been prepared and studied (Kratz et al., *Current Med. Chem.*, vol. 13, pp. 477-523, 2006; Jeffrey et al., *Bioorganic & Med. Chem. Letters*, vol. 16, pp. 358-362, 1996; Torgov et al., *Bioconj. Chem.*, vol. 16, pp. 717-721, 2005; Nagy et al., *Proc. Natl. Acad. Sci. USA*, vol. 97, pp. 829-834, 2000; Dubowchik et al., *Bioorg. & Med. Chem. Letters*, vol. 12, pp. 1529-1532, 2002; King et al., *J. Med. Chem.*, vol. 45, pp. 4336-4343, 2002; EP 0328147; U.S. Pat. No. 6,630,579). The antibody-drug conjugate BR96-doxorubicin reacts specifically with the tumor-associated antigen Lewis-Y and has been evaluated in phase I and II studies (Saleh et al., *J. Clin. Oncology*, vol. 18, pp. 2282-2292, 2000; Ajani et al., *Cancer Jour.*, vol. 6, pp. 78-81, 2000; Tolcher et al., *J. Clin. Oncology*, vol. 17, pp. 478-484, 1999).

[0279] PNU-159682 is a potent metabolite (or derivative) of nemorubicin (Quintieri et al., *Clinical Cancer Research*, vol. 11, pp. 1608-1617, 2005). Nemorubicin is a semisynthetic analog of doxorubicin with a 2-methoxymorpholino group on the glycoside amino of doxorubicin and has been under clinical evaluation (Grandi et al. *Cancer Treat. Rev.* vol.17, pp. 133-138, 1990; Ripamonti et al. *Brit. J. Cancer*, vol. 65, pp. 703-707, 1992), including phase II/III trials for hepatocellular carcinoma (Sun et al., *Proceedings of the American Society for Clinical Oncology*, vol. 22, Abs1448, 2003; Quintieri, *Proceedings of the American Association of Cancer Research*, vol. 44: 1st Ed, Abs 4649, 2003; Pacciarini et al., *Jour. Clin. Oncology*, vol. 24, p. 14116, 2006).

[0280] Anthracyclines, including PNU-159682, may be conjugated to antibodies through several linkage sites and a variety of linkers (US 2011/0076287; WO2009/099741; US 2010/0034837; WO 2010/009124), including the linkers described herein.

[0281] The linker of PNU-159682 maleimide acetal-Ab is acid-labile, while the linkers of PNU-159682-val-cit-PAB-Ab, PNU-159682-val-cit-PAB-spacer-Ab, and PNU-159682-val-cit-PAB-spacer(R¹R²)-Ab are protease cleavable.

6) Other Drug Moieties

[0282] Drug moieties also include geldanamycin (Mandler et al., *J. Nat. Cancer Inst.*, vol. 92, pp. 1573-1581, 2000; Mandler et al., *Bioorganic & Med. Chem. Letters*, vol. 10, pp. 1025-1028, 2000; Mandler et al., *Bioconjugate Chem.*, vol. 13, pp. 786-791, 2002); and enzymatically active toxins and fragments thereof, including, but not limited to, diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii

proteins, dianthin proteins, *Phytolaca americana* proteins (PAPI, PAPII, and PAP-S), *momordica charantia* inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, enomycin and the tricothecenes. See, e.g., WO 93/21232.

[0283] Drug moieties also include compounds with nucleolytic activity (e.g., a ribonuclease or a DNA endonuclease).

[0284] In certain embodiments, an immunoconjugate may comprise a highly radioactive atom. A variety of radioactive isotopes are available for the production of radioconjugated antibodies. Examples include At^{211} , I^{131} , I^{125} , Y^{90} , Re^{186} , Re^{188} , Sm^{153} , Bi^{212} , P^{32} , Pb^{212} and radioactive isotopes of Lu. In some embodiments, when an immunoconjugate is used for detection, it may comprise a radioactive atom for scintigraphic studies, for example Tc^{99} or I^{123} , or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, MRI), such as zirconium-89, iodine-123, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Zirconium-89 may be complexed to various metal chelating agents and conjugated to antibodies, e.g., for PET imaging (WO 2011/056983).

[0285] The radio- or other labels may be incorporated in the immunoconjugate in known ways. For example, a peptide may be biosynthesized or chemically synthesized using suitable amino acid precursors comprising, for example, one or more fluorine-19 atoms in place of one or more hydrogens. In some embodiments, labels such as Tc^{99} , I^{123} , Re^{186} , Re^{188} and In^{111} can be attached via a cysteine residue in the antibody. In some embodiments, yttrium-90 can be attached via a lysine residue of the antibody. In some embodiments, the IODOGEN method (Fraker et al., *Biochem. Biophys. Res. Commun.*, vol. 80, pp. 49-57, 1978) can be used to incorporate iodine-123. "Monoclonal Antibodies in Immunoscintigraphy" (Chatal, CRC Press 1989) describes certain other methods.

[0286] In certain embodiments, an immunoconjugate may comprise an antibody conjugated to a prodrug-activating enzyme. In some such embodiments, a prodrug-activating enzyme converts a prodrug (e.g., a peptidyl chemotherapeutic agent, see WO 81/01145) to an active drug, such as an anti-cancer drug. Such immunoconjugates are useful, in some embodiments, in antibody-dependent enzyme-mediated prodrug therapy ("ADEPT"). Enzymes that may be conjugated to an antibody include, but are not limited to, alkaline phosphatases, which are useful for converting phosphate-containing prodrugs into free drugs; arylsulfatases, which are useful for converting sulfate-containing prodrugs into free drugs; cytosine deaminase, which is useful for converting non-toxic 5-fluorocytosine into the anti-cancer drug, 5-fluorouracil; proteases, such as *serratia* protease, thermolysis, subtilisin, carboxypeptidases and cathepsins

(such as cathepsins B and L), which are useful for converting peptide-containing prodrugs into free drugs; D-alanylcarboxypeptidases, which are useful for converting prodrugs that contain D-amino acid substituents; carbohydrate-cleaving enzymes such as β -galactosidase and neuraminidase, which are useful for converting glycosylated prodrugs into free drugs; β -lactamase, which is useful for converting drugs derivatized with β -lactams into free drugs; and penicillin amidases, such as penicillin V amidase and penicillin G amidase, which are useful for converting drugs derivatized at their amine nitrogens with phenoxyacetyl or phenylacetyl groups, respectively, into free drugs. In some embodiments, enzymes may be covalently bound to antibodies by recombinant DNA techniques well known in the art. See, e.g., Neuberger et al., *Nature*, vol. 312, pp. 604-608, 1984.

c) Drug Loading

[0287] Drug loading is represented by p , the average number of drug moieties per antibody in a molecule of Formula I. Drug loading may range from 1 to 20 drug moieties (D) per antibody. ADCs of Formula I include collections of antibodies conjugated with a range of drug moieties, from 1 to 20. The average number of drug moieties per antibody use in the preparation of ADCs from conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of ADCs in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous ADCs where p is a certain value from ADCs with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

[0288] For some antibody-drug conjugates, p may be limited by the number of attachment sites on the antibody. For example, where the attachment is a cysteine thiol, as in certain exemplary embodiments above, an antibody may have only one or several cysteine thiol groups, or may have only one or several sufficiently reactive thiol groups through which a linker may be attached. In certain embodiments, higher drug loading, e.g. $p > 5$, may cause aggregation, insolubility, toxicity, or loss of cellular permeability of certain antibody-drug conjugates. In certain embodiments, the average drug loading for an ADC ranges from 1 to about 8; from about 2 to about 6; or from about 3 to about 5. Indeed, it has been shown that for certain ADCs, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5 (U.S. Pat. No. 7,498,298).

[0289] In certain embodiments, fewer than the theoretical maximum of drug moieties are conjugated to an antibody during a conjugation reaction. An antibody may contain, for example, lysine residues that do not react with the drug-linker intermediate or linker reagent, as discussed below. Generally, antibodies do not contain many free and reactive cysteine

thiol groups which may be linked to a drug moiety; indeed most cysteine thiol residues in antibodies exist as disulfide bridges. In certain embodiments, an antibody may be reduced with a reducing agent such as dithiothreitol (DTT) or tricarboylethylphosphine (TCEP), under partial or total reducing conditions, to generate reactive cysteine thiol groups. In certain embodiments, an antibody is subjected to denaturing conditions to reveal reactive nucleophilic groups such as lysine or cysteine.

[0290] The loading (drug/antibody ratio) of an ADC may be controlled in different ways, and for example, by: (i) limiting the molar excess of drug-linker intermediate or linker reagent relative to antibody, (ii) limiting the conjugation reaction time or temperature, and (iii) partial or limiting reductive conditions for cysteine thiol modification.

[0291] It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate or linker reagent, then the resulting product is a mixture of ADCs with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by a dual ELISA antibody assay, which is specific for antibody and specific for the drug. Individual ADCs may be identified in the mixture by mass spectroscopy and separated by HPLC, e.g. hydrophobic interaction chromatography (see, e.g., McDonagh et al., *Prot. Engr. Design & Selection*, vol. 19, pp. 299-307, 2006; Hamblett et al., *Clin. Cancer Res.*, vol. 10, pp. 7063-7070, 2004). In certain embodiments, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

d) Certain Methods of Preparing Immunoconjugates

[0292] An immunoconjugate that is an ADC of Formula I may be prepared by several routes employing organic chemistry reactions, conditions, and reagents known to those skilled in the art, including: (1) reaction of a nucleophilic group of an antibody with a bivalent linker reagent to form Ab-L via a covalent bond, followed by reaction with a drug moiety D; and (2) reaction of a nucleophilic group of a drug moiety with a bivalent linker reagent, to form D-L, via a covalent bond, followed by reaction with a nucleophilic group of an antibody.

Exemplary methods for preparing an ADC of Formula I via the latter route are described in U.S. Pat. No. 7,498,298.

[0293] Nucleophilic groups on antibodies include, but are not limited to: (i) N-terminal amine groups, (ii) side chain amine groups, e.g. lysine, (iii) side chain thiol groups, e.g. cysteine, and (iv) sugar hydroxyl or amino groups where the antibody is glycosylated. Amine, thiol, and hydroxyl groups are nucleophilic and capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters

such as NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; and (iii) aldehydes, ketones, carboxyl, and maleimide groups.

Certain antibodies have reducible interchain disulfides, i.e. cysteine bridges. Antibodies may be made reactive for conjugation with linker reagents by treatment with a reducing agent such as DTT (dithiothreitol) or tricarboylethylphosphine (TCEP), such that the antibody is fully or partially reduced. Each cysteine bridge will thus form, theoretically, two reactive thiol nucleophiles. Additional nucleophilic groups can be introduced into antibodies through modification of lysine residues, e.g., by reacting lysine residues with 2-iminothiolane (Traut's reagent), resulting in conversion of an amine into a thiol. Reactive thiol groups may also be introduced into an antibody by introducing one, two, three, four, or more cysteine residues (e.g., by preparing variant antibodies comprising one or more non-native cysteine amino acid residues).

[0294] Antibody-drug conjugates of the invention may also be produced by reaction between an electrophilic group on an antibody or antibody fragment, such as an aldehyde or ketone carbonyl group, with a nucleophilic group on a linker reagent or drug. Useful nucleophilic groups on a linker reagent include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. In one embodiment, an antibody is modified to introduce electrophilic moieties that are capable of reacting with nucleophilic substituents on the linker reagent or drug. In another embodiment, the sugars of glycosylated antibodies may be oxidized, e.g. with periodate oxidizing reagents, to form aldehyde or ketone groups which may react with the amine group of linker reagents or drug moieties. The resulting imine Schiff base groups may form a stable linkage, or may be reduced, e.g. by borohydride reagents to form stable amine linkages. In one embodiment, reaction of the carbohydrate portion of a glycosylated antibody with either galactose oxidase or sodium meta-periodate may yield carbonyl (aldehyde and ketone) groups in the antibody that can react with appropriate groups on the drug (Hermanson, *Bioconjugate Techniques*). In another embodiment, antibodies containing N-terminal serine or threonine residues can react with sodium meta-periodate, resulting in production of an aldehyde in place of the first amino acid (Geoghegan & Stroh, *Bioconjugate Chem.*, vol. 3, pp. 138-146, 1992; U.S. Pat. No. 5,362,852). Such an aldehyde can be reacted with a drug moiety or linker nucleophile.

[0295] Exemplary nucleophilic groups on a drug moiety include, but are not limited to: amine, thiol, hydroxyl, hydrazide, oxime, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide groups capable of reacting to form covalent bonds with electrophilic groups on linker moieties and linker reagents including: (i) active esters such as

NHS esters, HOBt esters, haloformates, and acid halides; (ii) alkyl and benzyl halides such as haloacetamides; (iii) aldehydes, ketones, carboxyl, and maleimide groups.

[0296] Nonlimiting exemplary cross-linker reagents that may be used to prepare ADCs are described herein in the section titled “Exemplary Linkers.” Methods of using such cross-linker reagents to link two moieties, including a proteinaceous moiety and a chemical moiety, are known in the art. In some embodiments, a fusion protein comprising an antibody and a cytotoxic agent may be made, e.g., by recombinant techniques or peptide synthesis. A recombinant DNA molecule may comprise regions encoding the antibody and cytotoxic portions of the conjugate either adjacent to one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the conjugate.

[0297] In yet another embodiment, an antibody or antibody fragment may be conjugated to a “receptor” (such as streptavidin) for utilization in tumor pre-targeting wherein the antibody/antibody fragment-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a “ligand” (e.g., avidin) which is conjugated to a cytotoxic agent (e.g., a drug or radionucleotide).

C. Methods and Compositions for Diagnostics and Detection

[0298] In certain embodiments, any of the anti-ROR2 antibodies or antibody fragments provided herein may be used for detecting the presence of ROR2 in a biological sample. The term “detecting” as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as breast, pancreas, esophagus, lung and/or brain cells or tissue.

[0299] A further aspect of the invention relates to an anti-ROR2 antibody or antibody fragment of the invention for diagnosing and/or monitoring a cancer or another disease in which ROR2 expression levels are increased or decreased from a normal physiological level at at least one location in the body.

[0300] In a preferred embodiment, antibodies or antibody fragments of the invention may be labelled with a detectable molecule or substance, such as a fluorescent molecule, a radioactive molecule or any other label known in the art as above described. For example, an antibody or antibody fragment of the invention may be labelled with a radioactive molecule. For example, suitable radioactive molecules include but are not limited to radioactive atoms used for scintigraphic studies such as ¹²³I, ¹²⁴I, ¹¹¹In, ¹⁸⁶Re, and ¹⁸⁸Re. Antibodies or antibody fragments of the invention may also be labelled with a spin label for nuclear magnetic resonance (NMR) imaging, such as iodine-123, iodine-131, indium-111, fluorine-19, carbon-

13, nitrogen-15, oxygen-17, gadolinium, manganese or iron. Following administration of the antibody, the distribution of the radiolabeled antibody within the patient is detected. Any suitable known method can be used. Some non-limiting examples include, computed tomography (CT), position emission tomography (PET), magnetic resonance imaging (MRI), fluorescence, chemiluminescence and sonography.

[0301] Antibodies or antibody fragments of the invention may be useful for diagnosing and staging of cancer and diseases associated with ROR2 overexpression. Cancers associated with ROR2 overexpression may include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer (NSCLC), gastric cancer, pancreatic cancer, glial cell tumors such as glioblastoma and neurofibromatosis, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, melanoma, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, sarcomas such as fibrosarcoma and osteosarcoma, hematological cancers (leukemias), astrocytomas, and various types of head and neck cancer or other ROR2 expressing or overexpressing hyperproliferative diseases.

[0302] Antibodies or antibody fragments of the invention may be useful for diagnosing diseases other than cancers for which ROR2 expression is increased or decreased. Both the (soluble or cellular ROR2 forms can be used for such diagnoses. Typically, such diagnostic methods involve use of a biological sample obtained from the patient. As used herein the term “biological sample” encompasses a variety of sample types obtained from a subject that can be used in a diagnostic or monitoring assay. Biological samples include but are not limited to blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or a tissue culture or cells derived therefrom, and the progeny thereof. For example, biological samples include cells obtained from a tissue sample collected from an individual suspected of having a cancer associated with ROR2 overexpression, and in preferred embodiments from glioma, gastric, lung, pancreatic, breast, prostate, renal, hepatic and endometrial. Biological samples encompass clinical samples, cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

[0303] In a particular embodiment, the invention is a method of diagnosing a cancer associated with ROR2 overexpression in a subject by detecting ROR2 on cells from the subject using the antibody of the invention. In particular, said method may include steps of:

(a) contacting a biological sample of a subject with an antibody or antibody fragment according to the invention under conditions suitable for the antibody or antibody fragment to form complexes with cells in the biological sample that express ROR2; and

(b) detecting and/or quantifying said complexes, whereby detection of said complexes is indicative of a cancer associated with ROR2 overexpression.

[0304] In order to monitor the progress of a cancer, the method according to the invention may be repeated at different times, in order to determine if antibody binding to the samples increases or decreases, wherefrom it can be determined if the cancer has progressed, regressed or stabilized.

[0305] In a particular embodiment, the invention is a method of diagnosing a disease associated with the expression or overexpression of ROR2 or a decrease or increase of the soluble form of ROR2. Examples of such diseases may include human immune disorders, thrombotic diseases (thrombosis and atherothrombosis), and cardiovascular diseases

[0306] In one embodiment, an anti-ROR2 antibody or antibody fragment for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of ROR2 in a biological sample is provided. In a further aspect, a method of quantifying the amount of ROR2 in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-ROR2 antibody or antibody fragment as described herein under conditions permissive for binding of the anti-ROR2 antibody or antibody fragment to ROR2, and detecting whether a complex is formed between the anti-ROR2 antibody or antibody fragment and ROR2. Such a method may be carried out *in vitro* or *in vivo*. In one embodiment, an anti-ROR2 antibody or antibody fragment is used to select subjects eligible for therapy. In some embodiments, the therapy will include administration of an anti-ROR2 antibody or antibody fragment to the subject.

[0307] In certain embodiments, labeled anti-ROR2 antibodies or antibody fragments are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, e.g., through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes ^{32}P , ^{14}C , ^{125}I , ^3H , and ^{131}I , fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luciferases, e.g., firefly luciferase and bacterial luciferase (U.S. Pat. No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β -galactosidase, glucoamylase, lysozyme, saccharide oxidases, e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

D. Pharmaceutical Formulations

[0308] The anti-ROR2 antibodies or antibody fragments have cell killing activity. This cell killing activity extends to multiple different types of cell lines. Further, these antibodies or antibody fragments, once conjugated to a cytotoxic agent, can reduce tumor size and may exhibit reduced toxicity. See Example 2 of this application. Thus, the anti-ROR2 antibodies, fragments or immunoconjugates thereof may be useful for treating proliferative diseases associated with ROR2 expression. The antibodies, fragments or immunoconjugates may be used alone or in combination with any suitable agent or other conventional treatments.

[0309] The anti-ROR2 antibody or antibody fragment may be used to treat diseases associated with ROR2 expression, overexpression or activation. There are no particular limitations on the types of cancer or tissue that can be treated other than the requirement for ROR2 expression. Examples include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer (NSCLC), gastric cancer, pancreatic cancer, glial cell tumors such as glioblastoma and neurofibromatosis, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, melanoma, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer, renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, sarcomas such as fibrosarcoma and osteosarcoma, hematological cancers (leukemias), astrocytomas, and various types of head and neck cancer. More typical cancers for treatment are glioma, gastric, lung, pancreatic, breast, prostate, renal, hepatic and endometrial cancers.

[0310] Anti-ROR2 antibodies or antibody fragments are potential activators of the innate immune response and thus may be used in the treatment of human immune disorders, such as sepsis. The anti-ROR2 antibody or antibody fragment of the invention may also be used as adjuvants for immunization such as for vaccines and as anti-infection agents against, for example, bacteria, viruses and parasites.

[0311] Anti-ROR2 antibody or antibody fragment may be used to protect against, prevent or treat thrombotic diseases such as venous and arterial thrombosis and atherothrombosis. Anti-ROR2 antibody or antibody fragment may also be used to protect against, prevent or treat cardiovascular diseases as well as to prevent or inhibit the entry of viruses such as Lassa and Ebola viruses and to treat viral infections.

[0312] In each of the embodiments of the treatment methods described herein, the anti-ROR2 antibody, antibody fragment or anti-ROR2 antibody or antibody fragment immunoconjugate may be delivered in a manner consistent with conventional methodologies associated with management of the disease or disorder for which treatment is sought. In accordance with the disclosure herein, an effective amount of the antibody, antibody fragment or

immunoconjugate is administered to a subject in need of such treatment for a time and under conditions sufficient to prevent or treat the disease or disorder. Thus, an aspect of the invention relates to a method for treating a disease associated with the expression of ROR2 comprising administering to a subject in need thereof with a therapeutically effective amount of an antibody, antibody fragment or immunoconjugate of the invention.

[0313] For administration, the anti-ROR2 antibody, antibody fragment or immunoconjugate may be formulated as a pharmaceutical composition. The pharmaceutical composition including anti-ROR2 antibody, antibody fragment or immunoconjugate can be formulated according to known methods for preparing pharmaceutical compositions. In such methods, the therapeutic molecule is typically combined with a mixture, solution or composition containing a pharmaceutically acceptable carrier.

[0314] A pharmaceutically acceptable carrier is a material that can be tolerated by a recipient patient. Sterile phosphate-buffered saline is one example of a pharmaceutically acceptable carrier. Other suitable pharmaceutically acceptable carriers are well-known to those in the art. (See, e.g., Gennaro (ed.), Remington's Pharmaceutical Sciences (Mack Publishing Company, 19th ed. 1995)) Formulations may further include one or more excipients, preservatives, solubilizers, buffering agents, albumin to prevent protein loss on vial surfaces, etc.

[0315] The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and sex of the patient, etc. These considerations can be taken into account by a skilled person to formulate suitable pharmaceutical compositions. The pharmaceutical compositions of the invention can be formulated for topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular administration and the like.

[0316] Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition of, for example, sterilized water or physiological saline, permit the constitution of injectable solutions.

[0317] In some embodiments, tonicity agents, sometimes known as “stabilizers” are present to adjust or maintain the tonicity of a liquid in a composition. When used with large, charged biomolecules such as proteins and antibodies, they are often termed “stabilizers” because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter- and intra-molecular interactions. Tonicity agents can be present in any amount of from 0.1% to 25% by weight, preferably 1 to 5% of the pharmaceutical

composition. Preferred tonicity agents include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[0318] Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) and agents preventing denaturation or adherence to the container wall. Such excipients may include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinositol, myoinositol, galactose, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (e.g., xylose, mannose, fructose, glucose; disaccharides (e.g., lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[0319] Non-ionic surfactants or detergents (also known as “wetting agents”) may be employed to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Non-ionic surfactants may be present in a concentration range of about 0.05 mg/ml to about 1.0 mg/ml, preferably about 0.07 mg/ml to about 0.2 mg/ml.

[0320] Suitable non-ionic surfactants include polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188, etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), laurmacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl cellulose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl sulfate, dioctyle sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride

[0321] The doses used for the administration can be adapted as a function of various parameters, and in particular as a function of the mode of administration used, of the relevant pathology, or alternatively of the desired duration of treatment. To prepare pharmaceutical compositions, an effective amount of the antibody or antibody fragment may be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

[0322] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

[0323] Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in a water suitably mixed with a surfactant. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

[0324] The anti-ROR2 antibody or antibody fragment can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

[0325] The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

[0326] Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with one or more of the other ingredients enumerated above, as may be required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a

sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[0327] The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of dimethyl sulfoxide (DMSO) as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area.

[0328] Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

[0329] For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

[0330] The antibodies or antibody fragments may be formulated within a therapeutic mixture to deliver about 0.0001 to 10.0 milligrams, or about 0.001 to 5 milligrams, or about 0.001 to 1 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose. Multiple doses can also be administered at selected time intervals.

[0331] In addition to the compounds formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; time release capsules; and any other form currently used.

[0332] In certain embodiments, the use of liposomes and/or nanoparticles is contemplated for the introduction of antibodies or antibody fragments into host cells. The formation and use of liposomes and/or nanoparticles are known to those of skill in the art.

[0333] Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) are generally designed using polymers able to degrade *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and such particles may be easily made.

[0334] Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μm . Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core. The physical

characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations

[0335] Pharmaceutical formulations containing an anti-ROR2 antibody or antibody fragment as described herein are prepared by mixing such antibody or antibody fragment having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG).

[0336] Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and

2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

[0337] Exemplary lyophilized antibody formulations are described in U.S. Pat. No. 6,267,958. Aqueous antibody formulations include those described in U.S. Pat. No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

[0338] The formulation herein may also contain more than one active ingredient as necessary for the particular indication being treated. Preferably, ingredients with complementary activities that do not adversely affect each other may be combined into a single formulation. For example, it may be desirable to provide an EGFR antagonist (such as erlotinib), an anti-angiogenic agent (such as a VEGF antagonist which may be an anti-VEGF antibody) or a chemotherapeutic agent (such as a taxoid or a platinum agent) in addition to the anti-ROR2 antibody, antibody fragment or immunoconjugate of the present invention. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

[0339] Active ingredients may be encapsulated in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization. For example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacrylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions may be employed. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

[0340] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody or antibody fragment, which matrices may be in the form of shaped articles, e.g. films, or microcapsules.

[0341] The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

E. Therapeutic Methods and Compositions

[0342] Any of the anti-ROR2 antibodies or antibody fragments provided herein may be used in therapeutic methods. In one aspect, an anti-ROR2 antibody or antibody fragment for use as a medicament is provided. In further aspects, an anti-ROR2 antibody or antibody fragment for use in treating cancer (e.g., breast cancer, non-small cell lung cancer, pancreatic cancer, brain cancer, cancer of pancreas, brain, kidney, ovary, stomach, leukemia, uterine endometrium, colon, prostate, thyroid, liver, sarcomas such as fibrosarcoma and

osteosarcoma, melanoma, and/or various head and neck cancers) is provided. In certain embodiments, an anti-ROR2 antibody or antibody fragment for use in a method of treatment is provided. In certain embodiments, the invention provides an anti-ROR2 antibody or antibody fragment for use in a method of treating an individual having cancer comprising administering to the individual an effective amount of the anti-ROR2 antibody or antibody fragment. In certain embodiments, the invention provides an anti-ROR2 antibody or antibody fragment for use in a method of treating an individual having an immune disorder (e.g., an autoimmune disorder), a cardiovascular disorder (e.g., atherosclerosis, hypertension, thrombosis), an infectious disease (e.g., Ebola virus, Marburg virus) or diabetes, comprising administering to the individual an effective amount of the anti-ROR2 antibody or antibody fragment. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In further embodiments, the invention provides an anti-ROR2 antibody or antibody fragment for use in inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function.

[0343] In certain embodiments, the invention provides an anti-ROR2 antibody or antibody fragment for use in a method of inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function in an individual comprising administering to the individual an effective of the anti-ROR2 antibody or antibody fragment to inhibit angiogenesis, inhibit cell proliferation, inhibit immune function, inhibit inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibit tumor vasculature development (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibit tumor stromal function. An “individual” according to any of the above embodiments is preferably a human.

[0344] In a further aspect, the invention provides for the use of an anti-ROR2 antibody or antibody fragment in the manufacture or preparation of a medicament. In one embodiment, the medicament is for treatment of cancer (in some embodiments, breast cancer, non-small cell lung cancer, pancreatic cancer, brain cancer, cancer of the pancreas, brain, kidney, ovary, stomach, leukemia, uterine endometrium, colon, prostate, thyroid, liver, sarcomas such as fibrosarcoma and osteosarcoma, melanoma, and/or various head and neck cancers). In a further embodiment, the medicament is for use in a method of treating cancer comprising

administering to an individual having cancer an effective amount of the medicament. In a further embodiment, the medicament is for use in a method of treating an immune disorder (e.g., an autoimmune disorder), a cardiovascular disorder (e.g., atherosclerosis, hypertension, thrombosis), an infectious disease (e.g., Ebola virus, Marburg virus) or diabetes, comprising administering to the individual an effective amount of the anti-ROR2 antibody or antibody fragment. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, e.g., as described below. In a further embodiment, the medicament is for inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function. In a further embodiment, the medicament is for use in a method of inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function in an individual comprising administering to the individual an amount effective of the medicament to inhibit angiogenesis, inhibit cell proliferation, promote immune function, induce inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibit tumor vasculature development (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibit tumor stromal function. An “individual” according to any of the above embodiments may be a human.

[0345] In a further aspect, the invention provides a method for treating ROR2-expressing tumors in an individual with cancer. In one embodiment, the method comprises administering to such individual having cancer an effective amount of an anti-ROR2 antibody or antibody fragment. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An “individual” according to any of the above embodiments may be a human.

[0346] In this aspect of the present invention, methods of treating ROR2-expressing tumors are provided. In some embodiments, the methods comprise administering an immunoconjugate that includes the anti-ROR2 antibody or antibody fragment of the invention.

[0347] In one embodiment of this aspect, the methods of treating ROR2-expressing tumors comprise administering an immunoconjugate that includes the antibody or antibody fragment of the invention, conjugated to an agent selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent, and a cytotoxic agent. The immunoconjugate is an

antibody-drug conjugate (ADC) in which a conditionally active biologic (CAB) anti-ROR2 antibody or antibody fragment is conjugated to one or more drug moieties via a cleavable linker (CAB- ROR2-ADC). For example, the CAB- ROR2-ADC may be BA3021-cleavable linker-MMAE_(n), in which the drug moiety is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive.

[0348] In a specific aspect, the methods of treating the ROR2-expressing tumor comprises administering to a human subject in need of such treatment, a BA3021-cleavable linker-MMAE_(n), where the BA3021 is an antibody or antibody fragment having a heavy chain variable region that includes CDRs H1, H2, and H3 having an amino acid sequence of SEQ ID NO. 16; and a light chain variable region that includes a CDRs L1, L2, and L3 having an amino acid sequence of SEQ ID NO. 21; MMAE is monomethyl auristatin E (MMAE), and (n) is an integer between 1 and 4, inclusive.

[0349] In some embodiments, the present invention provides a therapeutic regimen with a CAB- ROR2-ADC such as the BA3021-cleavable linker-MMAE_(n), administered at doses of about 0.3 mg/kg to about 3.3 mg/kg administered either once or twice every 21 day or 3 week period, or once or twice every 14 day or 2 week period. For instance, BA3021-cleavable linker-MMAE_(n) may be administered at doses of about 0.3 mg/kg to about 3.3 mg/kg once every three weeks (Q3W) or at doses ranging from 1.5 mg/kg to 1.8 mg/kg twice every three weeks such as on days 1 and 8 (2Q3W).

[0350] In certain embodiments, the present invention provides a therapeutic regimen with a CAB-ROR2-ADC such as the BA3021-cleavable linker-MMAE_(n), administered at doses of about 0.3 mg/kg to about 1.8 mg/kg administered either once or twice every 21 day or 3 week period, or once or twice every 14 day or 2 week period. For instance, the BA3021-cleavable linker-MMAE_(n) may be administered at doses of about 0.3 mg/kg to about 1.8 mg/kg administered twice every 14 day or 2 week period such as on days 1 and 8 of every 14 day or 2 week period. Preferably, the mAbBA3011-cleavable linker-MMAE_(n) is administered at doses of about 0.8 mg/kg to about 1.8 mg/kg, either once or twice every 21 day period, such as on days 1 and 8 or every 21 day period, or once or twice every 14 day period such as on days 1 and 8 every 14 day period. In one embodiment, the CAB-ROR2-ADC is administered at doses of 1.8 mg/kg on days 1 and 8 of each 21 day period for one or more consecutive 21 day periods.

[0351] Such a therapeutic regimen is surprisingly efficacious, as the antibody-drug conjugate of the present invention administered at such doses and at such intervals, provides a surprisingly high response rate and an acceptable toxicity or tolerability profile. Accordingly, the present methods provide a safe and effective dosing regimen for administering a CAB-

ROR2-ADC antibody-drug conjugate to a subject. In some embodiments, the dosing regimen increases the subject's probability of responding to the therapy as compared to other dosing regimens. In some embodiments, the dosing regimen does not increase the subject's probability of suffering from an adverse event (including a dose limiting toxicity) as compared to other dosing regimens. The present invention also provides maintenance therapy following the dosing regimen.

[0352] In yet another embodiment, the method of treating a ROR2-expressing tumor comprises administering to a human subject in need of such treatment, a pharmaceutical composition including a CAB- ROR2-ADC such as the BA3021-cleavable linker-MMAE_(n) and a pharmaceutically acceptable carrier, in which the pharmaceutical composition is administered at a dose of 1.8 mg/kg of the human subject weight every 14 days by intravenous infusion.

[0353] CAB-ROR2-ADCs such as BA3021-cleavable linker-MMAE_(n) of the present invention, preferentially bind under defined physiological conditions associated with different diseases and tissues. For example, in cancer, the unique cell metabolism described by Warburg (Warburg 1924; Warburg 1956) contributes to a characteristic tumor microenvironment (TME) such as, low pH and high lactate. The BA3021-cleavable linker-MMAE_(n) of the present invention takes advantage of the unique TME and selectively binds to its target when in close proximity to a tumor expressing ROR2. The activated binding property of BA3021-cleavable linker-MMAE_(n) is reversible, such that there are no permanent changes as it transitions from diseased normal to diseased tissue microenvironments.

[0354] In yet another embodiment, the ROR2-expressing tumor has a tumor membrane percent score (TmPS) of at least 1, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, or at least 95. In one embodiment, the ROR2-expressing tumor has a tumor membrane P score of at least 70, at least 75, at least 80, at least 85, at least 90, or at least 95 percent.

[0355] In a further aspect, the invention provides a method for treating an immune disorder (e.g., an autoimmune disorder), a cardiovascular disorder (e.g., atherosclerosis, hypertension, thrombosis), an infectious disease (e.g., Ebola virus, Marburg virus) or diabetes. In one such embodiment, the method further comprises administering to the individual an effective amount of at least one additional therapeutic agent, as described below. An "individual" according to any of the above embodiments may be a human.

[0356] In a further aspect, the invention provides a method for inhibiting angiogenesis, inhibiting cell proliferation, inhibiting immune function, inhibiting inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibiting tumor vasculature (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibiting tumor stromal function in an individual. In one embodiment, the method comprises administering to the individual an effective amount of an anti-ROR2 antibody or antibody fragment to inhibit angiogenesis, inhibit cell proliferation, promote immune function, induce inflammatory cytokine secretion (e.g., from tumor-associated macrophages), inhibit tumor vasculature development (e.g., intratumoral vasculature or tumor-associated vasculature), and/or inhibit tumor stromal function. In one embodiment, an “individual” is a human.

[0357] In a further aspect, the invention provides pharmaceutical formulations comprising any of the anti-ROR2 antibodies or antibody fragments provided herein, e.g., for use in any of the above therapeutic methods. In one embodiment, a pharmaceutical formulation comprises any of the anti-ROR2 antibodies or antibody fragments provided herein and a pharmaceutically acceptable carrier. In another embodiment, a pharmaceutical formulation comprises any of the anti-ROR2 antibodies or antibody fragments provided herein and at least one additional therapeutic agent, e.g., as described below.

[0358] In each and every treatment described above, the antibodies or antibody fragments of the invention can be used alone, as immunoconjugates or in combination with other agents in a therapy. For instance, an antibody of the invention may be co-administered with at least one additional therapeutic agent. In certain embodiments, an additional therapeutic agent is an anti-angiogenic agent. In certain embodiments, an additional therapeutic agent is a VEGF antagonist (in some embodiments, an anti-VEGF antibody, for example bevacizumab). In certain embodiments, an additional therapeutic agent is an EGFR antagonist (in some embodiment, erlotinib). In certain embodiments, an additional therapeutic agent is a chemotherapeutic agent and/or a cytostatic agent. In certain embodiments, an additional therapeutic agent is a taxoid (e.g., paclitaxel) and/or a platinum agent (e.g., carboplatinum). In certain embodiments the additional therapeutic agent is an agents that enhances the patient's immunity or immune system.

[0359] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same or separate formulations), and separate administration, in which case, administration of the antibody or antibody fragment can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent and/or adjuvant. Antibodies or antibody fragments can also be used in combination with radiation therapy.

[0360] Antibodies or antibody fragments may be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners. The antibody or antibody fragment need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question. The effective amount of such other agents depends on the amount of antibody or antibody fragment present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate.

[0361] For the prevention or treatment of disease, the appropriate dosage of an antibody or antibody fragment (when used alone or in combination with one or more other additional therapeutic agents) will depend on the type of disease to be treated, the type of antibody or antibody fragment, the severity and course of the disease, whether the antibody or antibody fragment is administered for preventive or therapeutic purposes, previous therapy, the patient's clinical history and response to the antibody or antibody fragment, and the discretion of the attending physician. The antibody or antibody fragment is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 1 $\mu\text{g/kg}$ to 40 mg/kg of antibody or antibody fragment can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 1 $\mu\text{g/kg}$ to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. Such doses may be administered intermittently, e.g. every week or every three weeks (e.g. such that the patient receives from about two to about twenty, or e.g. about six doses of the antibody or antibody fragment). An initial higher loading dose, followed by one or more lower doses may be administered. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

[0362] It is understood that any of the above formulations or therapeutic methods may be carried out using an antibody fragment or an immunoconjugate of the invention in place of or in addition to an anti-ROR2 antibody.

[0363] Enhancing the host's immune function to combat tumors is the subject of increasing interest. Conventional methods include (i) APC enhancement, such as (a) injection into the tumor of DNA encoding foreign MHC alloantigens, or (b) transfecting biopsied tumor cells with genes that increase the probability of immune antigen recognition (e.g., immune stimulatory cytokines, GM-CSF, co-stimulatory molecules B7.1, B7.2) of the tumor, (iii) adoptive cellular immunotherapy, or treatment with activated tumor-specific T-cells.

Adoptive cellular immunotherapy includes isolating tumor-infiltrating host T-lymphocytes, expanding the population in vitro, such as through stimulation by IL-2 or tumor or both.

Additionally, isolated T-cells that are dysfunctional may be also be activated by in vitro application of the anti-PD-L1 antibodies of the invention. T-cells that are so-activated may then be readministered to the host. One or more of these methods may be used in combination with administration of the antibody, antibody fragment or immunoconjugate of the present invention.

[0364] Traditional therapies for cancer include the following: (i) radiation therapy (e.g., radiotherapy, X-ray therapy, irradiation) or the use of ionizing radiation to kill cancer cells and shrink tumors. Radiation therapy can be administered either externally via external beam radiotherapy (EBRT) or internally via brachytherapy; (ii) chemotherapy, or the application of cytotoxic drug which generally affect rapidly dividing cells; (iii) targeted therapies, or agents which specifically affect the deregulated proteins of cancer cells (e.g., tyrosine kinase inhibitors imatinib, gefitinib; monoclonal antibodies, photodynamic therapy); (iv) immunotherapy, or enhancement of the host's immune response (e.g., vaccine); (v) hormonal therapy, or blockade of hormone (e.g., when tumor is hormone sensitive), (vi) angiogenesis inhibitor, or blockade of blood vessel formation and growth, and (vii) palliative care, or treatment directed to improving the quality of care to reduce pain, nausea, vomiting, diarrhea and hemorrhage. Pain medication such as morphine and oxycodone, anti-emetics such as ondansetron and aprepitant, can permit more aggressive treatment regimens.

[0365] In the treatment of cancer, any of the previously described conventional treatments for the treatment of cancer immunity may be conducted, prior, subsequent or simultaneous with the administration of the anti-ROR2 antibodies or antibody fragments. Additionally, the anti-ROR2 antibodies or antibody fragments may be administered prior, subsequent or simultaneous with conventional cancer treatments, such as the administration of tumor-binding antibodies (e.g., monoclonal antibodies, toxin-conjugated monoclonal antibodies) and/or the administration of chemotherapeutic agents.

F. Dosing Regimen

[0366] The present invention provides a dosing regimen for the treatment of ROR2-expressing tumors. The dosing regimen comprises a dose of a polypeptide, an antibody or antibody fragment, or an immunoconjugate of the invention as described herein of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, about 0.4 mg/kg body weight to about 3.3 mg/kg body weight, about 0.5 mg/kg body weight to about 3.3 mg/kg body weight, about 0.6 mg/kg body weight to about 3.3 mg/kg body weight, about 0.7 mg/kg body weight to about 3.3 mg/kg body weight, about 0.8 mg/kg body weight to about 3.3 mg/kg body weight, about 0.9 mg/kg body weight to about 3.3 mg/kg body weight, about 1.0 mg/kg body weight to about 3.3 mg/kg body weight, about 1.1 mg/kg body weight to about 3.3 mg/kg body weight, about 1.2 mg/kg body weight to about 3.3 mg/kg body weight, about 1.3 mg/kg body weight to about 3.3 mg/kg body weight, about 1.4 mg/kg body weight to about 3.3 mg/kg body weight, about 1.5 mg/kg body weight to about 3.3 mg/kg body weight, about 1.6 mg/kg body weight to about 3.3 mg/kg body weight, about 1.7 mg/kg body weight to about 3.3 mg/kg body weight, about 1.8 mg/kg body weight to about 3.3 mg/kg body weight, about 1.9 mg/kg body weight to about 3.3 mg/kg body weight, about 2.0 mg/kg body weight to about 3.3 mg/kg body weight, about 2.1 mg/kg body weight to about 3.3 mg/kg body weight, about 2.2 mg/kg body weight to about 3.3 mg/kg body weight, about 2.3 mg/kg body weight to about 3.3 mg/kg body weight, about 2.4 mg/kg body weight to about 3.3 mg/kg body weight, about 2.5 mg/kg body weight to about 3.3 mg/kg body weight, about 2.6 mg/kg body weight to about 3.3 mg/kg body weight, about 2.7 mg/kg body weight to about 3.3 mg/kg body weight, about 2.8 mg/kg body weight to about 3.3 mg/kg body weight, about 2.9 mg/kg body weight to about 3.3 mg/kg body weight, about 3.0 mg/kg body weight to about 3.3 mg/kg body weight, about 3.1 mg/kg body weight to about 3.3 mg/kg body weight, about 3.2 mg/kg body weight to about 3.3 mg/kg body weight, about 0.3 mg/kg body weight to about 1.8 mg/kg body weight, about 0.4 mg/kg body weight to about 1.8 mg/kg body weight, about 0.5 mg/kg body weight to about 1.8 mg/kg body weight, about 0.6 mg/kg body weight to about 1.8 mg/kg body weight, about 0.7 mg/kg body weight to about 1.8 mg/kg body weight, about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, about 0.9 mg/kg body weight to about 1.8 mg/kg body weight, about 1.0 mg/kg body weight to about 1.8 mg/kg body weight, about 1.1 mg/kg body weight to about 1.8 mg/kg body weight, about 1.4 mg/kg body weight to about 1.8 mg/kg body weight, about 1.5 mg/kg body weight to about 1.8 mg/kg body weight, about 1.6 mg/kg body weight to about 1.8 mg/kg body weight, or about 1.7 mg/kg body weight to about 1.8 mg/kg body weight, for at least a two week (e.g., 14 day period) or three week period (e.g., 21 day period). More preferably from about 1.5 mg/kg

body weight to about 1.8 mg/kg body weight for at least a two week (e.g., 14 day) period or a three week period (e.g., 21 day) period. Most preferably from about 1.5 mg/kg body weight to about 1.8 mg/kg body weight for at least a two week (e.g., 14 day) period. The (bi)weekly dose can either be administered as a single (bi)weekly dose (once a week) or by split delivery (e.g., two or more times per (bi)week). In some embodiments, the (bi)weekly dose of the polypeptide, an antibody or antibody fragment, or an immunoconjugate of the invention as described herein will be 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, or 3.3 mg/kg of the subject's body weight.

[0367] In certain embodiments, the dosing regimen comprises a dose of an anti-ROR2 antibody or antibody fragment or immunoconjugate that includes the anti-ROR2 antibody or antibody fragment of the invention, as described herein of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, about 0.4 mg/kg body weight to about 3.3 mg/kg body weight, about 0.5 mg/kg body weight to about 3.3 mg/kg body weight, about 0.6 mg/kg body weight to about 3.3 mg/kg body weight, about 0.7 mg/kg body weight to about 3.3 mg/kg body weight, about 0.8 mg/kg body weight to about 3.3 mg/kg body weight, about 0.9 mg/kg body weight to about 3.3 mg/kg body weight, about 1.0 mg/kg body weight to about 3.3 mg/kg body weight, about 1.1 mg/kg body weight to about 3.3 mg/kg body weight, about 1.2 mg/kg body weight to about 3.3 mg/kg body weight, about 1.3 mg/kg body weight to about 3.3 mg/kg body weight, about 1.4 mg/kg body weight to about 3.3 mg/kg body weight, about 1.5 mg/kg body weight to about 3.3 mg/kg body weight, about 1.6 mg/kg body weight to about 3.3 mg/kg body weight, about 1.7 mg/kg body weight to about 3.3 mg/kg body weight, about 1.8 mg/kg body weight to about 3.3 mg/kg body weight, about 1.9 mg/kg body weight to about 3.3 mg/kg body weight, about 2.0 mg/kg body weight to about 3.3 mg/kg body weight, about 2.1 mg/kg body weight to about 3.3 mg/kg body weight, about 2.2 mg/kg body weight to about 3.3 mg/kg body weight, about 2.3 mg/kg body weight to about 3.3 mg/kg body weight, about about 2.4 mg/kg body weight to about 3.3 mg/kg body weight, about 2.5 mg/kg body weight to about 3.3 mg/kg body weight, about 2.6 mg/kg body weight to about 3.3 mg/kg body weight, about 2.7 mg/kg body weight to about 3.3 mg/kg body weight, about 2.8 mg/kg body weight to about 3.3 mg/kg body weight, about 2.9 mg/kg body weight to about 3.3 mg/kg body weight, about 3.0 mg/kg body weight to about 3.3 mg/kg body weight, about 3.1 mg/kg body weight to about 3.3 mg/kg body weight, about 3.2 mg/kg body weight to about 3.3 mg/kg body weight, about 0.3 mg/kg body weight to about 1.8 mg/kg body weight, about 0.4 mg/kg body weight to about 1.8 mg/kg body weight, about 0.5 mg/kg body weight to about 1.8 mg/kg body weight, about 0.6 mg/kg body weight to about 1.8 mg/kg

body weight, about 0.7 mg/kg body weight to about 1.8 mg/kg body weight, about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, about 0.9 mg/kg body weight to about 1.8 mg/kg body weight, about 1.0 mg/kg body weight to about 1.8 mg/kg body weight, about 1.1 mg/kg body weight to about 1.8 mg/kg body weight about 1.4 mg/kg body weight to about 1.8 mg/kg body weight, about 1.5 mg/kg body weight to about 1.8 mg/kg body weight, about 1.6 mg/kg body weight to about 1.8 mg/kg body weight, or about 1.7 mg/kg body weight to about 1.8 mg/kg body weight, for at least a two week (e.g., 14 day period) or three week period (e.g., 21 day period). More preferably from about 1.5 mg/kg body weight to about 1.8 mg/kg body weight for at least a two week (e.g., 14 day) period or a three week period (e.g., 21 day) period. Most preferably from about 1.5 mg/kg body weight to about 1.8 mg/kg body weight for at least a two week (e.g., 14 day) period. The (bi)weekly dose can either be administered as a single (bi)weekly dose (once a week) or by split delivery (e.g., two or more times per (bi)week). In some embodiments, the (bi)weekly dose of the anti-ROR2 antibody or antibody fragment or immunoconjugate that includes the anti-ROR2 antibody or antibody fragment of the invention, as described herein will be 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, or 3.3 mg/kg of the subject's body weight.

[0368] In certain embodiments, the dosing regimen comprises a dose of an antibody-drug conjugate as described herein of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, about 0.4 mg/kg body weight to about 3.3 mg/kg body weight, about 0.5 mg/kg body weight to about 3.3 mg/kg body weight, about 0.6 mg/kg body weight to about 3.3 mg/kg body weight, about 0.7 mg/kg body weight to about 3.3 mg/kg body weight, about 0.8 mg/kg body weight to about 3.3 mg/kg body weight, about 0.9 mg/kg body weight to about 3.3 mg/kg body weight, about 1.0 mg/kg body weight to about 3.3 mg/kg body weight, about 1.1 mg/kg body weight to about 3.3 mg/kg body weight, about 1.2 mg/kg body weight to about 3.3 mg/kg body weight, about 1.3 mg/kg body weight to about 3.3 mg/kg body weight, about 1.4 mg/kg body weight to about 3.3 mg/kg body weight, about 1.5 mg/kg body weight to about 3.3 mg/kg body weight, about 1.6 mg/kg body weight to about 3.3 mg/kg body weight, about 1.7 mg/kg body weight to about 3.3 mg/kg body weight, about 1.8 mg/kg body weight to about 3.3 mg/kg body weight, about 1.9 mg/kg body weight to about 3.3 mg/kg body weight, about 2.0 mg/kg body weight to about 3.3 mg/kg body weight, about 2.1 mg/kg body weight to about 3.3 mg/kg body weight, about 2.2 mg/kg body weight to about 3.3 mg/kg body weight, about 2.3 mg/kg body weight to about 3.3 mg/kg body weight, about about 2.4 mg/kg body weight to about 3.3 mg/kg body weight, about 2.5 mg/kg body weight to about 3.3 mg/kg body weight, about 2.6 mg/kg body weight to about 3.3 mg/kg body weight, about

2.7 mg/kg body weight to about 3.3 mg/kg body weight, about 2.8 mg/kg body weight to about 3.3 mg/kg body weight, about 2.9 mg/kg body weight to about 3.3 mg/kg body weight, about 3.0 mg/kg body weight to about 3.3 mg/kg body weight, about 3.1 mg/kg body weight to about 3.3 mg/kg body weight, about 3.2 mg/kg body weight to about 3.3 mg/kg body weight, about 0.3 mg/kg body weight to about 1.8 mg/kg body weight, about 0.4 mg/kg body weight to about 1.8 mg/kg body weight, about 0.5 mg/kg body weight to about 1.8 mg/kg body weight, about 0.6 mg/kg body weight to about 1.8 mg/kg body weight, about 0.7 mg/kg body weight to about 1.8 mg/kg body weight, about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, about 0.9 mg/kg body weight to about 1.8 mg/kg body weight, about 1.0 mg/kg body weight to about 1.8 mg/kg body weight, about 1.1 mg/kg body weight to about 1.8 mg/kg body weight, about 1.4 mg/kg body weight to about 1.8 mg/kg body weight, about 1.5 mg/kg body weight to about 1.8 mg/kg body weight, about 1.6 mg/kg body weight to about 1.8 mg/kg body weight, or about 1.7 mg/kg body weight to about 1.8 mg/kg body weight, for at least a two week (e.g., 14 day period) or three week period (e.g., 21 day period). More preferably from about 1.5 mg/kg body weight to about 1.8 mg/kg body weight for at least a two week (e.g., 14 day) period or a three week period (e.g., 21 day) period. Most preferably from about 1.5 mg/kg body weight to about 1.8 mg/kg body weight for at least a two week (e.g., 14 day) period. The (bi)weekly dose can either be administered as a single (bi)weekly dose (once a (bi)week) or by split delivery (e.g., two or more times per (bi)week). In some embodiments, the (bi)weekly dose of the antibody drug conjugate will be 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, or 3.3 mg/kg of the subject's body weight.

[0369] In certain embodiments, the dose is administered, as a split delivery or as a single weekly dose, for at least one two week (e.g., 14 day) treatment cycle or for at least one three week (e.g., 21 day) treatment cycle. In some embodiments, the dose will be administered as a single dose on days 1 and 8 of a 14 day treatment cycle. Preferably, the dose, as a split delivery or as a single dose, is administered for two or more 14 day treatment cycles, even more preferably for three or more, four or more, five, or even six or more treatment cycles. In some embodiments, the dose is administered for no more than 3, no more than 4, no more than 5, or no more than 6 treatment cycles. In some embodiments, the dose will be administered as a weekly dose on days 1 and 8 of a 21 day treatment cycle. Preferably, the dose, as a split delivery or as a single dose, is administered for two or more 21 day treatment cycles, even more preferably for three or more, four or more, five, or even six or more treatment cycles. In some embodiments, the dose is administered for no more than 3, no

more than 4, no more than 5, or no more than 6 treatment cycles. In some embodiments, there will a period of rest between treatment cycles.

[0370] For example, in some embodiments, the dosing regimen will be a total weekly dose of the antibody-drug conjugate of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, about 0.4 mg/kg body weight to about 3.3 mg/kg body weight, about 0.5 mg/kg body weight to about 3.3 mg/kg body weight, about 0.6 mg/kg body weight to about 3.3 mg/kg body weight, about 0.7 mg/kg body weight to about 3.3 mg/kg body weight, about 0.8 mg/kg body weight to about 3.3 mg/kg body weight, about 0.9 mg/kg body weight to about 3.3 mg/kg body weight, about 1.0 mg/kg body weight to about 3.3 mg/kg body weight, about 1.1 mg/kg body weight to about 3.3 mg/kg body weight, about 1.2 mg/kg body weight to about 3.3 mg/kg body weight, about 0.3 mg/kg body weight to about 1.8 mg/kg body weight, about 0.4 mg/kg body weight to about 1.8 mg/kg body weight, about 0.5 mg/kg body weight to about 1.8 mg/kg body weight, about 0.6 mg/kg body weight to about 1.8 mg/kg body weight, about 0.7 mg/kg body weight to about 1.8 mg/kg body weight, about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, about 0.9 mg/kg body weight to about 1.8 mg/kg body weight, about 1.0 mg/kg body weight to about 1.8 mg/kg body weight, about 1.1 mg/kg body weight to about 1.8 mg/kg body weight, about 1.4 mg/kg body weight to about 1.8 mg/kg body weight, about 1.5 mg/kg body weight to about 1.8 mg/kg body weight, for at least one weekly (e.g., 7 day) treatment cycle. In some embodiments, the treatment cycle will be greater than 7 days. In some embodiments, the treatment will be for at least two treatment cycles with a one week period of rest between each of the treatment cycles. In some embodiments, the treatment will be greater than 21 days. The weekly dose can be administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week).

[0371] In some preferred embodiments, the dosing regimen will be a total biweekly dose of the antibody-drug conjugate of from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, about 0.9 mg/kg body weight to about 1.8 mg/kg body weight, about 1.0 mg/kg body weight to about 1.8 mg/kg body weight, about 1.1 mg/kg body weight to about 1.8 mg/kg body weight, about 1.2 mg/kg body weight to about 1.8 mg/kg body weight, about 1.3 mg/kg body weight to about 1.8 mg/kg body weight, about 1.4 mg/kg body weight to about 1.8 mg/kg body weight, or about 1.5 mg/kg body weight to about 1.8 mg/kg body weight for at least one biweekly (e.g., 14 day) treatment cycle. In some embodiments, the treatment cycle will be greater than 14 days. In some embodiments, the treatment will be for at least two treatment cycles with a two week period of rest between each of the treatment cycles (e.g., six single weekly doses during an eight week period). In some embodiments, the treatment

cycle will be greater than 21 days. The biweekly dose can be administered as a single biweekly dose (once a biweek) or by split delivery (e.g., two or more times per biweek).

[0372] In some embodiments, the weekly dose of the antibody drug conjugate will be about 0.3 mg/kg body weight to about 1.8 mg/kg body weight administered at least once for two weeks as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 0.4 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 0.5 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 0.6 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 0.7 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 0.8 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 0.9 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 1.0 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 1.1 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 1.2 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 1.3 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 1.4 mg/kg body weight

administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 1.5 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 1.6 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 1.7 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be about 1.8 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for two weeks will be 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, or 3.3 mg/kg of the subject's body weight of the subject's body weight.

[0373] In some embodiments, the weekly dose of the antibody drug conjugate will be about 0.3 mg/kg body weight to about 3.3 mg/kg body weight administered at least once for three weeks as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 0.4 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 0.5 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 0.6 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 0.7 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 0.8 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 0.9 mg/kg body weight administered

[illegible]

In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 2.2 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 2.3 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 2.4 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 2.5 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 2.6 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 2.7 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 2.8 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 2.9 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 3.0 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 3.1 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be about 3.2 mg/kg body weight administered as a single weekly dose (once a week) or by split delivery (e.g., two or more times per week). In some embodiments, the weekly dose of the antibody drug conjugate administered at least once for three weeks will be 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, or 3.3 mg/kg of the subject's body weight of the subject's body weight.

[0374] The two week (14 day period) treatment cycle with a one week period of rest between treatment cycles, can also be referred to as a 3 week (21 day) treatment cycle where the antibody-drug conjugate is delivered 2 out of 3 weeks in the 3 week treatment cycle.

Likewise, the three week (21 day period) treatment cycle with a one week period of rest between treatment cycles, can also be referred to as a 4 week (28 day) treatment cycle where the antibody-drug conjugate is delivered 3 out of 4 weeks in the 4 week treatment cycle.

Accordingly, in some embodiments, the dose is administered weekly, as a split delivery or as a single weekly dose, 2 out of 3 weeks in a 3 week treatment cycle, or the dose is administered weekly, as a split delivery or as a single weekly dose, 3 out of 4 weeks in a 4 week treatment cycle. In some embodiments, the dose will be administered as a single weekly dose on days 1 and 8 of a 14 day treatment cycle, the dose will be administered as a single weekly dose on days 1 and 8 of a 21 day treatment cycle, or the dose will be administered as a single weekly dose on days 1 and 8 of a 28 day treatment cycle. Preferably, the weekly dose, as a split delivery or as a single weekly dose, is administered for two or more four week treatment cycles, even more preferably for three or more, four or more, five or more, or even six or more four week treatment cycles (e.g., 2, 3, 4, 5, or 6 consecutive treatment cycles). In some embodiments, the weekly dose is administered for no more than 3, no more than 4, no more than 5, or no more than 6 treatment cycles.

[0375] For example, in some embodiments, the dosing regimen will be a weekly dose, as a split delivery or as a single weekly dose, for a total weekly dose of from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight, about 0.9 mg/kg body weight to about 1.8 mg/kg body weight, about 1.0 mg/kg body weight to about 1.8 mg/kg body weight, about 1.1 mg/kg body weight to about 1.8 mg/kg body weight, about 1.2 mg/kg body weight to about 1.8 mg/kg body weight, about 1.3 mg/kg body weight to about 1.8 mg/kg body weight, about 1.4 mg/kg body weight to about 1.8 mg/kg body weight, about 1.5 mg/kg body weight to about 1.8 mg/kg body weight, about 1.6 mg/kg body weight to about 1.8 mg/kg body weight, about 1.7 mg/kg body weight to about 1.8 mg/kg body weight, or about 1.8 mg/kg body weight, of the antibody-drug conjugate, 1 out of 2 weeks for at least a one two week treatment cycle, or 2 out of 3 weeks for at least two three week treatment cycles.

[0376] For example, in some embodiments, the dosing regimen will be a weekly dose, as a split delivery or as a single weekly dose, for a total weekly dose of from about 0.8 mg/kg body weight to about 3.3 mg/kg body weight, about 0.9 mg/kg body weight to about 3.3 mg/kg body weight, about 1.0 mg/kg body weight to about 3.3 mg/kg body weight, about 1.1 mg/kg body weight to about 3.3 mg/kg body weight, about 1.2 mg/kg body weight to about 3.3 mg/kg body weight, about 1.3 mg/kg body weight to about 3.3 mg/kg body weight, about

1.4 mg/kg body weight to about 3.3 mg/kg body weight, about 1.5 mg/kg body weight to about 3.3 mg/kg body weight, about 1.6 mg/kg body weight to about 3.3 mg/kg body weight, about 1.7 mg/kg body weight to about 3.3 mg/kg body weight, about 1.8 mg/kg body weight to about 3.3 mg/kg body weight, about 1.9 mg/kg body weight to about 3.3 mg/kg body weight, about 2.0 mg/kg body weight to about 3.3 mg/kg body weight, about 2.1 mg/kg body weight to about 3.3 mg/kg body weight, about 2.2 mg/kg body weight to about 3.3 mg/kg body weight, about 2.3 mg/kg body weight to about 3.3 mg/kg body weight, about 2.4 mg/kg body weight to about 3.3 mg/kg body weight, about 2.5 mg/kg body weight to about 3.3 mg/kg body weight, about 2.6 mg/kg body weight to about 3.3 mg/kg body weight, about 2.7 mg/kg body weight to about 3.3 mg/kg body weight, about 2.8 mg/kg body weight to about 3.3 mg/kg body weight, about 2.9 mg/kg body weight to about 3.3 mg/kg body weight, about 3.0 mg/kg body weight to about 3.3 mg/kg body weight, about 3.1 mg/kg body weight to about 3.3 mg/kg body weight, about 3.2 mg/kg body weight to about 3.3 mg/kg body weight, or about 3.3 mg/kg body weight, of the antibody-drug conjugate, 2 out of 3 weeks, for at least one three week treatment cycle. In some embodiments, the weekly dose of the antibody drug conjugate administered 2 out of 3 weeks will be 0.8, 0.9, 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, or 3.3 mg/kg of the subject's body weight.

[0377] In some embodiments, the dosing regimen will be a weekly dose, as a split delivery or as a single weekly dose, for a total weekly dose of from about 0.8 mg/kg body weight to about 3.3 mg/kg body weight, about 0.9 mg/kg body weight to about 3.3 mg/kg body weight, about 1.0 mg/kg body weight to about 3.3 mg/kg body weight, about 1.1 mg/kg body weight to about 3.3 mg/kg body weight, about 1.2 mg/kg body weight to about 3.3 mg/kg body weight, about 1.3 mg/kg body weight to about 3.3 mg/kg body weight, about 1.4 mg/kg body weight to about 3.3 mg/kg body weight, about 1.5 mg/kg body weight to about 3.3 mg/kg body weight, about 1.6 mg/kg body weight to about 3.3 mg/kg body weight, about 1.7 mg/kg body weight to about 3.3 mg/kg body weight, about 1.8 mg/kg body weight to about 3.3 mg/kg body weight, about 1.9 mg/kg body weight to about 3.3 mg/kg body weight, about 2.0 mg/kg body weight to about 3.3 mg/kg body weight, about 2.1 mg/kg body weight to about 3.3 mg/kg body weight, about 2.2 mg/kg body weight to about 3.3 mg/kg body weight, about 2.3 mg/kg body weight to about 3.3 mg/kg body weight, about 2.4 mg/kg body weight to about 3.3 mg/kg body weight, about 2.5 mg/kg body weight to about 3.3 mg/kg body weight, about 2.6 mg/kg body weight to about 3.3 mg/kg body weight, about 2.7 mg/kg body weight to about 3.3 mg/kg body weight, about 2.8 mg/kg body weight to about 3.3 mg/kg body weight, about 2.9 mg/kg body weight to about 3.3 mg/kg body weight, about 3.0 mg/kg body

weight to about 3.3 mg/kg body weight, about 3.1 mg/kg body weight to about 3.3 mg/kg body weight, about 3.2 mg/kg body weight to about 3.3 mg/kg body weight, or about 3.3 mg/kg body weight, of the antibody-drug conjugate, administered 3 out of 4 weeks, for at least two four week treatment cycles.

[0378] In some embodiments, the dosing regimen will be a weekly dose, as a split delivery or as a single weekly dose, for a total weekly dose of from about 0.8 mg/kg body weight to about 3.3 mg/kg body weight, about 0.9 mg/kg body weight to about 3.3 mg/kg body weight, about 1.0 mg/kg body weight to about 3.3 mg/kg body weight, about 1.1 mg/kg body weight to about 3.3 mg/kg body weight, about 1.2 mg/kg body weight to about 3.3 mg/kg body weight, about 1.3 mg/kg body weight to about 3.3 mg/kg body weight, about 1.4 mg/kg body weight to about 3.3 mg/kg body weight, about 1.5 mg/kg body weight to about 3.3 mg/kg body weight, about 1.6 mg/kg body weight to about 3.3 mg/kg body weight, about 1.7 mg/kg body weight to about 3.3 mg/kg body weight, about 1.8 mg/kg body weight to about 3.3 mg/kg body weight, about 1.9 mg/kg body weight to about 3.3 mg/kg body weight, about 2.0 mg/kg body weight to about 3.3 mg/kg body weight, about 2.1 mg/kg body weight to about 3.3 mg/kg body weight, about 2.2 mg/kg body weight to about 3.3 mg/kg body weight, about 2.3 mg/kg body weight to about 3.3 mg/kg body weight, about 2.4 mg/kg body weight to about 3.3 mg/kg body weight, about 2.5 mg/kg body weight to about 3.3 mg/kg body weight, about 2.6 mg/kg body weight to about 3.3 mg/kg body weight, about 2.7 mg/kg body weight to about 3.3 mg/kg body weight, about 2.8 mg/kg body weight to about 3.3 mg/kg body weight, about 2.9 mg/kg body weight to about 3.3 mg/kg body weight, about 3.0 mg/kg body weight to about 3.3 mg/kg body weight, about 3.1 mg/kg body weight to about 3.3 mg/kg body weight, about 3.2 mg/kg body weight to about 3.3 mg/kg body weight, or about 3.3 mg/kg body weight, of the antibody-drug conjugate administered 2 out of 3 weeks, for one, two, three, four, or five four week treatment cycles (e.g., four single weekly doses in a six week time period, six single weekly doses in a nine week time period, or eight single weekly doses in a twelve week time period).

[0379] In some embodiments, the dosing regimen will be a weekly dose, as a split delivery or as a single weekly dose, for a total weekly dose of from about 0.8 mg/kg body weight to about 3.3 mg/kg body weight, about 0.9 mg/kg body weight to about 3.3 mg/kg body weight, about 1.0 mg/kg body weight to about 3.3 mg/kg body weight, about 1.1 mg/kg body weight to about 3.3 mg/kg body weight, about 1.2 mg/kg body weight to about 3.3 mg/kg body weight, about 1.3 mg/kg body weight to about 3.3 mg/kg body weight, about 1.4 mg/kg body weight to about 3.3 mg/kg body weight, about 1.5 mg/kg body weight to about 3.3 mg/kg body weight, about 1.6 mg/kg body weight to about 3.3 mg/kg body weight, about 1.7 mg/kg

body weight to about 3.3 mg/kg body weight, about 1.8 mg/kg body weight to about 3.3 mg/kg body weight, about 1.9 mg/kg body weight to about 3.3 mg/kg body weight, about 2.0 mg/kg body weight to about 3.3 mg/kg body weight, about 2.1 mg/kg body weight to about 3.3 mg/kg body weight, about 2.2 mg/kg body weight to about 3.3 mg/kg body weight, about 2.3 mg/kg body weight to about 3.3 mg/kg body weight, about 2.4 mg/kg body weight to about 3.3 mg/kg body weight, about 2.5 mg/kg body weight to about 3.3 mg/kg body weight, about 2.6 mg/kg body weight to about 3.3 mg/kg body weight, about 2.7 mg/kg body weight to about 3.3 mg/kg body weight, about 2.8 mg/kg body weight to about 3.3 mg/kg body weight, about 2.9 mg/kg body weight to about 3.3 mg/kg body weight, about 3.0 mg/kg body weight to about 3.3 mg/kg body weight, about 3.1 mg/kg body weight to about 3.3 mg/kg body weight, about 3.2 mg/kg body weight to about 3.3 mg/kg body weight, or about 3.3 mg/kg body weight, of the antibody-drug conjugate, optionally, 3 out of every 4 weeks, for one, two, three, four, or five four weekly treatment cycles (e.g., six single weekly doses in an eight week time period, nine single weekly doses in a twelve week time period, or twelve single weekly doses in a sixteen week time period). In some embodiments, each dose of the antibody drug conjugate will be 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, or 3.3 mg/kg of the subject's body weight.

[0380] In some preferred embodiments, the dosing regimen will be a biweekly dose, as a split delivery or as a single biweekly dose, for a total biweekly dose of from about 0.8 mg/kg body weight to about 3.3 mg/kg body weight, about 0.9 mg/kg body weight to about 3.3 mg/kg body weight, about 1.0 mg/kg body weight to about 3.3 mg/kg body weight, about 1.1 mg/kg body weight to about 3.3 mg/kg body weight, about 1.2 mg/kg body weight to about 3.3 mg/kg body weight, about 1.3 mg/kg body weight to about 3.3 mg/kg body weight, about 1.4 mg/kg body weight to about 3.3 mg/kg body weight, about 1.5 mg/kg body weight to about 3.3 mg/kg body weight, about 1.6 mg/kg body weight to about 3.3 mg/kg body weight, about 1.7 mg/kg body weight to about 3.3 mg/kg body weight, about 1.8 mg/kg body weight to about 3.3 mg/kg body weight, about 1.9 mg/kg body weight to about 3.3 mg/kg body weight, about 2.0 mg/kg body weight to about 3.3 mg/kg body weight, about 2.1 mg/kg body weight to about 3.3 mg/kg body weight, about 2.2 mg/kg body weight to about 3.3 mg/kg body weight, about 2.3 mg/kg body weight to about 3.3 mg/kg body weight, about 2.4 mg/kg body weight to about 3.3 mg/kg body weight, about 2.5 mg/kg body weight to about 3.3 mg/kg body weight, about 2.6 mg/kg body weight to about 3.3 mg/kg body weight, about 2.7 mg/kg body weight to about 3.3 mg/kg body weight, about 2.8 mg/kg body weight to about 3.3 mg/kg body weight, about 2.9 mg/kg body weight to about 3.3 mg/kg body weight, about 3.0

mg/kg body weight to about 3.3 mg/kg body weight, about 3.1 mg/kg body weight to about 3.3 mg/kg body weight, about 3.2 mg/kg body weight to about 3.3 mg/kg body weight, or about 3.3 mg/kg body weight, of the antibody-drug conjugate, optionally, 3 out of every 4 biweeks, for one, two, three, four, or five four biweekly treatment cycles (e.g., six single biweekly doses in an fourteen week time period, nine single biweekly doses in a twenty week time period, or twelve single weekly doses in a twenty six week time period). In some embodiments, each dose of the antibody drug conjugate administered biweekly will be 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, or 3.3 mg/kg of the subject's body weight.

[0381] Following or during one or more treatment cycles (e.g., during days 14-21 of the second treatment cycle or during days 21-28 of the second treatment cycle), the subject can be evaluated (e.g., through clinical or diagnostic testing) to determine whether the subject should remain on the treatment schedule. For example, following or during one or more 28 day treatment cycles (e.g., 1, 2, 3, 4, 5, or 6 28 day treatment cycles), the subject can be evaluated (e.g., a clinical and/or diagnostic evaluation). Depending on the evaluation, the subject will discontinue treatment, continue on treatment with additional treatment cycles, or commence maintenance therapy by continuing treatment until progression or unacceptable toxicity. If the subject continues treatment, the subject can be further evaluated following one or more additional treatment cycles. Depending on each successive evaluation, the subject will discontinue treatment, continue on treatment with additional treatment cycles, or commence maintenance therapy by continuing treatment until progression or unacceptable toxicity.

[0382] The present invention encompasses embodiments in which the subject remains on the treatment cycle (e.g., the two week treatment cycle or the three week treatment cycle) following an evaluation indicating that the subject has no detectable cancer, for example, following a diagnostic test that is negative for the ROR2-expressing cancer (i.e., the diagnostic test is unable to detect any cancer in the subject). For example, in some embodiments, the subject will remain on the treatment cycle for at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more treatment cycles following such an evaluation. In some embodiments, the subject will remain on the treatment cycle for at least two but no more than 3, no more than 4, no more than 5, or no more than 6 treatment cycles. One example of a diagnostic test used for determining the presence and severity of cancers is positron emission tomography (PET).

[0383] In some embodiments, the subject will commence maintenance therapy by continuing treatment until progression or unacceptable toxicity following one or more, preferably two or more, (e.g, following 1, 2, 3, 4, 5, or 6) treatment cycles (e.g., the four week treatment cycle).

In some embodiments, the subject will commence maintenance therapy by continuing treatment until progression or unacceptable toxicity following an evaluation indicating that the subject has little or no detectable cancer, e.g., following an evaluation indicating that the subject has had a complete response. As used herein, maintenance therapy refers to therapy with the antibody-drug conjugate but at a reduced administration schedule at either the same or different dosages by continuing treatment until progression or unacceptable toxicity. During maintenance therapy, the antibody-drug conjugate is preferably administered at least once every two week treatment period, once every three week treatment period, on days 1 and 8 of every two week treatment period, or on days 1 and 8 of every three week treatment period. Following these maintenance therapy cycles, the subject can be further evaluated (e.g., through clinical or diagnostic testing) to determine whether the subject should remain on the maintenance therapy, continue with regular treatment or discontinue treatment. In some embodiments, maintenance therapy will be once every two weeks to four weeks, or every three weeks to six weeks. The dosage of the antibody drug conjugate administered during maintenance therapy can range, for example, from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, preferably from about 0.6 mg/kg body weight to about 1.8 mg/kg body weight, preferably from about 1.2 mg/kg body weight to about 2.0 mg/kg body weight, more preferably from about 1.5 mg/kg body weight to about 2.4 mg/kg body weight per dose, with 1.8 mg/kg body weight being an exemplary dose.

[0384] In some embodiments, following conclusion of the weekly or biweekly treatment at a dosage of the antibody drug conjugate of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, more preferably a dosage of from about 1.5 mg/kg body weight to about 1.8 mg/kg body weight and evaluation, the subject will begin a maintenance therapy which comprises administration of the antibody-drug conjugate once every two to four weeks or once every three to six weeks, at a dosage of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, preferably from about 0.6 mg/kg body weight to about 1.8 mg/kg body weight, preferably from about 1.2 mg/kg body weight to about 2.0 mg/kg body weight, more preferably from about 1.5 mg/kg body weight to about 2.4 mg/kg body weight with a dose of about 1.8 mg/kg body weight being preferred, preferably administered by biweekly administration.

[0385] In some embodiments, following conclusion of the treatment (e.g., for one, two, three, four or five treatment cycles), the subject will begin a once every two week administration schedule (e.g., treatment on day 1 of a two week maintenance therapy cycle) of the antibody drug conjugate at a dosage of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, preferably from about 0.6 mg/kg body weight to about 1.8 mg/kg body weight,

preferably from about 1.2 mg/kg body weight to about 2.0 mg/kg body weight, more preferably from about 1.5 mg/kg body weight to about 2.4 mg/kg body weight with about 1.8 mg/kg body weight being preferred.

[0386] In some embodiments, following conclusion of the treatment (e.g., for one, two, three, four or five treatment cycles), the subject will begin a once every three week administration schedule (e.g., treatment on day 1 of a three week maintenance therapy cycle) of the antibody drug conjugate at a dosage of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, preferably from about 0.6 mg/kg body weight to about 1.8 mg/kg body weight, preferably from about 1.2 mg/kg body weight to about 2.0 mg/kg body weight, more preferably from about 1.5 mg/kg body weight to about 2.4 mg/kg body weight with about 1.8 mg/kg body weight being preferred.

[0387] The present invention encompasses embodiments wherein a subject will be administered a weekly dose, as a split delivery or as a single weekly dose, of the antibody drug conjugate for a total weekly dose of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, from about 0.6 mg/kg body weight to about 1.8 mg/kg body weight, from about 1.2 mg/kg body weight to about 2.0 mg/kg body weight, from about 1.5 mg/kg body weight to about 2.4 mg/kg body weight with about 1.8 mg/kg body weight being preferred, 2 out of 3 weeks, for one, two, three, four, five, or six 21 day treatment cycles followed by administration of a dose every two to four weeks, preferably a dose every two weeks, of antibody drug conjugate at a dose of from about 0.4 mg/kg body weight to about 2 mg/kg body weight, from about 0.6 mg/kg body weight to about 2.0 mg/kg body weight, or from about 0.8 mg/kg body weight to about 1.8 mg/kg body weight for 2 or more maintenance therapy cycles. In some embodiments, the weekly administration will be for 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more treatment cycles and the biweekly administration will be for 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more maintenance therapy cycles. In some embodiments, the (bi)weekly administration cycle will be for no more than 2, 3, 4, 5, or 6 treatment cycles.

[0388] The present invention encompasses embodiments wherein a subject will be administered a weekly dose, as a split delivery or as a single weekly dose, of the antibody drug conjugate for a total weekly dose of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, about 0.6 mg/kg body weight to about 1.8 mg/kg body weight, about 1.2 mg/kg body weight to about 2.0 mg/kg body weight, about 1.5 mg/kg body weight to about 2.4 mg/kg body weight, 3 out of 4 weeks, for one, two, three, four, five, or six 28 day treatment cycles followed by administration of a dose every three to six weeks, preferably a dose every three weeks, of antibody drug conjugate at a dose of from about 0.3 mg/kg body weight to about 3.3 mg/kg body weight, from about 0.6 mg/kg body weight to about 1.8

mg/kg body weight, or from about 1.2 mg/kg body weight to about 2.0 mg/kg body weight for 2 or more maintenance therapy cycles. In some embodiments, the weekly administration cycle will be for 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more treatment cycles and the once every three week administration schedule will be for 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more maintenance therapy cycles. In some embodiments, the once every three week administration cycle will be for no more than 2, 3, 4, 5, or 6 treatment cycles.

[0389] The present invention encompasses embodiments wherein a subject will be administered a weekly dose, as a split delivery or as a single weekly dose, of the antibody drug conjugate at a total weekly dose of dose of from about 0.8 mg/kg of the subject's body weight to about 1.8 mg/kg 2 out of 3 weeks (e.g., on days 1 and 8 of a 21 day treatment cycle) for one, two, three, four, five, or six 21 day treatment cycles followed by administration of a a dose every two to four weeks, preferably, a dose every two weeks, of antibody drug conjugate of about 1.8 mg/kg per body weight for 2 or more maintenance therapy cycles (e.g., a dose of about 1.8 mg/kg per body weight every two weeks for two or more two week maintenance therapy cycles). Preferably, the biweekly dose of antibody drug conjugate is about 0.8 mg/kg per body weight for 2 or more maintenance therapy cycles (e.g., a dose of about 0.8 mg/kg per body weight every two weeks for two or more two week maintenance therapy cycles).

[0390] The present invention encompasses embodiments wherein a subject will be administered a weekly dose, as a split delivery or as a single weekly dose, of the antibody drug conjugate at a total weekly dose of from about 0.8 mg/kg of the subject's body weight to about 1.8 mg/kg 3 out of 4 weeks (e.g., on days 1 and 8 of a 28 day treatment cycle) for one, two, three, four, five, or six 28 day treatment cycles followed by administration of a dose every three to six weeks. The dose once every three weeks of the antibody drug conjugate may be a dose of about 1.8 mg/kg per body weight for 2 or more maintenance therapy cycles (e.g., a dose of about 1.8 mg/kg per body weight once every three weeks for two or more three week maintenance therapy cycles). The dose once every three weeks of antibody drug conjugate may be a dose of about 0.8 mg/kg per body weight for 2 or more maintenance therapy cycles (e.g., a dose of about 0.8 mg/kg per body weight once every three weeks for two or more three week maintenance therapy cycles).

[0391] The present invention encompasses embodiments wherein the subject is treated with a mAbBA301-cleavable linker-MMAE_(n) antibody-drug conjugate of the present invention but at a schedule other than the (bi)weekly dosing regimen (e.g., administration of the antibody drug conjugate at a dose of about 1.8 mg/kg body weight every one or two weeks for one or more two week therapy cycles, or once every three weeks for one or more three week therapy

cycles) and is switched to a weekly dosing regimen as described herein for no more than 1, 2, 3, 4, 5, or 6 treatment cycles. Following the weekly dosing regimen, the patient can optionally commence maintenance therapy as described herein.

[0392] The antibody-drug conjugate is preferably administered as a monotherapy. By the term “monotherapy” it is meant that the antibody drug conjugate is the only anti-cancer agent administered to the subject during the treatment cycle. However, other therapeutic agents can be administered to the subject as described herein. For example, a programmed death receptor-1 (PD-1) blocking antibody or a granulocyte colony stimulating factor or analog thereof may be co-administered with the antibody drug conjugate. Additionally, anti-inflammatory agents or other agents administered to a subject with cancer to treat symptoms associated with cancer, but not the underlying cancer itself, including, for example inflammation, pain, weight loss, and general malaise, can be administered during the period of monotherapy. A subject being treated by the present methods will preferably have completed any prior treatment with anti-cancer agents before administration of the antibody drug conjugate. In some embodiments, the subject will have completed any prior treatment with anti-cancer agents at least 1 week (preferably 2, 3, 4, 5, 6, 7, or 8 weeks) prior to treatment with the antibody drug conjugate. The subject will also, preferably, not be treated with any additional anti-cancer agents for at least 2 weeks (preferably at least 3, 4, 5, 6, 7, or 8 weeks) following completion of the first treatment cycle with the antibody drug conjugate and preferably for at least 2 weeks (preferably at least 3, 4, 5, 6, 7, or 8 weeks) following completion of the last dose of the antibody drug conjugate. The methods of the present invention encompass administering an anti-ROR2 antibody or antibody fragment or immunoconjugate comprising an anti-ROR2 antibody or antibody fragment of the present invention, to a subject for the treatment of ROR2-expressing tumors.

[0393] In some embodiments, after administration of immunoconjugate comprising an anti-ROR2 antibody or antibody fragment of the present invention, to a subject and binding of the anti-ROR2 antibody to an ROR2-expressing tumor cell, the antibody-drug conjugate internalizes into the cell, and the drug is released. For example, the methods of the present invention encompass administering an BA3021-cleavable linker-MMAE_(n) antibody-drug conjugate, to a subject for the treatment of a ROR2-expressing tumors. Following binding, the BA3021-cleavable linker-MMAE_(n) antibody-drug conjugate is internalized into the tumor cell where the peptide linker is cleaved by proteases to release MMAE. Delivery of the MMAE specifically to tumor cells expressing ROR2 is expected to prevent further tumor cell proliferation and result in tumor shrinkage.

[0394] The subjects to be treated with the methods of the present invention are those that have been diagnosed with a ROR2-expressing cancer or are suspected of having a ROR2-expressing cancer. Diagnosis can be by methods known in the art, including, for example, tissue biopsy.

G. Articles of Manufacture and Kits

[0395] In another aspect of the invention, an article of manufacture containing an anti-ROR2 antibody or antibody fragment and other materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is an antibody or antibody fragment of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody or antibody fragment; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0396] It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-ROR2 antibody or antibody fragment.

[0397] Finally, the invention also provides kits comprising at least one antibody or antibody fragment of the invention. Kits containing polypeptide, antibodies or antibody fragments, or antibody drug conjugate of the invention find use in detecting ROR2 expression (increase or decrease), or in therapeutic or diagnostic assays. Kits of the invention can contain an antibody

coupled to a solid support, e.g., a tissue culture plate or beads (e.g., sepharose beads). Kits can be provided which contain antibodies for detection and quantification of ROR2 *in vitro*, e.g. in an ELISA or a Western blot. Such antibody useful for detection may be provided with a label such as a fluorescent or radiolabel.

[0398] The kits further contain instructions on the use thereof. In some embodiments, the instructions comprise instructions required by the U.S. Food and Drug Administration for *in vitro* diagnostic kits. In some embodiments, the kits further comprise instructions for diagnosing the presence or absence of cerebrospinal fluid in a sample based on the presence or absence of ROR2 in said sample. In some embodiments, the kits comprise one or more antibodies or antibody fragments. In other embodiments, the kits further comprise one or more enzymes, enzyme inhibitors or enzyme activators. In still other embodiments, the kits further comprise one or more chromatographic compounds. In yet other embodiments, the kits further comprise one or more compounds used to prepare the sample for spectroscopic assay. In further embodiments, the kits further comprise comparative reference material to interpret the presence or absence of ROR2 according to intensity, color spectrum, or other physical attribute of an indicator.

H. Plasma Membrane Scoring of ROR2 in Tumor (Tumor Membrane P Score)

[0399] In tumor cells, ROR2 is primarily localized to the plasma membrane but can also be observed in the cytoplasm.

[0400] The present invention uses a scoring approach to compare ROR2 staining in serial sections of each sample. Using this approach, ROR2 plasma membrane staining is scored only in tumor cells to provide ROR2 tumor scoring (hereinafter “tumor membrane percent score” or (TmPS)).

[0401] The approaches used for scoring ROR2 may be detected by methods, including but not limited to, immunohistochemistry (IHC) in formalin-fixed, paraffin-embedded (FFPE) tumor samples as described below. All samples are also stained with hematoxylin and eosin (H&E) for morphological assessment to assist in scoring.

[0402] ROR2 plasma membrane expression in tumor is scored semi-quantitatively for plasma membrane staining (full or partial) and for cytoplasmic staining (diffuse or granular). Plasma membrane and cytoplasmic reactivity are scored separately. For ROR2 plasma membrane staining, the main components to the scoring are percentages at differential intensities, H-Scores, and Percent Scores $\geq 1+$ as described below. For ROR2 cytoplasmic staining, the main components to the scoring are percentages at differential intensities and H-Scores.

[0403] When scoring tumor tissues for ROR2, numeric scoring excludes any surrounding staining in stroma, areas of non-tumor, and adjacent normal tissue. Ischemic or necrotic areas are not scored in any indication. Percentage scores are assigned to describe the penetrance of plasma membrane staining per sample. Percentages are estimated and reported as an increment, including, but not limited to, one of the following increments: 0, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 97, 98, 99, or 100%. In certain embodiments of the present invention, the ROR2-expressing tumor has a TmPS of at least 1, at least 5, at least 10, at least 15, at least 20, at least 25, at least 30, at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 70, at least 75, at least 80, at least 85, at least 90, or at least 95%.

[0404] To gain a full understanding of ROR2 expression on the plasma membrane in tumors exclusive of macrophages across cancer indications, both standard Percent Score and H-Score approaches are used to capture the pattern of reactivity observed.

[0405] Both approaches require recording the percentage of tumor cells with ROR2 staining at a corresponding differential intensity using a four-point scale semi-quantitatively (0, 1+, 2+, 3+). On this scale, 0 = null, negative, or non-specific staining, 1+ = low or weak staining, 2+ = medium or moderate staining, and 3+ = high or strong staining.

Percent Score Method

[0406] Percent Scores are calculated by summing the percentages of intensities at either $\geq 1+$, $\geq 2+$ or $\geq 3+$. Thus, scores range from 0 to 100.

Percent Score $\geq 1+$ = (% at 1+) + (% at 2+) + (% at 3+)

Percent Score $\geq 2+$ = (% at 2+) + (% at 3+)

Percent Score $\geq 3+$ = (% at 3+)

H-Score Method

[0407] The H-Score is calculated by summing the percentage of cells with intensity of expression (brown staining) multiplied by their corresponding differential intensity on a four-point semi quantitative scale (0, 1+, 2+, 3+). Thus, scores range from 0 to 300.

H-Score = [(% at <1) $\times$ 0] + [(% at 1+) $\times$ 1] + [(% at 2+) $\times$ 2] + [(% at 3+) $\times$ 3]

[0408] For analysis of ROR2 expression in human cancer tissues, evaluation is semi-quantitative with numeric scoring data provided as a measure of reactivity. Samples are considered “positive” for ROR2 if they display $\geq 1+$ intensity plasma membrane staining in $\geq 10\%$ of tumor cells.

[0409] The following examples are illustrative, but not limiting, of the soft gelatin capsules of the present disclosure. Other suitable modifications and adaptations of the variety of conditions and parameters normally encountered in the field, and which are obvious to those skilled in the art, are within the scope of the disclosure.

EXAMPLES

[0410] Example 1: Conditionally active biological (CAB) antibodies against ROR2

[0411] Antibodies against human ROR2 were produced in this Example. A humanized antibody against ROR2 was used as the wild-type antibody to generate the CAB antibodies against ROR2. The DNAs encoding the wild-type antibody (heavy chain and light chain variable regions) were evolved to generate mutant antibody heavy chain and light chain variable region libraries. The mutant heavy chain and light chain variable regions in the libraries were screened for selective binding affinity to human ROR2 at pH 6.0 over pH 7.4 by ELISA (FIGS. 2A-2B and 3A-3B). Simultaneously, the expression level of the mutant antibodies was also optimized for the purpose of providing higher yields in a subsequent manufacturing process. The screening was done in serum using a FLAG tag because there were human antibodies in the serum which might cause false positives for the screening. The generated conditionally active antibodies were found to have a higher binding affinity to ROR2 at pH 6.0 than their binding affinity to the ROR2 at pH 7.4 (Tables 1 and 2).

[0412] The CAB antibodies did not show aggregation in a buffer as demonstrated in FIG. 4. A CAB antibody was analyzed by size exclusion chromatography. As shown in FIG. 4, only one peak was detected, demonstrating little or no aggregation of the antibody. Figure 5 shows that some of the selected mutant antibodies (scFv) demonstrated a higher binding affinity to ROR2 at pH 6.0 than the binding affinity to ROR2 at pH 7.4. In addition, Figure 5 also shows that raising the temperature from room temperature to 60 °C did not significantly alter the selectivity of the antibodies.

[0413] The conditionally active antibodies all exhibited high expression levels as shown in Table 4 below, with the column “Clone” indicating the antibodies and the expression level being shown in the second column in mg/ml.

[0414] The clones of these antibodies were sent to a service provider with a requested expression level representing an expected expression level (“amount ordered”). However, the actual expression levels of several of these antibodies (“amount delivered”) were very high and exceeded the expected expression levels. At least three clones had expression levels that exceeded the expected expression level (BAP048.7.067-HC-FLAG, BAP048.7-C02D09-FLAG, and BAP048.7-A03F01-FLAG).

Table 4. Antibodies with high expression levels

Clone	[mg/mL]	estimated yield	actual yield
BAP048.1-06-07	4.9	100	137
BAP048.7-06-07	3.7	100	94
BAP048.7-C02D09	3.8	30	57
BAP048.7-A03F01	4.4	30	65
BAP048.7-A03C05	4.2	30	36

[0415] The conditionally active antibodies were also assayed using surface plasmon resonance (SPR) to measure their on and off rates for binding to ROR2. The SPR assay can be used to measure on and off rates for protein binding. The *in vivo* on and off rate (in animals and humans) of the conditionally active antibodies is an important feature.

[0416] It was observed that the conditionally active antibodies have a good binding activity at pH 6.0 and little or no binding activity at pH 7.4 (FIGS. 6A-6B). The SPR assay showed that these same conditionally active antibodies were highly selective at pH 6.0 as compared to pH 7.4 (FIGS. 6A-6B).

[0417] Example 2: Anti-ROR2 antibodies conjugated to a model toxin

[0418] The anti-ROR2 antibody of the present invention was conjugated to a model toxin (e.g., paclitaxel) to produce a conditionally active antibody-drug conjugate (ROR2-CAB-ADC).

[0419] Tumors were induced in mice by injection of MDA-MB-436 tumor cells to produce xenografted mice. The ROR2-CAB-ADC was then injected into the xenografted mice at a dose of 0.3 or 1 mg/kg once a week for 2 weeks. The controls used in this study included PBS buffer as vehicle and the toxin alone (paclitaxel). The study showed that the ROR2-CAB-ADC provided a significantly greater reduction in the size of the tumor, in comparison with the controls (FIGS. 7A-7C). The results for the 0.3 mg/kg dosage group are presented in FIG. 7B and the results for the 1 mg/kg dosage group are presented in FIG. 7C. This study showed that the anti-ROR2 antibody conjugated with toxin is effective in reducing tumor size.

[0420] Example 3. pH dependent binding affinity of conditionally active antibody BAP048

[0421] The binding affinity of one of the conditionally active antibodies identified by the present invention, BAP048 (or BAP048.7 as shown in some of the figures), was tested by pH titration. The wild-type antibody was used as control. As shown in FIG. 8, the conditionally

active antibody BAP048 is more active at pH lower than pH 6.5, but less active at pH 7.0. The wild-type antibody does not show pH dependency for its binding affinity.

[0422] Example 4. Cross-species binding affinity of conditionally active antibody BAP048

[0423] The conditionally active antibody BAP048 was selected for conditional binding affinity of the human ROR2. This conditionally active antibody was tested for its binding affinity to three targets: human ROR2 (hROR2), cynomolgus ROR2 (cynoROR2), and mouse ROR2 (mROR2) using ELISA (FIG. 9). The conditionally active antibody BAP048 shown almost identical binding affinity to the human and cynomolgus ROR2, but significantly lower binding affinity to mouse ROR2. The EC₅₀ of the conditionally active antibody BAP048 for the three targets were calculated to be 201.4 ng/ml for human ROR2, 327.1 ng/ml for cynomolgus ROR2, and 7653 ng/ml for the mouse ROR2.

[0424] Example 5. Cell killing by conditionally active antibody BAP048-MMAE conjugates

[0425] The conditionally active antibody BAP048 was conjugated to chemotherapy drug Monomethyl auristatin E (MMAE) to produce an antibody drug conjugate (ADC). The ADC was tested on HEK293 cells that express human ROR2 on the cell surface. Negative control used for this test was an affinity matching antibody but not specific for human ROR2. The negative control was also conjugated to MMAE.

[0426] The BAP048 ADC was tested under two pH: 6.0 and 7. The percentage of cells remained alive after the treatment, relative to the cells treated by the negative control was presented in FIG. 10. It was observed that the BAP048 ADC displayed cell killing activity at pH 6.0 at a concentration below 100 ng/ml, while at pH 7.4, the BAP048 ADC showed cell killing at about 1000 ng/ml, a difference of effective concentration about 10 fold.

[0427] The cell killing activity of the BAP048 ADC was also tested on LCLC103H cells and HT1080 cells. For LCLC103H cells, which are a human lung cancer line, the cell killing activity of the BAP048 ADC was tested at pH 6.0, 6.2, and 6.4 (FIGS. 11A-11C). The difference of the cell killing activity of the BAP048 ADC was not significantly different among the three tested pH values, with the IC₅₀ at 1.819 ng/ml (pH 6.0), 1.232 ng/ml (pH 6.2), and 3.318 ng/ml (pH 6.5).

[0428] For HT1080 cells, which are a fibrosarcoma cell line, the cell killing activity of the BAP048 ADC was also tested at pH 6.0, 6.2, and 6.4 (FIGS. 12A-12C). The difference of the cell killing activity of the BAP048 ADC was not significantly different among the three

tested pH values, with the IC₅₀ at 2.897 ng/ml (pH 6.0), 3.138 ng/ml (pH 6.2), and 2.601 ng/ml (pH 6.5).

[0429] Example 6. Treatment of tumor in mice by conditionally active antibody BAP048-MMAE conjugates

[0430] The lung cancer cells LCLC103H were injected into mice to generate mouse xenografts (tumors in mice). The conditionally active antibody BAP048 was conjugated to MMAE through a linker thiobridge to generate an ADC for injection to the mice at two concentrations 1 mg/kg and 6 mg/kg. The negative control was the buffer (vehicle) without the ADC. One dose group was injected at 1 mg/kg dose weekly for three doses, and the other dose group was injected at 6 mg/kg dose every 4 days for 4 doses.

[0431] The volumes of the tumors were measured during the study. It was observed that at 6 mg/kg dose group, the tumor size shrank significantly by day 30 by the BAP048-MMAE ADC. At the 1 mg/kg dose group, the tumor growth was slowed by the BAP048-MMAE ADC, in comparison with the vehicle control (FIG. 13).

[0432] Example 7. Effect of linker in conditionally active antibody BAP048-MMAE conjugates

[0433] The effect of the linkers of the conditionally active antibody BAP048 MMAE conjugation was tested using two different linkers: mc-vc-PAB and mc-PEG8-vc (FIG. 14). The same negative control (vehicle) was used. These two BAP048-MMAE conjugates were injected into the mouse xenografts at a single dose of 1 mg/ml. The size of the tumors was measured during the study. It was observed that though both BAP048-MMAE conjugates significantly slowed the growth of the tumors in comparison with the negative control, the difference between the two BAP048-MMAE conjugates was not significant (FIG. 14). Thus, it appears the affect of the linkers on the BAP048-MMAE conjugates is not significant.

[0434] Example 8. Treatment of tumor in mice by conditionally active antibody BAP048

[0435] This example is similar to Example 6, except the cells used to induce the tumors in the mice are different. The two cell lines used in this example are HT1080 cells (a fibrosarcoma cell line) and MDA-MB-436 cells (a breast cancer cell line).

[0436] For the mouse xenograft induced by HT1080, the conditionally active antibody BAP048 was conjugated to MMAE with the linker of mc-vc-PAB. Three dose groups were used: 6 mg/kg every 4 days for 4 doses, 10 mg/kg every week for 3 doses, and 10 mg/kg

every 4 days for 4 doses (FIG. 15A). Two controls were used: one is the vehicle without antibody or MMAE, the other is the BAP048 antibody without conjugated MMAE.

[0437] It was observed that the tumor grew steadily with the two control groups. The growth of the tumor volume was slowed significantly by the conditionally active antibody BAP048-MMAE conjugates. The inhibition of the tumor growth was dose dependent, with the highest dose group, 10 mg/kg every 4 days for 4 doses, showed the largest reduction in tumor growth (FIG. 15A).

[0438] For the mouse xenograft induced by MDA-MB-436, the conditionally active antibody BAP048 was also conjugated to MMAE with the linker of mc-vc-PAB. There was only one dose group: 6 mg/kg every 4 days for 4 doses, with the vehicle (no antibody or MMAE) as the control (FIG. 15B). It was observed that the tumor grew steadily with the control group. The growth of the tumor volume was reduced significantly by the conditionally active antibody BAP048-MMAE conjugates (FIG. 15B).

[0439] It is to be understood, however, that even though numerous characteristics and advantages of the present invention have been set forth in the foregoing description, together with details of the structure and function of the invention, the disclosure is illustrative only, and changes may be made in detail, especially in matters of shape, size and arrangement of parts within the principles of the invention to the full extent indicated by the broad general meanings of the terms in which the appended claims are expressed.

[0440] Example 9. Phase I/II Study With BA3021

[0441] A Phase 1 dose escalation clinical trial was conducted using BA3021 (SEQ IDs 16 & 21-MMAE) in patients with locally advanced unresectable or metastatic solid tumors including NSCLC and melanoma, who were refractory or resistant to standard therapies. Cohorts were treated with doses of the BA3021 ranging from 0.3 mg/kg to 3.3 mg/kg once every three weeks (Q3W) or doses ranging from 1.5 mg/kg to 1.8 mg/kg twice every three weeks on days 1 and 8 (2Q3W).

[0442] 59 subjects were enrolled into 9 dose cohorts: 0.3 mg/kg Q3W (1 subject), 0.6 mg/kg Q3W (1 subject), 1.2 mg/kg Q3W (1 subject), 1.8 mg/kg Q3W (3 subjects), 2.4 mg/kg Q3W (16 subjects), 3.0 mg/kg Q3W (19 subject), 3.3 mg/kg Q3W (5 subjects), 1.2 mg/kg 2Q3W (3 subjects), 1.5 mg/kg 2Q3W (3 subjects), and 1.8 mg/kg 2Q3W (7 subjects). The solid tumor types enrolled in this study were: soft tissue sarcoma (40 subjects), NSCLC (6 subjects), melanoma (2 subjects), pancreatic (2 subjects), non-TNBC (2 subjects), colorectal, TNBC, GIST, urachus, ampulla of vatter, rectal carcinoid and head and neck (1 subject each).

[0443] As shown in FIG. 16, treatment with BA3021 resulted in a complete response in one patient with metastatic melanoma. This patient remained progression free more than two years after initiating therapy. In addition, two patients with NSCLC (~31% and ~49% tumor reduction) and one patient with advanced head and neck cancer (~54% tumor reduction) showed significant partial responses.

[0444] Two metastatic melanoma patients were enrolled in the initial part of the trial (FIG. 17). The ROR2 positive patient achieved a significant durable partial response. Furthermore, this patient, who had previously experienced treatment failure with both nivolumab and nivolumab plus ipilimumab, achieved complete response and remained without disease progression more than two years after initiating ozuriftamab vedotin (BA3021). The metastatic melanoma patient who achieved a complete response experienced clearance of one of two metastatic lung lesions (FIG. 18). Moreover, a pretreatment biopsy of an involved, abnormally enlarged cervical lymph node showed active melanoma. Subsequently, an on-treatment biopsy of the same node demonstrated no evidence of melanoma.

[0445] Of the six NSCLC patients enrolled in the dose escalation phase, two patients achieved a durable partial response and a third experienced tumor reduction to a lesser degree. The two NSCLC patients with partial responses to BA3021 had ROR2 TmPS of at least 70%. ROR2 TmPS was not characterized for the third patient who also experienced tumor shrinkage. Another patient with late-stage NSCLC and bone metastases and a ROR2 TmPS of 100%, treated with a suboptimal dose of the BA3021 (1.2mg/kg 2Q3W), experienced tumor shrinkage prior to progression of their metastatic bone lesions. All NSCLC patients who enrolled in this trial had previously been treated with PD-1 therapy.

[0446] In addition, one head and neck cancer patient achieved a partial response with a 54% reduction in tumor size.

[0447] BA3021 was generally well-tolerated. Adverse events (AEs) that appeared to be related to on-target injury of normal, ROR2-expressing tissues was not observed. Reported toxicities consistent with off-target effects of free MMAE were consistent with those described with other marketed MMAE-based ADCs.

[0448] In the Phase 1 trial, the Grade 3 or greater AEs or serious adverse events (SAEs) deemed related to BA3021 were consistent with MMAE-based toxicity and could generally be classified as either reversible myelosuppression (AEs: neutropenia, anemia), transient liver enzyme elevations (AEs: AST/ALT increased) or metabolic disturbances (AEs: hyponatremia, hypokalemia). There were a total of 23 (39%) patients who experienced an SAE, 11 (18.6%) of which were serious treatment emergent adverse events (TEAEs) that were considered related to treatment.

[0449] At the Phase 2 dosing levels (1.8mg/kg Q2W), BA3021 was well tolerated with few patients having treatment-related Grade 3-4 AEs (for 1.8 mg 1Q3W: 33.3% (1/3) anemia; for 1.8 mg 2Q3W: 42.9% (3/7) fatigue, hyponatremia and hyperglycemia) and few patients having SAEs (1.8mg/kg Q3W: 0 SAE (0%); 1.8mg/kg 2Q3W: 3 SAEs (43%; infected biloma, pyrexia and hyperglycemia). Out of these SAEs, 2 (28.6%) were deemed related to treatment by the investigator (1.8mg/kg Q3W: 0 SAEs (0%); 1.8mg/kg 2Q3W: 2 SAEs (28.6%; pyrexia and hyperglycemia). None of the related AEs or SAEs led to treatment discontinuation.

[0450] Table 5 - Overview of adverse events in BA3021 Phase 1 trial for patients administered 1.8mg/kg Q3W (d1) or 2Q3W (d1,8) (safety population)

Characteristic	BA3021 1.8 mg/kg (Q3W) (N=3)	BA3021 1.8 mg/kg (2Q3W) (N=7)
Any AEs	3 (100%)	7 (100%)
Related AEs with CTCAE Grade 3 or 4 ¹	1 (33%)	3 (43%)
Any related serious AEs ¹	0	2 (29%)
Related AEs leading to death ¹	0	0
Related AEs leading to treatment discontinuation ¹	0	0

1. As assessed by the investigator. Missing responses are counted as related.

[0451] At a dose of 3mg/kg Q3W, two patients experienced dose-limiting toxicities: one with Grade 3 dyspnea (self-resolved without intervention) and the other with Grade 4 febrile neutropenia (in a subject that did not receive prophylactic pegfilgrastim as directed) which resolved on day 2 of hospitalization.

[0452] Example 10. Tumor Membrane P Score

[0453] Immunohistochemical (IHC) staining for ROR2 (receptor tyrosine kinase-like Orphan Receptor 2) used monoclonal Mouse IgG clone H-1 from Santa Cruz Biotechnology (Cat # sc-374174) for detection of ROR2 in formalin-fixed, paraffin-embedded (FFPE) tissues (Table 6 below).

[0454] Table 6 – Antibody Specifications and IHC Assay Conditions

Antibody	ROR2
Source	Santa Cruz Biotechnology
Catalog No.	sc-374174
Vendor Lot No.	I2718
QualTek Lot No.	NT2794
Species/ Isotype	Mouse IgG1
Clone	H-1
Immunogen	Amino acids 868-943 at the C- terminus of human ROR2
Suggested Dilution	0.75ug/mL
Incubation Time	1-hour
Pre-treatment	SHIER 7, No Enzyme Steam Heat 20 min
TechMate Protocol	MIP
Detection System	Polink-2 Plus HRP Mouse
Chromogen	DAB

[0455] The IHC staining for ROR2 used the Customer Specific Protocol (CSP) described below on a TechMate staining platform.

[0456] • Step 1: FFPE tissue blocks were cut at 4-5 µm thickness and sections mounted onto positively-charged, capillary gap glass slides. Slides were baked (60°C, dry heat) prior to use.

Slide Preparation

[0457] a. Microtomy was performed. Four to five-micron (4-5 µm) sections were mounted onto Fisher Biotech 22-230-900 Probe-On Plus microscope slides.

[0458] b. Slides baked for at least 1 hour at 60°C (dry-heat) and allowed to cool a minimum of 15 minutes at room temperature prior to initiation of Step 2.

[0459] • Step 2: Tissue sections are de-waxed using organic solvents (xylene, 100%, four changes) and an alcohol series (100%, 70%, 30% ethanol) descending to distilled water to sufficiently hydrate the tissues and allow proper binding of the primary antibody and other detection reagents.

De-wax/Pre-Antigen Retrieval

- a.** Four (4) changes of room temperature (25°C) absolute xylene for 5min each [no agitation]
- b.** Two (2) changes of room temperature (25°C) absolute alcohol for 2min each [no agitation]
- c.** Two (2) changes of room temperature (25°C) 70% alcohol for 2min each [no agitation]

- d. Two (2) changes of room temperature (25°C) 30% alcohol for 2min each [no agitation]
 - e. Two (2) changes of room temperature (25°C) distilled water for rinsing [min. 16 dips in-out]
 - f. Slides immersed in room temperature (25°C) distilled water (transfer to antigen retrieval)
- [0460] • Step 3:** Antigen retrieval was performed after tissue sections were dewaxed. This step used a steam heat induced epitope recovery (SHIER) solution that was drawn into the capillary gap formed between paired microscope slides with a commercial steamer (20 minutes above 97°C) as a heat source (for description please see Ladner *et al*, Cancer Res.; vol. 60, pp 3493-3503, 2000).

Steam Heat Antigen Retrieval

- a. Commercial steamer pre-heated above 98°C
- b. Heat induced antigen/epitope retrieval using SHIER 2 (Dako S1700, citrate-based, pH 6.0-6.2) or SHIER 1 (QualTek formulation, citrate-based, pH5.6-6.1) above 98°C for 20 minutes (Black & Decker HS1000 model steamer or equivalent). Up to ten (10) slides were face-paired with clean blank slides in TechMate reagent trays containing exactly 10 mL antigen/epitope retrieval solution to capillary draw reagent up and over the tissues.
- c. Post-retrieval cool for 5 minutes, slide pairs firmly inserted into a TechMate slide holder and drained of SHIER 1/SHIER 2 with an absorbent wick pad.
- d. Wash two (2) times manually using capillary action (drain-draw) with Tris-buffered saline containing 0.02% v/v Tween-20 detergent (TBST, formulated as 20X stock solution by QualTek according to SOP MFB003 and used as 1X solution following dilution with distilled/deionized water [stored 4°C]).

[0461] • Step 4: Samples were tested by IHC according to the general procedure outlined in Table 7 below using the TechMate instrumentation platform and the MIP program (which does not include enzymatic digestion) or the MIPE program (which includes digestion with Proteinase K at a 1:40 dilution). Sequential detection of antibodies is employed during IHC with a high level of specificity for the antigen or for the primary antibody. The location of the primary antibody is ultimately visualized by the application of a colorimetric chromogen (DAB) that precipitates a discrete insoluble reaction product at the site of antigen in the presence horseradish peroxidase (HRP). Nuclei are counterstained using hematoxylin (blue stain) to assess cell and tissue morphology.

[0462] Table 7 – IHC Procedure – TechMate Protocol

TechMate Sequence	Reagent
1	Blocking Reagent – 15 min
2	Primary Antibody – 1 hour
3	Secondary Antibody – 25 min
4	Hydrogen Peroxide Block – 3 x 2.5 min
5	Conjugated Horseradish Peroxidase (HRP) – 25 min
6	DAB Chromogen – 3 x 5 min
7	Hematoxylin Counterstain – 1 min

Immunohistochemistry

[0463] Mouse Polink2+ HRP reagents (Golden Bridge International [GBI]; Cat #: D37-110) are stored ready-to-use at 2-8°C, with all procedures below automated at room temperature (25°C) on the TechMate running QualTek MIPE Procedure. Reagent changes (washes, incubations) take place by capillary action (drain-draw) using absorbent wick pads (drain) and TechMate reagent trays (draw)

- a. Wash three (3) times with TBST.
- b. Goat Blocking Reagent (QML), 15 min
- c. Wash one (1) time with TBST.
- d. ROR2 (0.75 µg/mL) antibody clone H-1(Santa Cruz Biotechnology/sc-374174) freshly diluted in QualTek reagent manufacturing buffer (RMB: 0.01M Phosphate, 0.151M NaCl, 1% w/v BSA, 0.1% v/v ProClin 300, 0.2% v/v Tween-20, 1% v/v normal goat serum, pH 7.2, as formulated by QualTek according to SOP MFB002) from 0.1 mg/mL working stock (4°C, also in RMB) for an hour
- e. Wash five (5) times with TBST.
- f. Mouse Polink2+ secondary (part of GBI Kit Cat #: D37-110), 25min.
- g. Wash two (2) times with TBST wash buffer.
- h. Peroxidase block (3% USP H2O2, with ~0.02% v/v Tween-20 added), 3X 2.5min (7.5min total) with intervening reagent drain.
- i. Wash three (3) times with TBST.
- j. Mouse Polink2+ HRP conjugated polymer (part of GBI Kit Cat #: D37-110), 25min
- k. Wash five (5) times with TBST.

l. GBI (Cat #: C09-12) DAB Chromogen (reagent made freshly at conclusion of polymer incubation, using 40µl DAB chromogen concentrate per 1mL supplied substrate buffer), 3X 5min (15min total) with intervening reagent drain and one (1) wash in TBST.

m. Wash four (4) times with TBST

n. Hematoxylin counterstain (1:5), 1min

o. Wash six (6) times with TBST.

p. Slides immersed in room temperature (25°C) distilled water (transfer to coverslip area).

[0464] • Step 5: Slides were unpaired, rinsed in distilled water, dehydrated in an alcohol series (70%, 95%, 100% ethanol) and in organic solvent (xylene, 100%, four changes), then permanently coverslipped, using CytoSeal (or equivalent), for interpretation and storage.

Slides were examined under a microscope to assess staining.

[0465] SHIER 7(Citra Plus) solution was used for unmasking the epitopes of ROR2 in FFPE tissues. SHIER 1 (Citrate-based, pH 5.6-6.1) solution was used for unmasking the epitopes of CD68. After heat induced epitope retrieval, the process steps were automated with TechMate Instrument (Roche Diagnostics) running QML workmate software v3.96. This automated platform uses a capillary gap process for all reagent changes, up to and including counterstaining, and intervening buffer washes. All steps were carried out at room temperature (25°C).

[0466] Reagent Manufacturing Buffer [RMB; made by QualTek's Santa Barbara lab (QML-SB)] with Goat Serum was used to prepare working dilutions of primary antibodies and negative control antibodies. Target recognition for at the site of antigen-primary antibody interaction in FFPE sections used reagents from Polink-2 Plus HRP kits from GBI Labs designed for detection of Mouse primary antibodies. Refer to Table 6 for antibody specifications and optimized IHC assay conditions for ROR2.

Dehydration/Coverslipping

a. Two (2) changes of room temperature (25°C) 95% alcohol for rinsing [min. 16 dips in-out].

b. Six (6) changes of room temperature (25°C) absolute alcohol for rinsing [min. 16 dips in-out].

c. Four (4) changes of room temperature (25°C) absolute xylene for rinsing [min. 16 dips in-out].

d. Coverslip with Thermo Scientific 8312 Cytoseal XYL or equivalent non-aqueous semi-permanent mounting media.

Internal Process Controls

[0467] Species-match positive controls (standard antibodies) with established signal strength in control tissues were used in each run to confirm proper detection reagent performance. The positive control used throughout the duration of this project was LCA (derived in Mouse) run on formalin-fixed, paraffin-embedded (FFPE) control tonsil tissues or CK (cytokeratin) (derived in Mouse) run on FFPE colon cancer control tissues.

[0468] Mixed cancer multi-tissue blocks (QMTB273) from a tissue bank that included a range of ROR2 expression levels were used as positive controls for ROR2 in each IHC run. Mouse IgG1 isotype-match negative controls for the corresponding biomarker assay conditions were used to determine any nonspecific staining inherent in the detection reagents or tissues and to define any potential background reactivity from these sources.

Test Tissues

[0469] For assay concordance between laboratories, a total of 12 different formalin-fixed, paraffin embedded (FFPE) sarcoma, ovarian cancer, and lung cancer (Non-Small Cell Lung cancer (NSCLC) tissues from a tissue bank were tested. For CLIA sensitivity testing, ROR2 testing was evaluated in FFPE tissue samples for the following cancer indications: Sarcoma (Soft Tissue, 30 samples), Ovarian Cancer (31 samples), Non-Small Cell Lung cancer (NSCLC) (38 samples), and Breast Cancer (Triple Negative Breast Carcinoma or TNBC, 38 samples). All samples were from a tissue bank. Detailed information on each sample is included in the sensitivity scoring table in the Results section. A subset of these cancer samples was used for validation testing of the ROR2 IHC assay in each cancer indication.

[0470] For ROR2 specificity testing, FDA multi-normal human tissue microarray (TMA) slides from Pantomics, Inc (Cat # MNO961) were obtained. The TMA (designated as P1478Q0035) contained 96 different samples derived from 35 different organs or sites. Specifically, 35 types of normal tissues (each in triplicates) as listed in Table 8 below, based on FDA recommendation for antibody cross-reactivity testing, were fixed in 10% neutral buffered formalin for 24 hours and processed using SOPs. Sections were picked onto Superfrost Plus or Startfrost Adhesive slides.

[0471] Table 8

Tissue	Pathology
Adrenal gland	Normal and/or hyperplasia
Bladder	Normal
Bone marrow	Normal
Eye	Normal
Breast	Normal
Cerebellum	Normal
Cerebral cortex	Normal
Fallopian tube	Normal
Esophagus	Normal
Stomach	Normal
Small intestine	Normal
Colon	Normal
Rectum	Normal
Heart	Normal
Lung	Normal
Kidney	Normal
Liver	Normal
Ovary	Normal
Pancreas	Normal
Parathyroid	Normal
Pituitary gland	Normal
Placenta	Normal
Prostate	Normal
Skin	Normal
Spinal cord	Normal
Spleen	Normal
Skeletal muscle	Normal

Table 8 (continued)	Pathology
Tissue	
Testis	Normal
Thymus	Normal and/or hyperplasia
Thyroid	Normal
Tonsil	Tonsilitis
Ureter	Normal
Uterus, cervix	Normal

Scoring Scheme

[0472] Comparison scoring for ROR2 staining in serial sections of each sample was performed as described in the Plasma Membrane Scoring of ROR2 in Tumor (Tumor Membrane P Score) section above.

Results

[0473] ROR2 IHC Assay Concordance (Part A)

[0474] A total of 6 different FFPE samples consisting of 2 each of Sarcoma (Synovial), Ovarian Cancer, and Non-Small Cell Lung cancer (NSCLC) were used for concordance testing between laboratories. The samples represented a range of ROR2 plasma membrane tumor cell staining.

[0475] The tissues for concordance testing were stained using the non-GLP ROR2 IHC assays at a facility. They were also stained as serial sections (one section of tissue per slide with minimal loss of material between preparations) at a second facility by a different operator using the assays described in Table 6 and above. All assay testing was performed using a TechMate automated staining platform.

[0476] For ROR2, scoring was performed by recording the percentage of tumor cells with plasma membrane staining at differential intensities from 0-3+ as described herein. Comparisons between the same samples run at different laboratories were made based on Percent Scores $\geq 1+$ [(% at 1+) + (% at 2+) x 1 + (% at 3+)].

[0477] When comparing ROR2 Percent Scores between samples stained at different laboratories, the following concordance parameters were set: scores within +/- 20% of each other are deemed concordant; and 85% of the samples tested must be concordant for approved assay transfer. Under these conditions, acceptable concordance was noted

throughout the set of Sarcoma, Ovarian Cancer, and NSCLC tissues when run at the two different laboratories.

[0478] Scoring data that compared the samples stained at each facility were obtained (Table 10). Table 10 includes the difference between ROR2 Percent Score $\geq 1+$ between each facility sample were obtained. A total of 12 samples were run at both laboratories. Of these 12 samples, all were considered concordant (within 20% difference) for ROR2. This yielded 100% sample set concordance between laboratories for ROR2 IHC assay.

[0479] According to the assay transfer scoring data obtained (Table 9) and the established criteria for concordance, the ROR2 IHC assays were deemed successfully transferred.

[0480] Table 9 – Reagent Summary

Reagent	Source or Vendor	Part # or Cat #
Probe-On Plus Microscope Slides	Fisher	22-230-900
Ethanol	Fisher	A962P-4
Xylene	Fisher	X5-4
SHIER 7	BioGenex	HK081-20K
Goat RMB	QML-SB	400001
TBS Buffer	QML-SB	300010
Normal Goat Block	QML-SB	300003
Polink-2 Plus HRP Detection Kit Mouse	GBI Labs	D37-110
Hydrogen Peroxide	Supervalu (OTC)	Equaline
DAB	GBI Labs	C09-100
Hematoxylin	QML-SB	100005
Cytoseal 60	Thermo/Fisher Scientific	8310-4, 23-244256

[0481] Part B: Sensitivity Screening for ROR2 Cancers

[0482] The optimized IHC assays for ROR2 in Table 7 were used along with the general IHC procedure in Table 7 and reagents in Table 6 to screen serial sections (one section of tissue per slide with minimal loss of material between preparations) of human tumor tissues from different cancer indications.

[0483] Table 10 – IHC Assay Concordance for ROR2

Sample Information

Row	Comparison	Slide No.	Tissue Type	Q/MTB
1	non-CLIA IHC Assay	569	Synovial Sarcoma	QMTB312-05
2	IHC Assay for CLIA	16		
3	non-CLIA IHC Assay	569	Synovial Sarcoma	QMTB312-07
4	IHC Assay for CLIA	16		
5	non-CLIA IHC Assay	566	Ovarian CA	QMTB308-01
6	IHC Assay for CLIA	15		
7	non-CLIA IHC Assay	566	Ovarian CA	QMTB308-08
8	IHC Assay for CLIA	15		
9	non-CLIA IHC Assay	572	NSCLC	QMTB398-04
10	IHC Assay for CLIA	17		
11	non-CLIA IHC Assay	572	NSCLC	QMTB398-09

Table 10 (continued) – IHC Assay Concordance for ROR2
ROR2 (H-1) Plasma Membrane Tumor Staining

Row	Tumor Differential Intensities				% Score ≥1+	*POS/NEG	Difference
	0	1+	2+	3+			
1	0	0	0	100	100	POS	0.0
2	0	0	0	100	100	POS	
3	100	0	0	0	0	NEG	0.0
4	100	0	0	0	0	NEG	
5	30	0	0	70	70	POS	0.0
6	30	0	50	20	70	POS	
7	75	0	5	20	25	POS	0.0
8	75	0	5	20	25	POS	
9	30	20	30	20	70	POS	0.0
10	30	20	20	30	70	POS	
11	50	40	10	0	50	POS	10.0

[0484] * ROR2 Cut-Off: $\geq 1+$ intensity in $\geq 10\%$ of tumor cells = Positive (POS)

[0485] The cancer indications and the number of samples analyzed in each for CAP/CLIA sensitivity screening were as follows:

[0486] 30 samples of Sarcoma (Soft Tissue Sarcoma including Spindle Cell Sarcoma, Leiomyosarcoma, Myxoid Liposarcoma, and Synovial Sarcoma), 31 samples of Ovarian Cancer, 38 samples of Non-Small Cell Lung cancer (NSCLC), and 38 samples of Triple Negative Breast Cancer (TNBC). All tissues for sensitivity testing were formalin-fixed, paraffin-embedded (FFPE) human cancer specimens.

[0487] Cancer tissues that previously showed a range of ROR2 reactivity served as a positive control/quality control (QC) to demonstrate appropriate staining during the current tumor screen. Standard species-match positive controls (Mouse CK) and isotype-match negative controls (Mouse IgG1) were included during testing and reacted as expected. Samples were also stained with hematoxylin and eosin (H&E) for morphological assessment to assist in scoring.

[0488] ROR2 is reactive in a subset of tumor cells. It primarily localizes to the plasma membrane but can also be present in the cytoplasm. All tissues in the tumor screen were evaluated by the Medical Director of QualTek Laboratories (a board-certified pathologist). ROR2 plasma membrane staining in tumor was evaluated using Percent Scores [sum of percentages of intensities $\geq 1+$, $\geq 2+$, and $\geq 3+$ with values ranging from 0 to 100] and H-Scores [sum of each percentage score (0-100%) multiplied by its corresponding intensity score (0, 1+, 2+, 3+) with values ranging from 0 to 300]. Cytoplasmic tumor cell staining for ROR2 was evaluated using H-Scores as described in the Plasma Membrane Scoring of ROR2 in Tumor (Tumor Membrane P Score) section above.

[0489] The ROR2 tumor screen was performed to understand the range of staining intensities and abundance (penetrance) of reactivity across a representative sample set from different cancer indications. Scoring results for ROR2 CLIA sensitivity were obtained for the cancer indications above.

[0490] Any ROR2 staining observed in stroma or adjacent normal tissue while analyzing the cancer samples is noted in the scoring table. Scoring results for ROR2 in the evaluable cancer samples are shown in this report in Table 11 for Sarcoma, Table 12 for Ovarian Cancer, Table 13 for NSCLC, and Table 14 for TNBC. In the Sarcoma indication, most of the samples tested had moderate or high ROR2 plasma membrane staining. In the Ovarian Cancer, NSCLC, and TNBC indications, most the samples tested had low ROR2 plasma membrane staining. However, in each of these indications, examples of negative, low, moderate, and high ROR2 expression were observed.

[0491] Representative images of a range of ROR2 expression levels are shown in FIG. 19 for Sarcoma, FIG. 20 for Ovarian Cancer, FIG. 21 for NSCLC, and FIG. 22 for TNBC. Mouse IgG1 isotype-match negative controls were included on each tissue sample tested in the sensitivity screen and were nonreactive. Representative images of these Mouse IgG serum negative controls in each cancer indication are shown in panel D of FIGS. 19-22.

[0492] Table 11 – ROR2 in Sarcoma for CAP/CLIA Sensitivity Testing

Sample Information
cROR2 (H-1) Plasma
Membrane
Tumor Staining

Row	Tissue Type	Q/MTB	Unique ID	Pathologic Diagnosis	% at Differential Intensities			
					0	1+	2+	3+
1	Sarcoma	QMTB313-01	Q0010	Spindle cell sarcoma	0	20	30	50
2	Sarcoma	QMTB313-02	Q0174	Spindle cell sarcoma	100	0	0	0
3	Sarcoma	QMTB313-03	Q0222	Spindle cell sarcoma	20	0	30	50
4	Sarcoma	QMTB313-04	Q6410	Spindle cell sarcoma	30	20	30	20
5	Sarcoma	QMTB313-05	Q3176	Spindle cell sarcoma	0	90	10	0
6	Sarcoma	QMTB313-06	Q5834	Spindle cell sarcoma	50	10	10	30
7	Sarcoma	QMTB313-07	Q3203	Spindle cell sarcoma	60	0	20	20
8	Sarcoma	QMTB313-08	Q3901	Leiomyosarcoma	30	0	20	50
9	Sarcoma	QMTB313-09	Q4971	Leiomyosarcoma	NET	NET	NET	NET
10	Sarcoma	Q3169-13	N/A	Osteosarcoma	NET	NET	NET	NET
11	Sarcoma	Q3170-01	N/A	Osteosarcoma	NET	NET	NET	NET
12	Sarcoma	Q3180-01	N/A	Myxoid liposarcoma	40	30	30	0
13	Sarcoma	Q3205-02	N/A	Synovial sarcoma	20	0	0	80
14	Sarcoma	Q4860	N/A	Leiomyosarcoma	NET	NET	NET	NET
15	Sarcoma	Q6912	N/A	Leiomyosarcoma	30	0	0	70
16	Sarcoma	Q6913	N/A	Leiomyosarcoma	NET	NET	NET	NET
17	Sarcoma	Q6914	N/A	Leiomyosarcoma	10	20	20	50
18	Sarcoma	Q6915	N/A	Leiomyosarcoma	20	10	20	50
19	Sarcoma	Q9396	N/A	Synovial sarcoma	70	0	0	30
20	Sarcoma	Q9399	N/A	Synovial sarcoma	30	40	20	10
21	Sarcoma	Q9400	N/A	Synovial sarcoma	NET	NET	NET	NET
22	Sarcoma	Q9403	N/A	Synovial sarcoma	0	0	0	100
23	Sarcoma	Q9406	N/A	Synovial sarcoma	50	10	10	30
24	Sarcoma	Q9416	N/A	Synovial sarcoma	10	40	20	30
25	Sarcoma	Q9451	N/A	Synovial sarcoma	0	10	20	70
26	Sarcoma	Q9452	N/A	Synovial sarcoma	0	10	20	70
27	Sarcoma	Q9453	N/A	Synovial sarcoma	50	10	20	20
28	Sarcoma	Q9454	N/A	Synovial sarcoma	50	30	10	10
29	Sarcoma	Q9455	N/A	Synovial sarcoma	100	0	0	0

Table 11 (continued)– ROR2 in Sarcoma for CAP/CLIA Sensitivity Testing**Sample Information****ROR2 (H-1) Plasma
Membrane
Tumor Staining**

Row	Tissue Type	Q/MTB	Unique ID	Pathologic Diagnosis	% at Differential Intensities			
					0	1+	2+	3+
30	Sarcoma	Q9456	N/A	Myxoid liposarcoma	100	0	0	0
31	Sarcoma	Q9457	N/A	Myxoid liposarcoma	100	0	0	0
32	<i>Sarcoma</i>	<i>Q9458</i>	<i>N/A</i>	<i>Myxoid liposarcoma</i>	<i>NET</i>	<i>NET</i>	<i>NET</i>	<i>NET</i>
33	Sarcoma	Q9459	N/A	Myxoid liposarcoma	100	0	0	0
34	Sarcoma	Q9468	N/A	Synovial sarcoma	60	0	0	40
35	Sarcoma	Q9469	N/A	Synovial sarcoma	100	0	0	0
36	Sarcoma	Q9470	N/A	Myxoid liposarcoma	30	0	40	30
37	Sarcoma	Q9471	N/A	Myxoid liposarcoma	70	30	0	0

Table 11 (continued)– ROR2 in Sarcoma for CAP/CLIA Sensitivity Testing**ROR2 (H-1) Plasma membrane
Tumor Staining****ROR2 (H-1) Cytoplasmic
Tumor Staining**

Row	H-Score	% Score ≥1+	*POS/NEG	% at Differential Intensities				H-Score
				0	1+	2+	3+	
1	230	100	POS	0	20	30	50	230
2	0	0	NEG	100	0	0	0	0
3	210	80	POS	20	0	30	50	210
4	140	70	POS	30	20	30	20	140
5	110	100	POS	0	90	10	0	110
6	120	50	POS	50	10	10	30	120
7	100	40	POS	60	0	20	20	100
8	190	70	POS	30	0	20	50	190
9	NET	NET	NET	NET	NET	NET	NET	NET
10	NET	NET	NET	NET	NET	NET	NET	NET
11	NET	NET	NET	NET	NET	NET	NET	NET
12	90	60	POS	40	30	30	0	90
13	240	80	POS	20	0	0	80	240
14	NET	NET	NET	NET	NET	NET	NET	NET
15	210	70	POS	30	0	0	70	210
16	NET	NET	NET	NET	NET	NET	NET	NET
17	210	90	POS	10	20	20	50	210
18	200	80	POS	20	10	20	50	200
19	90	30	POS	70	0	0	30	90
20	110	70	POS	30	40	20	10	110
21	NET	NET	NET	NET	NET	NET	NET	NET
22	300	100	POS	0	0	0	100	300
23	120	50	POS	50	10	10	30	120
24	170	90	POS	10	40	20	30	170
25	260	100	POS	0	10	20	70	260
26	260	100	POS	0	10	20	70	260
27	110	50	POS	50	10	20	20	110
28	80	50	POS	50	30	10	10	80
29	0	0	NEG	100	0	0	0	0
30	0	0	NEG	100	0	0	0	0
31	0	0	NEG	100	0	0	0	0
32	NET	NET	NET	NET	NET	NET	NET	NET
33	0	0	NEG	100	0	0	0	0
34	120	40	POS	100	0	0	0	0
35	0	0	NEG	100	0	0	0	0
36	170	70	POS	30	0	40	30	170
37	30	30	POS	70	30	0	0	30

* ROR2 Cut-Off: ≥1+ intensity in ≥10% of tumor cells = Positive (POS)

[0493] Table 12 – ROR2 in Ovarian Cancer for CAP/CLIA Sensitivity Testing

Sample Information					ROR2 (H-1) Plasma Membrane Tumor Staining			
Row	Tissue Type	Q/MTB	Unique ID	Pathologic Diagnosis	% at Differential Intensities			
					0	1+	2+	3+
1	Ovarian Cancer	QMTB310-01	Q6458	Ovarian Carcinoma	NET	NET	NET	NET
2	Ovarian Cancer	QMTB310-02	Q6413	Adenoca, no mucinous	100	0	0	0
3	Ovarian Cancer	QMTB310-03	Q6414	Adenoca, mucinous	50	0	20	30
4	Ovarian Cancer	QMTB310-04	Q7504	Adenoca, no mucinous	100	0	0	0
5	Ovarian Cancer	QMTB310-05	Q7505	Adenoca, serous, no mucinous	100	0	0	0
6	Ovarian Cancer	QMTB310-06	Q6415	Adenoca, mucinous	100	0	0	0
7	Ovarian Cancer	QMTB400-01	Q1740	High grade serous Ovarian Ca	100	0	0	0
8	Ovarian Cancer	QMTB400-02	Q7500-04	High grade serous Ovarian Ca	100	0	0	0
9	Ovarian Cancer	QMTB400-03	Q7502-05	High grade serous Ovarian Ca	99	0	0	1
10	Ovarian Cancer	QMTB400-04	Q7503	High grade serous Ovarian Ca	100	0	0	0
11	Ovarian Cancer	QMTB400-05	Q7505-04	High grade serous Ovarian Ca	90	2	3	5
12	Ovarian Cancer	QMTB400-06	Q5488-06	High grade serous Ovarian Ca	95	5	0	0
13	Ovarian Cancer	QMTB400-07	Q1265-04	High grade serous Ovarian Ca	100	0	0	0
14	Ovarian Cancer	QMTB400-08	Q1271-03	High grade serous Ovarian Ca	100	0	0	0
15	Ovarian Cancer	QMTB400-09	Q3232-02	High grade serous Ovarian Ca	100	0	0	0
16	Ovarian Cancer	Q1261-02	N/A	Seromucinous tumor	100	0	0	0
17	Ovarian Cancer	Q1262-02	N/A	High grade serous Ovarian Ca	90	0	0	10
18	Ovarian Cancer	Q1266-03	N/A	High grade serous Ovarian Ca	100	0	0	0
19	Ovarian Cancer	Q1267-03	N/A	Low grade serous Ovarian Ca	100	0	0	0
20	Ovarian Cancer	Q1269-02	N/A	Granulosa cell tumor	NET	NET	NET	NET

Table 12 (continued)– ROR2 in Ovarian Cancer for CAP/CLIA Sensitivity Testing

Sample Information					ROR2 (H-1) Plasma Membrane Tumor Staining			
Row	Tissue Type	Q/MTB	Unique ID	Pathologic Diagnosis	% at Differential Intensities			
					0	1+	2+	3+
21	Ovarian Cancer	Q1270-05	N/A	Borderline mucinous tumor	100	0	0	0
22	Ovarian Cancer	Q1272-04	N/A	Ovarian Carcinoma	100	0	0	0
23	Ovarian Cancer	Q1283-01	N/A	<i>Granulosa cell tumor</i>	NET	NET	NET	NET
24	Ovarian Cancer	Q1284-01	N/A	High grade serous Ovarian Ca	100	0	0	0
25	Ovarian Cancer	Q2015-02	N/A	High grade serous Ovarian Ca	99	0	0	1
26	Ovarian Cancer	Q2016-02	N/A	<i>High grade serous Ovarian Ca</i>	NET	NET	NET	NET
27	Ovarian Cancer	Q3214-01	N/A	Serous adenocarcinoma	95	0	0	5
28	Ovarian Cancer	Q3247-01	N/A	Serous adenocarcinoma	100	0	0	0
29	Ovarian Cancer	Q3255-02	N/A	Ovarian Carcinoma	100	0	0	0
30	Ovarian Cancer	Q5488-02	N/A	High grade serous Ovarian Ca	30	10	30	30
31	Ovarian Cancer	Q6415	N/A	<i>Mucinous ovarian carcinoma</i>	NET	NET	NET	NET
32	Ovarian Cancer	Q6946-01	N/A	Serous Adenocarcinoma	80	0	0	20
33	Ovarian Cancer	Q6948-02	N/A	Serous Adenocarcinoma	100	0	0	0
34	Ovarian Cancer	Q7499-02	N/A	Serous Ovarian Carcinoma	50	10	20	20
35	Ovarian Cancer	Q7501-02	N/A	Malignant mixed epithelial	99	0	0	0
36	Ovarian Cancer	Q7506-01	N/A	<i>Clear cell adenocarcinoma</i>	NET	NET	NET	NET
37	Ovarian Cancer	Q7539-01	N/A	Serous adenocarcinoma	10	70	10	10

Table 12 (continued) – ROR2 in Ovarian Cancer for CAP/CLIA Sensitivity Testing

ROR2 (H-1) Plasma Membrane
Tumor StainingROR2 (H-1) Cytoplasmic
Tumor Staining

Row	H-Score	% Score ≥1+	*POS/NEG	% at Differential Intensities				H-Score
				0	1+	2+	3+	
1	NET	NET	NET	NET	NET	0	NET	NET
2	0	0	NEG	100	0	0	0	0
3	130	50	POS	100	0	0	0	0
4	0	0	NEG	100	0	0	0	0
5	0	0	NEG	100	0	0	0	0
6	0	0	NEG	100	0	0	0	0
7	0	0	NEG	100	0	0	0	0
8	0	0	NEG	100	0	0	0	0
9	3	1	NEG	70	30	40	0	110
10	0	0	NEG	100	0	0	0	0
11	23	10	POS	100	0	0	0	0
12	5	5	NEG	100	0	0	0	0
13	0	0	NEG	100	0	0	0	0
14	0	0	NEG	100	0	0	0	0
15	0	0	NEG	100	0	0	0	0
16	0	0	NEG	100	0	0	0	0
17	30	10	POS	100	0	0	0	0
18	0	0	NEG	100	0	0	0	0
19	0	0	NEG	100	0	0	0	0
20	NET	NET	NET	NET	NET	0	NET	NET
21	0	0	NEG	100	0	0	0	0
22	0	0	NEG	100	0	0	0	0
23	NET	NET	NET	NET	NET	0	NET	NET
24	0	0	NEG	100	0	0	0	0
25	3	1	NEG	100	0	0	0	0
26	NET	NET	NET	NET	NET	0	NET	NET
27	15	5	NEG	100	0	0	0	0
28	0	0	NEG	70	0	30	0	60
29	0	0	NEG	90	10	0	0	10
30	160	70	POS	100	0	0	0	0
31	NET	NET	NET	NET	NET	NET	NET	NET
32	60	20	POS	100	0	0	0	0
33	0	0	NEG	100	0	0	0	0
34	110	50	POS	100	0	0	0	0
35	0	0	NEG	100	0	0	0	0
36	NET	NET	NET	NET	NET	NET	NET	NET
37	120	90	POS	100	0	0	0	0

* ROR2 Cut-Off: ≥1+ intensity in ≥10% of tumor cells = Positive (POS)

Table 13 – ROR2 in Ovarian Cancer for CAP/CLIA Sensitivity Testing

Sample Information

ROR2 (H-1) Plasma
Membrane
Tumor Staining

Row	Tissue Type	Q/MTB	Unique ID	Pathologic Diagnosis	% at Differential Intensities			
					0	1+	2+	3+
1	NSCLC	QMTB249-01	Q5040	Adenoca, PD	100	0	0	0
2	NSCLC	QMTB249-02	Q5041	Adenoca, MD	100	0	0	0
3	NSCLC	QMTB249-03	Q5043	Adenoca, PD	100	0	0	0
4	NSCLC	QMTB249-04	Q0958	Adenoca, WD	100	0	0	0
5	NSCLC	QMTB249-05	Q1035	Adenoca, PD	100	0	0	0
6	NSCLC	QMTB249-06	Q1041	Adenoca, PD	100	0	0	0
7	NSCLC	QMTB249-07	Q1044	Adenoca, MD	100	0	0	0
8	NSCLC	QMTB249-08	Q1045	Adenoca, WD	NET	NET	NET	NET
9	NSCLC	QMTB397-01	Q0953-01	Squamous Cell Carcinoma	100	0	0	0
10	NSCLC	QMTB397-02	Q6949-01	Squamous Cell Carcinoma	75	20	5	0
11	NSCLC	QMTB397-03	Q9369-02	Squamous Cell Carcinoma	100	0	0	0
12	NSCLC	QMTB397-04	Q6953-02	Squamous Cell Carcinoma	100	0	0	0
13	NSCLC	QMTB397-05	Q6955-01	Squamous Cell Carcinoma	100	0	0	0
14	NSCLC	QMTB397-06	Q6956-01	Squamous Cell Carcinoma	95	5	0	0
15	NSCLC	QMTB397-07	Q6958-03	Squamous Cell Carcinoma	100	0	0	0
16	NSCLC	QMTB397-08	Q6959-01	Squamous Cell Carcinoma	100	0	0	0
17	NSCLC	QMTB397-09	Q7031-02	Squamous Cell Carcinoma	0	20	30	50
18	NSCLC	Q0292 -01	N/A	Non-Small Cell Lung Ca	100	0	0	0
19	NSCLC	Q1015-01	N/A	Squamous Cell Carcinoma	NET	NET	NET	NET
20	NSCLC	Q1022-1	N/A	Adenocarcinoma	100	0	0	0
21	NSCLC	Q1023-7	N/A	Non-Small Cell Lung Ca	NET	NET	NET	NET
22	NSCLC	Q1051-01	N/A	Adenocarcinoma	100	0	0	0
23	NSCLC	Q1946-04	N/A	Non-Small Cell Lung Ca	100	0	0	0
24	NSCLC	Q1951-01	N/A	Adenocarcinoma	100	0	0	0

Table 13 (continued)– ROR2 in Ovarian Cancer for CAP/CLIA Sensitivity Testing

Sample Information					ROR2 (H-1) Plasma Membrane Tumor Staining			
Row	Tissue Type	Q/MTB	Unique ID	Pathologic Diagnosis	% at Differential Intensities			
					0	1+	2+	3+
25	NSCLC	Q2584-1	N/A	Non-Small Cell Lung Ca	100	0	0	0
26	NSCLC	Q2585	N/A	Non-Small Cell Lung Ca	100	0	0	0
27	NSCLC	Q2651-01	N/A	Adenocarcinoma	NET	NET	NET	NET
28	NSCLC	Q2654-6	N/A	Metastatic Carcinoma	NET	NET	NET	NET
29	NSCLC	Q4038-01	N/A	Squamous Cell Carcinoma	100	0	0	0
30	NSCLC	Q4041-01	N/A	Non-Small Cell Lung Ca	100	0	0	0
31	NSCLC	Q4044-04	N/A	Squamous Cell Carcinoma	90	0	10	0
32	NSCLC	Q4048-01	N/A	Squamous Cell Carcinoma	100	0	0	0
33	NSCLC	Q4051-01	N/A	Squamous Cell Carcinoma	100	0	0	0
34	NSCLC	Q4053	N/A	Papillary Carcinoma	100	0	0	0
35	NSCLC	Q4057-02	N/A	Adenocarcinoma	100	0	0	0
36	NSCLC	Q4062-01	N/A	Squamous Cell Carcinoma	100	0	0	0
37	NSCLC	Q4064-01	N/A	Squamous Cell Carcinoma	100	0	0	0
38	NSCLC	Q4065-01	N/A	Squamous Cell Carcinoma	100	0	0	0
39	NSCLC	Q6996-02	N/A	Adenocarcinoma	100	0	0	0
40	NSCLC	Q4070-02	N/A	Squamous Cell Carcinoma	100	0	0	0
41	NSCLC	Q4071-01	N/A	Squamous Cell Carcinoma	95	0	0	5
42	NSCLC	Q4092-2	N/A	Non-Small Cell Lung Ca	100	0	0	0
43	NSCLC	Q6949-01	N/A	Squamous Cell Carcinoma	30	20	20	30

Table 13 (continued)– ROR2 in Ovarian Cancer for CAP/CLIA Sensitivity Testing

ROR2 (H-1) Plasma Membrane Tumor Staining				ROR2 (H-1) Cytoplasmic Tumor Staining				
Row	H-Score	% Score ≥1+	*POS/NEG	% at Differential Intensities				H-Score
				0	1+	2+	3+	
1	0	0	NEG	100	0	0	0	0
2	0	0	NEG	100	0	0	0	0
3	0	0	NEG	100	0	0	0	0
4	0	0	NEG	100	0	0	0	0
5	0	0	NEG	75	20	5	0	30
6	0	0	NEG	100	0	0	0	0
7	0	0	NEG	100	0	0	0	0
8	NET	NET	NET	NET	NET	NET	NET	NET
9	0	0	NEG	100	0	0	0	0
10	30	25	POS	100	0	0	0	0
11	0	0	NEG	100	0	0	0	0
12	0	0	NEG	100	0	0	0	0
13	0	0	NEG	95	5	0	0	5
14	5	5	NEG	100	0	0	0	0
15	0	0	NEG	100	0	0	0	0
16	0	0	NEG	100	0	0	0	0
17	230	100	POS	100	0	0	0	0
18	0	0	NEG	100	0	0	0	0
19	NET	NET	NET	NET	NET	NET	NET	NET
20	0	0	NEG	100	0	0	0	0
21	NET	NET	NET	NET	NET	NET	NET	NET
22	0	0	NEG	100	0	0	0	0
23	0	0	NEG	100	0	0	0	0
24	0	0	NEG	100	0	0	0	0
25	0	0	NEG	100	0	0	0	0
26	0	0	NEG	100	0	0	0	0
27	NET	NET	NET	NET	NET	NET	NET	NET
28	NET	NET	NET	NET	NET	NET	NET	NET
29	0	0	NEG	100	0	0	0	0
30	0	0	NEG	100	0	0	0	0
31	20	10	POS	100	0	0	0	0
32	0	0	NEG	100	0	0	0	0
33	0	0	NEG	100	0	0	0	0
34	0	0	NEG	100	0	0	0	0
35	0	0	NEG	100	0	0	0	0
36	0	0	NEG	100	0	0	0	0
37	0	0	NEG	100	0	0	0	0

Table 13 (continued)– ROR2 in Ovarian Cancer for CAP/CLIA Sensitivity Testing

ROR2 (H-1) Plasma Membrane Tumor Staining				ROR2 (H-1) Cytoplasmic Tumor Staining				
Row	H-Score	% Score ≥1+	*POS/NEG	% at Differential Intensities				H-Score
				0	1+	2+	3+	
38	0	0	NEG	100	0	0	0	0
39	0	0	NEG	100	0	0	0	0
40	0	0	NEG	100	0	0	0	0
41	15	5	NEG	90	5	5	0	15
42	0	0	NEG	100	0	0	0	0
43	150	70	POS	100	0	0	0	0

* ROR2 Cut-Off: ≥1+ intensity in ≥10% of tumor cells = Positive (POS)

[0494] Table 14 – ROR2 in TNBC for CAP/CLIA Sensitivity Testing**Sample Information****ROR2 (H-1) Plasma
Membrane
Tumor Staining**

Row	Tissue Type	Q/MTB	Unique ID	Pathologic Diagnosis	% at Differential Intensities			
					0	1+	2+	3+
1	TNBC	Q9250	N/A	Triple Negative Breast Ca	80	0	20	0
2	TNBC	Q9251	N/A	Triple Negative Breast Ca	100	0	0	0
3	TNBC	Q9253	N/A	Triple Negative Breast Ca	100	0	0	0
4	TNBC	Q9254	N/A	Triple Negative Breast Ca	100	0	0	0
5	TNBC	Q9255	N/A	Triple Negative Breast Ca	30	10	20	40
6	TNBC	Q9257	N/A	Triple Negative Breast Ca	100	0	0	0
7	TNBC	Q9260	N/A	Triple Negative Breast Ca	90	0	10	0
8	TNBC	Q9261	N/A	Triple Negative Breast Ca	100	0	0	0
9	TNBC	Q9263	N/A	Triple Negative Breast Ca	100	0	0	0
10	TNBC	Q9265	N/A	Triple Negative Breast Ca	100	0	0	0
11	TNBC	Q9268	N/A	Triple Negative Breast Ca	30	10	30	30
12	TNBC	Q9269	N/A	Triple Negative Breast Ca	100	0	0	0
13	TNBC	Q9270	N/A	Triple Negative Breast Ca	100	0	0	0
14	TNBC	Q9274	N/A	Triple Negative Breast Ca	100	0	0	0
15	TNBC	Q9275	N/A	Triple Negative Breast Ca	100	0	0	0
16	TNBC	Q9276	N/A	Triple Negative Breast Ca	100	0	0	0
17	TNBC	Q9277	N/A	Triple Negative Breast Ca	100	0	0	0
18	TNBC	Q9278	N/A	Triple Negative Breast Ca	100	0	0	0
19	TNBC	Q9279	N/A	Triple Negative Breast Ca	100	0	0	0

Table 14 (continued) – ROR2 in TNBC for CAP/CLIA Sensitivity Testing**Sample Information****ROR2 (H-1) Plasma
Membrane
Tumor Staining**

Row	Tissue Type	Q/MTB	Unique ID	Pathologic Diagnosis	% at Differential Intensities			
					0	1+	2+	3+
20	TNBC	Q9280	N/A	Triple Negative Breast Ca	100	0	0	0
21	TNBC	Q9281	N/A	Triple Negative Breast Ca	70	0	0	30
22	TNBC	Q9282	N/A	Triple Negative Breast Ca	100	0	0	0
23	TNBC	Q9283	N/A	Triple Negative Breast Ca	100	0	0	0
24	TNBC	Q9285	N/A	Triple Negative Breast Ca	100	0	0	0
25	TNBC	Q9286	N/A	Triple Negative Breast Ca	40	0	30	30
26	TNBC	Q9287	N/A	Triple Negative Breast Ca	10	40	10	40
27	TNBC	Q9288	N/A	Triple Negative Breast Ca	100	0	0	0
28	TNBC	Q9289	N/A	Triple Negative Breast Ca	30	30	10	30
29	TNBC	Q9325	N/A	Triple Negative Breast Ca	95	5	0	0
30	TNBC	Q9330	N/A	Triple Negative Breast Ca	100	0	0	0
31	TNBC	Q9331	N/A	Triple Negative Breast Ca	100	0	0	0
32	TNBC	Q9332	N/A	Triple Negative Breast Ca	100	0	0	0
33	TNBC	Q9333	N/A	Triple Negative Breast Ca	10	10	30	50
34	TNBC	Q9334	N/A	Triple Negative Breast Ca	100	0	0	0
35	TNBC	Q9335	N/A	Triple Negative Breast Ca	85	0	0	15
36	TNBC	Q9336	N/A	Triple Negative Breast Ca	100	0	0	0
37	TNBC	Q9337	N/A	Triple Negative Breast Ca	100	0	0	0
38	TNBC	Q9338	N/A	Triple Negative Breast Ca	80	20	0	0

Table 14 (continued)– ROR2 in TNBC for CAP/CLIA Sensitivity Testing

ROR2 (H-1) Plasma Membrane Tumor Staining				ROR2 (H-1) Cytoplasmic Tumor Staining				
Row	H-Score	% Score ≥1+	*POS/NEG	% at Differential Intensities				H-Score
				0	1+	2+	3+	
1	40	20	POS	100	0	0	0	0
2	0	0	NEG	100	0	0	0	0
3	0	0	NEG	100	0	0	0	0
4	0	0	NEG	100	0	0	0	0
5	170	70	POS	100	0	0	0	0
6	0	0	NEG	100	0	0	0	0
7	20	10	POS	100	0	0	0	0
8	0	0	NEG	100	0	0	0	0
9	0	0	NEG	100	0	0	0	0
10	0	0	NEG	100	0	0	0	0
11	160	70	POS	100	0	0	0	0
12	0	0	NEG	100	0	0	0	0
13	0	0	NEG	100	0	0	0	0
14	0	0	NEG	100	0	0	0	0
15	0	0	NEG	100	0	0	0	0
16	0	0	NEG	100	0	0	0	0
17	0	0	NEG	100	0	0	0	0
18	0	0	NEG	100	0	0	0	0
19	0	0	NEG	100	0	0	0	0
20	0	0	NEG	100	0	0	0	0
21	90	30	POS	70	0	0	30	90
22	0	0	NEG	100	0	0	0	0
23	0	0	NEG	100	0	0	0	0
24	0	0	NEG	100	0	0	0	0
25	150	60	POS	100	0	0	0	0
26	180	90	POS	100	0	0	0	0
27	0	0	NEG	100	0	0	0	0
28	140	70	POS	70	30	0	0	30
29	5	5	NEG	100	0	0	0	0
30	0	0	NEG	100	0	0	0	0
31	0	0	NEG	100	0	0	0	0
32	0	0	NEG	100	0	0	0	0
33	220	90	POS	100	0	0	0	0
34	0	0	NEG	100	0	0	0	0
35	45	15	POS	100	0	0	0	0
36	0	0	NEG	100	0	0	0	0
37	0	0	NEG	100	0	0	0	0
38	20	20	POS	100	0	0	0	0

* ROR2 Cut-Off: ≥1+ intensity in ≥10% of tumor cells = Positive (POS)

H-Score and Percent Score Analysis for Sensitivity Screen

[0495] The sensitivity screen for ROR2 is intended to assist in determining a cut-off for ROR2 positivity for use in clinical testing. According to this cut-off, samples are considered “positive” for ROR2 if they display $\geq 1+$ intensity plasma membrane staining in $\geq 10\%$ of tumor cells. This positivity cut-off was applied to the Sarcoma, Ovarian Cancer, NSCLC, and TNBC samples in the CLIA sensitivity screen.

[0496] According to the defined cut-off criteria, the following percentage of cases in each indication was ROR2- positive: Sarcoma- 80.0%, Ovarian Cancer- 22.6%, NSCLC- 10.5%, TNBC- 28.9%. A chart summarizing this data is shown in Table 15 below. FIG. 23 displays the proportion of cases in each cancer indication that were positive or negative for ROR2, according to the Percent Score cut-off, using a stacked column graph.

[0497] **Table 15 – Percentage of ROR2 Positive/Negative Cases by Indication**

Breakdown of ROR2 (H-1) POS/NEG Status by Cancer Indication					
ROR2 Positivity Cut-Off: $\geq 1+$ Intensity in $\geq 10\%$ Tumor Cells					
Tumor Type	Evaluable Cases	POSITIVE		NEGATIVE	
		No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication
Sarcoma	30	24	80.0%	6	20.0%
Ovarian Cancer	31	7	22.6%	24	77.4%
NSCLC	38	4	10.5%	34	89.5%
TNBC	38	11	28.9%	27	71.1%

[0498] The ROR2 scoring results were also divided by additional reactivity thresholds for Percent Score $\geq 1+$ (Table 16). The ROR2 cut-off ($\geq 1+$ Staining in $\geq 10\%$ of Tumor Cells) is included in this table. Other theoretical thresholds for Percent Score $\geq 1+$ are shown in $\geq 1\%$, $\geq 10\%$, $\geq 25\%$, $\geq 50\%$, $\geq 75\%$ and $\geq 90\%$ of tumor cells. These thresholds include the number and percent of samples in each indication that would be considered positive under these criteria. They are included for comparison to the determined cut-off for ROR2 in the CLIA sensitivity screen.

[0499] The cancer tissues in the CLIA sensitivity screen were also analyzed for ROR2 plasma membrane tumor cell expression using H-Scores as described in the Plasma Membrane Scoring of ROR2 in Tumor (Tumor Membrane P Score) section above.

[0500] Table 17 presents the number and percent of cases in each cancer indication that met the following H-Score cut-offs for ROR2 plasma membrane tumor staining: ≥ 1 , ≥ 50 , ≥ 100 , ≥ 150 , ≥ 200 , ≥ 250 . Table 16 also includes average H-Scores across the samples tested in each indication. The average H-Scores for ROR2 reactivity were also compared in the bar graph shown in FIG. 24.

[0501] According to H-Score analysis for ROR2 plasma membrane tumor staining, average H-Scores were low (<35 on a 0-300 scale) for each cancer indication tested except Sarcoma. The average H-Scores for each indication were as follows: Sarcoma 129.0, Ovarian Cancer 21.3, NSCLC 11.8, and TNBC 32.6. H-Score calculations were presented in this report to aid in comparative evaluation of samples and indications in the CLIA sensitivity screen.

[0502] Table 16–ROR2 Reactivity Grouped by Thresholds of $\geq 1+$ Staining

Breakdown of ROR2 (H-1) Plasma Membrane Tumor Reactivity Data at Intensities $\geq 1+$								
Row	Tumor Type	Evaluable Cases	$\geq 1+$ Staining in $\geq 1\%$ of Tumor Cells		$\geq 1+$ Staining in $\geq 10\%$ of Tumor Cells		$\geq 1+$ Staining in $\geq 25\%$ of Tumor Cells	
			No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication
1	Sarcoma	30	24	80%	24	80%	24	80%
2	Ovarian Cancer	31	11	35%	7	23%	4	13%
3	NSCLC	38	6	16%	4	11%	3	8%
4	TNBC	38	12	32%	11	29%	7	18%

Row	$\geq 1+$ Staining in $\geq 50\%$ of Tumor Cells		$\geq 1+$ Staining in $\geq 75\%$ of Tumor Cells		$\geq 1+$ Staining in $\geq 90\%$ of Tumor Cells	
	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication
1	20	67%	10	33%	7	23%
2	4	13%	1	3%	1	3%
3	2	5%	1	3%	1	3%
4	6	16%	2	5%	2	5%

Table 17 –ROR2 Plasma Membrane Reactivity Grouped by H-Score Thresholds
Breakdown of ROR2 (H-1) Plasma Membrane Tumor Reactivity Data by H-Scores

Row	Tumor Type	Evaluable Cases	H-Score ≥ 1		H-Score ≥ 50		H-Score ≥ 100	
			No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication
1	Sarcoma	30	24	80%	23	77%	20	67%
2	Ovarian Cancer	31	11	35%	5	16%	4	13%
3	NSCLC	38	6	16%	2	5%	2	5%
4	TNBC	38	12	32%	7	18%	6	16%

Row	H-Score ≥ 150		H-Score ≥ 200		H-Score ≥ 250		Average H-Score
	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	No. of Cases	Pct. of Indication	
1	12	40%	9	30%	3	10%	129.0
2	1	3%	0	0%	0	0%	21.3
3	2	5%	1	3%	0	0%	11.8
4	5	13%	1	3%	0	0%	32.6

Specificity Testing of ROR2 in Normal Tissues (Part C)

[0503] The specificity of ROR2 to its targets was tested using the IHC methods as described in Tables 6 and 7. Specificity testing was performed using 96 different tissues from an FDA-recommended multi-normal human tissue microarray (TMA). The TMA (P1497Q0001) was purchased from Pantomics, Inc (Cat # MNO961, multi-normal human tissues, FDA, 96 samples, 35 organs/sites from 3 individuals, 1.5mm) as fully described in Table 8. Sections of all normal tissue samples were histology-stained with hematoxylin and eosin (H&E) and IHC-stained with ROR2 and Mouse IgG1.

[0504] All stained normal tissues were assessed using the H-Score method for ROR2 plasma membrane staining described in the Tumor Membrane P Score section above to evaluate normal tissue components (as opposed to tumor). Scoring data for this specificity test also included ROR2 positive/negative status based on a cut-off of $\geq 1+$ intensity staining in $\geq 10\%$ of normal cells. Specificity data for ROR2 in the normal tissue is presented in Table 18.

[0505] ROR2 showed nonreactive plasma membrane staining (100% at 0) in all normal human tissues tested; except for reactivity in reticulated crypt epithelium of tonsil (FIG. 25F) (previously observed with ROR2 clone H-1) and occasional epithelial staining in thyroid (FIG. 25E) and uterus (which may be related to the increased ROR2 expression previously observed in fetal tissue. As such, this specificity testing suggested that the ROR2 H-1 antibody and IHC test was highly specific to targets in tumor cells.

[0506] FIG. 25 shows a panel of representative images with negative ROR2 expression in normal tissues of human kidney (FIG. 25A), liver (FIG. 25B), small intestine (FIG. 25C), and

brain (FIG. 25D). The mouse IgG1 isotype-match negative control was run on the normal TMA tissue panel and was nonreactive in all samples.

[0507] Table 18 – Specificity Testing for ROR2 in Normal Tissues

Row	Slide No.	TMA	Sample Q#	Core ID	Sex	Age	Anatomic Site
1	375	FDA Normal TMA	P1497Q0001	A01	M	45	Adrenal gland
2	375	<i>FDA Normal TMA</i>	<i>P1497Q0001</i>	<i>A02</i>	<i>F</i>	37	<i>Adrenal gland</i>
3	375	FDA Normal TMA	P1497Q0001	A03	F	61	Adrenal gland
4	375	FDA Normal TMA	P1497Q0001	A04	F	51	Bladder, urinary
5	375	FDA Normal TMA	P1497Q0001	A05	M	36	Bladder, urinary
6	375	FDA Normal TMA	P1497Q0001	A06	M	61	Bladder, urinary
7	375	FDA Normal TMA	P1497Q0001	A07	M	16	Bone, bone marrow
8	375	FDA Normal TMA	P1497Q0001	A04	F	51	Bladder, urinary
9	375	<i>FDA Normal TMA</i>	<i>P1497Q0001</i>	<i>A09</i>	<i>F</i>	70	<i>Eye</i>
10	375	FDA Normal TMA	P1497Q0001	A10	F	34	Breast
11	375	<i>FDA Normal TMA</i>	<i>P1497Q0001</i>	<i>A11</i>	<i>F</i>	43	<i>Breast</i>
12	375	FDA Normal TMA	P1497Q0001	A12	F	45	Breast
13	375	FDA Normal TMA	P1497Q0001	B01	M	46	Brain, cerebellum
14	375	FDA Normal TMA	P1497Q0001	B02	M	49	Brain, cerebellum
15	375	FDA Normal TMA	P1497Q0001	B03	M	3	Brain, cerebellum
16	375	FDA Normal TMA	P1497Q0001	B04	M	49	Brain, cerebral cortex
17	375	FDA Normal TMA	P1497Q0001	B05	F	5	Brain, cerebral cortex
18	375	FDA Normal TMA	P1497Q0001	B06	M	46	Brain, cerebral cortex
19	375	FDA Normal TMA	P1497Q0001	B07	F	45	Fallopian tube
20	375	FDA Normal TMA	P1497Q0001	B08	F	23	Fallopian tube
21	375	<i>FDA Normal TMA</i>	<i>P1497Q0001</i>	<i>B09</i>	<i>F</i>	48	<i>Fallopian tube</i>
22	375	FDA Normal TMA	P1497Q0001	B10	M	62	Esophagus
23	375	FDA Normal TMA	P1497Q0001	B11	M	55	Esophagus
24	375	FDA Normal TMA	P1497Q0001	B12	F	61	Esophagus
25	375	FDA Normal TMA	P1497Q0001	C01	M	59	Stomach
26	375	FDA Normal TMA	P1497Q0001	C02	F	50	Stomach
27	375	FDA Normal TMA	P1497Q0001	C03	F	48	Stomach
28	375	FDA Normal TMA	P1497Q0001	C04	M	80	Intestine, small intestine
29	375	FDA Normal TMA	P1497Q0001	C05	M	17	Intestine, small intestine
30	375	FDA Normal TMA	P1497Q0001	C06	F	76	Intestine, small intestine
31	375	FDA Normal TMA	P1497Q0001	C07	F	69	Intestine, colon
32	375	FDA Normal TMA	P1497Q0001	C08	F	27	Intestine, colon
33	375	FDA Normal TMA	P1497Q0001	C09	M	34	Intestine, colon
34	375	FDA Normal TMA	P1497Q0001	C10	M	53	Intestine, rectum
35	375	FDA Normal TMA	P1497Q0001	C11	M	68	Intestine, rectum

Table 18 (continued)– Specificity Testing for ROR2 in Normal Tissues

Sample Information		ROR2 Plasma Membrane Staining					
Row	Pathology	Differential Intensities				% Score ≥1+	*POS/NEG
		0	1+	2+	3+		
1	Normal, hyperplasia	100	0	0	0	0	NEG
2	<i>Normal</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>
3	Normal	100	0	0	0	0	NEG
4	Normal	100	0	0	0	0	NEG
5	Normal	100	0	0	0	0	NEG
6	Normal	100	0	0	0	0	NEG
7	Normal	100	0	0	0	0	NEG
8	Normal	100	0	0	0	0	NEG
9	<i>Normal</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>
10	Normal breast	100	0	0	0	0	NEG
11	<i>Normal</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>
12	Normal	100	0	0	0	0	NEG
13	Normal	100	0	0	0	0	NEG
14	Normal	100	0	0	0	0	NEG
15	Normal	100	0	0	0	0	NEG
16	Normal	100	0	0	0	0	NEG
17	Normal	100	0	0	0	0	NEG
18	Normal	100	0	0	0	0	NEG
19	Normal	100	0	0	0	0	NEG
20	Normal	100	0	0	0	0	NEG
21	<i>Normal</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>	<i>N/A</i>
22	Normal	100	0	0	0	0	NEG
23	Normal	100	0	0	0	0	NEG
24	Normal	100	0	0	0	0	NEG
25	Normal	100	0	0	0	0	NEG
26	Normal	100	0	0	0	0	NEG
27	Normal	100	0	0	0	0	NEG
28	Normal	100	0	0	0	0	NEG
29	Normal	100	0	0	0	0	NEG
30	Normal	100	0	0	0	0	NEG
31	Normal colon	100	0	0	0	0	NEG
32	Normal	100	0	0	0	0	NEG
33	Normal	100	0	0	0	0	NEG
34	Normal	100	0	0	0	0	NEG
35	Normal	100	0	0	0	0	NEG

* ROR2 Cut-Off: ≥1+ intensity in ≥10% of cells = Positive (POS)

Table 18 (continued) – Specificity Testing for ROR2 in Normal Tissues

Row	Slide No.	TMA	Sample Q#	Core ID	Sex	Age	Anatomic Site
36	375	FDA Normal TMA	P1497Q0001	C12	M	70	Intestine, rectum
37	375	<i>FDA Normal TMA</i>	<i>P1497Q0001</i>	<i>D01</i>	<i>M</i>	<i>46</i>	<i>Heart</i>
38	375	FDA Normal TMA	P1497Q0001	D02	F	5	Heart
39	375	FDA Normal TMA	P1497Q0001	D03	M	65	Heart
40	375	FDA Normal TMA	P1497Q0001	D04	F	51	Kidney
41	375	FDA Normal TMA	P1497Q0001	D05	M	39	Kidney
42	375	FDA Normal TMA	P1497Q0001	D06	M	30	Kidney
43	375	FDA Normal TMA	P1497Q0001	D07	F	51	Kidney
44	375	FDA Normal TMA	P1497Q0001	D08	M	39	Kidney
45	375	FDA Normal TMA	P1497Q0001	D09	M	30	Kidney
46	375	FDA Normal TMA	P1497Q0001	D10	M	41	Liver
47	375	FDA Normal TMA	P1497Q0001	D11	M	42	Liver
48	375	FDA Normal TMA	P1497Q0001	D12	M	35	Liver
49	375	<i>FDA Normal TMA</i>	<i>P1497Q0001</i>	<i>E01</i>	<i>M</i>	<i>19</i>	<i>Lung</i>
50	375	FDA Normal TMA	P1497Q0001	E02	M	59	Lung
51	375	FDA Normal TMA	P1497Q0001	E03	M	72	Lung
52	375	<i>FDA Normal TMA</i>	<i>P1497Q0001</i>	<i>E04</i>	<i>F</i>	<i>53</i>	<i>Ovary</i>
53	375	FDA Normal TMA	P1497Q0001	E05	F	45	Ovary
54	375	FDA Normal TMA	P1497Q0001	E06	F	35	Ovary
55	375	FDA Normal TMA	P1497Q0001	E07	F	5	Pancreas
56	375	FDA Normal TMA	P1497Q0001	E08	M	1	Pancreas
57	375	FDA Normal TMA	P1497Q0001	E09	F	58	Pancreas
58	375	FDA Normal TMA	P1497Q0001	E10	F	62	Parathyroid
59	375	FDA Normal TMA	P1497Q0001	E11	F	5	Pituitary gland
60	375	FDA Normal TMA	P1497Q0001	E12	M	46	Pituitary gland
61	375	FDA Normal TMA	P1497Q0001	F01	F	23	Placenta
62	375	FDA Normal TMA	P1497Q0001	F02	F	27	Placenta
63	375	FDA Normal TMA	P1497Q0001	F03	F	23	Placenta
64	375	FDA Normal TMA	P1497Q0001	F04	M	68	Prostate
65	375	FDA Normal TMA	P1497Q0001	F05	M	82	Prostate
66	375	FDA Normal TMA	P1497Q0001	F06	M	64	Prostate
67	375	FDA Normal TMA	P1497Q0001	F07	F	47	Skin
68	375	<i>FDA Normal TMA</i>	<i>P1497Q0001</i>	<i>F08</i>	<i>M</i>	<i>50</i>	<i>Skin</i>
69	375	<i>FDA Normal TMA</i>	<i>P1497Q0001</i>	<i>F09</i>	<i>M</i>	<i>46</i>	<i>Spinal cord</i>
70	375	FDA Normal TMA	P1497Q0001	F10	M	65	Spinal cord
71	375	<i>FDA Normal TMA</i>	<i>P1497Q0001</i>	<i>F11</i>	<i>F</i>	<i>21</i>	<i>Spleen</i>
72	375	FDA Normal TMA	P1497Q0001	F12	M	55	Spleen
73	375	FDA Normal TMA	P1497Q0001	G01	M	44	Skeletal muscle

Table 18 (continued) – Specificity Testing for ROR2 in Normal Tissues

Row	Slide No.	TMA	Sample Q#	Core ID	Sex	Age	Anatomic Site
74	375	FDA Normal TMA	P1497Q0001	G02	M	46	Skeletal muscle
75	375	FDA Normal TMA	P1497Q0001	G03	M	59	Skeletal muscle
76	375	FDA Normal TMA	P1497Q0001	G04	M	77	Testis
77	375	FDA Normal TMA	P1497Q0001	G05	M	34	Testis
78	375	FDA Normal TMA	P1497Q0001	G06	M	30	Testis
79	375	FDA Normal TMA	P1497Q0001	G07	M	13	Thymus
80	375	FDA Normal TMA	P1497Q0001	G08	F	2.5	Thymus
81	375	FDA Normal TMA	P1497Q0001	G09	F	15	Thymus
82	375	FDA Normal TMA	P1497Q0001	G10	F	64	Thyroid
83	375	FDA Normal TMA	P1497Q0001	G11	F	52	Thyroid
84	375	FDA Normal TMA	P1497Q0001	G12	F	39	Thyroid
85	375	FDA Normal TMA	P1497Q0001	H01	F	12	Tonsil
86	375	FDA Normal TMA	P1497Q0001	H02	M	6	Tonsil
87	375	FDA Normal TMA	P1497Q0001	H03	M	20	Tonsil
88	375	FDA Normal TMA	P1497Q0001	H04	F	51	Ureter
89	375	FDA Normal TMA	P1497Q0001	H05	M	56	Ureter
90	375	FDA Normal TMA	P1497Q0001	H06	F	56	Ureter
91	375	FDA Normal TMA	P1497Q0001	H07	F	48	Uterus, cervix
92	375	FDA Normal TMA	P1497Q0001	H08	F	48	Uterus, cervix
93	375	FDA Normal TMA	P1497Q0001	H09	F	51	Uterus, cervix
94	375	FDA Normal TMA	P1497Q0001	H10	F	40	Uterus, endometrium
95	375	FDA Normal TMA	P1497Q0001	H11	F	50	Uterus, endometrium
96	375	FDA Normal TMA	P1497Q0001	H12	F	49	Uterus, endometrium

* ROR2 Cut-Off: $\geq 1+$ intensity in $\geq 10\%$ of cells = Positive (POS)

Table 18 (continued) – Specificity Testing for ROR2 in Normal Tissues

Sample Information		ROR2 Plasma Membrane Staining					
Row	Pathology	Differential Intensities				% Score ≥1+	*POS/NEG
		0	1+	2+	3+		
36	Normal	100	0	0	0	0	NEG
37	Normal	N/A	N/A	N/A	N/A	N/A	N/A
38	Normal	100	0	0	0	0	NEG
39	Normal	100	0	0	0	0	NEG
40	Normal	100	0	0	0	0	NEG
41	Normal	100	0	0	0	0	NEG
42	Normal	100	0	0	0	0	NEG
43	Normal kidney cortex	100	0	0	0	0	NEG
44	Normal	100	0	0	0	0	NEG
45	Normal	100	0	0	0	0	NEG
46	Normal	100	0	0	0	0	NEG
47	Normal & cell swelling	100	0	0	0	0	NEG
48	Normal	100	0	0	0	0	NEG
49	Normal	N/A	N/A	N/A	N/A	N/A	N/A
50	Normal	100	0	0	0	0	NEG
51	Normal	100	0	0	0	0	NEG
52	Normal	N/A	N/A	N/A	N/A	N/A	N/A
53	Normal	100	0	0	0	0	NEG
54	Normal	100	0	0	0	0	NEG
55	Normal	100	0	0	0	0	NEG
56	Normal	100	0	0	0	0	NEG
57	Normal	100	0	0	0	0	NEG
58	Adenoma	100	0	0	0	0	NEG
59	Normal	100	0	0	0	0	NEG
60	Normal	100	0	0	0	0	NEG
61	Normal	100	0	0	0	0	NEG
62	Normal	100	0	0	0	0	NEG
63	Normal	100	0	0	0	0	NEG
64	Normal, hyperplasia	100	0	0	0	0	NEG
65	Normal	100	0	0	0	0	NEG
66	Normal, hyperplasia	100	0	0	0	0	NEG
67	Normal	100	0	0	0	0	NEG
68	Normal	N/A	N/A	N/A	N/A	N/A	N/A
69	Normal	N/A	N/A	N/A	N/A	N/A	N/A
70	Normal	100	0	0	0	0	NEG
71	Normal	N/A	N/A	N/A	N/A	N/A	N/A
72	Normal	100	0	0	0	0	NEG
73	Normal	100	0	0	0	0	NEG

* ROR2 Cut-Off: ≥1+ intensity in ≥10% of cells = Positive (POS)

Table 18 (continued) – Specificity Testing for ROR2 in Normal Tissues

Sample Information		ROR2 Plasma Membrane Staining					
Row	Pathology	Differential Intensities				% Score ≥1+	*POS/NEG
		0	1+	2+	3+		
74	Normal	100	0	0	0	0	NEG
75	Normal	100	0	0	0	0	NEG
76	Normal	100	0	0	0	0	NEG
77	Normal	100	0	0	0	0	NEG
78	Normal	100	0	0	0	0	NEG
79	Normal, hyperplasia	100	0	0	0	0	NEG
80	Normal	100	0	0	0	0	NEG
81	Normal	100	0	0	0	0	NEG
82	Normal	100	0	0	0	0	NEG
83	Normal	90	5	5	0	10	POS
84	Normal	95	5	0	0	5	NEG
85	Tonsillitis	100	0	0	0	0	NEG
86	Tonsillitis	100	0	0	0	0	NEG
87	Tonsillitis	90	5	5	0	10	POS
88	Normal	100	0	0	0	0	NEG
89	Normal	100	0	0	0	0	NEG
90	Normal	100	0	0	0	0	NEG
91	Normal	100	0	0	0	0	NEG
92	Normal	100	0	0	0	0	NEG
93	Normal	100	0	0	0	0	NEG
94	Normal	100	0	0	0	0	NEG
95	Normal	N/A	N/A	N/A	N/A	N/A	N/A
96	Normal	0	20	30	50	100	POS

* ROR2 Cut-Off: ≥1+ intensity in ≥10% of cells = Positive (POS)

ROR2 Precision and Reproducibility Testing (Part D)

[0508] The results from the sensitivity screen helped identify appropriate tissues for testing the precision and reproducibility of the ROR2 IHC assay in the Sarcoma, Ovarian Cancer, Non-Small Cell Lung cancer (NSCLC), and Triple Negative Breast Cancer (TNBC) indications. For the ROR2 assay validation, 4 tumor samples per indication that showed a range of ROR2 plasma membrane staining in tumor cells were selected for use.

[0509] Each sample for each indication was run in triplicate for ROR2 according to the IHC assays in Table 6 and the protocol described above in a single run (precision). In two separate runs, performed on non-consecutive days, the same samples were run in triplicate

with ROR2 by both the same and a different operator (reproducibility). All replicate slides were prepared as serial sections (one section of tissue per slide with minimal loss of material between slide preparations).

[0510] Intra-assay (precision) and inter-assay (reproducibility) was determined using a 3-run series with 3 replicate sections (per run) of each of the 4 selected tumor samples for ROR2, resulting in a set of 9 replicates for each sample. Two operators ran the assays using different TechMate instruments (Operator 1, Run 1; Operator 1, Run 2; Operator 2, Run 3). Positive, standard, and negative controls included in each run reacted as expected.

[0511] All replicates stained during validation were reviewed and scored. ROR2 plasma membrane staining in tumor tissue was evaluated using Percent Scores $\geq 1+$. For the validation test, the cut-off as described hereinabove, was used to determine ROR2 positivity. A sample with 10% or more tumor cells staining at $\geq 1+$ intensity (Percent Score $\geq 1+$ that is ≥ 10) was called positive (POS). A sample was negative (NEG) if staining was present at $\geq 1+$ in 0-9% of tumor cells or if only $<1+$ staining was observed. For the precision and reproducibility test, a sample with 10% or more tumor cells staining at $\geq 1+$ intensity (Percent Score $\geq 1+$ that is ≥ 10) was called positive (POS). A sample was negative (NEG) if staining was present at $\geq 1+$ in 0-9% of tumor cells or if only $<1+$ staining was observed.

[0512] Overall, the samples for precision and reproducibility testing were comparable to the sections stained with ROR2 during sensitivity screening. Some scoring differences between these tests were observed (both higher and lower during sensitivity) but they were attributable to changes in tissue section depth and not to variation in the ROR2 assay. Each sample for ROR2 precision and reproducibility was deemed acceptable by the pathologist as compared to the sensitivity test. Full validation scoring results, including statistical analyses for the replicates, are shown in Table 19 for Sarcoma, Table 20 for Ovarian Cancer, Table 21 for NSCLC, and Table 22 for TNBC.

[0513] Similar cellular patterns of ROR2 reactivity were observed in all replicates in the pattern, percent, and intensity of tumor staining. This consistency is represented for ROR2 in FIGS. 26 and 27 for Sarcoma, FIGS. 28 and 29 for Ovarian Cancer, FIGS. 30 and 31 for NSCLC, and FIGS. 32 and 33 for TNBC.

[0514] Any minor or graded changes observed in ROR2 plasma membrane staining between replicates were attributable to slight changes in the amount of tumor in each serial section. Images of corresponding Mouse IgG1 negative controls are included in the bottom row of these figures.

Statistical Analysis and Confidence Interval Assessment

[0515] Acceptance/rejection of IHC assay validation was determined through evaluation of consistency in staining patterns, statistical analysis of semi-quantitative scores, and the percent of agreement/concordant estimates. Acceptance of the precision and reproducibility testing requires that the lower bounds of the selected 95% confidence interval (CI), computed by percent agreement, met or exceeded 85%. The general criteria also stated that the standard error of the mean (SEM) among replicates did not exceed 5, and the coefficient of variation (CV) did not exceed 20% (for samples with Percent Score values ≥ 10).

[0516] Summarized validation scoring results for ROR2 Percent Scores $\geq 1+$ percentage scores in tumor; including the mean, standard deviation (Std Dev), standard error of the mean (SEM), and coefficient of variation (CV) for each replicate set are presented in Table 23. A positivity cut-off for ROR2 of $\geq 1+$ intensity staining in $\geq 10\%$ of tumor cells was applied to the evaluation of these samples for precision and reproducibility analysis.

[0517] The SEM for each set of 9 replicates from the 4 cases tested for each biomarker did not exceed 1.7 and the CV did not exceed 16% (for cases with a Percent Score $\geq 1+ > 5$). The ROR2 Percent Score for TNBC sample Q9250 ranged from 2 to 5 among the replicates tested. This variation was caused by a reduction in the cluster of reactive cells as the FFPE tissue block was serially sectioned. It resulted in a CV value of 47% but with a very low SEM of 0.4 and a Standard Deviation of only 1.3.

[0518] When Percent Scores are low, differences between replicates translate into higher CV values by nature of the test. In cases where Percent Scores are < 10 , the sample can be considered acceptable. Sample Q9250 was deemed acceptable, and all other samples were within the defined limits.

[0519] Confidence interval assessment for ROR2 is shown in Table 24 and includes positive/negative staining agreement and mean, standard deviation, standard error of the mean (SEM), and pre-defined Z-value for the 95% confidence interval (CI). For this evaluation, the reference point used to calculate the CI was based on the staining result (positive or negative) for the majority of the ROR2 replicates. For example, 9/9 replicates for Sarcoma sample QMTB313-04, were positive for ROR2. If a QMTB313-04 replicate had been negative, it would have been against the majority and would have lowered the CI. However, no discordant results were found.

[0520] In total, pairwise comparisons from the ROR2 precision and reproducibility study resulted in 36 concordant and 0 discordant outcomes in Sarcoma, Ovarian Cancer, NSCLC,

and TNBC (Table 24). As such, 144/144 tests for ROR2 agreed with the appropriate majority.

Acceptance criteria were met for the ROR2 IHC assay with a 95%CI.

[0521] The ROR2 IHC assays that were used for detection in human Sarcoma, Ovarian Cancer, NSCLC, and TNBC indications are considered successfully validated for use in clinical testing.

Table 19 – Precision and Reproducibility of ROR2 in Sarcoma

Row	Run	Operator	Sample Information			
			Tissue Type	Q/MTB	Replicate	Slide No.
1	1	1	Sarcoma	QMTB313-04	1	461
					2	462
					3	463
2	2	1	Sarcoma	QMTB313-04	1	478
					2	479
					3	480
3	3	2	Sarcoma	QMTB313-04	1	534
					2	535
					3	536
4	1	1	Sarcoma	QMTB313-07	1	461
					2	462
					3	463
5	2	1	Sarcoma	QMTB313-07	1	478
					2	479
					3	480
6	3	2	Sarcoma	QMTB313-07	1	534
					2	535
					3	536
7	1	1	Sarcoma	Q9403	1	464
					2	465
					3	466
8	2	1	Sarcoma	Q9403	1	481
					2	482
					3	483
9	3	2	Sarcoma	Q9403	1	537
					2	538
					3	539
10	1	1	Sarcoma	Q9456	1	411
					2	412
					3	413
11	2	1	Sarcoma	Q9456	1	484
					2	485
					3	486
12	3	2	Sarcoma	Q9456	1	540
					2	541
					3	542

Table 19 (continued)– Precision and Reproducibility of ROR2 in Sarcoma

Row	ROR2 Plasma Membrane Tumor Staining						ROR2 Statistical Analysis			
	0	1+	2+	3+	% Score ≥1+	*POS/NEG	% Score Mean	% Score Std Dev	% Score SEM	% Score CV (%)
1	10	20	20	50	90	POS	90.0	0.0	0.0	0%
	10	20	20	50	90	POS				
	10	20	20	50	90	POS				
2	10	20	20	50	90	POS				
	10	20	20	50	90	POS				
	10	20	20	50	90	POS				
3	10	30	20	40	90	POS				
	10	30	20	40	90	POS				
	10	20	20	50	90	POS				
4	30	0	20	50	70	POS	70.0	0.0	0.0	0%
	30	0	20	50	70	POS				
	30	0	20	50	70	POS				
5	30	0	20	50	70	POS				
	30	0	20	50	70	POS				
	30	0	20	50	70	POS				
6	30	0	20	50	70	POS				
	30	0	20	50	70	POS				
	30	0	20	50	70	POS				
7	0	0	0	100	100	POS	100.0	0.0	0.0	0%
	0	0	0	100	100	POS				
	0	0	0	100	100	POS				
8	0	0	0	100	100	POS				
	0	0	0	100	100	POS				
	0	0	0	100	100	POS				
9	0	0	0	100	100	POS				
	0	0	0	100	100	POS				
	0	0	0	100	100	POS				
10	100	0	0	0	0	NEG	0.0	0.0	0.0	0%
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
11	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
12	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				

[0522] Table 20 – Precision and Reproducibility of ROR2 in Ovarian Cancer

Row	Run	Operator	Sample Information			
			Tissue Type	Q/MTB	Replicate	Slide No.
1	1	1	Ovarian Cancer	QMTB400-01	1	414
					2	415
					3	467
2	2	1	Ovarian Cancer	QMTB400-01	1	487
					2	488
					3	489
3	3	2	Ovarian Cancer	QMTB400-01	1	543
					2	544
					3	545
4	1	1	Ovarian Cancer	QMTB400-05	1	414
					2	415
					3	467
5	2	1	Ovarian Cancer	QMTB400-05	1	487
					2	488
					3	489
6	3	2	Ovarian Cancer	QMTB400-05	1	543
					2	544
					3	545
7	1	1	Ovarian Cancer	Q5488-02	1	417
					2	418
					3	419
8	2	1	Ovarian Cancer	Q5488-02	1	490
					2	491
					3	492
9	3	2	Ovarian Cancer	Q5488-02	1	546
					2	547
					3	548
10	1	1	Ovarian Cancer	Q7499-02	1	421
					2	422
					3	472
11	2	1	Ovarian Cancer	Q7499-02	1	493
					2	494
					3	495
12	3	2	Ovarian Cancer	Q7499-02	1	549
					2	550
					3	551

Table 20 (continued) – Precision and Reproducibility of ROR2 in Ovarian Cancer

Row	ROR2 Plasma Membrane Tumor Staining						ROR2 Statistical Analysis			
	0	1+	2+	3+	% Score ≥1+	*POS/NEG	% Score Mean	% Score Std Dev	% Score SEM	% Score CV (%)
1	100	0	0	0	0	NEG	0.0	0.0	0.0	0%
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
2	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
3	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
4	90	0	0	10	10	POS	10.4	0.9	0.3	8%
	90	0	0	10	10	POS				
	90	0	0	10	10	POS				
5	90	0	0	10	10	POS				
	88	0	2	10	12	POS				
	88	0	2	10	12	POS				
6	90	0	0	10	10	POS				
	90	0	0	10	10	POS				
	90	0	0	10	10	POS				
7	20	20	20	40	80	POS	76.7	5.0	1.7	7%
	20	20	20	40	80	POS				
	20	20	20	40	80	POS				
8	20	20	20	40	80	POS				
	20	20	20	40	80	POS				
	20	20	20	40	80	POS				
9	30	20	10	40	70	POS				
	30	20	10	40	70	POS				
	30	20	10	40	70	POS				
10	20	30	20	30	80	POS	80.0	0.0	0.0	0%
	20	20	30	30	80	POS				
	20	30	20	30	80	POS				
11	20	30	20	30	80	POS				
	20	30	20	30	80	POS				
	20	30	20	30	80	POS				
12	20	30	20	30	80	POS				
	20	30	20	30	80	POS				
	20	30	20	30	80	POS				

[0523] Table 21 – Precision and Reproducibility of ROR2 in NSCLC

Row	Run	Operator	Sample Information			
			Tissue Type	Q/MTB	Replicate	Slide No.
1	1	1	NSCLC	QMTB397-02	1	423
					2	424
					3	425
2	2	1	NSCLC	QMTB397-02	1	496
					2	497
					3	498
3	3	2	NSCLC	QMTB397-02	1	552
					2	553
					3	554
4	1	1	NSCLC	QMTB397-07	1	423
					2	424
					3	425
5	2	1	NSCLC	QMTB397-07	1	496
					2	497
					3	498
6	3	2	NSCLC	QMTB397-07	1	552
					2	553
					3	554
7	1	1	NSCLC	Q6949-01	1	429
					2	430
					3	431
8	2	1	NSCLC	Q6949-01	1	502
					2	503
					3	504
9	3	2	NSCLC	Q6949-01	1	558
					2	559
					3	560
10	1	1	NSCLC	Q4044-04	1	426
					2	427
					3	428
11	2	1	NSCLC	Q4044-04	1	499
					2	500
					3	501
12	3	2	NSCLC	Q4044-04	1	555
					2	556
					3	557

Table 21 (continued) – Precision and Reproducibility of ROR2 in NSCLC

Row	ROR2 Plasma Membrane Tumor Staining						ROR2 Statistical Analysis			
	0	1+	2+	3+	% Score ≥1+	*POS/NEG	% Score Mean	% Score Std Dev	% Score SEM	% Score CV (%)
1	74	20	5	1	26	POS	24.7	3.9	1.3	16%
	74	20	5	1	26	POS				
	74	20	5	1	26	POS				
2	69	25	5	1	31	POS				
	73	20	5	2	27	POS				
	74	15	10	1	26	POS				
3	82	15	2	1	18	POS				
	79	15	5	1	21	POS				
	79	15	5	1	21	POS				
4	100	0	0	0	0	NEG	0.0	0.0	0.0	0%
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
5	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
6	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
7	30	30	20	20	70	POS	71.1	3.3	1.1	5%
	30	30	20	20	70	POS				
	20	30	20	30	80	POS				
8	30	30	20	20	70	POS				
	30	30	20	20	70	POS				
	30	30	20	20	70	POS				
9	30	30	20	20	70	POS				
	30	30	20	20	70	POS				
	30	30	20	20	70	POS				
10	90	5	5	0	10	POS	10.0	0.0	0.0	0%
	90	5	5	0	10	POS				
	90	5	5	0	10	POS				
11	90	5	5	0	10	POS				
	90	5	5	0	10	POS				
	90	5	5	0	10	POS				
12	90	5	5	0	10	POS				
	90	5	5	0	10	POS				
	90	5	5	0	10	POS				

[0524] Table 22 – Precision and Reproducibility of ROR2 in TNBC

Row	Run	Operator	Sample Information			
			Tissue Type	Q/MTB	Replicate	Slide No.
1	1	1	TNBC	Q9333	1	441
					2	442
					3	443
2	2	1	TNBC	Q9333	1	514
					2	515
					3	516
3	3	2	TNBC	Q9333	1	570
					2	571
					3	572
4	1	1	TNBC	Q9289	1	438
					2	439
					3	440
5	2	1	TNBC	Q9289	1	511
					2	512
					3	513
6	3	2	TNBC	Q9289	1	567
					2	568
					3	569
7	1	1	TNBC	Q9250	1	432
					2	433
					3	434
8	2	1	TNBC	Q9250	1	505
					2	506
					3	507
9	3	2	TNBC	Q9250	1	561
					2	562
					3	563
10	1	1	TNBC	Q9254	1	435
					2	436
					3	437
11	2	1	TNBC	Q9254	1	508
					2	509
					3	510
12	3	2	TNBC	Q9254	1	564
					2	565
					3	566

Table 22 (continued) – Precision and Reproducibility of ROR2 in TNBC

Row	ROR2 Plasma Membrane Tumor Staining						ROR2 Statistical Analysis			
	0	1+	2+	3+	% Score ≥1+	*POS/NEG	% Score Mean	% Score Std Dev	% Score SEM	% Score CV (%)
1	10	10	30	50	90	POS	90.0	0.0	0.0	0%
	10	10	30	50	90	POS				
	10	10	30	50	90	POS				
2	10	10	30	50	90	POS				
	10	10	30	50	90	POS				
	10	10	30	50	90	POS				
3	10	10	30	50	90	POS				
	10	10	30	50	90	POS				
	10	10	30	50	90	POS				
4	30	30	10	30	70	POS	70.0	0.0	0.0	0%
	30	30	10	30	70	POS				
	30	30	10	30	70	POS				
5	30	30	10	30	70	POS				
	30	30	10	30	70	POS				
	30	30	10	30	70	POS				
6	30	40	10	20	70	POS				
	30	40	10	20	70	POS				
	30	30	10	30	70	POS				
7	95	3	2	0	5	NEG	2.8	1.3	0.4	47%
	95	3	2	0	5	NEG				
	98	0	2	0	2	NEG				
8	98	0	2	0	2	NEG				
	98	0	2	0	2	NEG				
	97	1	2	0	3	NEG				
9	98	0	2	0	2	NEG				
	98	1	1	0	2	NEG				
	98	0	2	0	2	NEG				
10	100	0	0	0	0	NEG	0.0	0.0	0.0	0%
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
11	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
12	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				
	100	0	0	0	0	NEG				

[0525] Table 23 – Validation Statistical Summary for ROR2 in Cancer Indications

Precision & Reproducibility Statistics Summary					
Tissue Type	Q/MTB	ROR2			
		% Score Mean	% Score Std Dev	% Score SEM	% Score CV
Sarcoma	QMTB313-04	90.0	0.0	0.0	0%
Sarcoma	QMTB313-07	70.0	0.0	0.0	0%
Sarcoma	Q9403	100.0	0.0	0.0	0%
Sarcoma	Q9456	0.0	0.0	0.0	0%
Ovarian Cancer	QMTB400-01	0.0	0.0	0.0	0%
Ovarian Cancer	QMTB400-05	10.4	0.9	0.3	8%
Ovarian Cancer	Q5488-02	76.7	5.0	1.7	7%
Ovarian Cancer	Q7499-02	80.0	0.0	0.0	0%
NSCLC	QMTB397-02	24.7	3.9	1.3	16%
NSCLC	QMTB397-07	0.0	0.0	0.0	0%
NSCLC	Q6949-01	71.1	3.3	1.1	5%
NSCLC	Q4044-04	10.0	0.0	0.0	0%
TNBC	Q9333	90.0	0.0	0.0	0%
TNBC	Q9289	70.0	0.0	0.0	0%
TNBC	Q9250	2.8	1.3	0.4	47%
TNBC	Q9254	0.0	0.0	0.0	0%

[0526] Table 24 – Confidence Interval Validation Analysis for ROR2 in Cancer Indications

Precision & Reproducibility Confidence Interval Analysis								
Tissue Type	Q/MTB	ROR2 POS/NEG Agreement	95% Confidence Interval					
			Total N	Mean	Std Dev	SEM	Z-Value	95% CI
Sarcoma	QMTB313-04	9/9	36	100%	0	0	1.96	0
Sarcoma	QMTB313-07	9/9						
Sarcoma	Q9403	9/9						
Sarcoma	Q9456	9/9						
Ovarian Cancer	QMTB400-01	9/9	36	100%	0	0	1.96	0
Ovarian Cancer	QMTB400-05	9/9						
Ovarian Cancer	Q5488-02	9/9						
Ovarian Cancer	Q7499-02	9/9						
NSCLC	QMTB397-02	9/9	36	100%	0	0	1.96	0
NSCLC	QMTB397-07	9/9						
NSCLC	Q6949-01	9/9						
NSCLC	Q4044-04	9/9						
TNBC	Q9333	9/9	36	100%	0	0	1.96	0
TNBC	Q9289	9/9						
TNBC	Q9250	9/9						
TNBC	Q9254	9/9						

[0527] All documents mentioned herein are hereby incorporated by reference in their entirety or alternatively to provide the disclosure for which they were specifically relied upon. The applicant(s) do not intend to dedicate any disclosed embodiments to the public, and to the extent any disclosed modifications or alterations may not literally fall within the scope of the claims, they are considered to be part hereof under the doctrine of equivalents.

Table of Correspondence of SEQ ID NOS and Codes

SEQ ID NO/SEQ ID NOS	CORRESPONDING CODE
SEQ ID NO: 13	48.6-LC-WT in FIG. 3B and Table 1 of specification.
SEQ ID NO: 14	48.6-LC-V029E in FIG. 3B and Table 1 of specification.
combination of VL (SEQ ID NO: 15) and VH (SEQ ID NO: 20)	BAP048.7-humC02D09-CAB (#3947) in FIG. 5, ROR2-CAB1-ADC in FIGS. 7A-7C, and BAP048.7-C02D09 in Table 4 of specification.
combination of VL (SEQ ID NO: 16) and VH (SEQ ID NO: 22)	BAP048.7-humA03F01 (#3948) in FIG. 5, ROR2-CAB2-ADC in FIGS. 7A-7C, and BAP048.7-A03F01 in Table 4 of specification.
SEQ ID NO: 17	48.3-LC-WT in FIG. 3A
SEQ ID NO: 18	48.6-HC-WT in FIG. 2B and Table 2 of specification, and 048.1-2-11-VH of Fig. 2A in of U.S. Provisional Application No. 62/447,218 which is incorporated into the disclosure of the present application.
SEQ ID NO: 19	048.6-2-11-64-VH of Fig. 2A in of U.S. Provisional Application No. 62/447,218 which is incorporated into the disclosure of the present application.

SEQ ID NO/SEQ ID NOS	CORRESPONDING CODE
combination of VL (SEQ ID NO: 16) and VH (SEQ ID NO: 21)	BAP048.7-humA03C05-CAB (#3950) in FIG. 5, ROR2-CAB3-ADC in FIGS. 7A-7C, and BAP048.7-A03C05 in Table 4 of specification, and 048.7-HC03D05-VH in FIG. 2A of U.S. Provisional Application No. 62/447,218 which is incorporated into the disclosure of the present application.
SEQ ID NO: 23	48.3-HC-WT of FIG. 2A of the present specification, and 048.3-1-09-VH in FIG. 2B of U.S. Provisional Application No. 62/447,218 which is incorporated into the disclosure of the present application.
SEQ ID NO: 24	048.3-1-09-VH in FIG. 2B of U.S. Provisional Application No. 62/447,218 which is incorporated into the disclosure of the present application.
SEQ ID NO: 25	BAP048-5-humVH-R098H
SEQ ID NO: 26	BAP048-5-humVH-Y0H33E
SEQ ID NO: 27	BAP-048-5-humVL

SEQ ID NO/SEQ ID NOS	CORRESPONDING CODE
combination of VL (SEQ ID NO: 108) and VH (SEQ ID NO: 75)	BAP048.6-2-11-Chimera-CAB in FIG. 5 (#3949) of the present specification.
combination of VL (SEQ ID NO: 118) and VH (SEQ ID NO: 119)	BAP048.1-06-07 Table 4 and BAP048.7-06-07-HC-FLAG in Example 1 of the present specification.
combination of VL (SEQ ID NO: 16) and VH (SEQ ID NO: 21)	BAP048 CAB Antibody in FIG. 8 or BAP048.7 in Example 3, BAP048 or BAP048 ADC in FIGS. 9 and 10, FIGS. 11A-11C and 12A-12C, BAP048-MMAE conjugates or BAP048.7 in FIGS. 13, 14, 15A-15B of the present specification.

WHAT IS CLAIMED IS:

1. A method of treating cancer comprising a step of administering to a subject in need of such treatment, (i) a dose of from 0.3 mg/kg to 3.3 mg/kg of a weight of the subject every two or three weeks, (ii) a dose of from 0.3 mg/kg to 1.8 mg/kg of the weight of the subject every 2 weeks, or (iii) a dose of 1.8 mg/kg of weight of the subject on days 1 and 8 every 3 weeks, of a polypeptide that specifically binds to ROR2 protein, said polypeptide comprising a heavy chain variable region including three complementarity determining regions, said regions having H1, H2, and H3 sequences, wherein:
 - (a) the H1 sequence is GYTX₁TEX₂X₃X₄H (SEQ ID NO:1) or GYSITTGX₂₉YWN (SEQ ID NO:4);
 - (b) the H2 sequence is X₅X₆X₇X₈NNGGTGYNQKFKG (SEQ ID NO:2) or YITYDGSX₃₀NYNPSLKN (SEQ ID NO:5); and
 - (c) the H3 sequence is X₉X₁₀X₁₁SX₁₂YX₁₃YX₁₄X₁₅SYFX₁₆X₁₇X₁₈ (SEQ ID NO:3) or CSX₃₁X₃₂X₃₃X₃₄VX₃₅X₃₆X₃₇LDX₃₈ (SEQ ID NO:6);

wherein

X₁ is F or E,

X₂ is Y or D,

X₃ is T or C,

X₄ is M or D or E or Y,

X₅ is G or S,

X₆ is I or E,

X₇ is N or C or L or V,

X₈ is T or D or E,

X₉ is A or M or T,

X₁₀ is R or H,

X₁₁ is G or E,

X₁₂ is L or F,

X₁₃ is S or G,

X₁₄ is G or D,

X₁₅ is N or E,

X₁₆ is D or L,

X₁₇ is Y or C or T,

X₁₈ is W or L,

X₂₉ is Y or E or R or T,

X₃₀ is K or N,
 X₃₁ is R or G or H or W or Y,
 X₃₂ is F or C or N or Q,
 X₃₃ is E or S,
 X₃₄ is G or E or F or H or M or Q or S,
 X₃₅ is W or A or I or P or Q or T or V,
 X₃₆ is Y or G or N or Q,
 X₃₇ is G or S or T, and
 X₃₈ is Y or I; and

a light chain variable region including three complementarity determining regions L1, L2, and L3 sequences, wherein:

- (d) the L1 sequence is SATSSX₁₉X₂₀X₂₁MX₂₂ (SEQ ID NO:7) or
 RASESVDRYGNSX₃₉IH (SEQ ID NO:10);
- (e) L2 sequence is X₂₃TSNLAS (SEQ ID NO:8) or X₄₀TYX₄₁LES (SEQ ID NO:11);
 and
- (f) L3 sequence is QX₂₄X₂₅SX₂₆YPFX₂₇X₂₈ (SEQ ID NO:9) or
 QQX₄₂NX₄₃DPX₄₄TX₄₅ (SEQ ID NO:12);

wherein

X₁₉ is V or E,
 X₂₀ is S or D,
 X₂₁ is Y or C or D,
 X₂₂ is H or G or L,
 X₂₃ is G or C or H or P,
 X₂₄ is Q or E,
 X₂₅ is R or H,
 X₂₆ is S or D or G or I or Q or V,
 X₂₇ is T or D,
 X₂₈ is F or D or E,
 X₃₉ is F or S or T,
 X₄₀ is R or C or D or E or W,
 X₄₁ is N or D,
 X₄₂ is T or I or P,
 X₄₃ is E or V,
 X₄₄ is W or T, and

X₄₅ is F or T,

with the proviso that X₁ to X₂₈ cannot simultaneously be F, Y, T, M, G, I, N, T, A, R, G, L, S, G, N, D, Y, W, V, S, Y, H, G, Q, R, S, T, and F, respectively.

2. The method of claim 1, wherein the heavy chain variable region has an amino acid sequence selected from sequences of SEQ ID NOS: 18-26.
3. The method of any one of claims 1-2, wherein the light chain variable region has an amino acid sequence selected from SEQ ID NOS: 13-17 and 27.
4. The method of any one of claims 1-2, wherein the light chain variable region includes three complementarity determining regions L1, L2, and L3 having sequences of SEQ ID NOS: 10-12 respectively.
5. The method of any one of claims 1-4, wherein the X₂₉ is Y.
6. The method of any one of claims 1-4, wherein the X₂₉ is E.
7. The method of any one of claims 1-6, wherein the dose is from 0.3 mg/kg to 1.8 mg/kg of the weight of the subject every 2 weeks.
8. The method of any one of claims 1-6, wherein the subject has non-small cell lung cancer, and the dose is 1.8 mg/kg of the weight of the subject every 2 weeks.
9. The method of any one of claims 1-6, wherein the dose is 1.8 mg/kg of the weight of the subject on days 1 and 8 every 3 weeks.
10. The method of any one of claims 1-9, wherein the polypeptide is an anti-ROR2 antibody or antibody fragment.
11. The method of claim 10, wherein the antibody or antibody fragment has a higher binding affinity to ROR2 protein at a value of a condition in a tumor microenvironment in comparison with a different value of the same condition that occurs in a non-tumor microenvironment.

12. The method of claim 11, wherein the condition is pH.
13. The method of claim 12, wherein the pH in the tumor microenvironment is in a range of from 5.8 to 6.8 and the pH in the non-tumor microenvironment is in a range of 7.0 to 7.6.
14. The method of any one of claims 10-13, wherein the antibody or antibody fragment has a ratio of binding affinity to the ROR2 protein at a value of the condition in the tumor microenvironment to a binding affinity to the ROR2 protein at the different value of the same condition in the non-tumor microenvironment of at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.
15. The method of any one of claims 10-14, wherein the antibody or antibody fragment is a chimeric antibody, a multispecific antibody, or a humanized antibody.
16. The method of any one of claims 1-15, wherein the polypeptide, antibody or antibody fragment is administered to the subject as an immunoconjugate comprising the polypeptide, antibody or antibody fragment conjugated to at least one agent for treatment of cancer or a symptom experienced by said subject.
17. The method of claim 16, wherein the at least one agent is selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.
18. The method of any one of claims 16-17, wherein said immunoconjugate comprises at least two said agents.
19. The method of any one of claims 16-18, wherein the polypeptide, antibody or antibody fragment and the at least one agent are covalently bonded to a linker molecule.
20. The method of any one of claims 16-19, wherein the at least one agent is an antineoplastic agent.

21. The method of any one of claims 16-20, wherein the at least one agent is selected from maytansinoids, auristatins, dolastatins, calicheamicin, pyrrolobenzodiazepines, and anthracyclines.
22. The method of any one of claims 1-21, wherein the polypeptide, antibody, antibody fragment or immunoconjugate is administered as a composition comprising a pharmaceutically acceptable carrier.
23. The method of claim 22, wherein the composition further comprises a tonicity agent.
24. The method of any one of claims 1-22, wherein the subject has a ROR2-expressing cancer or previously had a ROR2-expressing cancer.
25. The method of any one of claims 1-23, wherein the subject has a ROR2-expressing tumor.
26. The method of claim 25, wherein the ROR2 expressing tumor has a tumor membrane P score of at least 1.
27. The method of any one of claims 1-26, wherein the cancer is selected from sarcoma, ovarian cancer, melanoma, non-small cell lung cancer, breast carcinoma, and a head and neck cancer.
28. The method of any one of claims 16-27, wherein the immunoconjugate is administered to the subject and the immunoconjugate comprises BA3021-cleavable linker-MMAE_n, wherein BA3021 is the antibody or antibody fragment having the heavy chain variable region comprising SEQ ID NO. 16; and the light chain variable region comprising SEQ ID NO. 21; and n is an integer of 1 to 4, inclusive.
29. The method of any one of claims 16-28, wherein the immunoconjugate is administered to the subject at a dose of 1.8 mg/kg of the weight of the subject in a single dose once every 2 weeks.

30. The method of any one of claims 16-28, wherein the immunoconjugate is administered to the subject at a dose of 1.8 mg/kg of the weight of the subject on days 1 and 8 of one or more consecutive 21 day periods.

31. The method of any one of claims 16-28, wherein the immunoconjugate is administered to the subject at a dose of 0.3 mg/kg to 3.3 mg/kg of a weight of the subject every two or three weeks.

32. The method of any one of claims 16-28, wherein the immunoconjugate is administered to the subject at a dose of 0.3 mg/kg to 1.8 mg/kg of a weight of the subject every 2 weeks.

33. The method of any one of claims 1-32, wherein the subject is a human.

34. Use of (i) a dose of 0.3 mg/kg to 3.3 mg/kg of a weight of a subject every two or three weeks, (ii) a dose of 0.3 mg/kg to 1.8 mg/kg of the weight of the subject every 2 weeks, or (iii) a dose of 1.8 mg/kg of the weight of the subject on days 1 and 8 every 3 weeks of an immunoconjugate for treatment of cancer, said immunoconjugate comprising a polypeptide, antibody or antibody fragment comprising a heavy chain variable region including three complementarity determining regions, said regions having H1, H2, and H3 sequences, wherein:

(a) the H1 sequence is GYTX₁TEX₂X₃X₄H (SEQ ID NO:1) or GYSITTGX₂₉YWN (SEQ ID NO:4);

(b) the H2 sequence is X₅X₆X₇X₈NNGGTGYNQKFKG (SEQ ID NO:2) or YITYDGSX₃₀NYNPSLKN (SEQ ID NO:5); and

(c) the H3 sequence is X₉X₁₀X₁₁SX₁₂YX₁₃YX₁₄X₁₅SYFX₁₆X₁₇X₁₈ (SEQ ID NO:3) or CSX₃₁X₃₂X₃₃X₃₄VX₃₅X₃₆X₃₇LDX₃₈ (SEQ ID NO:6);

wherein

X₁ is F or E,

X₂ is Y or D,

X₃ is T or C,

X₄ is M or D or E or Y,

X₅ is G or S,

X₆ is I or E,

X₇ is N or C or L or V,
 X₈ is T or D or E,
 X₉ is A or M or T,
 X₁₀ is R or H,
 X₁₁ is G or E,
 X₁₂ is L or F,
 X₁₃ is S or G,
 X₁₄ is G or D,
 X₁₅ is N or E,
 X₁₆ is D or L,
 X₁₇ is Y or C or T,
 X₁₈ is W or L,
 X₂₉ is Y or E or R or T,
 X₃₀ is K or N,
 X₃₁ is R or G or H or W or Y,
 X₃₂ is F or C or N or Q,
 X₃₃ is E or S,
 X₃₄ is G or E or F or H or M or Q or S,
 X₃₅ is W or A or I or P or Q or T or V,
 X₃₆ is Y or G or N or Q,
 X₃₇ is G or S or T, and
 X₃₈ is Y or I; and

a light chain variable region including three complementarity determining regions L1, L2, and L3 sequences, wherein:

- (d) the L1 sequence is SATSSX₁₉X₂₀X₂₁MX₂₂ (SEQ ID NO:7) or RASESVDRYGNSX₃₉IH (SEQ ID NO:10);
- (e) L2 sequence is X₂₃TSNLA (SEQ ID NO:8) or X₄₀TYX₄₁LES (SEQ ID NO:11);
and
- (f) L3 sequence is QX₂₄X₂₅SX₂₆YPFX₂₇X₂₈ (SEQ ID NO:9) or QQX₄₂NX₄₃DPX₄₄TX₄₅ (SEQ ID NO:12);

wherein

X₁₉ is V or E,
 X₂₀ is S or D,
 X₂₁ is Y or C or D,

X₂₂ is H or G or L,
X₂₃ is G or C or H or P,
X₂₄ is Q or E,
X₂₅ is R or H,
X₂₆ is S or D or G or I or Q or V,
X₂₇ is T or D,
X₂₈ is F or D or E,
X₃₉ is F or S or T,
X₄₀ is R or C or D or E or W,
X₄₁ is N or D,
X₄₂ is T or I or P,
X₄₃ is E or V,
X₄₄ is W or T, and
X₄₅ is F or T,

with the proviso that X₁ to X₂₈ cannot simultaneously be F, Y, T, M, G, I, N, T, A, R, G, L, S, G, N, D, Y, W, V, S, Y, H, G, Q, R, S, T, and F, respectively.

35. The use of claim 34, wherein the heavy chain variable region has an amino acid sequence selected from sequences of SEQ ID NOS: 18-26.

36. The use of any one of claims 34-35, wherein the light chain variable region has an amino acid sequence selected from SEQ ID NOS: 13-17 and 27.

37. The use of any one of claims 34-35, wherein the light chain variable region includes three complementarity determining regions L1, L2, and L3 having sequences of SEQ ID NOS: 10-12 respectively.

38. The use of any one of claims 34-36, wherein the X₂₉ is Y.

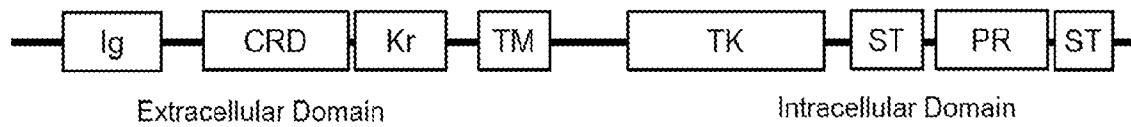
39. The use of any one of claims 34-37, wherein the X₂₉ is E.

40. The use of any one of claims 34-39, wherein the polypeptide is an anti-ROR2 antibody or antibody fragment.

41. The use of claim 40, wherein the antibody or antibody fragment has a higher binding affinity to ROR2 protein at a value of a condition in a tumor microenvironment in comparison with a different value of the same condition that occurs in a non-tumor microenvironment.
42. The use of claim 41, wherein the condition is pH.
43. The use of claim 42, wherein the pH in the tumor microenvironment is in a range of from 5.8 to 6.8 and the pH in the non-tumor microenvironment is in a range of 7.0 to 7.6.
44. The use of any one of claims 41-43, wherein the antibody or antibody fragment has a ratio of binding affinity to the ROR2 protein at a value of the condition in the tumor microenvironment to a binding affinity to the ROR2 protein at the different value of the same condition in the non-tumor microenvironment of at least about 1.5:1, at least about 2:1, at least about 3:1, at least about 4:1, at least about 5:1, at least about 6:1, at least about 7:1, at least about 8:1, at least about 9:1, at least about 10:1, at least about 20:1, at least about 30:1, at least about 50:1, at least about 70:1, or at least about 100:1.
45. The use of any of one of claims 40-44, wherein the antibody or antibody fragment is a chimeric antibody, a multispecific antibody, or a humanized antibody.
46. The use of any one of claims 34-45, wherein the polypeptide, antibody or antibody fragment is administered to the subject as an immunoconjugate comprising the polypeptide, antibody or antibody fragment conjugated to at least one agent for treatment of cancer or a symptom experienced by said subject.
47. The use of claim 46, wherein the at least one agent is selected from a chemotherapeutic agent, a radioactive atom, a cytostatic agent and a cytotoxic agent.
48. The use of any one of claims 46-47, wherein said immunoconjugate comprises at least two said agents.
49. The use of any one of claims 46-48, wherein the polypeptide, antibody or antibody fragment and the at least one agent are covalently bonded to a linker molecule.

50. The use of any one of claims 46-49, wherein the at least one agent is an antineoplastic agent.
51. The use of any one of claims 46-50, wherein the at least one agent is selected from maytansinoids, auristatins, dolastatins, calicheamicin, pyrrolobenzodiazepines, and anthracyclines.
52. The use of any one of claims 34-51, wherein the polypeptide, antibody, antibody fragment or immunoconjugate is administered as a composition comprising a pharmaceutically acceptable carrier.
53. The use of claim 52, wherein the composition further comprises a tonicity agent.
54. The use of any one of claims 34-53 wherein the subject has a ROR2-expressing cancer or previously had a ROR2-expressing cancer.
55. The use of any one of claims 34-53, wherein the subject has a ROR2-expressing tumor.
56. The use of claim 55, wherein the ROR2 expressing tumor has a tumor membrane P score of at least 1.
57. The use of any one of claims 34-56, wherein the cancer is selected from sarcoma, ovarian cancer, melanoma, non-small cell lung cancer, breast carcinoma, and a head and neck cancer.
58. The use of any one of claims 46-57, wherein the immunoconjugate is administered to the subject and the immunoconjugate comprises BA3021-cleavable linker-MMAE_n, wherein BA3021 is the antibody or antibody fragment having the heavy chain variable region comprising SEQ ID NO. 16; and the light chain variable region comprising SEQ ID NO. 21; and n is an integer of 1 to 4, inclusive.
59. The use of any one of claims 46-58, wherein the immunoconjugate is administered to the subject at a dose of 1.8 mg/kg of a weight of the subject in a single dose once every 2 weeks.

60. The use of any one of claims 46-58, wherein the immunoconjugate is administered to the subject at a dose of 0.3 mg/kg to 3.3 mg/kg of a weight of the subject every two or three weeks.
61. The use of any one of claims 46-58, wherein the immunoconjugate is administered to the subject at a dose of 0.3 mg/kg to 1.8 mg/kg of a weight of the subject every 2 weeks.
61. The use of any one of claims 34-58, wherein the subject has non-small cell lung cancer, and the dose is 1.8 mg/kg of the weight of the subject every 2 weeks.
62. The use of any one of claims 34-58, wherein the dose is 1.8 mg/kg on days 1 and 8 every three weeks.
63. The use of any one of claims 42-54, wherein the subject is a human.

**FIG. 1**

SEQ No.	
23	YNP
28	SLK
29	NR
30	IS
31	IT
32	ED
33	TS
34	KN
35	RI
36	SI
37	TD
38	TS
39	KN
40	RI
41	SI
42	TD
43	TS
44	KN
45	RI
46	SI
47	TD
48	TS
49	KN
50	RI
51	SI
52	TD
53	TS
54	KN
55	RI
56	SI
57	TD

Fig. 2A

Fig.	Fig.
2A-2	2A-1

Fig. 2A-1

	10	20	30	40	50
48. 3-LC-WT					
48. 3-LC-F036S	DIVLTQSPD	SLAVSLGRATISCRASESVDRYGNSEFIHWYLQKPGOPP	KLLI	VRYTYNLE	
48. 3-LC-F036T	.	.	S.	.	.
48. 3-LC-R054C	.	.	T.	.	C.
48. 3-LC-R054D	.	.	.	.	D.
48. 3-LC-R054E	.	.	.	E.	E.
48. 3-LC-R054W	.	.	.	W.	W.
48. 3-LC-N057D	.	.	.	.	D.
48. 3-LC-T095D	.	.	.	.	.
48. 3-LC-T095I	.	.	.	.	.
48. 3-LC-T095P	.	.	.	.	.
48. 3-LC-E097V	.	.	.	.	.
48. 3-LC-W100T	.	.	.	.	.
48. 3-LC-F102S	.	.	.	.	.

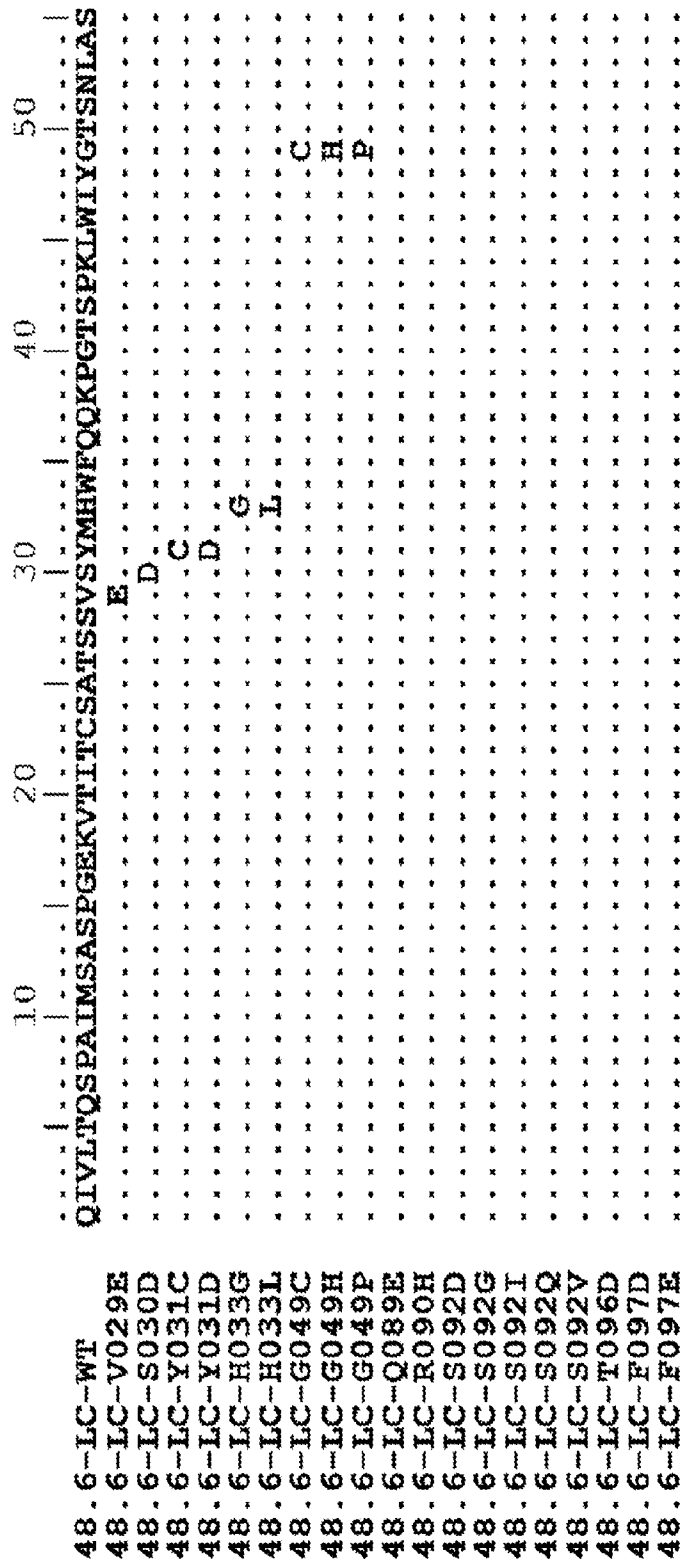


Fig. 3B-2

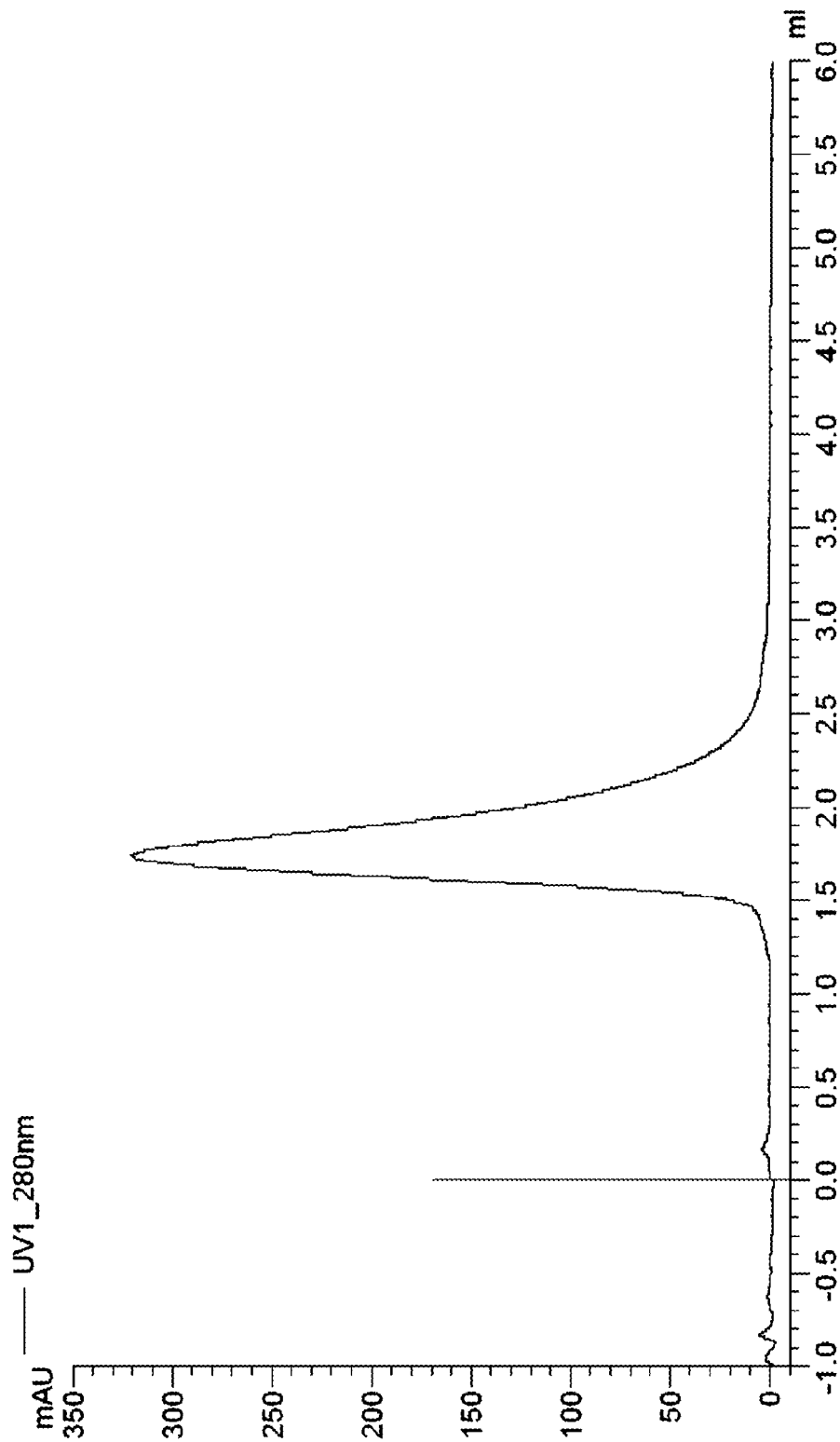
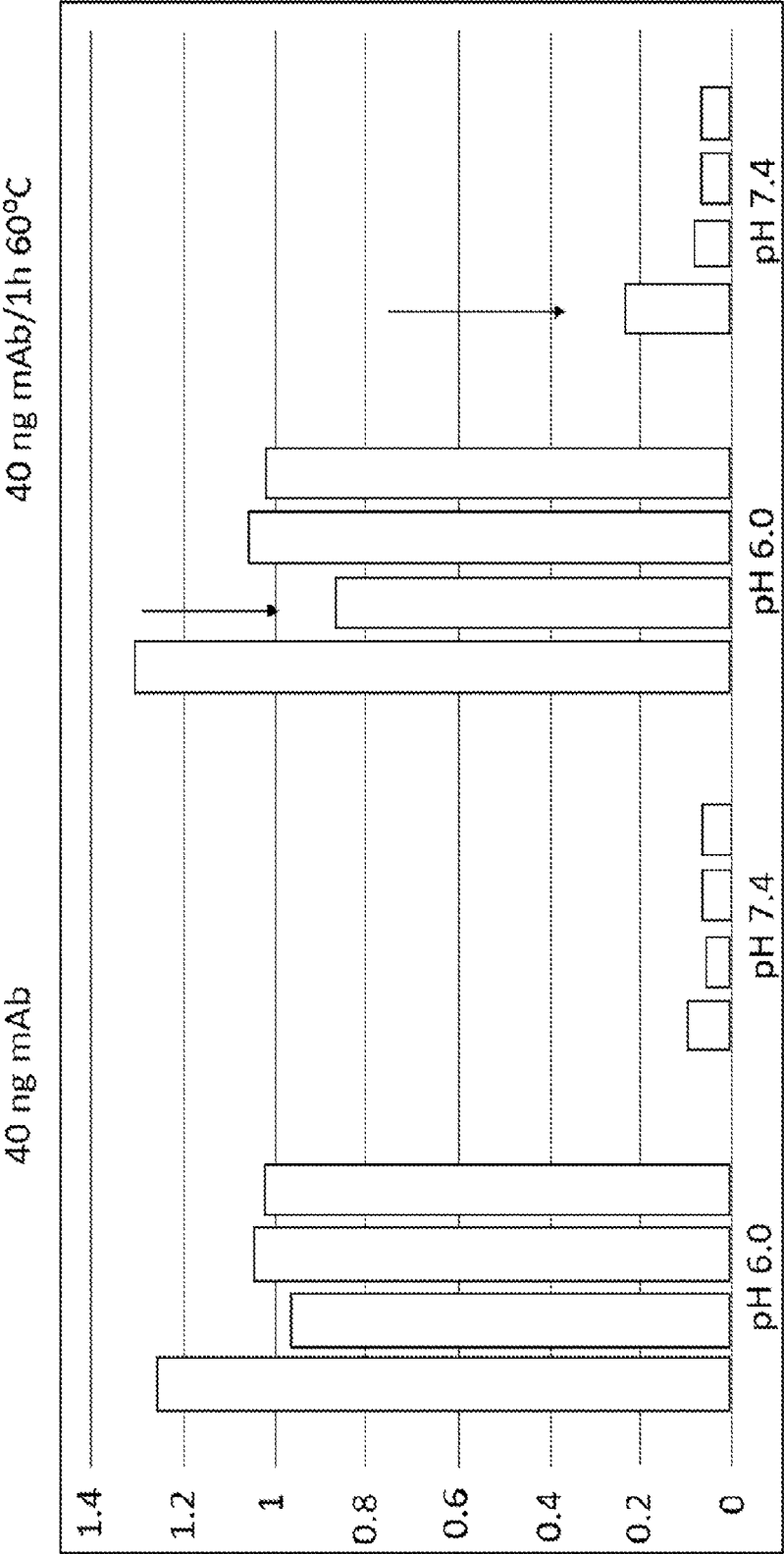


FIG. 4



There are 4 antibodies tested under each condition (i.e., 11 bars in each set of bars). The 11 bars under each condition from left to right represent respectively clones BAP048.6-2-11-Chimera-CAB, (#3949), BAP048.7-humC02D09-CAB)#3947), BAP048.7-humA03C05-CAB (#3950), and BAP048.7-humA03F01-CAB (#3948).

FIG. 5

Tumor environment (pH6.0)

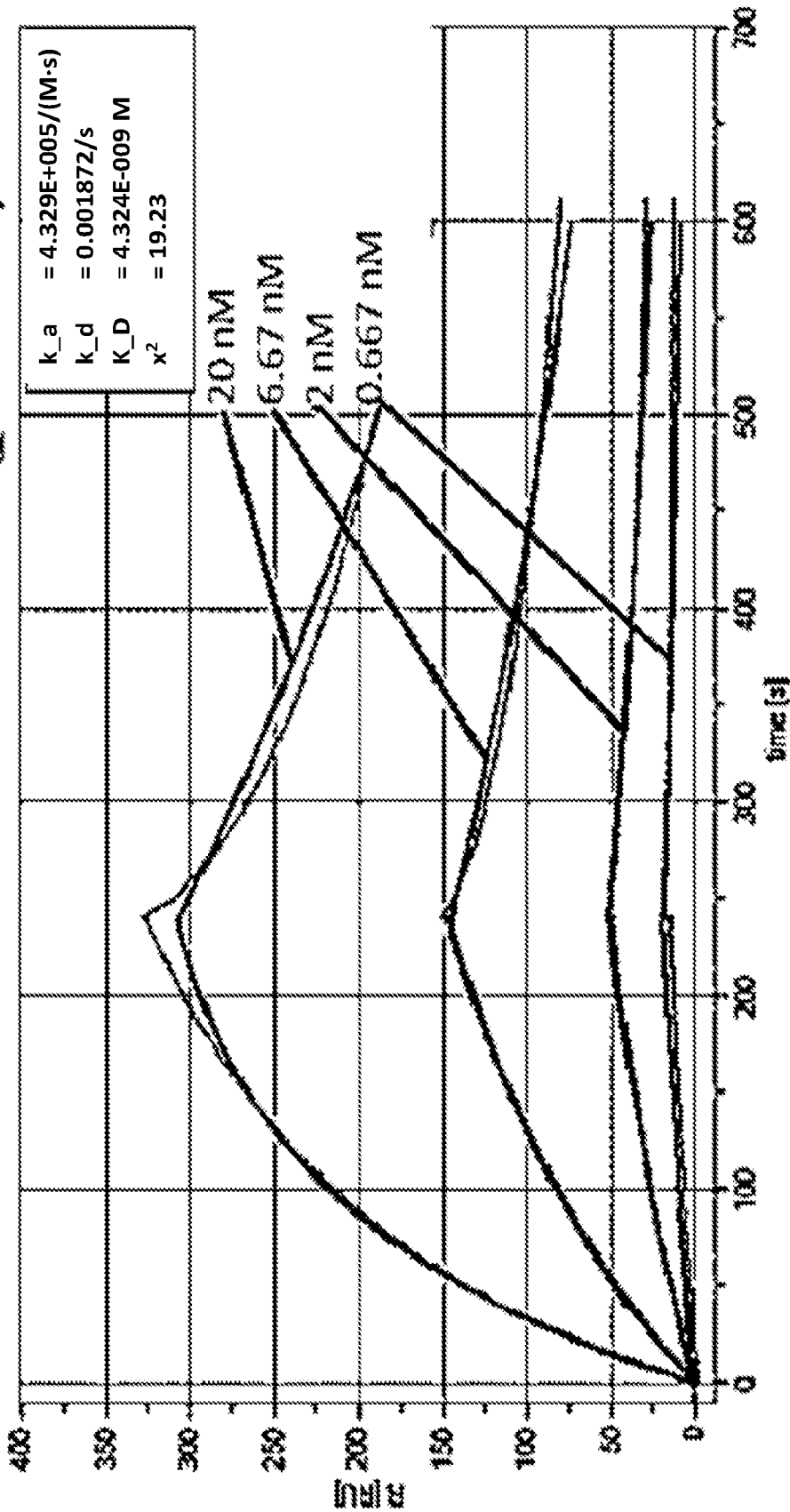


FIG. 6A

Normal environment (pH7.4)

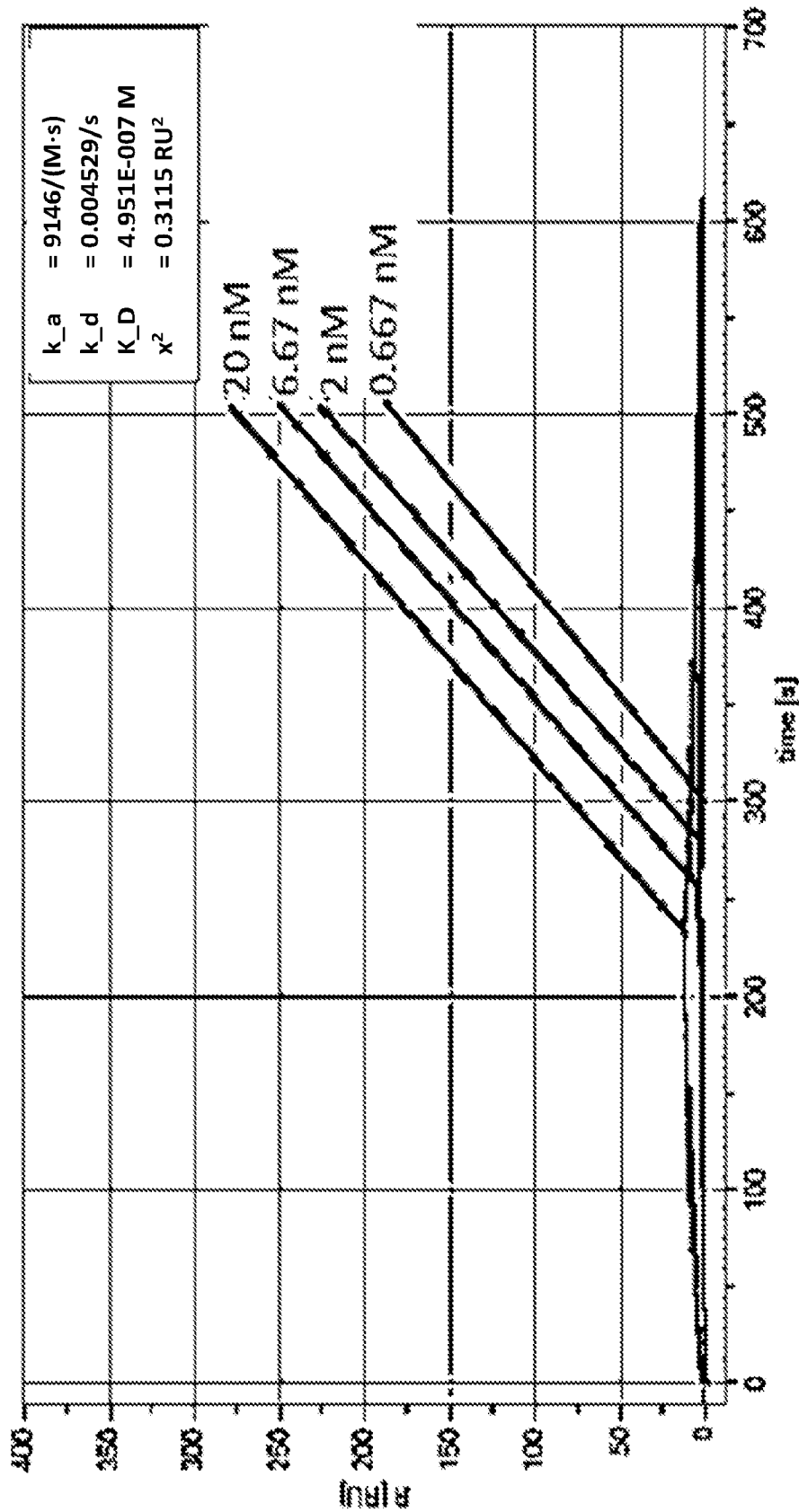


FIG. 6B

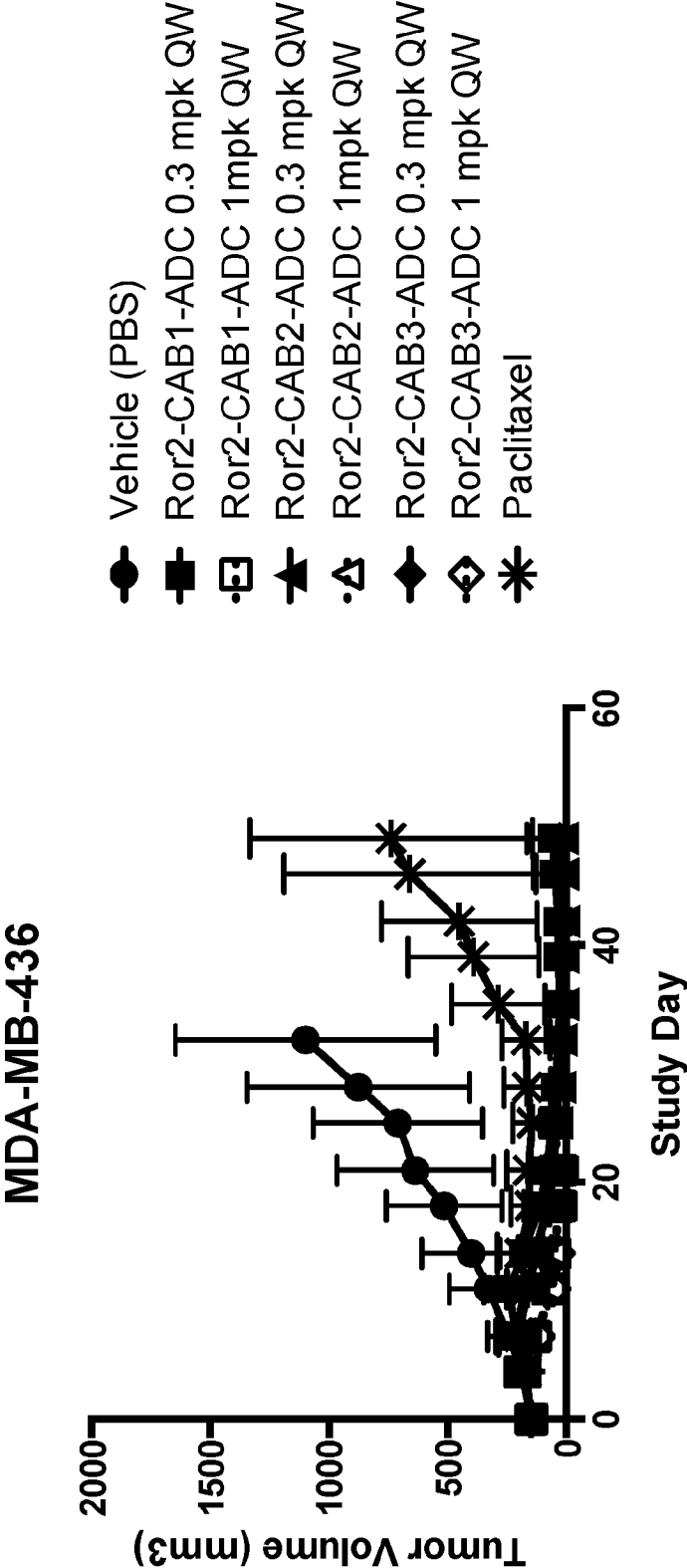


FIG. 7A

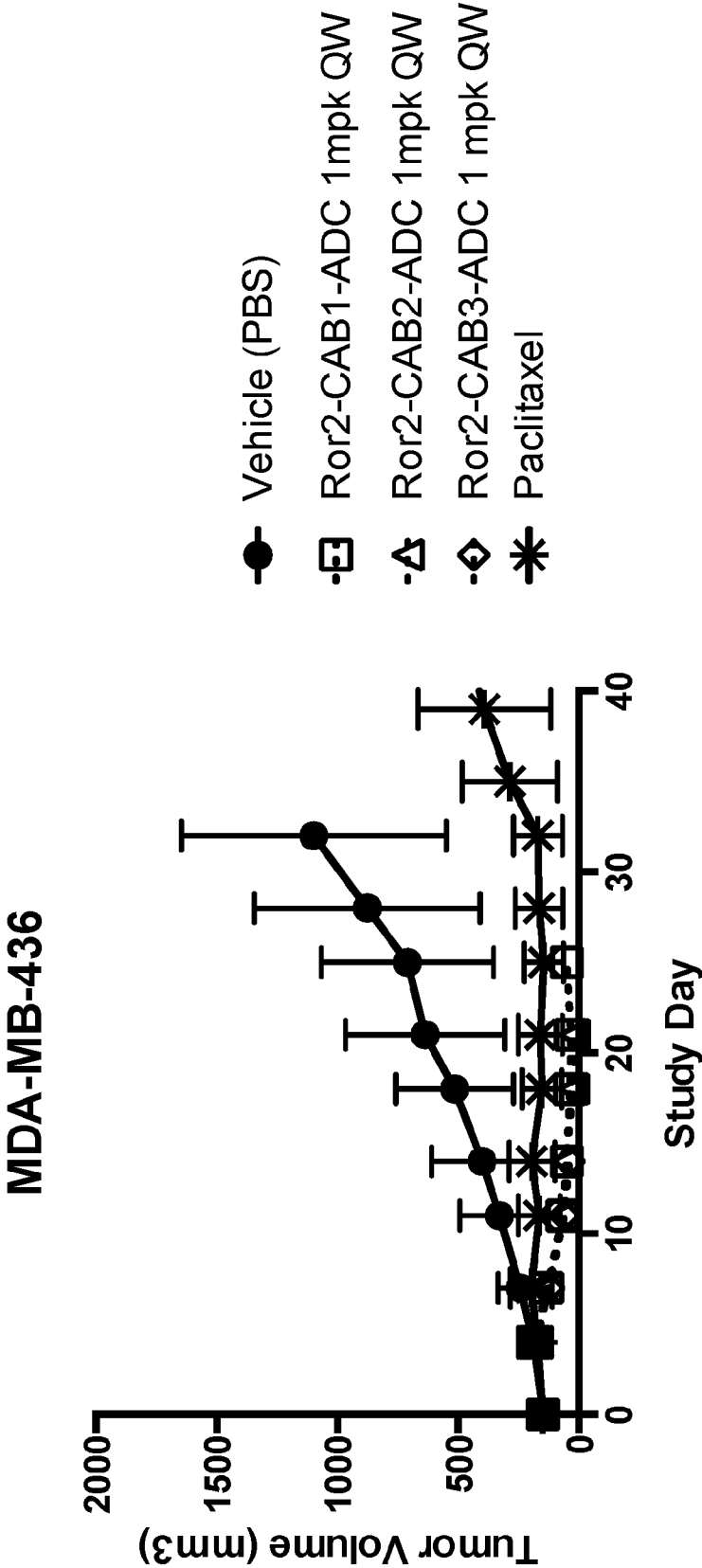


FIG. 7B

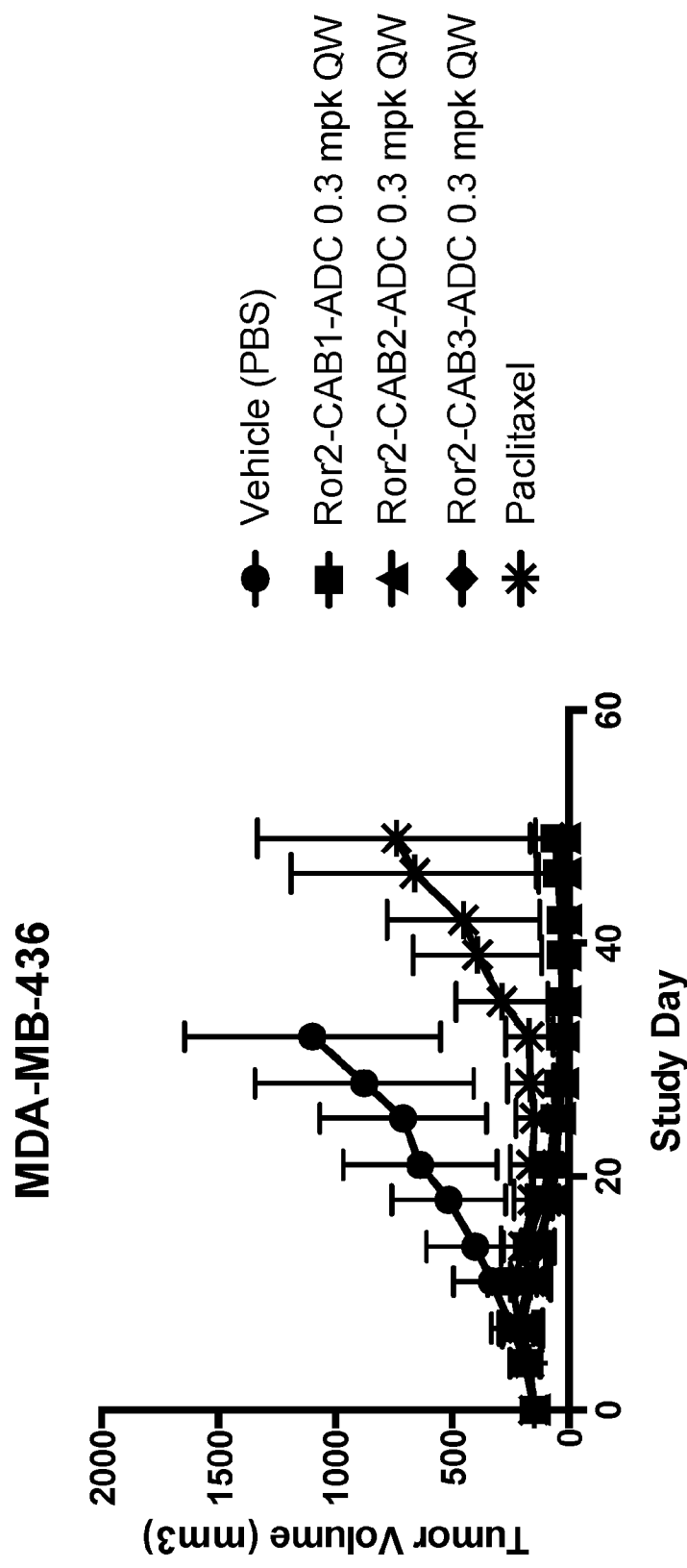
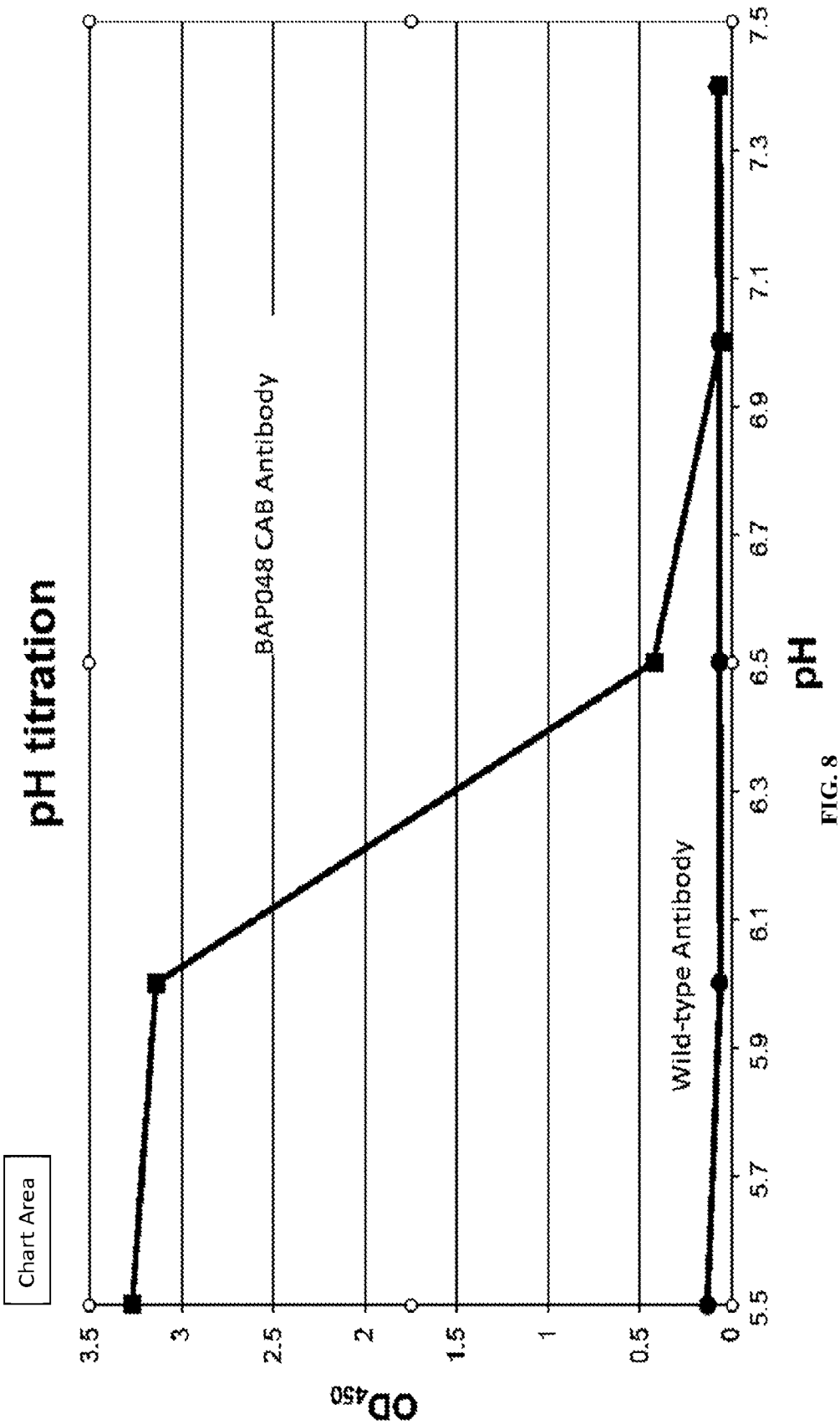
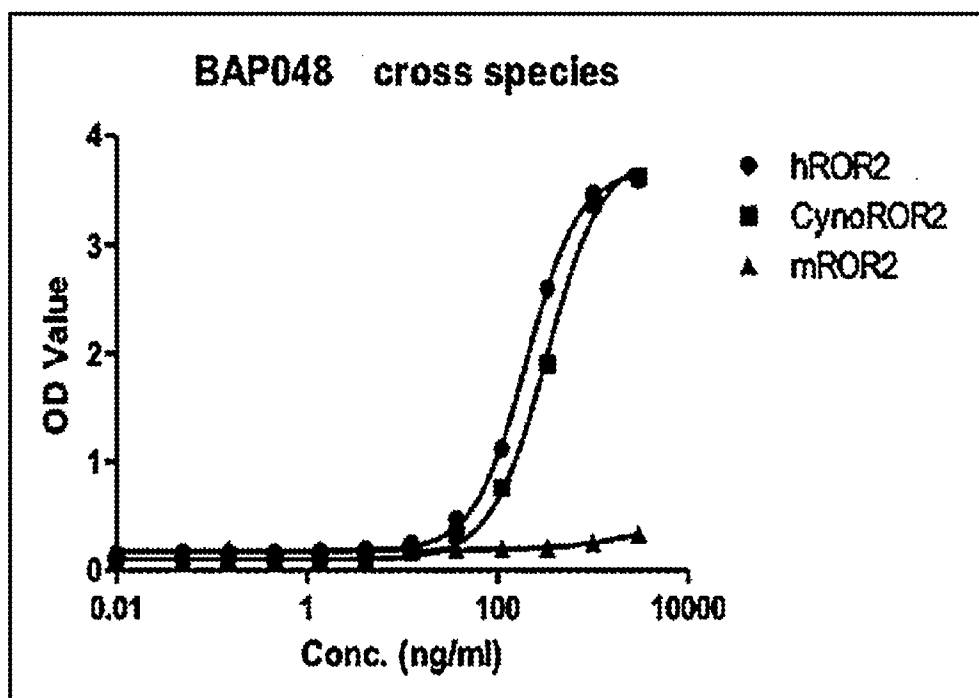


FIG. 7C





Antigen	hROR2	CynoROR2	mROR2
EC50 (ng/ml)	201.4	327.1	7653

FIG. 9

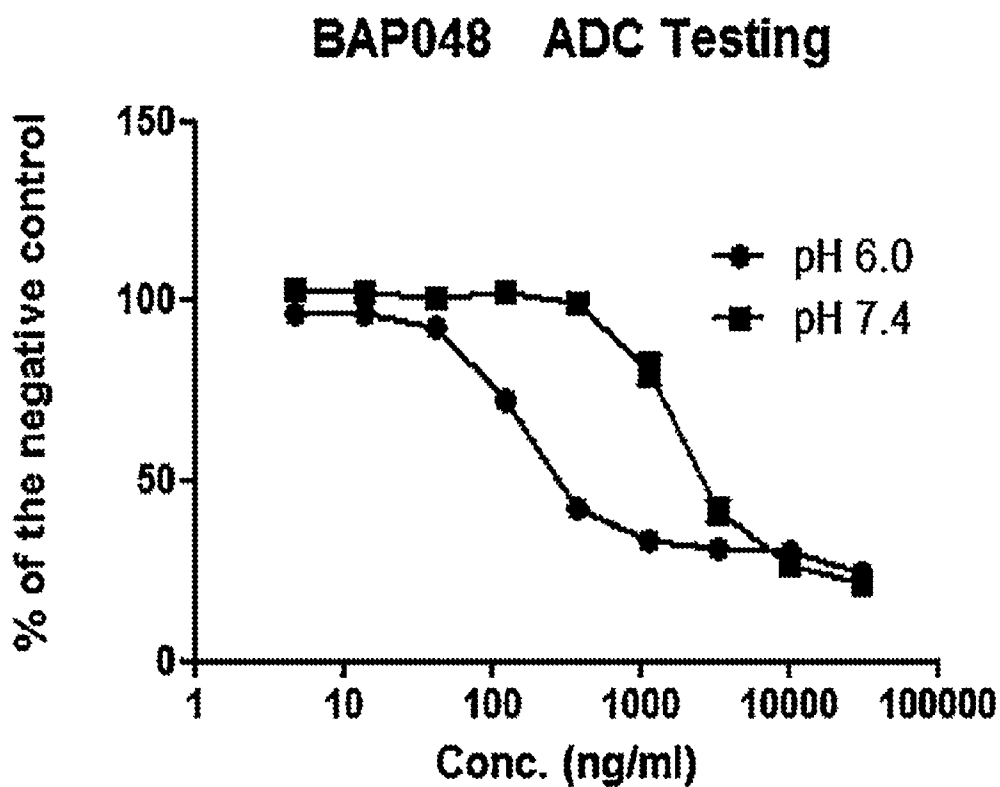


FIG. 10

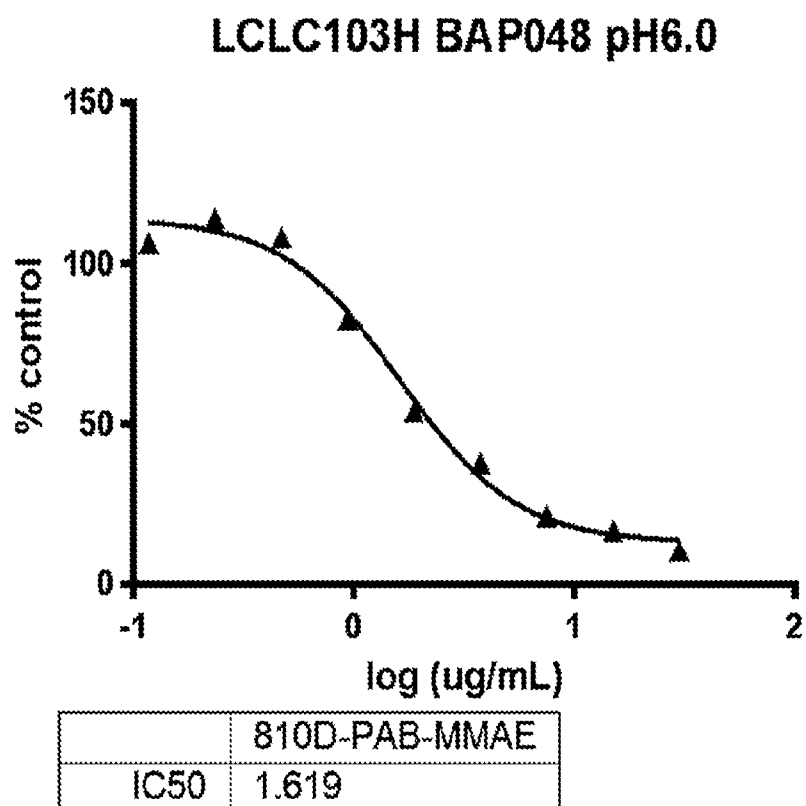


FIG. 11A

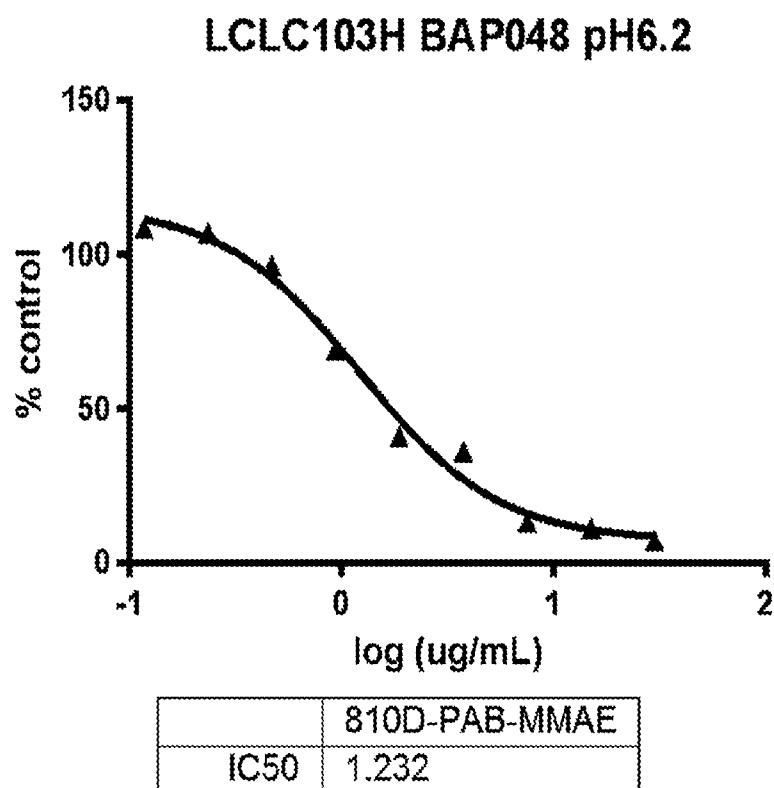


FIG. 11B

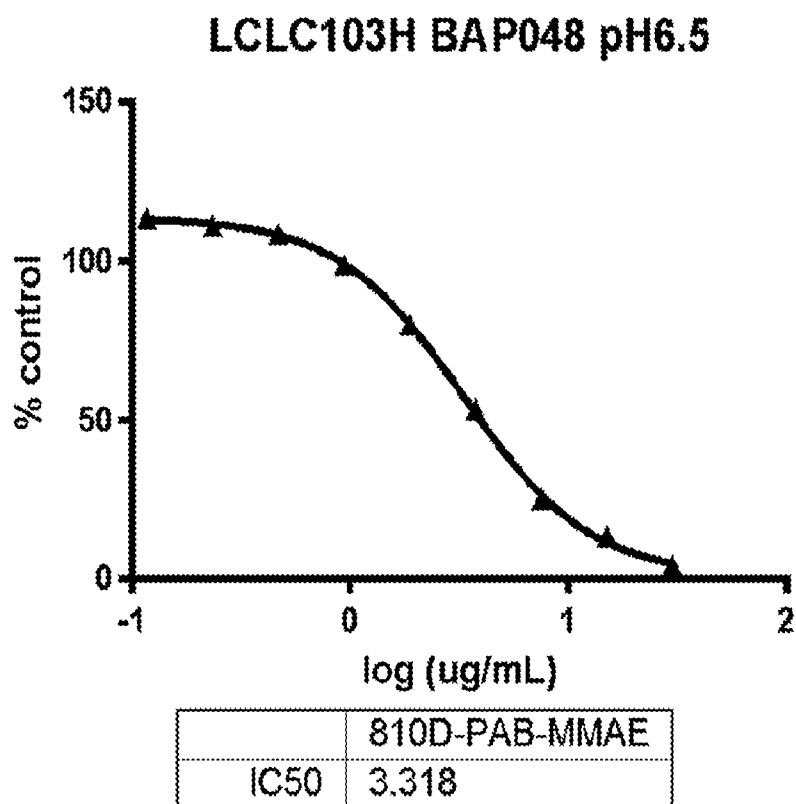


FIG. 11C

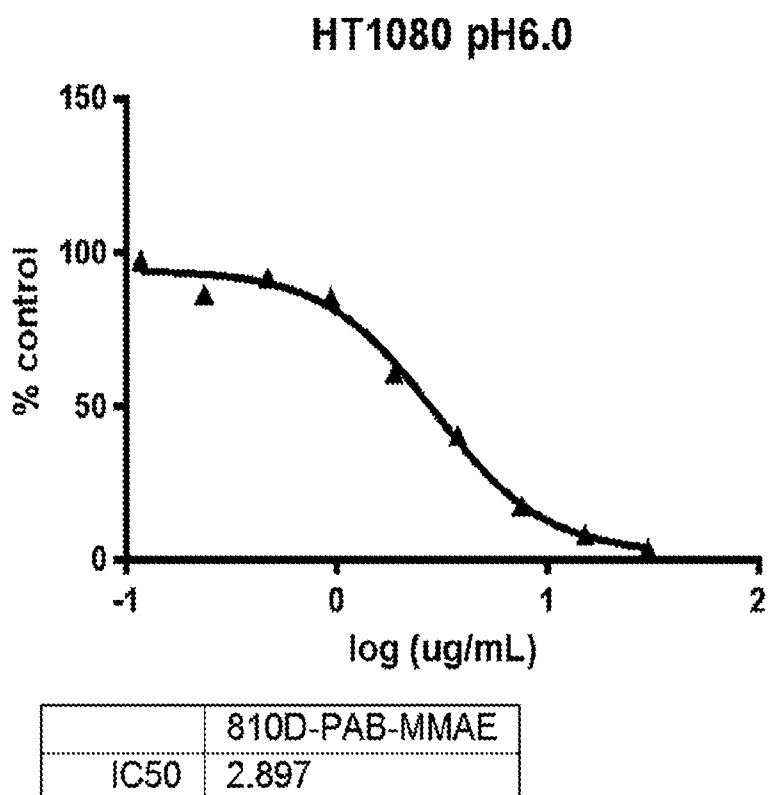


FIG. 12A

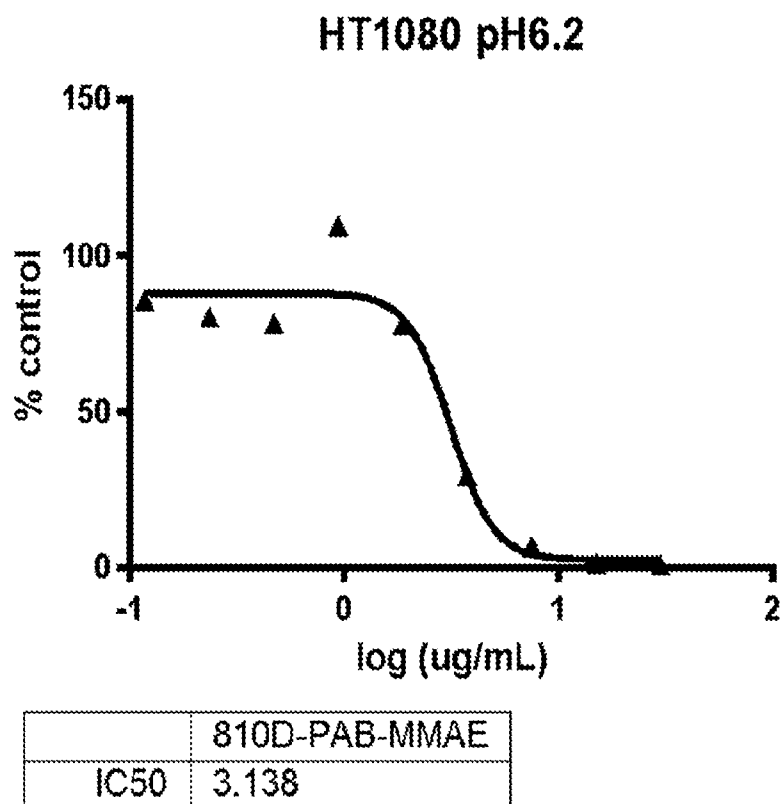


FIG. 12B

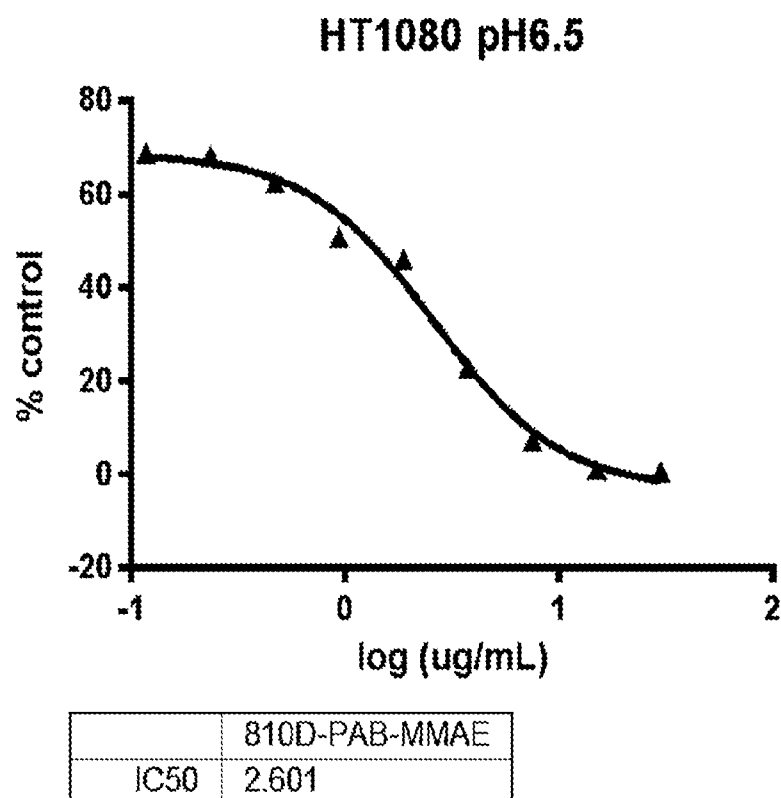


FIG. 12C

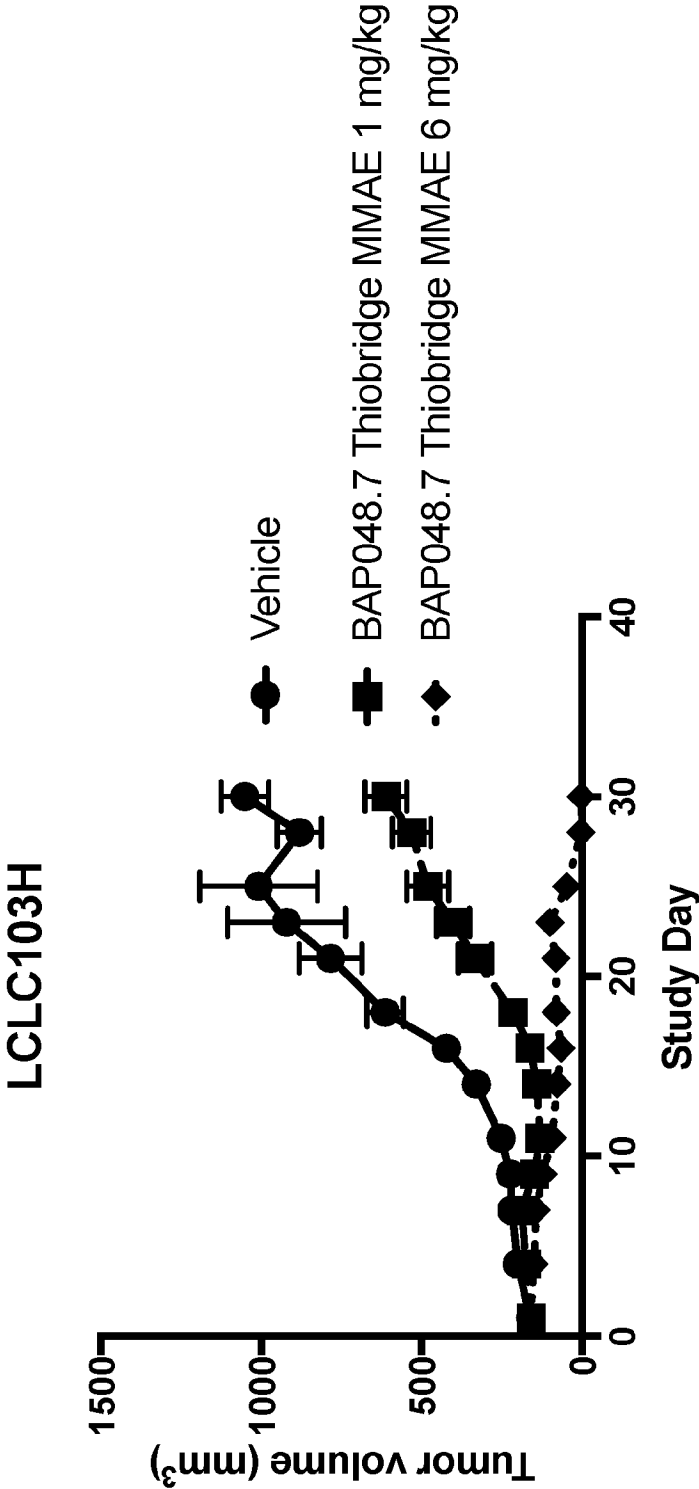


FIG. 13

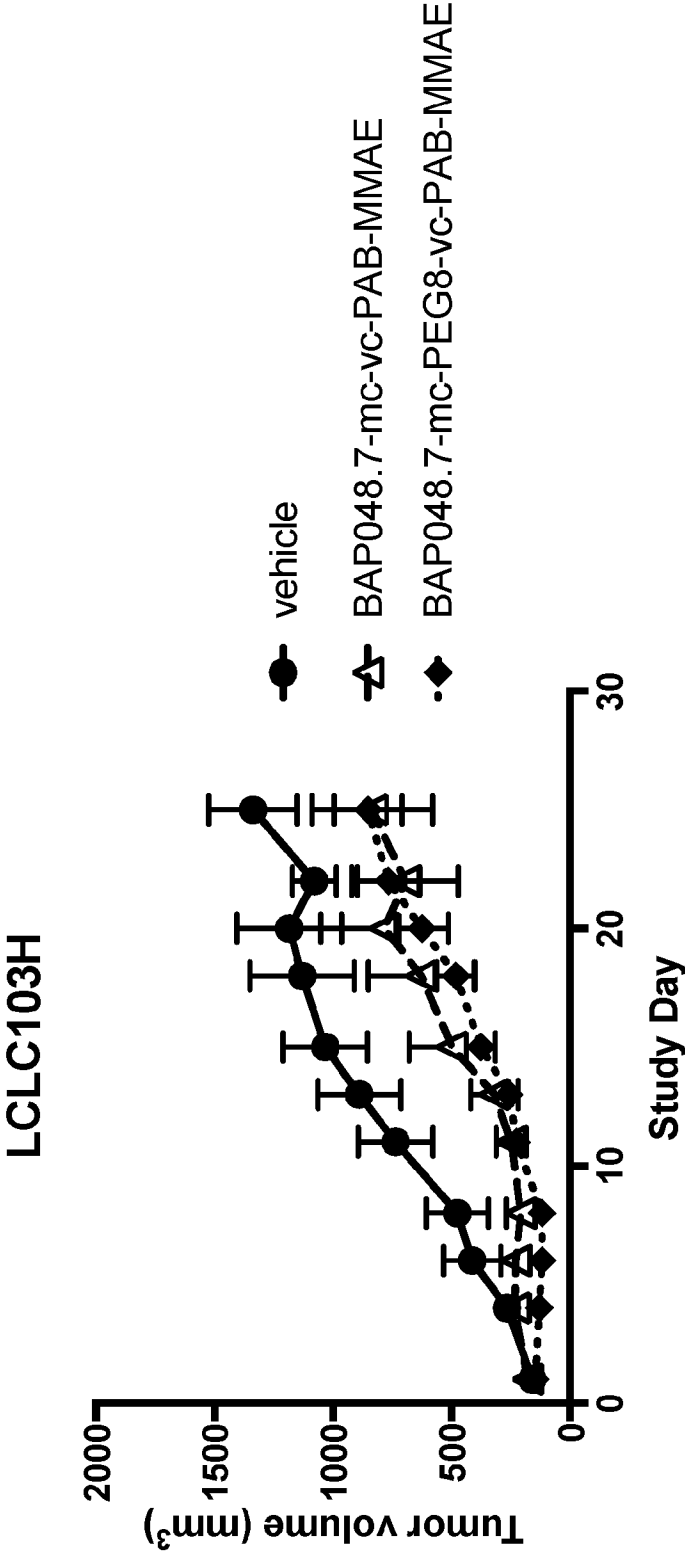


FIG. 14

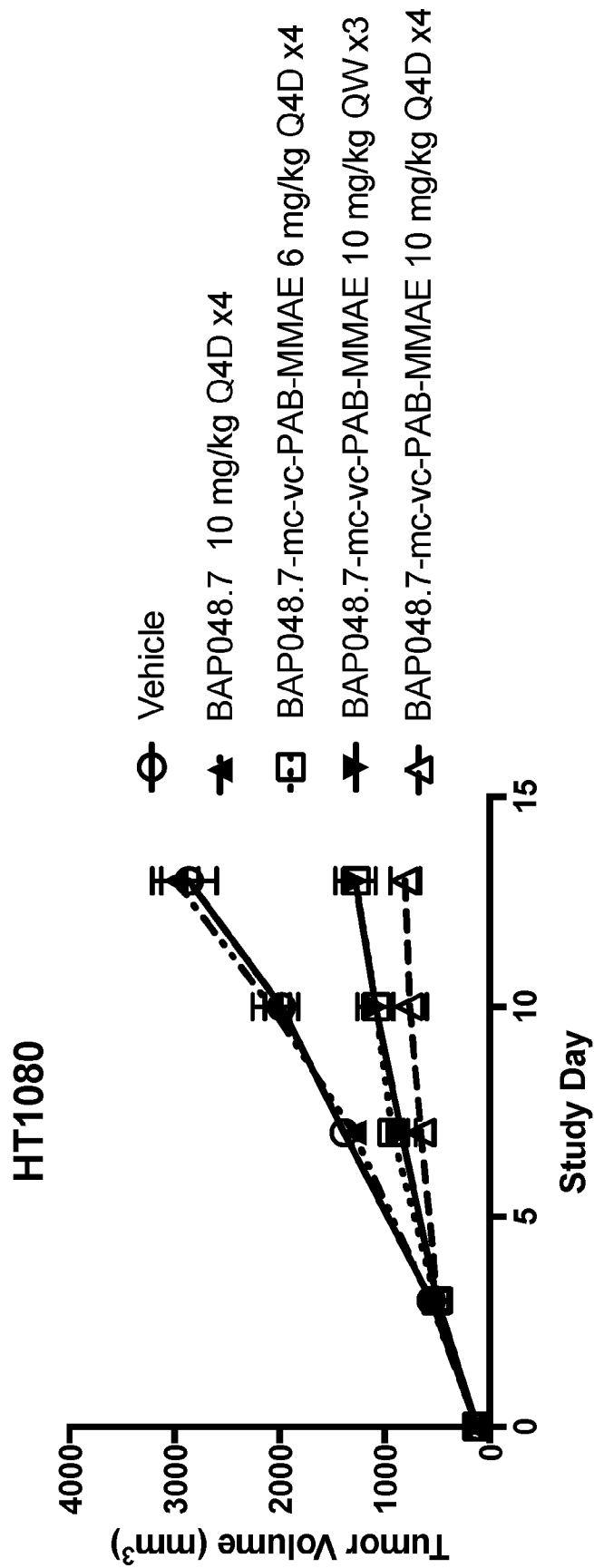


FIG. 15A

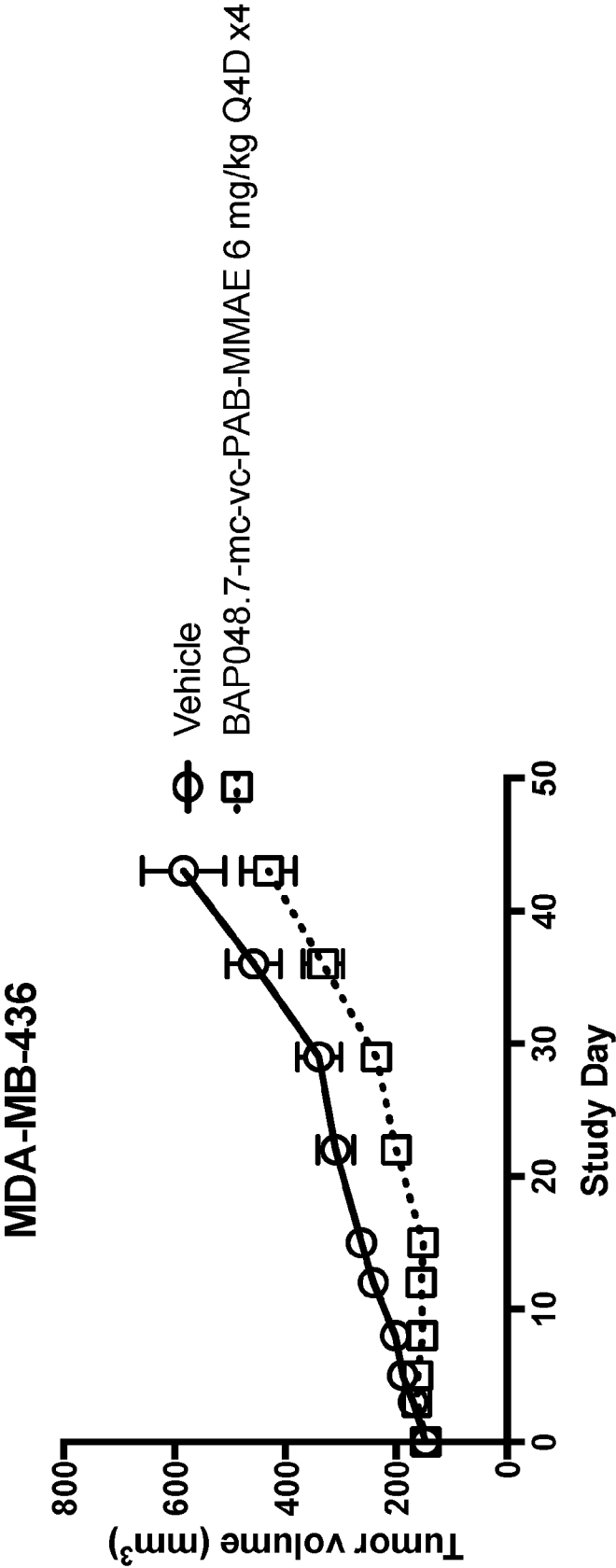


FIG. 15B

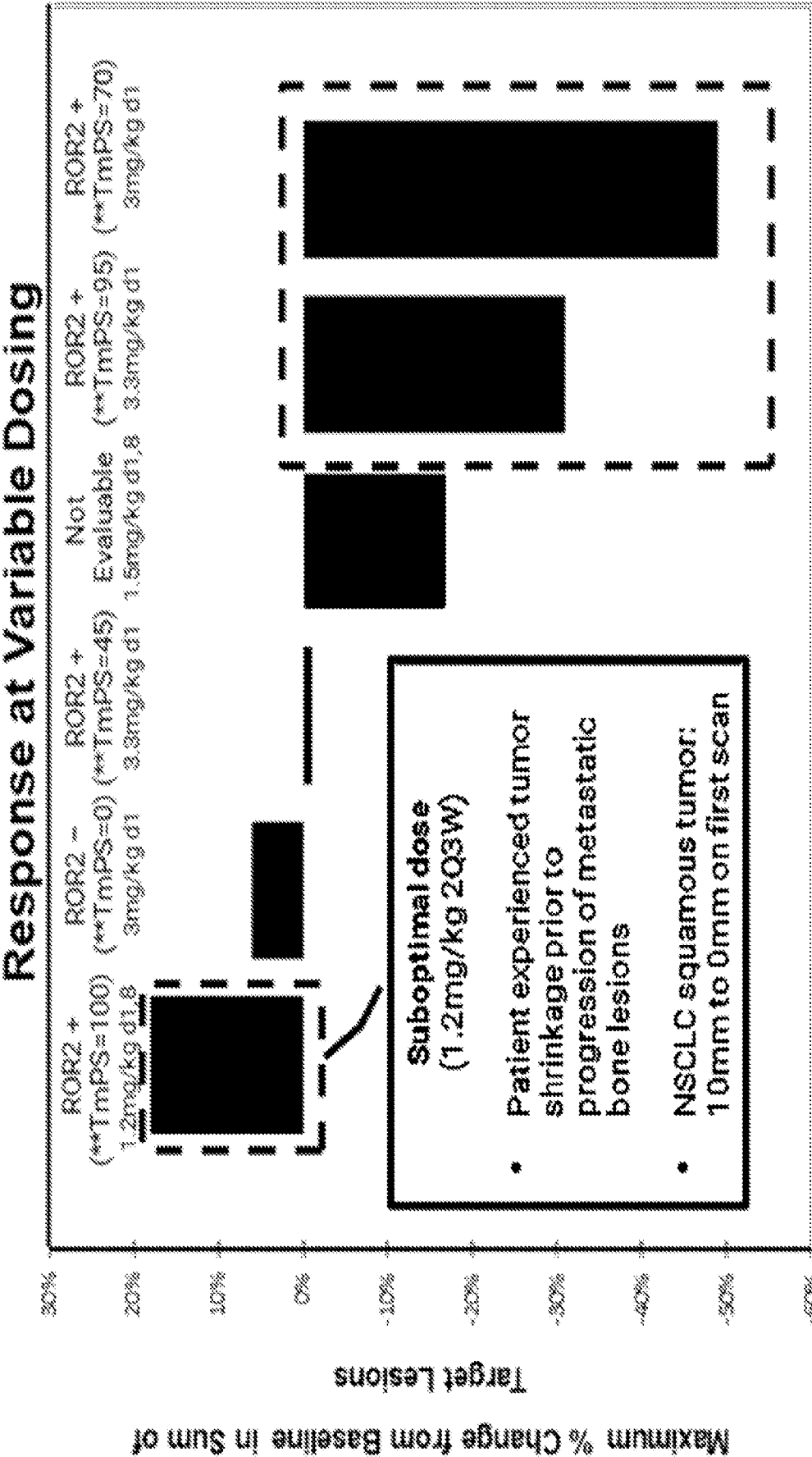


FIG. 16

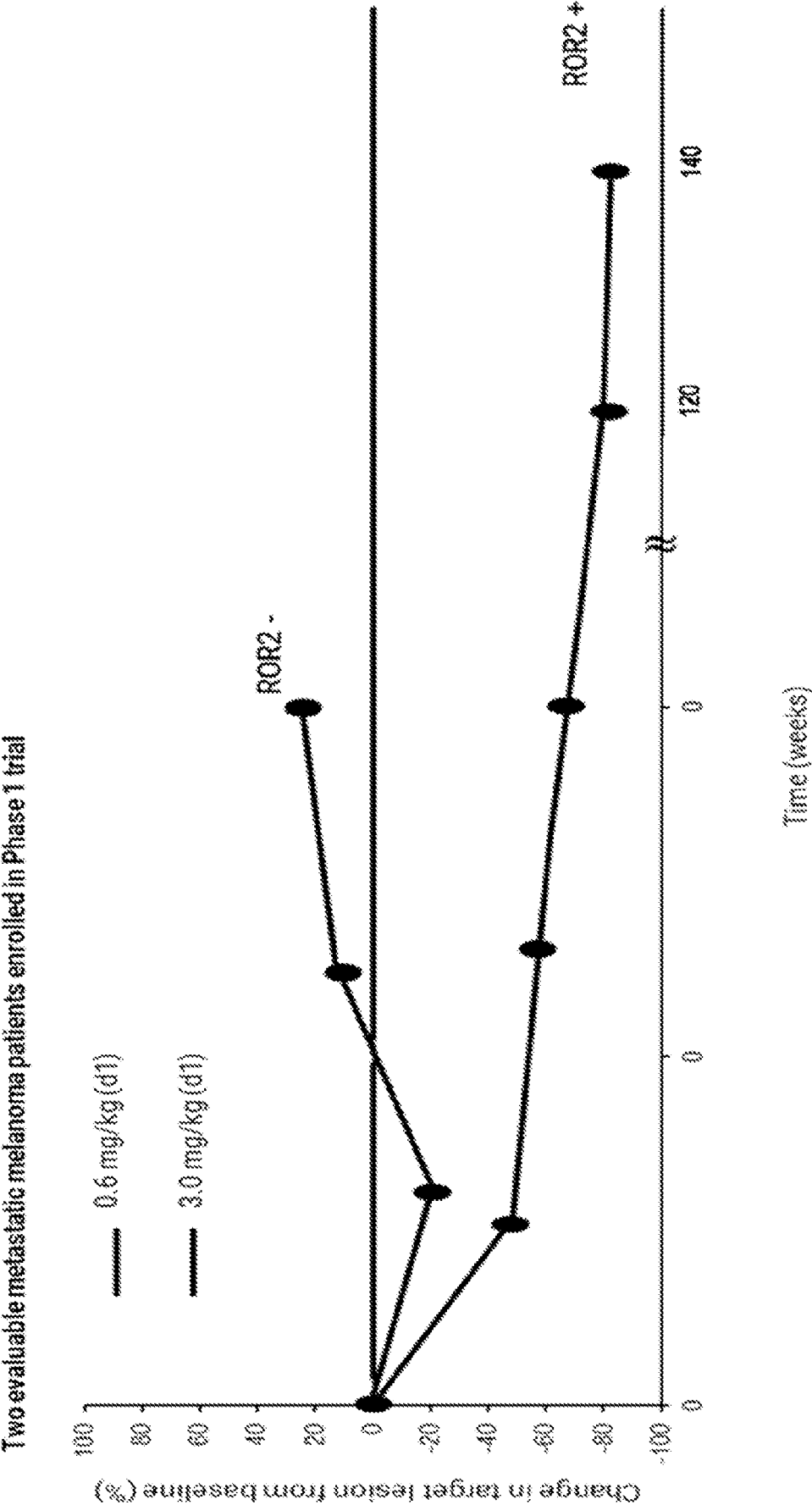
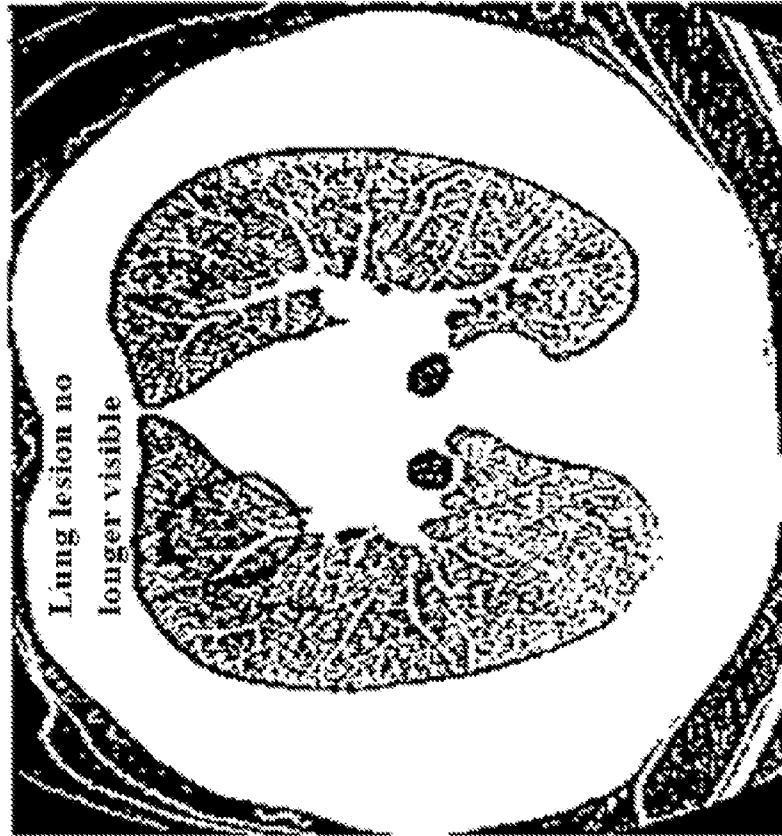


FIG. 17

FIG. 18B



Post-Treatment; Week 6 Scan

FIG. 18

FIG. 18A



Pre-treatment CT Scan

FIG 19B

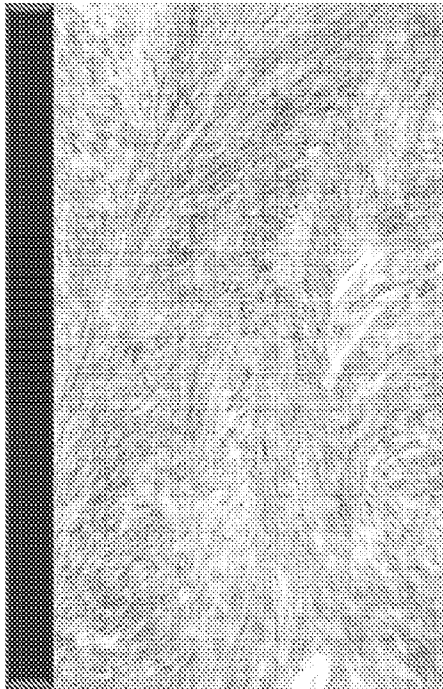


FIG 19D

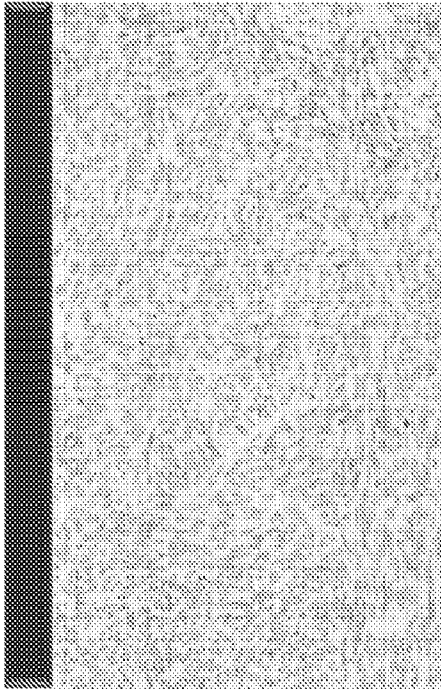


FIG 19A

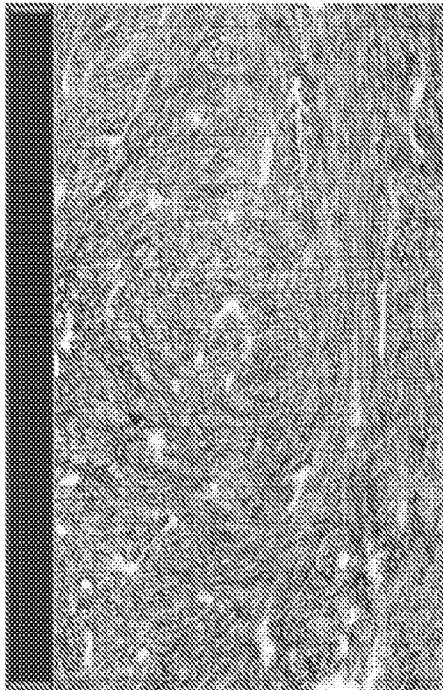


FIG 19C

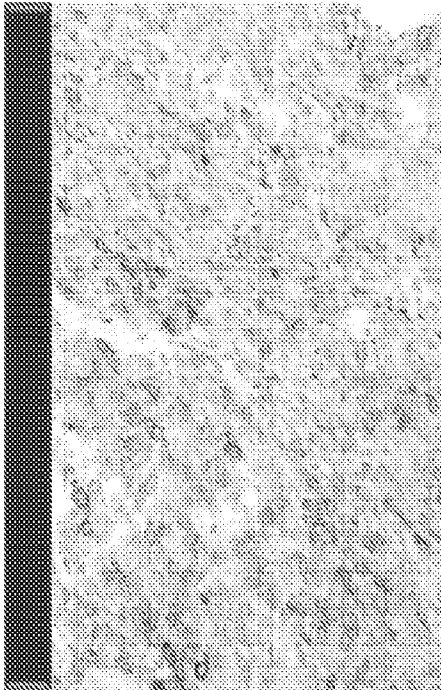


FIG. 19

FIG 20B

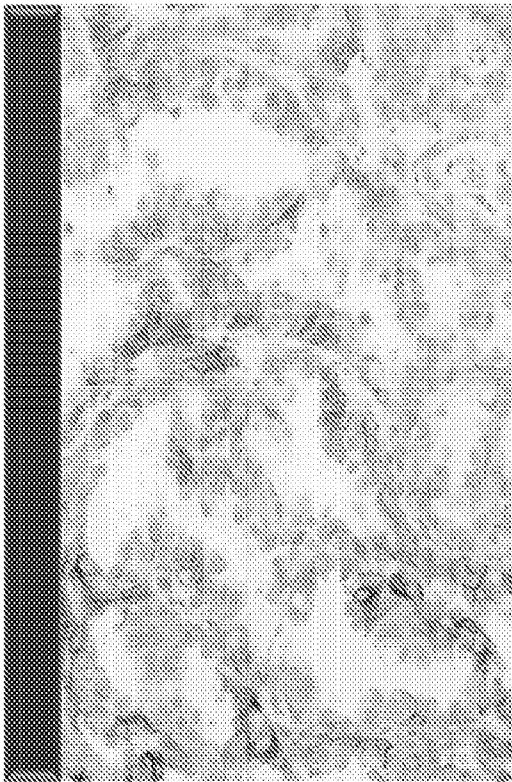


FIG 20D

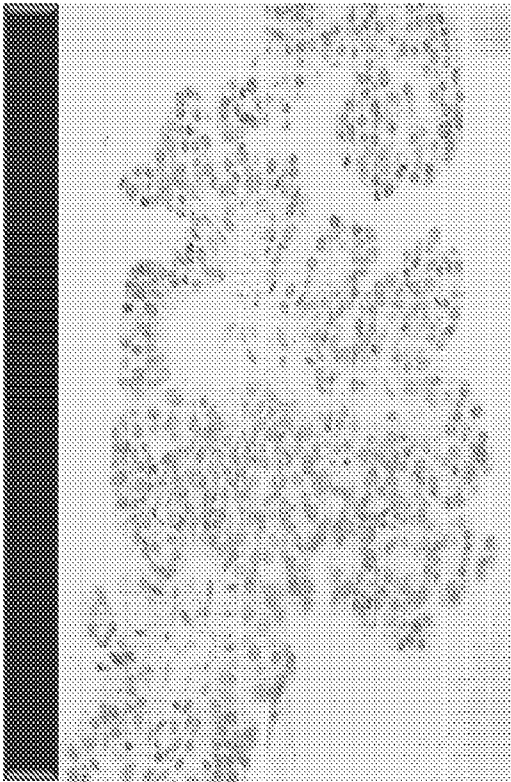


FIG 20A

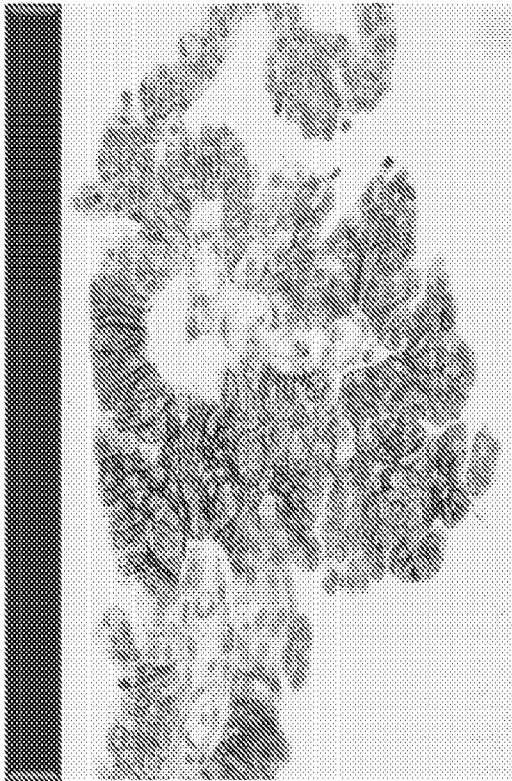


FIG 20C

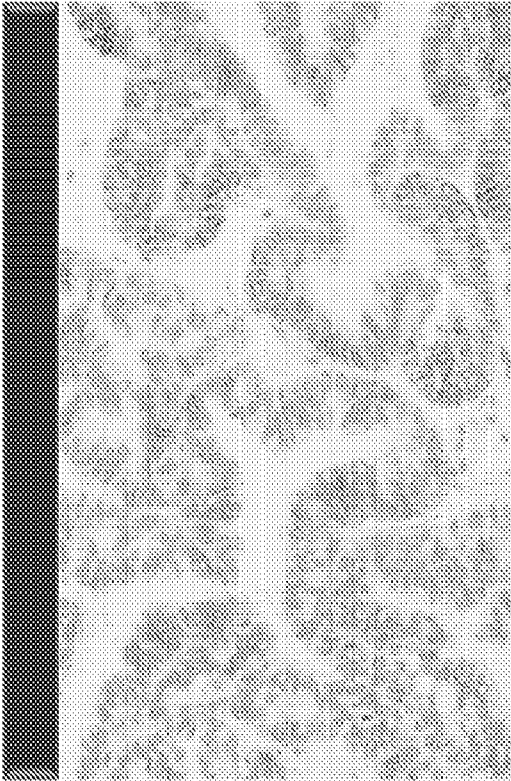


FIG. 20

FIG 21A

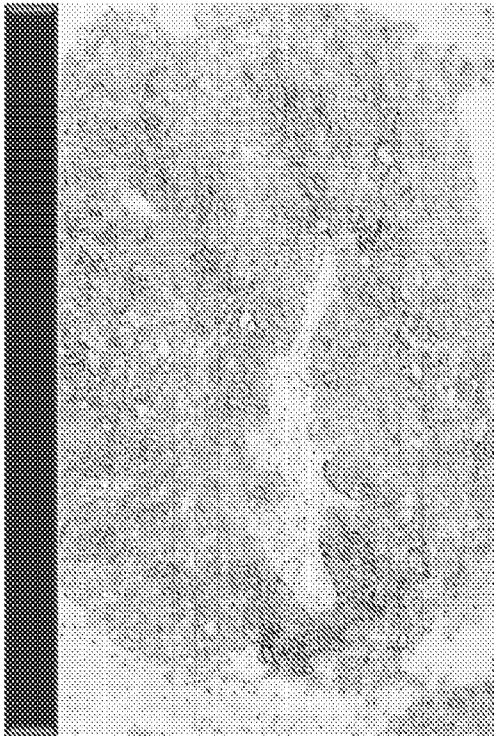


FIG 21B

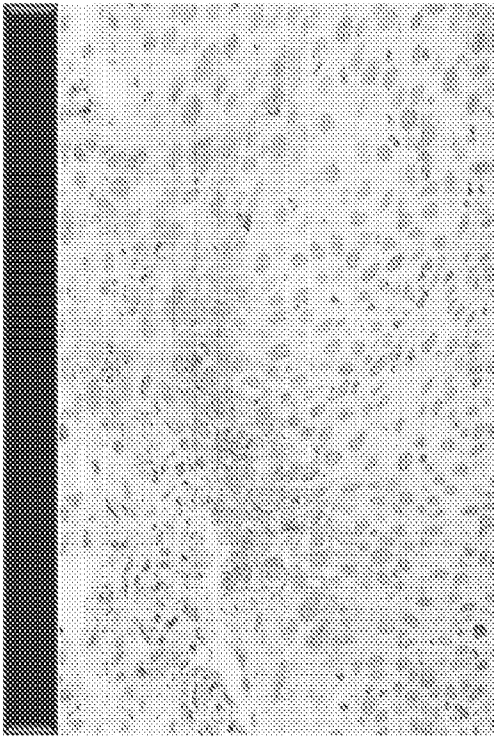


FIG 21C

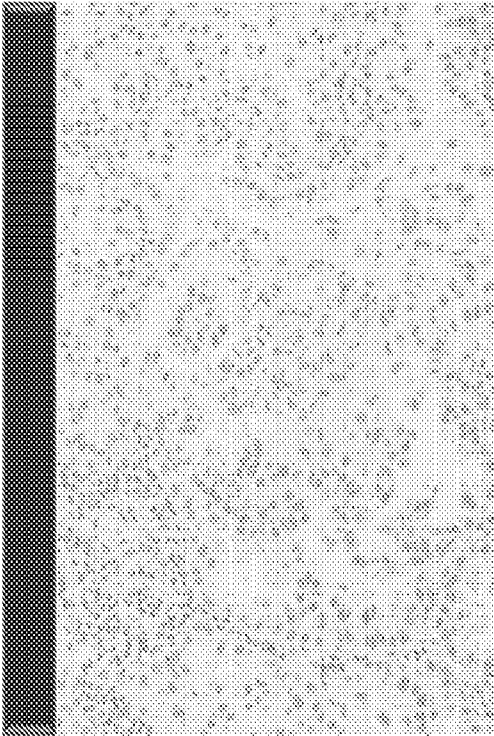


FIG 21D

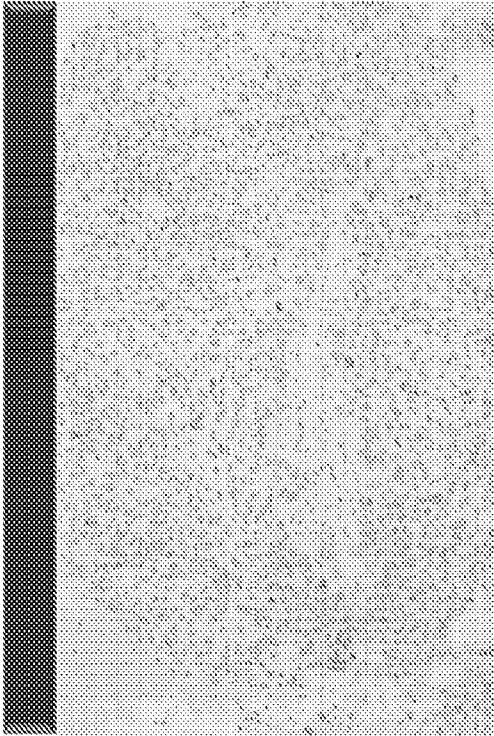


FIG. 21

FIG 22B

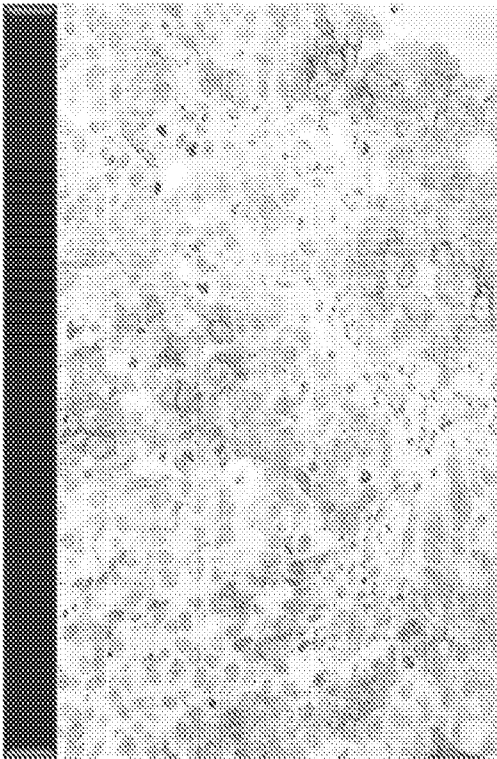


FIG 22D



FIG 22A

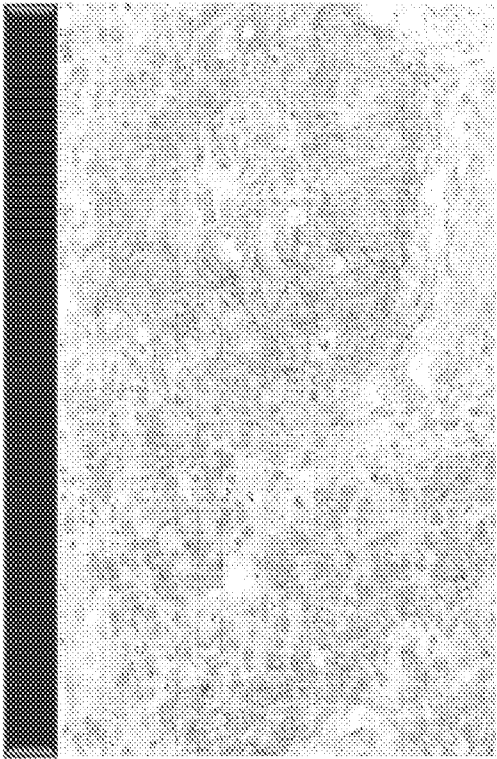


FIG 22C

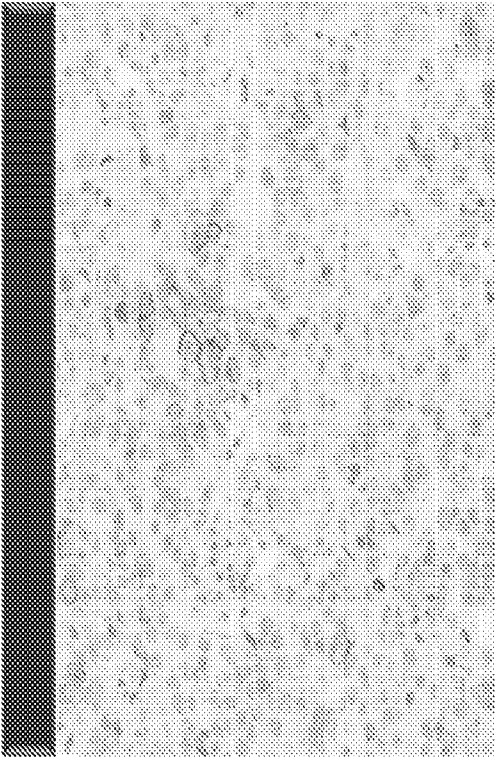
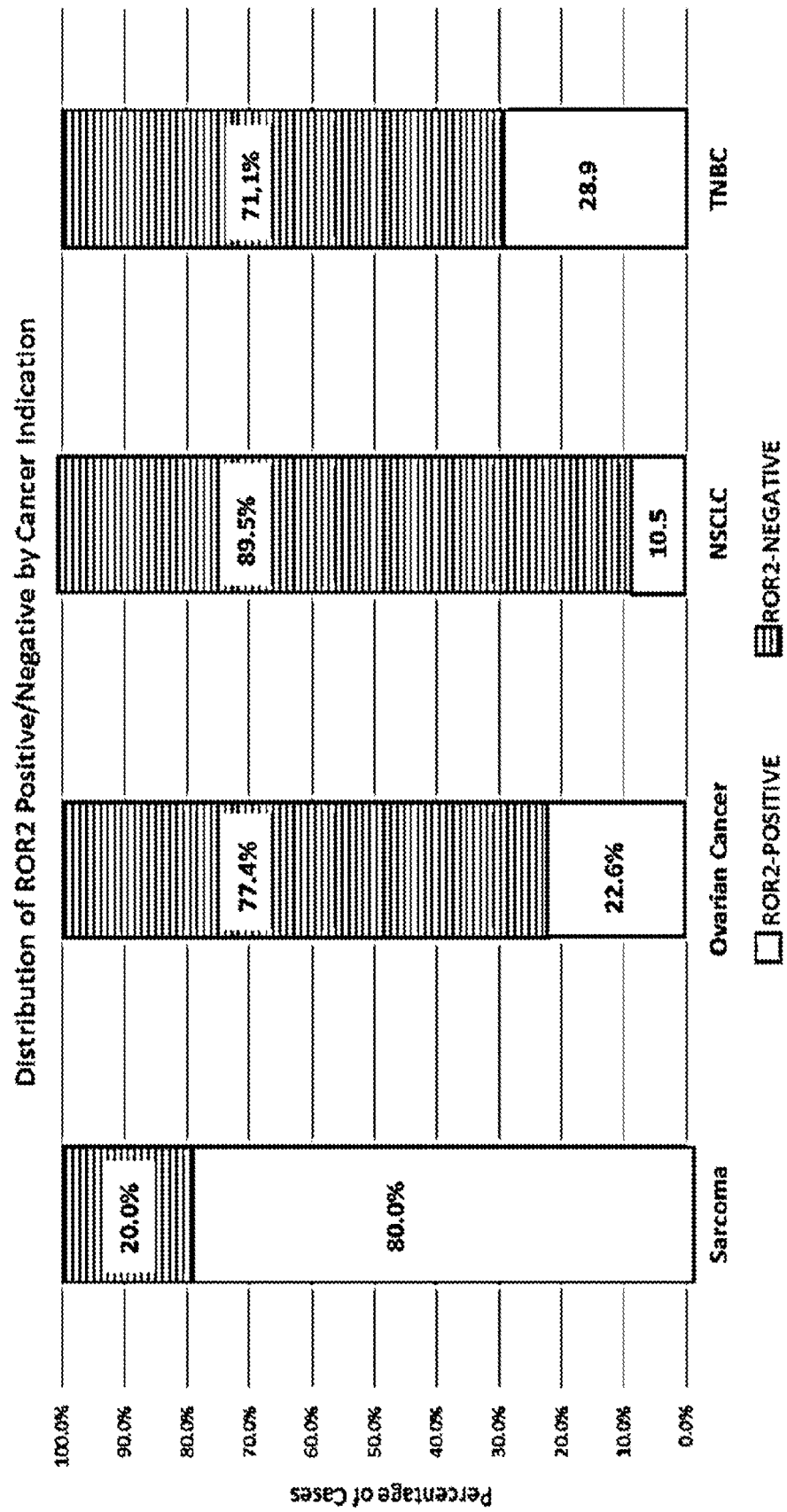


FIG. 22



Status based on Ror2 cut-off: $\geq 1+$ Intensity in $\geq 10\%$ tumor cells = POSITIVE

FIG. 23

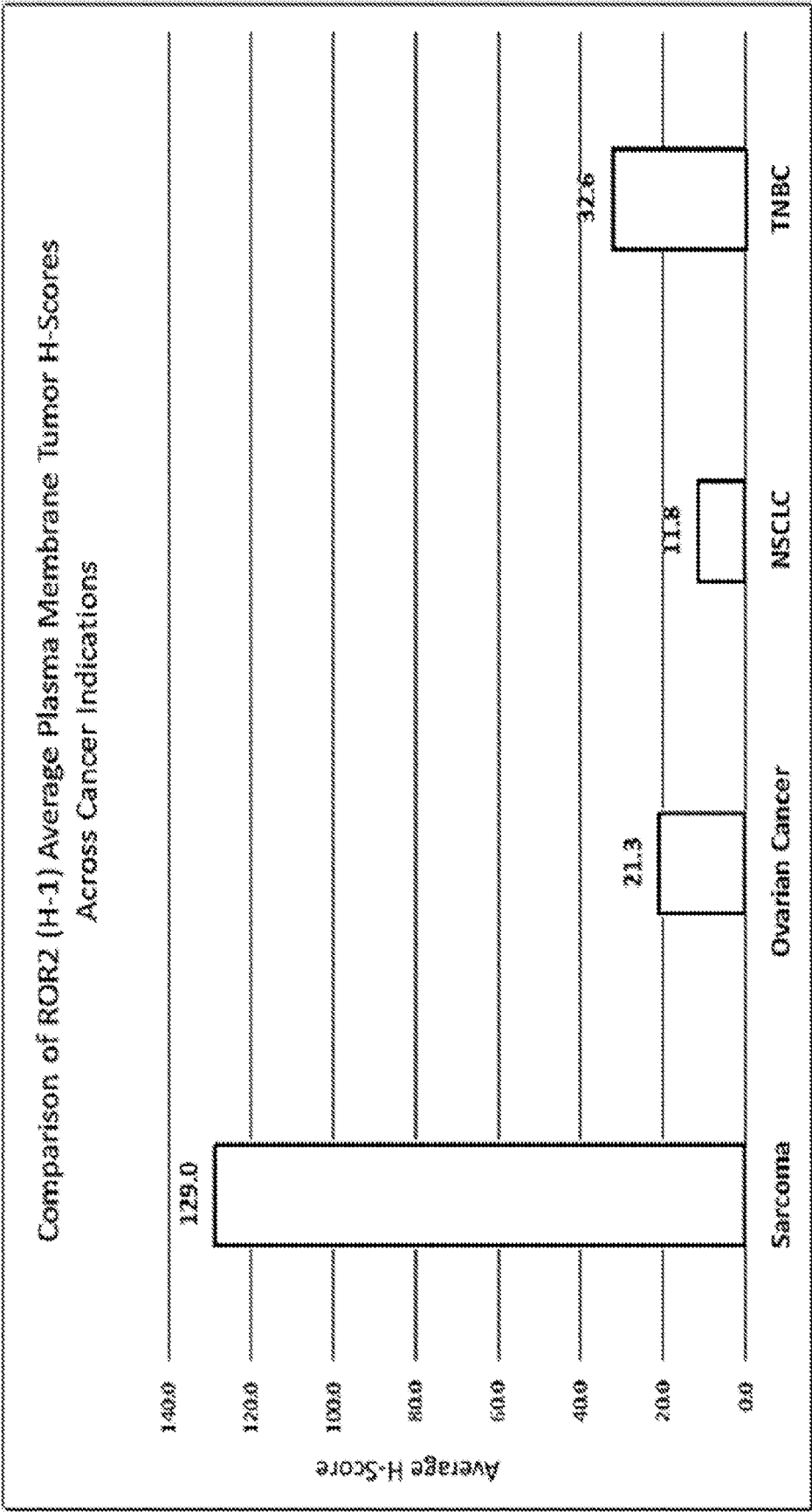


FIG. 24

FIG. 25

FIG. 25A



FIG. 25B

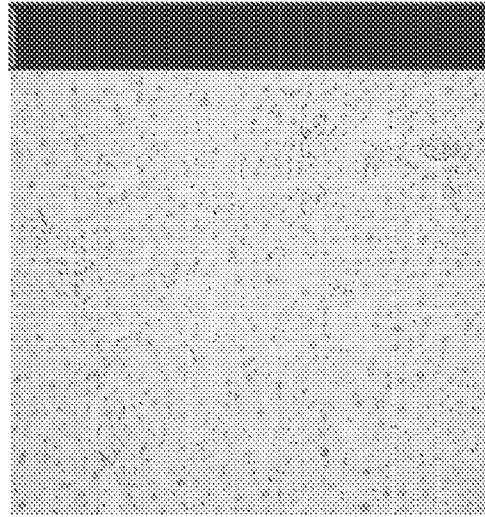


FIG. 25C

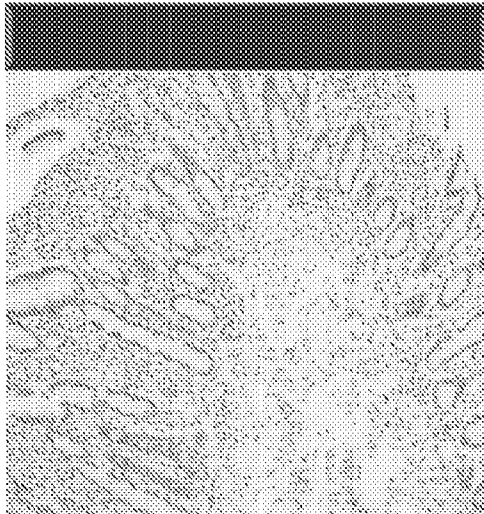


FIG. 25D

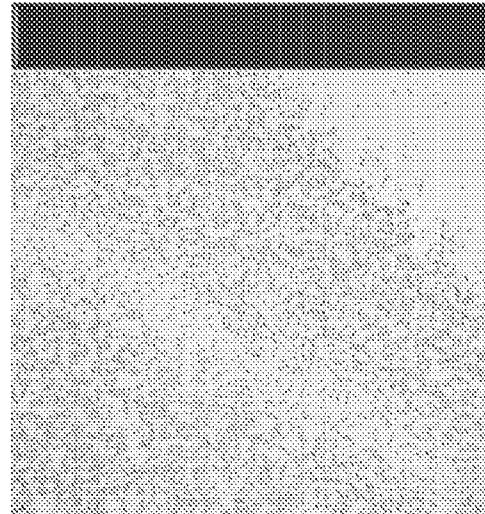


FIG. 25E

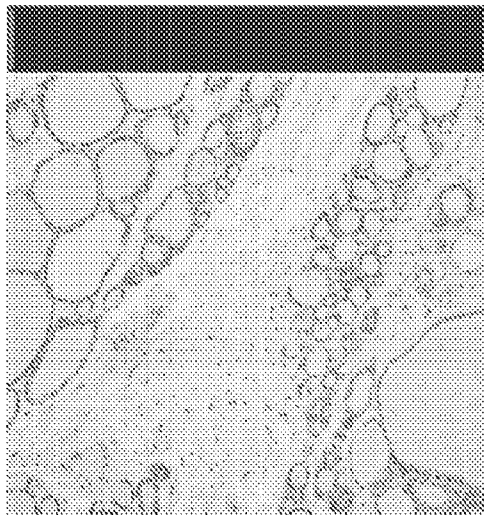


FIG. 25F



Quantitative

FIG. 26

Sarcoma Q9403, Top Three Rows: ROR2, Bottom Row: Mouse IgG1 Negative Control, 20X

Operator 1, Run 1, in Triplicate | Operator 1, Run 2, In Triplicate | Operator 2, Run 3, in Triplicate

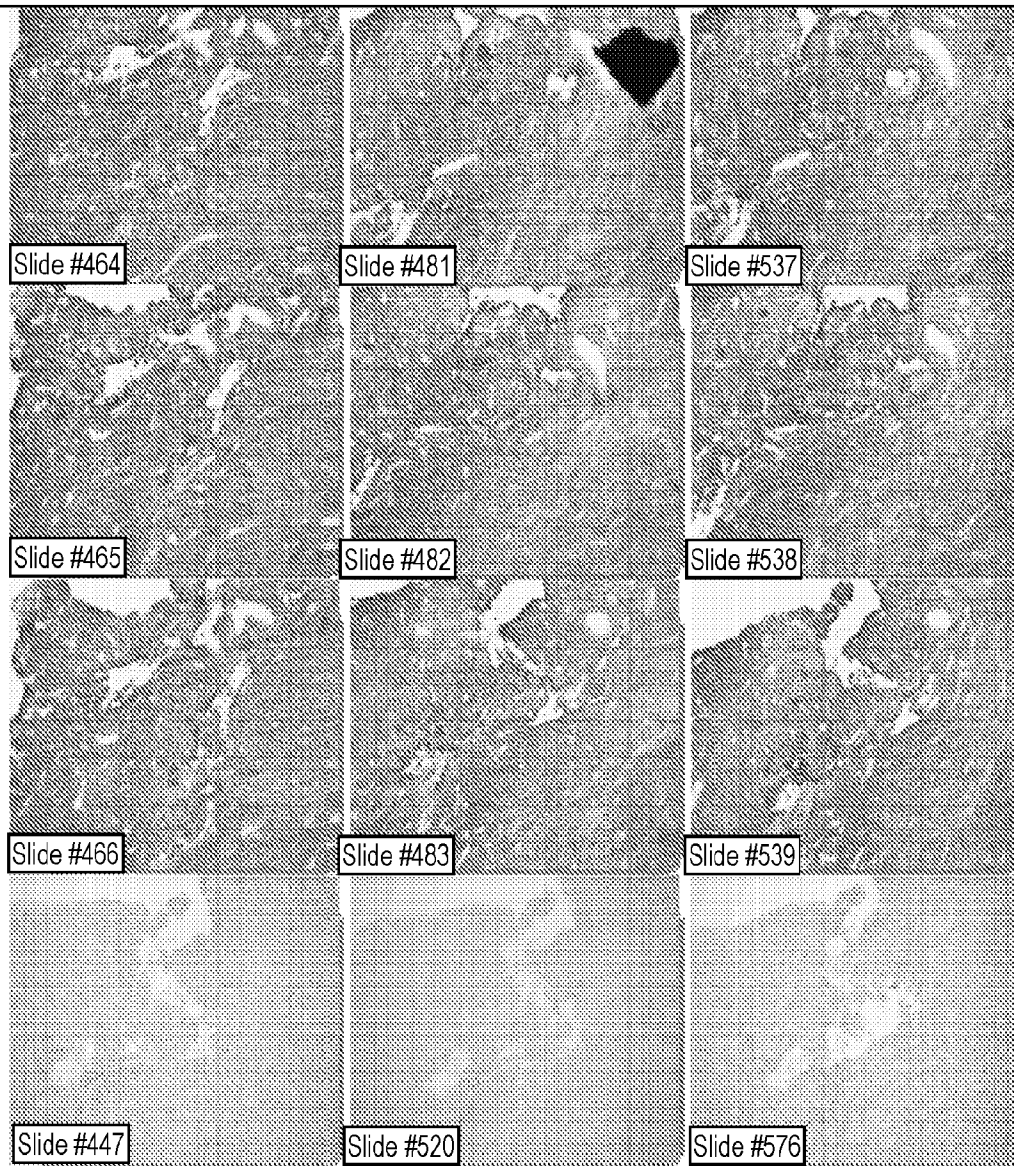


FIG. 27

Sarcoma QMTB313-07, Top Three Rows: ROR2, Bottom Row: Mouse IgG1 Negative Control, 20X

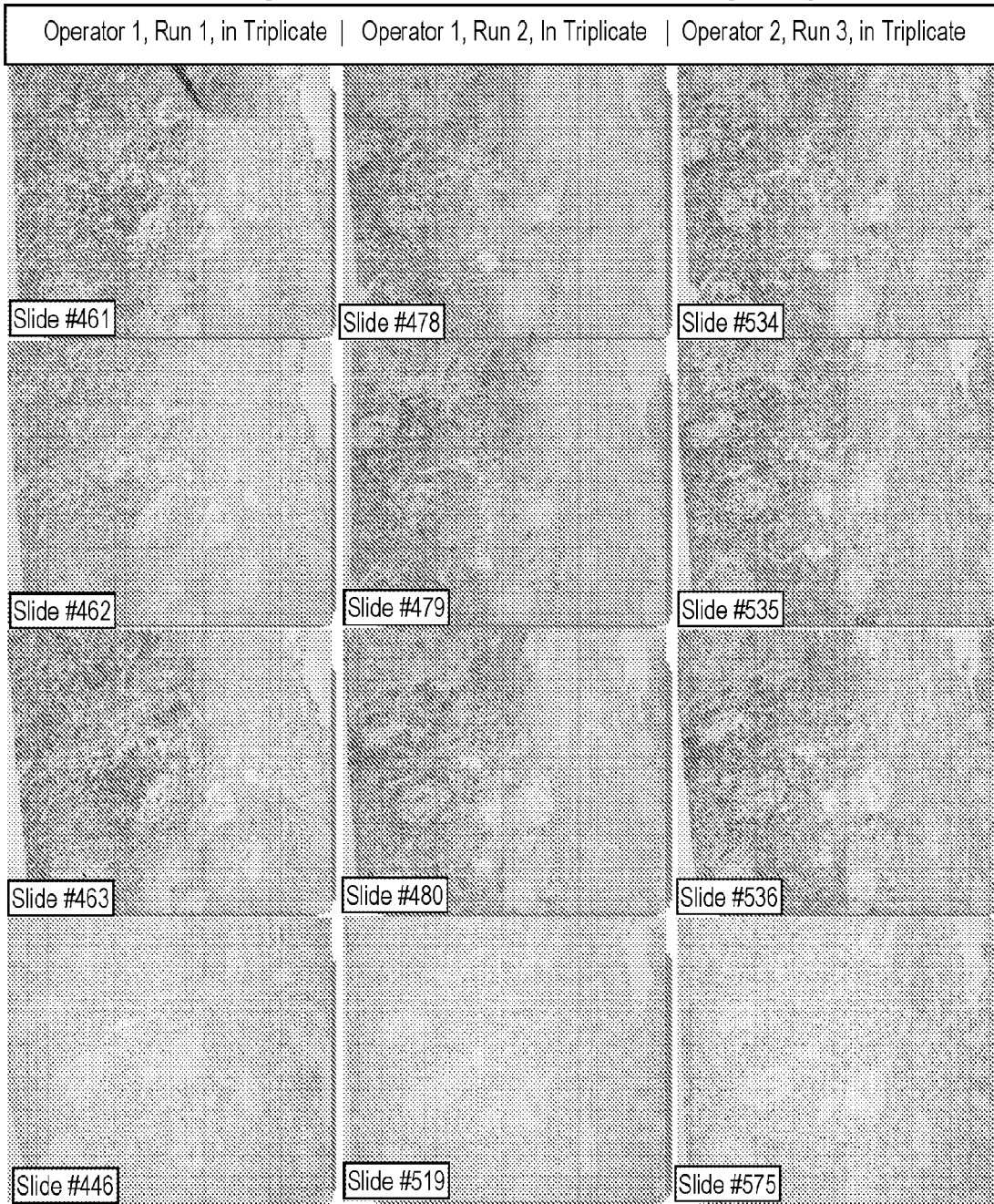


FIG. 28

Ovarian Cancer Q7499-02, Top Three Rows: ROR2, Bottom Row: Mouse IgG1 Negative Control, 20X

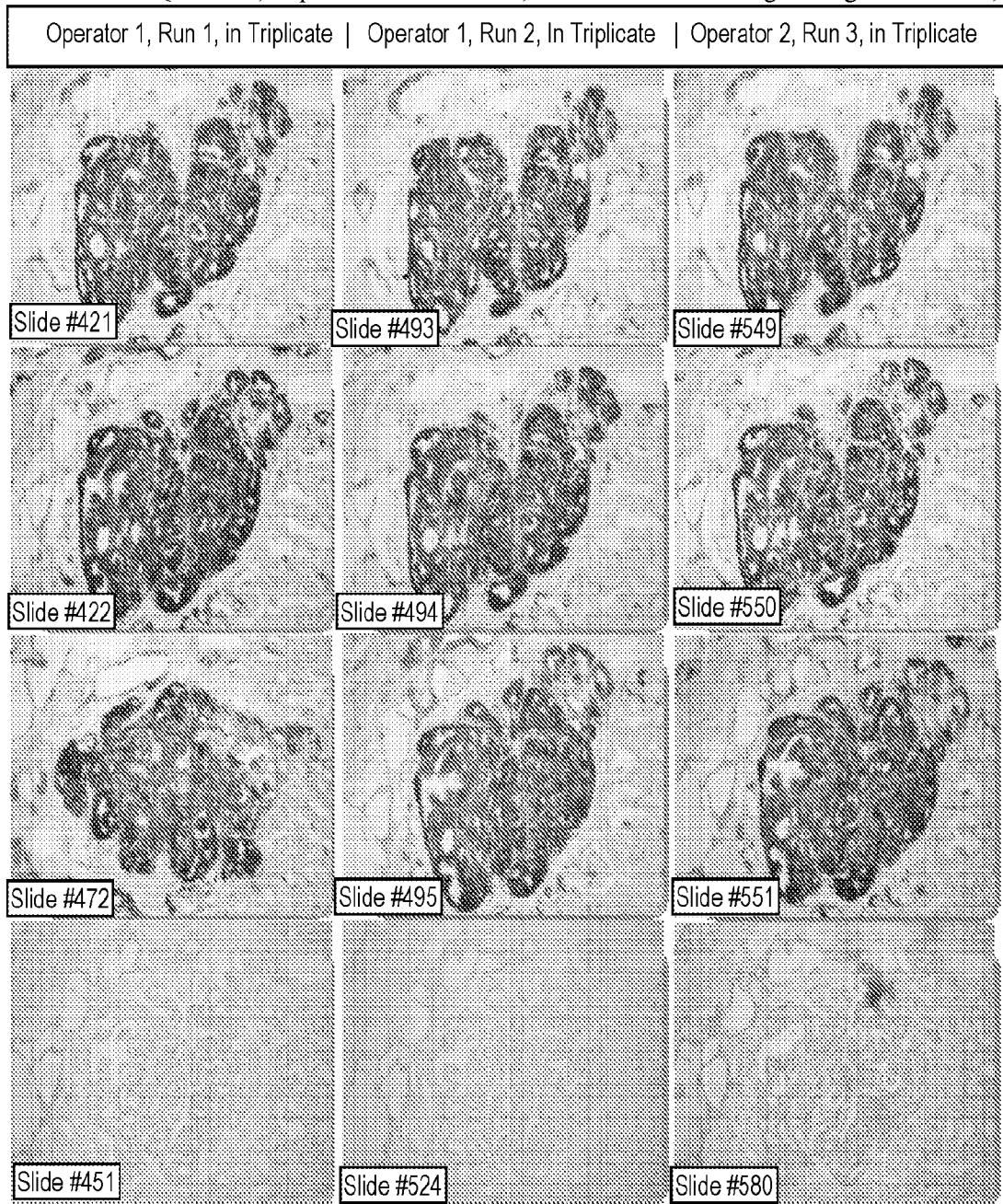


FIG. 29

Ovarian Cancer QMTB400-05, Top Three Rows: ROR2, Bottom Row: Mouse IgG1 Negative Control, 20X

Operator 1, Run 1, in Triplicate | Operator 1, Run 2, In Triplicate | Operator 2, Run 3, in Triplicate

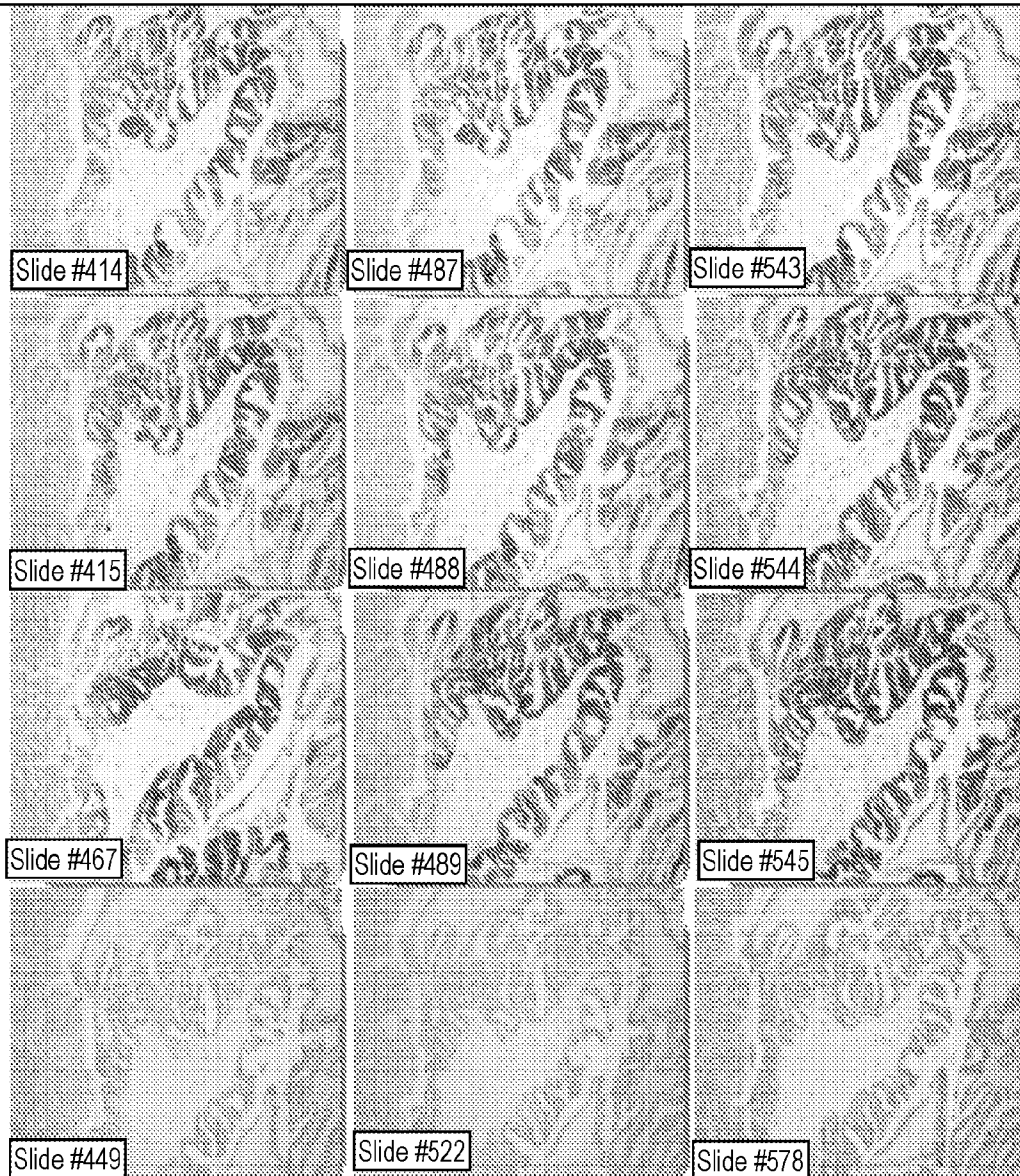


FIG. 30

NSCLC Q6949-01, Top Three Rows: ROR2, Bottom Row: Mouse IgG1 Negative Control, 20X

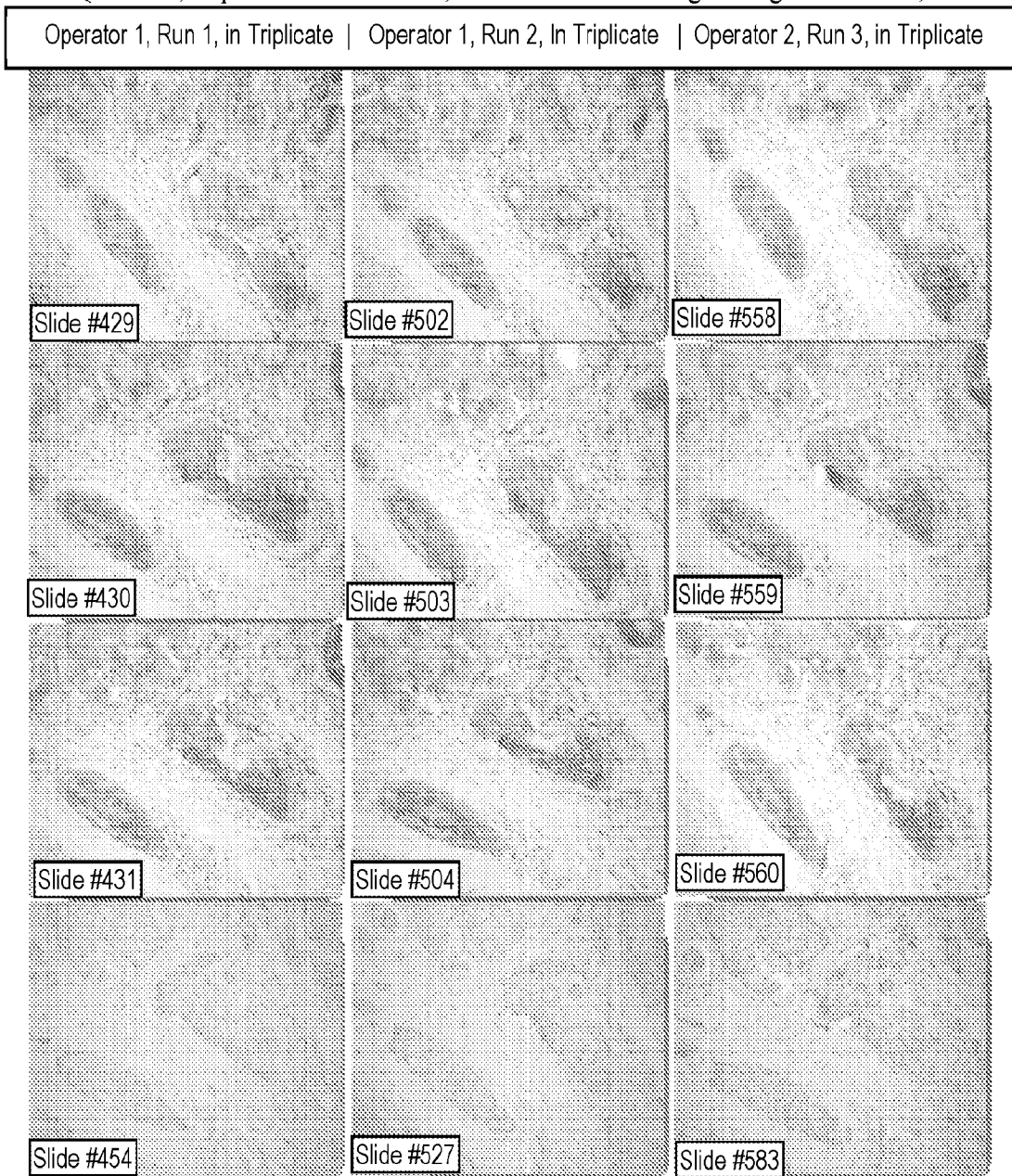


FIG. 31

SCLC Q4044-04, Top Three Rows: ROR2, Bottom Row: Mouse IgG1 Negative Control, 20X

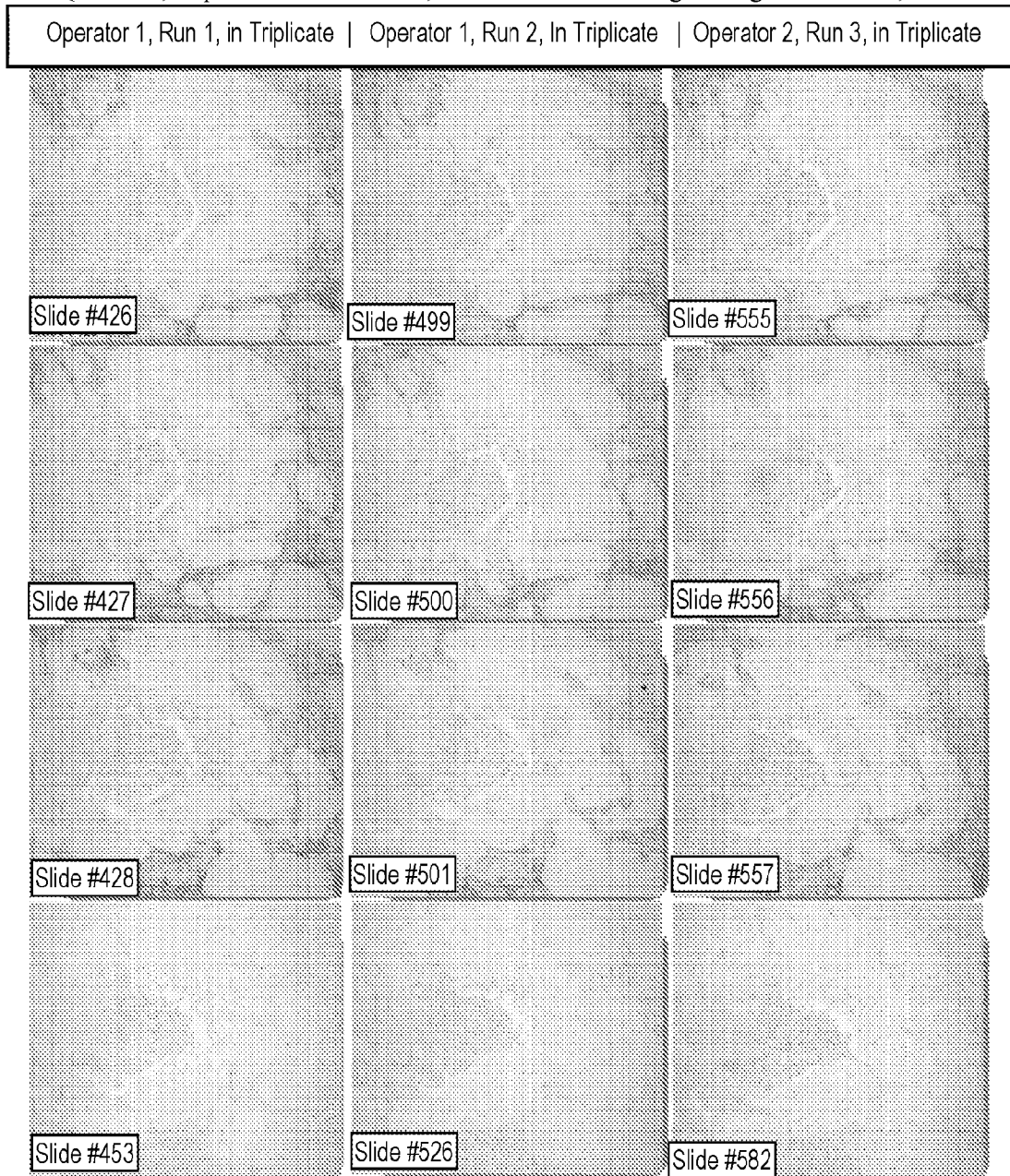


FIG. 32

TNBC Q9333, Top Three Rows: ROR2, Bottom Row: Mouse IgG1 Negative Control, 20X

Operator 1, Run 1, in Triplicate | Operator 1, Run 2, In Triplicate | Operator 2, Run 3, in Triplicate

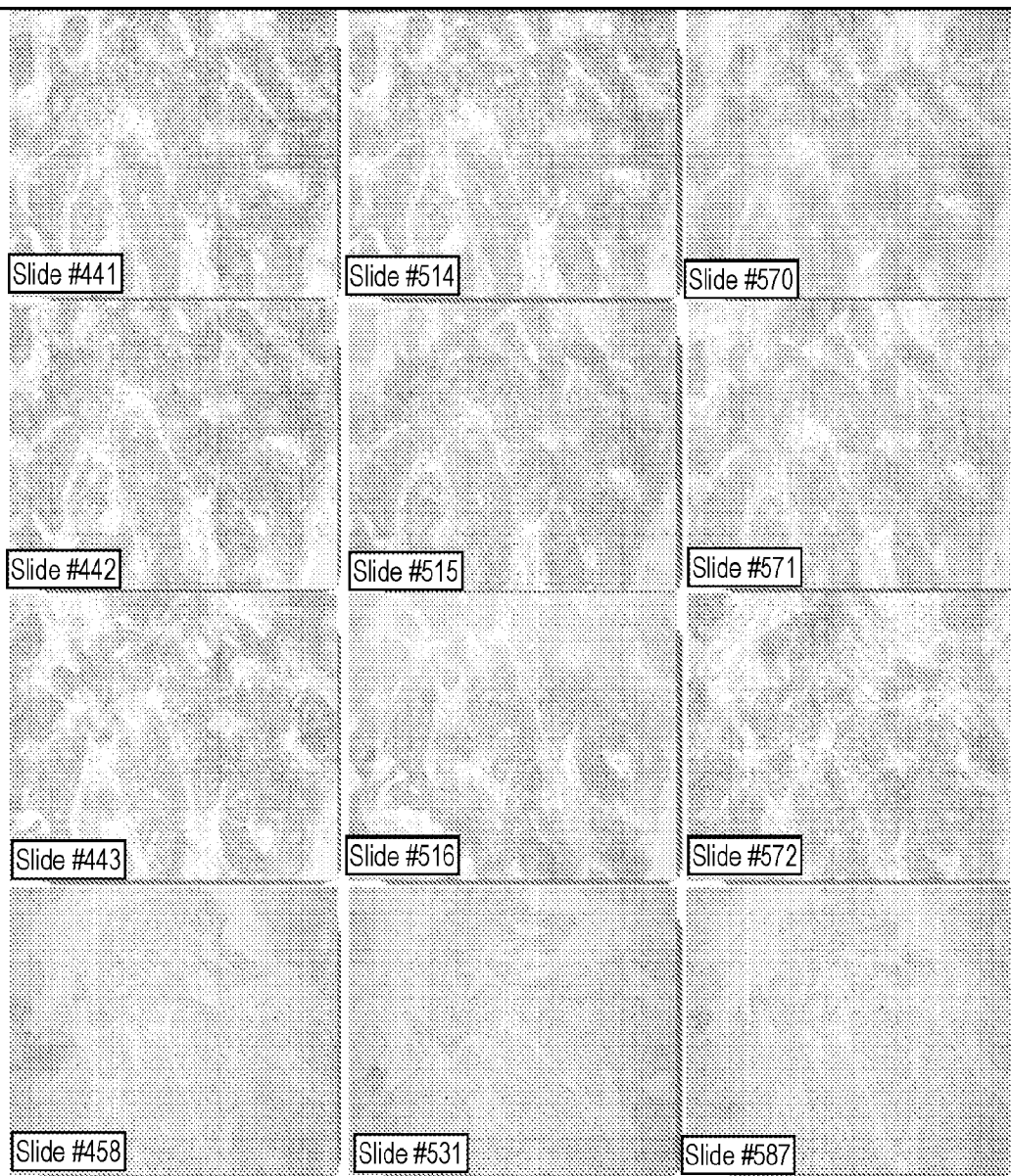
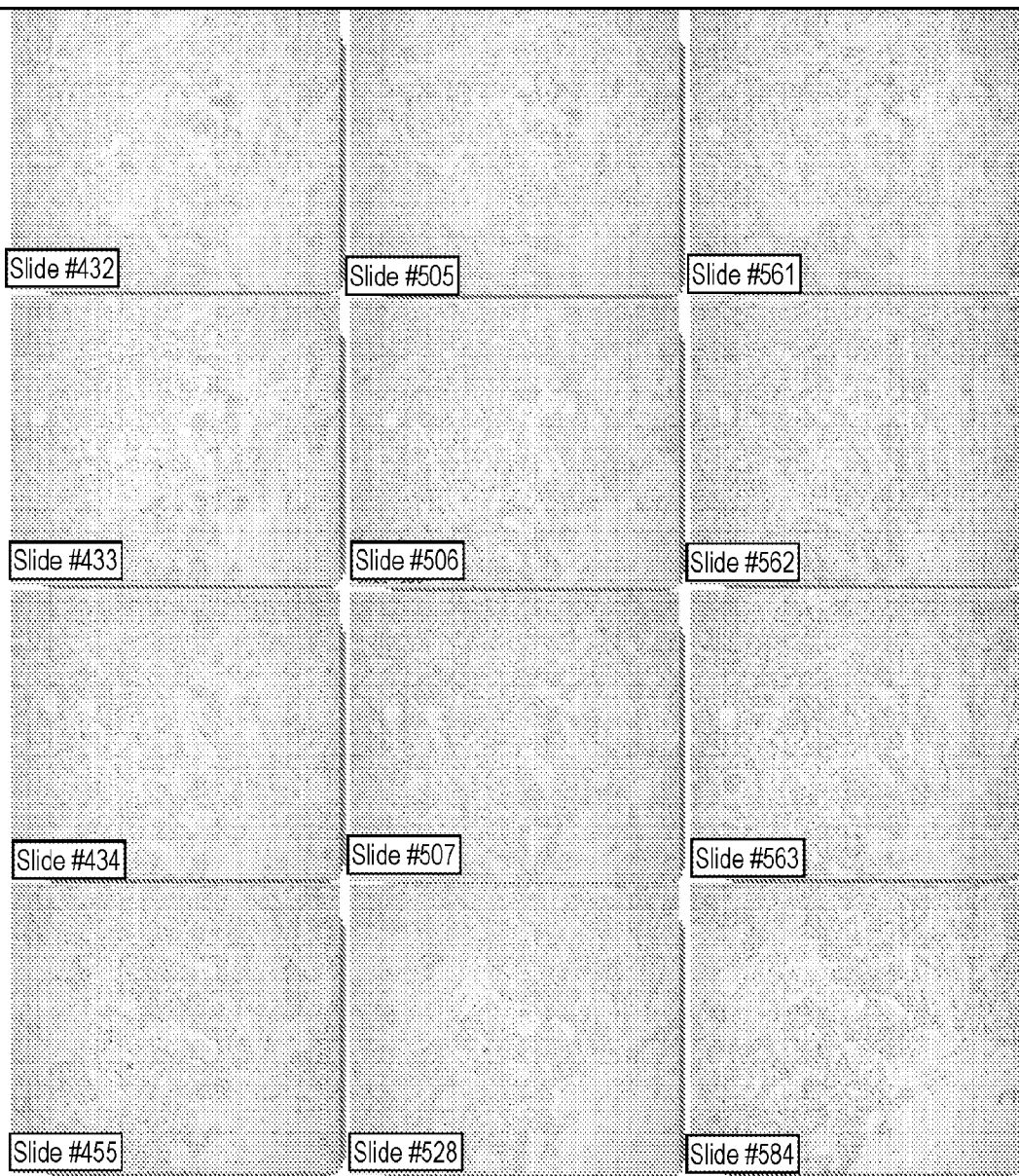


FIG. 33

TNBC Q9250, Top Three Rows: ROR2, Bottom Row: Mouse IgG1 Negative Control, 20X

Operator 1, Run 1, in Triplicate | Operator 1, Run 2, In Triplicate | Operator 2, Run 3, in Triplicate



Days 1 and 8 every 21-days
cycles ongoing

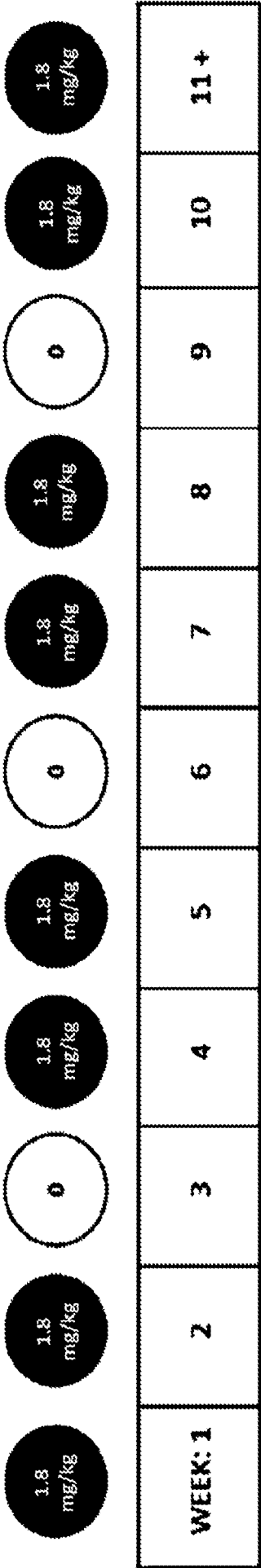


FIG. 34

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/063226

A. CLASSIFICATION OF SUBJECT MATTER C07K 16/28(2006.01)i; A61K 47/68(2017.01)i; A61P 35/00(2006.01)i 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 16/28(2006.01); A61K 47/68(2017.01); C12Q 1/68(2006.01); G01N 33/574(2006.01) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Korean utility models and applications for utility models Japanese utility models and applications for utility models Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) eKOMPASS(KIPO internal) & Keywords: ROR2(receptor tyrosine kinase-like orphan receptor 2), heavy chain variable region, light chain variable region, antibody, cancer		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017-197234 A1 (BIOATLA, LLC) 16 November 2017 (2017-11-16) abstract; example 2; paragraphs [0318]-[0320]; claims 1-25	34-37
A	WO 2016-142768 A1 (EUREKA THERAPEUTICS, INC. et al.) 15 September 2016 (2016-09-15) abstract; claims 1-39	34-37
A	WO 2021-102055 A1 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 27 May 2021 abstract; claims 1-86	34-37
A	US 2019-0031754 A1 (THE SCRIPPS RESEARCH INSTITUTE) 31 January 2019 (2019-01-31) abstract; claims 1-42	34-37
A	US 2014-0322234 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 30 October 2014 (2014-10-30) abstract; claims 1-24	34-37
☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "D" document cited by the applicant in the international application "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 15 June 2023		Date of mailing of the international search report 15 June 2023
Name and mailing address of the ISA/KR Korean Intellectual Property Office 189 Cheongsa-ro, Seo-gu, Daejeon 35208, Republic of Korea Facsimile No. +82-42-481-8578		Authorized officer HEO, Joo Hyung Telephone No. +82-42-481-5373

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/063226

Box No. I **Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13ter.1(a)),
☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/063226**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

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

1. ☒ Claims Nos.: **1-33**
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1-33 are directed to a treatment method of the human body by therapy or surgery and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.
2. ☒ Claims Nos.: **11-13,17,23,26,41-43,47,53,56**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Claims 11-13, 17, 23, 26, 41-43, 47, 53, 56 are regarded to be unclear because they refer to claims which do not comply with PCT Rule 6.4(a).
3. ☒ Claims Nos.: **5-10,14-16,18-22,24-25,27-33,38-40,44-46,48-52,54-55,57-61,61(1)-63**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US2023/063226

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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