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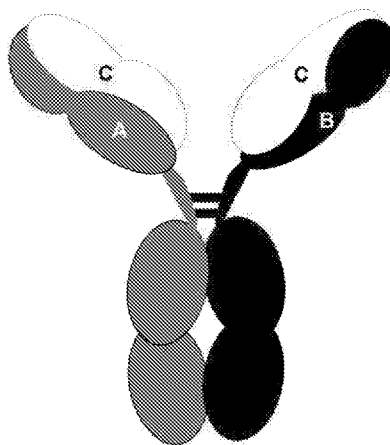
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FIG. 2



(57) Abstract: Provided are multi-specific antibodies that target both a cellular efflux pump and a cancer-associated antigen as well as pharmaceutical compositions, nucleic acids, recombinant expression vectors, cells, and kits that include or encode such multi-specific antibodies. Methods of treating a subject for a cancer that include administering to the subject a multi-specific antibody that targets both a cellular efflux pump and a cancer-associated antigen are also provided. Provided as well are methods of generating the described multi-specific antibodies and reagents related thereto, including genetically modified cell lines useful in the subject methods and methods of making such genetically modified cell lines.



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EFFLUX PUMP-CANCER ANTIGEN MULTI-SPECIFIC ANTIBODIES AND COMPOSITIONS, REAGENTS,
KITS AND METHODS RELATED THERETO

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority to U.S. Provisional Patent Application No. 62/967,478 filed on January 29, 2020 and to U.S. Provisional Patent Application No. 62/828,044 filed on April 2, 2019, which applications are incorporated herein by reference in their entirety.

INTRODUCTION

Drug resistance, a well-known phenomenon that results when diseases become tolerant to pharmaceutical treatments, is a major and increasing challenge in various fields of medicine, including oncology. Some methods of drug resistance are disease-specific, while others, such as drug efflux, which is observed in microbes and human drug-resistant cancers, are evolutionarily conserved. Although many types of cancers are initially susceptible to chemotherapy, over time they can develop resistance through these and other mechanisms, such as DNA mutations and metabolic changes that promote drug inhibition, degradation and enhanced efflux.

Efflux pumps (EP) are proteins expressed by virtually all living cells and have evolved to naturally expel various compounds from the cells. Members of the ATP-binding cassette (ABC) transporter family proteins are examples of EPs that enable drug efflux and are important, well-studied regulators at the plasma membranes of healthy cells. Though a transporter's structure varies from protein to protein (e.g., there are 49 known members of the ABC family in humans), they are all classified by the presence of two distinct domains—a highly conserved nucleotide binding domain and a more variable transmembrane domain. Multidrug resistance protein 1, encoded by the ATP Binding Cassette Subfamily B Member 1 (ABCB1) gene, was the first of these to be identified and has been studied extensively. Normal expression of MDR1 is increased in certain tissues (e.g., colon, liver, and kidney) when these tissues become neoplastic and increased expression in response to treatment with certain chemotherapeutics demonstrates that both intrinsic and extrinsic mechanisms of MDR1 overexpression are at play.

EPs enable cells and tumors to develop resistance to chemotherapeutic agents. Such resistance is frequently associated with enhanced efflux of the treatment molecules from the resistant cells. This chemotherapy resistance is termed multi drug resistance (MDR) when it applies to more than one chemotherapeutic agent. Various small molecule inhibitors have

been developed that target and inhibit EPs but none have been successful in the human clinical setting for a variety of reasons among which is their tendency to penetrate into and affect all cells in the body, including healthy cells that employ EPs for efflux of naturally occurring cellular toxins, regardless of the function of the cells or their efflux pumps.

Among cancer patients, where metastatic cancer cell populations have largely been killed and cleared from the patients by use of chemotherapy, it is not uncommon for a drug-resistant cancerous population of cells to emerge and spread without response to renewed treatment with the earlier therapy. In most cases, a different drug with a different mechanism of action is applied until, once again, another emergent population of drug-resistant cells and/or tumor develops.

SUMMARY

Provided are anti-MDR1 antibodies that may be used as multi-specific antibodies that target both MDR1 and a tumor-associated antigen (TAA) as well as pharmaceutical compositions, nucleic acids, recombinant expression vectors, cells, and kits that include or encode such multi-specific antibodies. The multi-specific antibodies include a common variable light (VL) chain that includes an antigen-binding site for MDR1, a first variable heavy (VH) chain that includes an antigen-binding site for MDR1, and a second VH chain that includes an antigen-binding site for the TAA. Methods of treating a subject for a cancer that include administering to the subject a multi-specific antibody that targets both MDR1 and a TAA are also provided. The treating may involve administering the multi-specific antibody alone or administering the multi-specific antibody and a chemotherapeutic agent. Provided as well are methods of generating the described multi-specific antibodies and reagents related thereto, including genetically modified cell lines useful in the subject methods and methods of making such genetically modified cell lines.

The bispecific antibodies provided herein bind to cancer cells expressing both MDR1 and the TAA while showing reduced binding to non-cancer cells expressing MDR1 and/or the TAA. In other words, the bispecific antibodies provided herein bind with low affinity to (1) cells expressing TAA where MDR1 expression is low or absent and (2) cells expressing MDR1 where TAA expression is low or absent, and with high affinity to cancer cells that express at least one or both MDR1 and CD47 at relatively high levels, i.e., levels higher than normal cells.

Also disclosed herein are anti-MDR1 antibodies that have reduced affinity for MDR1 as compared to the anti-MDR1 antibody 15D3 and anti-CD47 antibodies that have reduced affinity for CD47 as compared to the anti-CD47 antibody 5F9. These antibodies find use in treatment of cancer in a subject when co-administered and to increase chemosensitivity of cancer cells to chemotherapy.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1A provides a schematic depiction of an embodiment of a multi-specific antibody of the present disclosure as described herein.

FIG. 1B provides a schematic depiction showing binding of the embodiment of a multi-specific antibody depicted in FIG. 1A to cells expressing one or both of the targets to which the multi-specific antibody is targeted.

FIG. 2 provides a schematic of a bispecific monoclonal antibody as described herein.

FIG. 3A-3F provide FACS analysis of binding of labeled monoclonal antibodies against Pgp and CD47 (also referred to in some instances as “KT14”) on various cell lines, including naïve and recombinant cell lines expressing the targets as indicated.

FIG. 4A-4B provide FACS analysis of binding of bispecific antibody (HC-15D3:HC-5F9:LC-MRK16) binding to a variety of reagent cell lines indicating specific binding when both targets are co-expressed (e.g., 293T naïve cells) and significantly reduced binding when only one of the targets is present (e.g., 293 KT14-KO and KPB1-KD (Pgp is also referred to in some instances herein as “KPB1”)).

FIG. 5 provides chemosensitivity analysis showing naïve 293T and N6ADR cell line susceptibility to paclitaxel in the presence of various monovalent, Fab and bispecific antibody molecules described herein. The cells (naïve 293T cells (left panel) and N6ADR cells (right panel)) showed enhanced sensitivity in the presence of the 15D3.aCD47:MRK16 bispecific, e.g., as compared to treatment with chemotherapy only (“Chemo only”) or chemotherapy in combination with the other antibodies (15D3:MRK16 Fab; a15D3:MRK16 antibody; aCD47:MRK16 antibody; aCD47:MRK16 Fab; and 15D3:9F11 Fab). FIG. 5 demonstrates that in spite of the decreased affinity of the bispecific antibody for each individual target, the bispecific antibody enhances chemosensitivity of naïve 293T cells and Adriamycin resistant N6ADR cells.

FIG. 6A-6C provide re-representations of the data provided in FIG. 5, including side-by-side fitted curves of the naïve 293T cell and N6ADR cell data (FIG. 6A) and side-by-side depictions of the raw (left) and fitted (right) data for naïve 293T cells (FIG. 6B) and N6ADR cells (FIG. 6C).

FIG. 7 demonstrates a drug sensitizing effect of the 15D3.aCD47:MRK16 antibody when the antibody was used in combination with vincristine.

FIG. 8 provides a table showing binding and target cell killing of antibody light and heavy chain combinations tested, as described in the Examples section.

FIG. 9 shows effect of the indicated antibodies, without chemotherapy, on MES-SA-DX5 (Uterine sarcoma) tumor volume.

FIG. 10 shows effect of paclitaxel on tumor growth inhibition of NALM6 cells.

FIG. 11 shows effect of BisP1.1 on tumor growth inhibition of paclitaxel resistant NALM6ADR cells.

FIG. 12 shows effect of valspodar on tumor growth inhibition of paclitaxel resistant NALM6ADR cells.

FIG. 13 shows effect of BisP1.1 on body weight of mice implanted with paclitaxel resistant NALM6ADR cells.

FIG. 14 shows effect of valspodar on body weight of mice implanted with paclitaxel resistant NALM6ADR cells.

FIG. 15 shows effect of KBisP1.1 on survival of mice implanted with paclitaxel resistant NALM6ADR cells.

FIG. 16 shows expression of CD47 and ABCB1 on surface of A2780ADR cells.

FIG. 17 shows expression of CD47 and ABCB1 on surface of HeyT30 cells.

FIG. 18 shows reduction in A2780ADR tumor volume in vivo following administration of KBisP1.1 with or without Paclitaxel.

FIG. 19 shows reduction in HeyT30 tumor volume in vivo following administration of KBisP1.1 with or without Paclitaxel.

FIG. 20 shows that KbisP1.1 is more efficacious than the 15D3 antibody against N6ADR cell tumors *in vivo*.

Fig. 21 shows KbisP1.1 chemo-sensitization of multidrug resistant N6ADR tumor to Paclitaxel is dose-dependent, *in-vivo*.

FIGS. 22A-22B show that KBisP1.1 antibody exhibits no significant binding to human red blood cells (RBCs) (Fig. 22A) and cynomolgus RBCs (Fig. 22B) in contrast to the anti-CD47 5F9 antibody and that an anti-CD47 antibody comprising 5F9 HCs and MRK16 LCs ("KT14_MRK16") does not significantly bind to RBCs in contrast to the anti-CD47 5F9 antibody ("KT14").

FIG. 23 shows alignment of humanized 15D3 VH chain sequences.

FIG. 24 shows alignment of humanized MRKK16 chain sequences.

FIG. 25 shows humanized, muted Fc bispecific antibody chemosensitizes multidrug resistant MES-SA-DX5 tumor.

FIG. 26 shows that humanized KBisP1.1 antibody chemosensitizes multidrug resistant A2780ADR cell-generated tumors in mice.

FIG. 27A shows that the KBisP1.1 bispecific antibody with N297A substitution chemosensitizes multidrug resistant MES-SA/Dx5 cell tumors to paclitaxel in mice.

FIG. 27B shows that the KBisP1.1 N297A antibody (labeled as "N297A") chemosensitizes multidrug resistant A2780ADR cells to paclitaxel in vivo.

FIG. 28 shows that the KBisP1.1 antibody chemosensitizes multidrug resistant A2780ADR cells to paclitaxel in vivo.

FIG. 29 shows that the KBisP1.1 antibody chemosensitizes multidrug resistant MES-SA/Dx5 cells to paclitaxel in vivo.

FIG. 30 shows that the KBisP1.1 bispecific antibody chemosensitizes paclitaxel resistant CTG-2616 patient-derived breast tumors and resulted in tumor growth inhibition in vivo.

FIG. 31 provides a summary of binding of the indicated antibodies to CD47 measured by ELISA, to MDR1 measured by FACS, to PD-L1 measured by ELISA, and to EGFR, measured by ELISA or FACS.

FIG. 32A shows MFI for the listed antibodies measured using 293T cells overexpressing MDR1.

FIG. 32B shows MFI for the listed antibodies measured using 293T cells overexpressing EGFR.

FIG. 32C shows MFI for the listed antibodies measured using 293T cells overexpressing EGFR for a subset of antibodies show in Fig. 32B.

DEFINITIONS

The terms "antibodies" and "immunoglobulin" include antibodies or immunoglobulins of any isotype, fragments of antibodies which retain specific binding to antigen, including, but not limited to, Fab, Fv, scFv, and Fd fragments, chimeric antibodies, humanized antibodies, single-chain antibodies, including antibodies comprising only heavy chains (e.g. VHH camelid antibodies), bispecific antibodies, and fusion proteins comprising an antigen-binding portion of an antibody and a non-antibody protein. The antibodies may be detectably labeled, *e.g.*, with a radioisotope, an enzyme which generates a detectable product, a fluorescent protein, and the like. The antibodies may be further conjugated to other moieties, such as members of specific binding pairs, *e.g.*, biotin (member of biotin-avidin specific binding pair), and the like. The antibodies may also be bound to a solid support, including, but not limited to, polystyrene plates or beads, and the like. Also encompassed by the term are Fab', Fv, F(ab')₂, and or other antibody fragments that retain specific binding to antigen, and monoclonal antibodies. An antibody may be monovalent or bivalent. The antibodies used herein may be used to assay expression of a target antigens(s) on a cell surface, *e.g.*, in a cell sample or a tissue sample from a patient.

"Antibody fragments" comprise a portion of an intact antibody, for example, the antigen binding or variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies (Zapata et al., Protein Eng. 8(10): 1057-1062 (1995)); single-chain antibody molecules, including antibodies comprising only heavy chains (e.g. VHH camelid antibodies); and multispecific antibodies formed from

antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H - V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen and can form the antigen binding site, although at a lower affinity than the entire binding site comprising the three CDRs of each variable domain.

The "Fab" fragment also contains the constant domain of the light chain and the first constant domain (CH_1) of the heavy chain. Fab fragments differ from Fab' fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH_1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins can be assigned to different classes. There are five major classes of immunoglobulins: 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.

"Single-chain Fv", "sFv" or "scFv" antibody fragments comprise the V_H and V_L domains of antibody, wherein these domains are present in a single polypeptide chain. In some embodiments, the Fv polypeptide further comprises a polypeptide linker between the V_H and V_L domains, which enables the sFv to form the desired structure for antigen binding. For a review of sFv, see *Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994)*.

The term "diabodies" 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. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and *Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)*.

As used herein, the term "affinity" refers to the equilibrium constant for the reversible binding of two agents and is expressed as a dissociation constant (K_d). Affinity can be at least 1-fold greater, at least 2-fold greater, at least 3-fold greater, at least 4-fold greater, at least 5-fold greater, at least 6-fold greater, at least 7-fold greater, at least 8-fold greater, at least 9-fold greater, at least 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 60-fold greater, at least 70-fold greater, at least 80-fold greater, at least 90-fold greater, at least 100-fold greater, or at least 1000-fold greater, or more, than the affinity of an antibody for unrelated amino acid sequences. Affinity of an antibody to a target protein can be, for example, from about 100 nanomolar (nM) to about 0.1 nM, from about 100 nM to about 1 picomolar (pM), or from about 100 nM to about 1 femtomolar (fM) or more. As used herein, the term "avidity" refers to the resistance of a complex of two or more agents to dissociation after dilution. The terms "immunoreactive" and "preferentially binds" are used interchangeably herein with respect to antibodies and/or antigen-binding fragments.

The term "binding" refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. A MDR1-specific antibody binds specifically to an epitope within a MDR1 polypeptide. Non-specific binding would refer to binding with an affinity of less than about 10⁻⁷ M, e.g., binding with an affinity of 10⁻⁶ M, 10⁻⁵ M, 10⁻⁴ M, etc.

As used herein, the term "CDR" or "complementarity determining region" is intended to mean the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. CDRs have been described by Kabat et al., *J. Biol. Chem.* 252:6609-6616 (1977); Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991); by Chothia et al., *J. Mol. Biol.* 196:901-917 (1987); and MacCallum et al., *J. Mol. Biol.* 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table 1 as a comparison.

Table 1: CDR Definitions

	Kabat ¹	Chothia ²	MacCallum ³
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V _H CDR1	31-35	26-32	30-35
V _H CDR2	50-65	53-55	47-58
V _H CDR3	95-102	96-101	93-101
V _L CDR1	24-34	26-32	30-36
V _L CDR2	50-56	50-52	46-55
V _L CDR3	89-97	91-96	89-96

- 1 Residue numbering follows the nomenclature of Kabat et al., *supra*
- 2 Residue numbering follows the nomenclature of Chothia et al., *supra*
- 3 Residue numbering follows the nomenclature of MacCallum et al., *supra*

As used herein, the term “framework” when used in reference to an antibody variable region is intended to mean all amino acid residues outside the CDR regions within the variable region of an antibody. A variable region framework is generally a discontinuous amino acid sequence between about 100-120 amino acids in length but is intended to reference only those amino acids outside of the CDRs. As used herein, the term “framework region” is intended to mean each domain of the framework that is separated by the CDRs. A V_H chain can comprise three CDRs and four FRs arranged from N-terminus to C-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Similarly, a V_L chain can comprise three CDRs and four FRs arranged from N-terminus to C-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

As used herein the term antibody encompasses a tetramer of two heavy and two light chains, wherein the heavy and light chains are interconnected by, for example, disulphide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable regions of the heavy and light chains comprise binding regions that interact with antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues and factors, including various cells of the immune system and the first component of the complement system. The term "antibody" includes immunoglobulins of types IgA, IgG, IgE, IgD, IgM and subtypes thereof. In some embodiments, a subject antibody is an IgG isotype, e.g., IgG1.

As used herein the term "immunoglobulin" refers to a protein including one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes; and numerous immunoglobulin variable region genes. Full-length immunoglobulin light chains (about 25 kD or 214 amino acids) are encoded by a variable region gene at the N-terminus (about 110 amino acids) and a kappa or lambda constant region at the C-terminus. Full-length immunoglobulin heavy chains (about 50 kD or 446 amino acids) are encoded by a variable region gene at the N-terminus (about 116 amino acids) and one of the other aforementioned

constant region genes at the C-terminus, e.g. gamma (encoding about 330 amino acids). In some embodiments, a subject antibody comprises a whole immunoglobulin comprising full-length immunoglobulin heavy chain and a full-length immunoglobulin light chain.

The term "antigen-binding fragment" refers to one or more fragments of a full-length antibody that are capable of specifically binding to an antigen. Examples of binding fragments include (i) a Fab fragment (a monovalent fragment including, e.g., consisting of, the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment (a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment (including, e.g., consisting of, the VH and CH1 domains); (iv) a Fv fragment (including, e.g., consisting of, the VH and VL domains of a single arm of an antibody); (v) a dAb fragment (including, e.g., consisting of, the VH domain); (vi) an isolated CDR; (vii) a single chain Fv (scFv) (including, e.g., consisting of, the VH and VL domains of a single arm of an antibody joined by a synthetic linker using recombinant means such that the VH and VL domains pair to form a monovalent molecule); (viii) diabodies (including, e.g., consisting of, two scFvs in which the VH and VL domains are joined such that they do not pair to form a monovalent molecule; the VH of each one of the scFv pairs with the VL domain of the other scFv to form a bivalent molecule).

The term "chimeric" antibody 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.

A "human antibody" 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.

A "human consensus framework" is a framework (FR) which represents the most commonly occurring amino acid residues in a selection of human immunoglobulin variable light chain (VL) or variable heavy chain (VH) framework sequences. Generally, the selection of human immunoglobulin VL or VH 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 VL, the subgroup is subgroup kappa I as in Kabat et al., *supra*. In one embodiment, for the VH, the subgroup is subgroup III as in Kabat et al., *supra*.

A "humanized" antibody refers to a chimeric antibody comprising amino acid residues from non-human CDRs and amino acid residues from human frameworks (FRs). At least a portion of a humanized antibody constant region derived from a human antibody. In preferred embodiments of the antibody molecules disclosed herein, the constant region is from a human

IgG antibody, such as, human IgG1. In preferred embodiments, the antibody molecules disclosed herein include a heavy chain comprising a variable heavy chain region as provided herein and a human IgG1 constant region having the amino acid sequence set forth in UniProt: P01857-1, version 1. In preferred embodiments, the antibody molecules disclosed herein include a light chain comprising a variable light chain region as provided herein and a human light chain constant region. In preferred embodiments, the human light chain constant region is a human kappa light chain constant region. In certain aspects, the human IgG1 heavy chain constant region present in the subject antibodies may include mutations, e.g., substitutions to modulate Fc function. For example, the LALAPG effector function mutations (L234A, L235A, and P329G) or the N297A mutation may be introduced to reduce antibody dependent cellular cytotoxicity (ADCC). The numbering of the substitutions is based on the EU numbering system. The "EU numbering system" or "EU index" is generally used when referring to a residue in an immunoglobulin heavy chain constant region (e.g., the EU index reported in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). The "EU index as in Kabat" refers to the residue numbering of the human IgG 1 EU antibody.

A "humanized form" of an antibody, e.g., a non-human antibody, refers to an antibody that has undergone humanization.

The term "epitope" refers to a region of an antigen that is recognized by the immune system, for example by antibodies, B cells, or T cells. For example, the epitope is the specific region of the antigen to which an antibody binds.

An "isolated" antibody is one that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, the antibody will be purified (1) to greater than 90%, greater than 95%, or greater than 98%, by weight of antibody as determined by the Lowry method, for example, more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing or nonreducing conditions using Coomassie blue or silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. In some instances, isolated antibody will be prepared by at least one purification step.

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. A "chemotherapeutic agent," also

referred to an "antineoplastic agent," can be a cytotoxic agent which is used for treating a cancer or other disease or disorder.

As used herein, the terms "treatment," "treating," and the like, refer to obtaining a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a disease and/or adverse effect attributable to the disease. "Treatment," as used herein, covers any treatment of a disease in a mammal, including in a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., causing regression of the disease.

The terms "individual," "subject," "host," and "patient," used interchangeably herein, refer to a mammal, including, but not limited to, murines (rats, mice), non-human primates, humans, canines, felines, ungulates (e.g., equines, bovines, ovines, porcines, caprines), etc.

A "therapeutically effective amount" or "efficacious amount" refers to the amount of a target-specific antibody that, when administered to a mammal or other subject for treating a disease, is sufficient to affect such treatment for the disease. The "therapeutically effective amount" will vary depending on the antibody, the disease and its severity and the age, weight, etc., of the subject to be treated.

The term "refractory", used herein, refers to a disease or condition that does not respond to treatment. With regard to cancer, "refractory cancer", as used herein, refers to cancer that does not respond to treatment. A refractory cancer may be resistant at the beginning of treatment or it may become resistant during treatment. Refractory cancer may also be called resistant cancer.

A "biological sample" encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as polynucleotides. The term "biological sample" encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples.

Percent identity between a pair of sequences may be calculated by multiplying the number of matches in the pair by 100 and dividing by the length of the aligned region, including gaps. Identity scoring only counts perfect matches and does not consider the degree of

similarity of amino acids to one another. Only internal gaps are included in the length, not gaps at the sequence ends. Percent Identity = (Matches x 100)/Length of aligned region (with gaps)

The phrase “conservative amino acid substitution” refers to substitution of amino acid residues within the following groups: 1) L, I, M, V, F; 2) R, K; 3) F, Y, H, W, R; 4) G, A, T, S; 5) Q, N; and 6) D, E. Conservative amino acid substitutions may preserve the activity of the protein by replacing an amino acid(s) in the protein with an amino acid with a side chain of similar acidity, basicity, charge, polarity, or size of the side chain.

The term “vector” means any molecule or entity (e.g., nucleic acid, plasmid, bacteriophage or virus) used to transfer protein coding information into a host cell.

The term “expression vector” or “expression construct” refers to a vector that is suitable for transformation of a host cell and contains nucleic acid sequences that direct and/or control (in conjunction with the host cell) expression of one or more heterologous coding regions operatively linked thereto. An expression construct may include, but is not limited to, sequences that affect or control transcription, translation, and, if introns are present, affect RNA splicing of a coding region operably linked thereto.

The term “stimulation,” refers to a primary response induced by binding of a stimulatory molecule (e.g., a TCR/CD3 complex or CAR) with its cognate ligand (or tumor antigen in the case of a CAR) thereby mediating a signal transduction event, such as, but not limited to, signal transduction via the TCR/CD3 complex or signal transduction via the appropriate NK receptor or signaling domains of the CAR. Stimulation can mediate altered expression of certain molecules.

The term “stimulatory molecule,” refers to a molecule expressed by an immune cell (e.g., T cell, NK cell, B cell) that provides the cytoplasmic signaling sequence(s) that regulate activation of the immune cell in a stimulatory way for at least some aspect of the immune cell signaling pathway. In one aspect, the signal is a primary signal that is initiated by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, and which leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A primary cytoplasmic signaling sequence (also referred to as a “primary signaling domain”) that acts in a stimulatory manner may contain a signaling motif which is known as immunoreceptor tyrosine-based activation motif or ITAM. Examples of an ITAM containing cytoplasmic signaling sequence that is of particular use in the invention includes, but is not limited to, those derived from CD3 zeta, common FcR gamma (FCER1G), Fc gamma RIIa, FcR beta (Fc Epsilon R1b), CD3 gamma, CD3 delta, CD3 epsilon, CD79a, CD79b, DAP10, and DAP12.

The term a “costimulatory molecule” refers to a cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules are cell surface

molecules other than antigen receptors or their ligands that are contribute to an efficient immune response. Costimulatory molecules include, but are not limited to an MHC class I molecule, BTLA and a Toll ligand receptor, as well as OX40, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), and 4-1BB (CD137).

The term “autologous” refers to any material derived from the same individual to whom it is later to be re-introduced into the individual.

An “intracellular signaling domain,” as the term is used herein, refers to an intracellular portion of a molecule. The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CAR-T cell. Examples of immune effector function, e.g., in a CAR-T cell, include cytolytic activity and helper activity, including the secretion of cytokines.

“Immune effector cell,” as that term is used herein, refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes.

Guidance for substitutions, insertions, or deletions may be based on alignments of amino acid sequences of proteins from different species or from a consensus sequence based on a plurality of proteins having the same or similar function.

DETAILED DESCRIPTION

Provided are anti-MDR1 antibodies that may be used as multi-specific antibodies that target both MDR1 and a tumor-associated antigen (TAA) as well as pharmaceutical compositions, nucleic acids, recombinant expression vectors, cells, and kits that include or encode such multi-specific antibodies. The multi-specific antibodies include a common variable light (VL) chain that includes an antigen-binding site for MDR1, a first variable heavy (VH) chain that includes an antigen-binding site for MDR1, and a second VH chain that includes an antigen-binding site for the TAA. Methods of treating a subject for a cancer that include administering to the subject a multi-specific antibody that targets both MDR1 and a TAA are also provided. The treating may involve administering the multi-specific antibody alone or administering the multi-specific antibody and a chemotherapeutic agent. Provided as well are methods of generating the described multi-specific antibodies and reagents related thereto, including genetically modified cell lines useful in the subject methods and methods of making such genetically modified cell lines.

The bispecific antibodies provided herein bind to cancer cells expressing both MDR1 and the TAA while showing reduced binding to non-cancer cells expressing MDR1 and/or the

TAA. In other words, the bispecific antibodies provided herein bind with low affinity to (1) cells expressing TAA where MDR1 expression is low or absent and (2) cells expressing MDR1 where TAA expression is low or absent, and with high affinity to cancer cells that express at least one or both MDR1 and CD47 at relatively high levels, i.e., levels higher than normal cells.

Also disclosed herein are anti-MDR1 antibodies that have reduced affinity for MDR1 as compared to the anti-MDR1 antibody 15D3 and anti-CD47 antibodies that have reduced affinity for CD47 as compared to the anti-CD47 antibody 5F9. These antibodies find use in treatment of cancer in a subject when co-administered and to increase chemosensitivity of cancer cells to chemotherapy.

Before the present invention is described in greater detail, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Certain ranges are presented herein with numerical values being preceded by the term "about." The term "about" is used herein to provide literal support for the exact number that it precedes, as well as a number that is near to or approximately the number that the term precedes. In determining whether a number is near to or approximately a specifically recited number, the near or approximating unrecited number may be a number which, in the context in which it is presented, provides the substantial equivalent of the specifically recited number.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, representative illustrative methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and

describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present invention is not entitled to antedate such publication. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

It is noted that, as used herein and in the appended claims, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present invention. Any recited method can be carried out in the order of events recited or in any other order which is logically possible.

While the methods and compositions have or will be described for the sake of grammatical fluidity with functional explanations, it is to be expressly understood that the claims, unless expressly formulated under 35 U.S.C. §112(f), are not to be construed as necessarily limited in any way by the construction of “means” or “steps” limitations, but are to be accorded the full scope of the meaning and equivalents of the definition provided by the claims under the judicial doctrine of equivalents, and in the case where the claims are expressly formulated under 35 U.S.C. §112(f) are to be accorded full statutory equivalents under 35 U.S.C. §112(f).

ANTIBODIES

Bispecific Antibodies

The present disclosure provides a bispecific antibody molecule that binds multidrug resistance protein 1 (MDR1) and a tumor associated antigen (TAA), the antibody molecule comprising two identical variable light (VL) chains, a first variable heavy (VH) chain, and a second VH chain, where the VL chains each comprise an antigen-binding site for MDR1, the first VH chain comprises an antigen-binding site for MDR1, and the second VH chain comprises an antigen-binding site for the TAA, and wherein the second VH chain binds the TAA when paired with one of the light chains. The bispecific antibody molecule binds to cancer cells expressing both MDR1 and the TAA while showing reduced binding to non-cancer cells expressing MDR1 and/or the TAA. In other words, the bispecific antibodies provided herein

bind with low affinity to (1) cells expressing TAA where MDR1 expression is low or absent and (2) cells expressing MDR1 where TAA expression is low or absent, and with high affinity to cancer cells that express at least one or both MDR1 and CD47 at relatively high levels, i.e., levels higher than normal cells.

Relatively high levels refer to expression that is at least 1.5 times (e.g., at least 2X, at least 3X, at least 4X, at least 5X or more) the expression level in a normal cell of the same type as the cancer cell. Reduced affinity refers to binding affinity that is reduced by at least 10% (e.g. at least 20%, at least 30%, at least 40%, at least 50% or more) as compared to binding of the antibody molecule to a normal cell of the same type as the cancer cell. Reduced affinity also encompasses lack of detectable binding.

The term “antibody molecule” encompasses antibodies as defined herein and includes antigen-binding fragments thereof. In certain aspects, the antibody molecule includes two variable light (VL) and two variable heavy (VH) chain. In certain aspects, the antibody molecule includes heavy chain and light chain constant regions as well. The heavy and light chain constant regions may be from a human antibody, e.g., human IgG1 antibody. The human IgG1 heavy chain (HC) constant region may be modified to include mutations that reduce antibody dependent cellular cytotoxicity (ADCC). In addition, or alternatively, the two VH chains may each be conjugated to a different human IgG1 HC constant region where the individual human IgG1 HC constant region has substitutions that favour formation of dimers between the different human IgG1 HC constant regions. Such HC regions are described in further detail herein. In certain aspects, where the antibody molecule is a bispecific antibody molecule, one of the human IgG1 HC constant regions may include substitutions to introduce one or more amino acids having a positively-charged side chain and the other human IgG1 HC constant region may include substitutions to introduce one or more amino acids having a negatively-charged side chain to favour formation of dimers between the two different HCs.

In certain aspects, the antigen binding sites of the two VL chains comprise the light chain CDRs (LCDRs) of a VL chain having the sequence:

DVLMTQTPVSLSVSLGDAQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK, where X¹
is N, Q or S.

In certain aspects, the two VL chains comprise LCDRs 1-3 of the VL chain having the sequence:

- (i) DVLMTQTPVSLSVSLGDAQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKL
LIYKISNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTF
GGGKLEIK;

- (ii) DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPPRL
LIYKISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGYYCFQASHFPRTF
GQGTKLEIK; or
- (iii) DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPGQPPRL
LIYKISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGYYCFQASHFPRTF
GQGTKLEIK.

In some aspects, the antibody described herein does not comprise (i) two VL chains comprising LCDRs of the VL chain having the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPPRLLIYKISNRFSG
VPDRFSGSGAGTDFTLKISRVEAEDVGYYCFQASHFPRTFGQGTKLEIK; and a first VH
chain comprising HCDRs of the VH chain having the sequence:
EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGSTY
YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGLTVTS
S.

In certain aspects, the CDRs of VL and VH light chains may be defined based on the Kabat nomenclature.

In certain aspects, i) the LCDR1 comprises the sequence: RSSQSIVHSTGX¹TYLE;
(ii) the LCDR2 comprises the sequence: KISNRF; and (iii) the LCDR3 comprises the
sequence: FQASHFPRT; where X¹ is N, Q or S. These LCDRs are based on the Kabat
nomenclature.

In certain aspects, the two VL chains are humanized. In certain aspects, the two VL
chains are humanized to include framework regions from a human antibody.

In certain aspects, the two VL chains comprise a sequence that is at least 80%, at least
85%, at least 90%, at least 95%, or a 100% identical to the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGNTYLEWYQQRPGQPPRLLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGYYCFQASHFPRTFGGGTKLEIK;

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPPRLLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGYYCFQASHFPRTFGQGTKLEIK; or

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPGQPPRLLIYKISN
RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGYYCFQASHFPRTFGQGTKLEIK.

In particular aspects, the bispecific antibody molecule comprises two VL chains each
comprising the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGNTYLEWYQQRPGQPPRLLIYKISNRFSG
VPDRFSGSGAGTDFTLKISRVEAEDVGYYCFQASHFPRTFGGGTKLEIK.

In certain aspects, the bispecific antibody includes a light chain comprising the VL
chain as described herein and a light chain constant region. The light chain constant region

may be the human immunoglobulin kappa chain constant region having the amino acid sequence set forth in UniProtKB/Swiss-Prot: P01834.2.

In certain aspects, the bispecific antibody molecule includes the first VH chain where the VH chain comprises heavy chain CDRs 1-3 (HCDRs 1-3) of a VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGX²TY
YPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGTTLTVS
S, wherein X² is N, Q or S.

In certain aspects, the first VH chain comprises the HCDRs 1-3 of the VH chain having the sequence:

(i) EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNT
YYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGTTLTV
SS, or

(ii) EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGQ
TYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGTTLT
VSS, or

(iii) EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGS
TYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGTTLT
VSS.

In certain aspects, the HCDRs 1-3 of the VH chain are defined based on the Kabat nomenclature.

In certain aspects, the first VH chain comprises: (i) the HCDR1 comprising the sequence: RYTMS; (ii) the HCDR2 comprising the sequence: TISSGGGX²TYYPDSVKG; and (iii) the HCDR3 comprising the sequence: YGAGDAWFAY; where X² is N, Q or S. These HCDRs are based on the Kabat nomenclature.

In certain aspects, the first and/or second VH chain is humanized. In certain aspects, the VH chains are humanized to include framework regions from a human antibody.

In certain aspects, the first VH chain comprises the sequence:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGNTY
YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGTTLTVS
S;

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGQTY
YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGTTLTVS
S; or

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGSTY
YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGTTLTVS
S.

In certain aspects, the first VH chain comprises a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to the sequence:
 EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGNTY
 YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGTLLTVS
 S;
 EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGQTY
 YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGTLLTVS
 S;
 or
 EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGSTY
 YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGTLLTVS
 S.

In certain aspects, the bispecific antibody includes a first heavy chain comprising the first VH chain as described herein and a human IgG1 heavy chain constant region.

In certain aspects, the second VH chain of the bispecific antibody is derived from a monospecific antibody molecule which binds the TAA, and wherein the affinity of the bispecific antibody molecule for the TAA is at least 2-fold lower (e.g. at least 3-fold lower, at least 4-fold lower, at least 5-fold lower) than the affinity of the monospecific antibody molecule for the TAA from which the VH chain is derived. The affinity of the bispecific antibody and the monospecific antibody is measured using the same assay. Any suitable method for measuring antibody affinity may be utilized. In certain aspects, affinity may be measured by calculating the equilibrium constant for the reversible binding of the antibody to an antigen and is expressed as a dissociation constant (Kd). In certain aspects, Kd may be measured by ELISA.

In certain aspects, the second VH chain of the bispecific antibody is derived from a monospecific antibody molecule which binds the TAA, and wherein the half-maximal effective concentration (EC50) of the bispecific antibody molecule for the TAA is at least 2-fold higher (e.g. at least 3-fold higher, at least 4-fold higher, at least 5-fold higher) than the EC50 of the monospecific antibody molecule for the TAA from which the VH chain is derived. The EC50 of the bispecific antibody and the monospecific antibody is measured using the same assay. Any suitable method for measuring EC50 of an antibody may be utilized. The concentration that provides half maximal response (e.g., half of the maximum fluorescence intensity) is measured as the EC50.

The EC50 of a test antibody may be determined by flow cytometry or ELISA. For example, flow cytometry may involve contacting a cell expressing an antigen (e.g. human wild type MDR1 or a mutant MDR1) with the antibody in a flow cytometry buffer, where the antibody is serially diluted, and incubating at room temperature or 4°C for a period of time sufficient for the antibody to bind to the cells (e.g. 10 min-1 hr). After incubating, the cells may optionally be washed to remove and non-specifically bound antibody and/or the cells may be contacted

with a fluorescently labeled secondary antibody that specifically binds to the test antibody. After incubation, the fluorescently labeled secondary antibody may be removed and the cells washed. The washed cells may be sorted by flow cytometry and the number of cells bound to the fluorescently labeled secondary antibody counted. The concentration that provides half maximal response (e.g., half of the maximum fluorescence intensity) is measured as the EC50. In variations of the flow cytometry assay, the cell may be a 293T cell overexpressing MDR1.

The TAA may be any antigen that is known to be overexpressed in cancer cells. For example, the TAA may be an antigen that is not expressed at detectable levels in a normal cell and is expressed in cancer cells, where the normal and cancer cells are the same cell type, e.g., epithelial cells. For example, TAAs may be neoantigens that are a class of tumor antigens that arise from a tumor-specific mutation(s) which alters the amino acid sequence of encoded proteins as compared to the amino acid sequence of the unmutated protein. In other aspects, a TAA is an antigen that is expressed in normal cells but is expressed at higher levels in cancer cells. In certain aspects, the TAA may be CD47.

Anti-MDR1 and Anti-CD47 Bispecific Antibody

In certain aspects, the bispecific antibody molecule binds to CD47 and the second VH chain comprises the HCDRs of a VH chain comprising the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVITADTSASTAYMELSSLRSEDVAVYYCARGGYRAMDYWGQGTLVTVSS.

In certain aspects, the second VH chain comprises the HCDR1 comprising the sequence: NYNMH, the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD, and the HCDR3 comprising the sequence: GGYRAMDY. The HCDRs1-3 are defined based on the Kabat nomenclature.

In certain aspects, the second VH chain comprises a sequence that is at least 80% identical (e.g., at least 85% identical, at least 90% identical, or at least 95% identical) to the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVITADTSASTAYMELSSLRSEDVAVYYCARGGYRAMDYWGQGTLVTVSS.

In certain aspects, the second VH chain comprises the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVITADTSASTAYMELSSLRSEDVAVYYCARGGYRAMDYWGQGTLVTVSS.

In certain aspects, the bispecific antibody that binds to MDR1 and CD47 may include a humanized VH chain that includes an antigen binding site for CD47 having the sequence:

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYNMHWVRQAPGKGLEWMGTIYPG
 NDDTSYNQKFKDRVTISRDN SKNTLYLQMNSLRAEDTAVYYCARGGYRAMDYWGQGT
 LVSS;

EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYNMHWVRQMPGKGLEWMGTIYPG
 NDDTSYNQKFKDQVTISADKSISTAYLQWSSSLKASDTAMY CARGGYRAMDYWGQGT
 TVT VSS; or

QVQLVQSGSELKKPGASVKVSCASGYTFTNYNMHWVRQAPGQGLEWMGTIYPG
 NDDTSYNQKFKDRFVFSLDTSVSTAYLQISSSLKAEDTAVYYCARGGYRAMDYWGQGT
 TVT VSS.

Additional aspects of the bispecific antibody are described elsewhere in the application and include variations humanized versions of the sequences disclosed herein and/or substitutions in the Fc region to promoter formation of heterodimers between the first and the second VH chains.

Anti-MDR1 and Anti-PD-L1 Bispecific Antibody

In certain aspects, the TAA may be Programmed death-ligand 1 (PD-L1). PD-L1 is also known as cluster of differentiation 274 (CD274) or B7 homolog 1 (B7-H1). In certain aspects, the bispecific antibody molecule that binds to MDR1 and PD-L1 includes the common light chain and the first VH chain as described in the preceding sections and the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPY
 GGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGT
 LVT VSS

The HCDRs 1-3 defined as per Kabat nomenclature are:

HCDR1: DSWIH

HCDR2: WISPYGGSTYYADSVKG

HCDR3: RHWPGGFDY

The second VH chain of the bispecific antibody that binds to MDR1 and PD-L1 may have an amino acid sequence at least 80%, at least 90%, at least 95%, or a 100% identical to the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPY
 GGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGT
 LVT VSS

The second VH chain of the bispecific antibody that binds to MDR1 and PD-L1 may be present in heavy chain having an amino acid sequence at least 80%, at least 90%, at least 95%, or a 100% identical to the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAVISPY
GGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGGQTL
VTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YASTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE
EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

As described elsewhere herein, at least one, two, or all of the VL chain, the first VH chain, and the second VH may be humanized. In addition, the Fc regions of the VH chains may include substitutions to increase heterodimerization between the first and second VH chain. In certain aspects, the first heavy chain may be humanized and include the charged pair substitutions K392D and K409D and the second heavy chain and include the charged pair substitutions E356K and D399K.

Anti-MDR1 and Anti-EGFR Bispecific Antibody

In certain aspects, the TAA may be epidermal growth factor receptor (EGFR). The HCDRs1-3 for the second VH chain that includes an antigen-binding site for EGFR may be derived from the VH chain of the anti-EGFR antibody necitumumab or cetuximab. The heavy chain of necitumumab has the following sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYY
GSTDYNPSLKSRTMSVDTSKNQFSLKVNSTAAADTAVYYCARVSIFGVGTFDYWGQGT
LTVSSASTKGPSVLPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

The heavy chain of cetuximab has the following sequence:

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSG
NTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT
LVTSAASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK

In a first aspect, the anti-MDR1 anti-EGFR bispecific antibody includes the VL and first VH chain as described in the preceding sections and the second VH chain may include the HCDRs from VH region of necitumumab. The HCDRs defined as per Kabat nomenclature may have the following sequences:

HCDR1: SGDYYWS

HCDR2: YIYYSGSTDYNPSLKS

HCDR3: VSIFGVGTFDY

In certain aspects, the second VH chain may have an amino acid sequence at least 80%, at least 90%, at least 95%, or a 100% identical to the amino acid sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYS
GSTDYNPSLKSRTMSVDTSKNQFSLKVNSTAAADTAVYYCARVSIFGVGTFDYWGQGT
LVTVSS

The second VH chain of the bispecific antibody that binds to MDR1 and EGFR may be present in heavy chain having an amino acid sequence at least 80%, at least 90%, at least 95%, or a 100% identical to the amino acid sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYS
GSTDYNPSLKSRTMSVDTSKNQFSLKVNSTAAADTAVYYCARVSIFGVGTFDYWGQGT
LVTVSSASTKGPSVLPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSR
EEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSR
WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

In a second aspect, the anti-MDR1 anti-EGFR bispecific antibody includes the VL and first VH chain as described in the preceding sections and the second VH chain may include the HCDRs from VH region of cetuximab. The HCDRs defined as per Kabat nomenclature may have the following sequences:

HCDR1: NYGVH

HCDR2: VIWSGGNTDYNTPFTS

HCDR3: ALTYDYEFAY

In certain aspects, the second VH chain may have an amino acid sequence at least 80%, at least 90%, at least 95%, or a 100% identical to the amino acid sequence:

QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSG
NTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT
LVTVSSA

The second VH chain of the bispecific antibody that binds to MDR1 and EGFR may be present in heavy chain having an amino acid sequence at least 80%, at least 90%, at least 95%, or a 100% identical to the amino acid sequence:

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGG
NTDYNTPTFSTRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT
LVTSAASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQY
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDE
LTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQ
QGNVFSCSVMHEALHNHYTQKSLSLSPGK

The anti-MDR1 anti-EGFR bispecific antibodies described above include the same combination of VH and VL chains as the anti-MDR1 anti-CD47 bispecific antibodies and the anti-MDR1 anti-PD-L1 bispecific antibodies described above and may be referred to as 15D3 HC::MRK16 LC:: necitumumab HC and 15D3 HC::MRK16 LC:: cetuximab HC antibodies.

Also provided herein are bispecific antibody molecules that include a different combination of the common VL chain that includes an antigen-binding site for MDR1 and the first VH chain that includes an antigen-binding site for MDR1 but are not based on the MRK16 LC and/or the 15D3 HC as described above.

In certain aspects, an anti-MDR1 anti-EGFR bispecific antibody may have the following combination of heavy and light chains: (i) 15D3 HC :: cetuximab HC :: 15D3 LC; (ii) MRK16 HC:: cetuximab HC:: 15D3 LC; or (iii) MRK16 HC:: cetuximab HC:: MRK16 LC.

The HCDRs and the LCDRs, the VL and VH regions, and the heavy chain and light chains may have the same sequence as provided herein.

The first HC may include the charged pair substitutions K392D and K409D and the second heavy chain may include the charged pair substitutions E356K and D399K or vice versa.

In certain aspects, the bispecific antibodies of the present disclosure do not encompass the bispecific antibody, UIC2 DD HC:: cetuximab KK HC :: MRK16 LC. This bispecific antibody includes a VH chain comprising HCDRs1-3 of the anti-MDR1 antibody, UIC2. However, this antibody does not retain binding to MDR1 as measured by FACS using 293T cells overexpressing MDR1. See Fig. 31. The VH chain of UIC2 HC is as provided elsewhere herein.

In certain aspects, the bispecific antibody includes a second heavy chain comprising the second VH chain as described herein and a heavy chain constant region. The heavy chain may comprise the human IgG1 heavy chain constant region sequence.

In certain aspects, the bispecific antibody molecule specifically binds a cell expressing both MDR1 and the TAA and has greater than twice the affinity for a cell expressing both MDR1 and the TAA as compared to a cell expressing either MDR1 or the TAA.

In certain aspects, the bispecific antibody molecule is capable of increasing sensitivity of a cancer cell to treatment with a chemotherapeutic agent, where the half maximal inhibitory concentration (IC50) of the chemotherapeutic agent when co-administered with the antibody is at least 2 times lower (e.g. at least 3 times lower, at least 4 times lower, at least 5 times lower, at least 10 times lower, at least 20 times lower, or at least 30 times lower) than the IC50 of the chemotherapeutic agent when co-administered with an anti-MDR1 antibody comprising a VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNTYY
PDSVKGRFTVSRDNAMSSLYLQMSSLRSEDTALYYCARYGAGDAWFAYWGQGLTVTSA

; and a VL chain having the sequence:

DVLMQTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVSNRFSG
VPDRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHFPRTFGGGTRLEIK. In certain

aspects, the anti-MDR1 antibody may be the 15D3 antibody described in U.S. Patent No. 5,959,084.

In certain aspects, the bispecific antibody molecule binds to MDR1 with at least 2-fold lower affinity (e.g. at least 3 times lower, at least 4 times lower, at least 5 times lower, at least 10 times lower, at least 20 times lower, or at least 30 times lower affinity) than an anti-MDR1 antibody comprising a VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNTYY
PDSVKGRFTVSRDNAMSSLYLQMSSLRSEDTALYYCARYGAGDAWFAYWGQGLTVTSA;

and a VL chain having the sequence:

DVLMQTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVSNRFSG
VPDRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHFPRTFGGGTRLEIK. In certain

aspects, the anti-MDR1 antibody may the 15D3 antibody described in U.S. Patent No. 5,959,084.

In certain aspects, the bispecific antibody molecule when bound to a cell expressing a MDR1, inhibits efflux by the MDR1. Inhibition may be a decrease in efflux by at least 5%, at least 10%, at least 15%, at least 20%, at least 25%, at least 50%, or more, as compared to efflux by the MDR1 in absence of the bispecific antibody.

In certain aspects, the bispecific antibody molecule comprises a Fc domain that has been modified to reduce or abrogate binding of the antibody to one or more Fcγ receptors. In certain aspects, the IgG1 Fc domain may have one or more of the substitutions L234A, L235A, P329G and N297A/Q/G.

As summarized above, the present disclosure provides multi-specific antibodies having a domain that targets a cellular efflux pump and a domain that targets a cancer-associated antigen. Included are multi-specific antibodies that include a multidrug resistance protein 1 (MDR1)-binding domain and a leukocyte surface antigen CD47-binding domain. Multi-specific antibodies of the present disclosure specifically bind cells that express both MDR1 and CD47.

Accordingly, the multi-specific antibodies of the present disclosure target both MDR1 and CD47. MDR1, also known as P-glycoprotein 1 (Pgp), is an energy-dependent efflux pump responsible for decreased drug accumulation in multidrug-resistant cells that is expressed from the ATP binding cassette subfamily B member 1 (ABCB1). CD47, also known as integrin associated protein (IAP), is an immunoglobulin superfamily transmembrane protein that binds membrane integrins and also serves as a receptor for the ligands thrombospondin-1 (TSP-1) and signal-regulatory protein alpha (SIRPα) and is encoded by the CD47 gene. CD47 ligand binding can result in inhibition of phagocytosis and thus, as a target in immune therapy, masking of the CD47 extracellular domain prevents inhibition of immune-mediated killing of CD47-expressing cancer cells.

A schematic depiction of an embodiment of a multi-specific antibody of the present disclosure is provided in FIG. 1A. As depicted, the multi-specific antibody 100 includes a MDR1-binding domain 101 and a CD47-binding domain 102 and, optionally, all or a portion of an Fc domain 103. As illustrated in FIG. 1B, in the presence of a cell 104 that expresses MDR1 107, but where CD47 expression is low or absent, the multi-specific antibody 100 has low affinity for the cell. Correspondingly, in the presence of a cell 105 that expresses CD47 108, but where MDR1 expression is low or absent, the multi-specific antibody 100 has low affinity for the cell. However, in the presence of a cell 106 that expresses both MDR1 107 and CD47 108, the multi-specific antibody 100 has high affinity for the cell.

Thus, multi-specific antibodies of the present disclosure bind cells that express both MDR1 and CD47 with higher affinity than cells that express only MDR1 or CD47. Correspondingly, multi-specific antibodies of the present disclosure bind with much reduced affinity when low levels of the respective second target are present, e.g., as compared to when both first and second targets are present above low levels (e.g., at average, normal, and/or high levels). In some embodiments, the affinity with which the subject multi-specific antibodies bind cells that express both MDR1 and CD47 is greater than twice, including e.g., greater than 2.5 times, greater than 3 times, greater than 4 times, greater than 5 times, greater than 6

times, greater than 7 times, greater than 8 times, greater than 9 times, greater than 10 times, or more, as compared to the affinity with which the subject multi-specific antibodies bind cells that express either MDR1 or CD47 (or a low level of either MDR1 or CD47).

In some embodiments, the subject multi-specific antibodies may, when bound to a cell expressing MDR1, prevent the functioning of the cellular MDR1 protein. Accordingly, multi-specific antibodies of the present disclosure may inhibit efflux by the MDR1 protein, including e.g., where efflux is reduced by 5% or more, including e.g., 10% or more, 15% or more, 20% or more, 25% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70% or more, 80% or more, or 90% or more, as compared to efflux by MDR1 in the absence of the subject multi-specific antibody.

In some embodiments, the subject multi-specific antibodies may, when bound to a cell expressing CD47, prevent the functioning of the cellular CD47 protein. Accordingly, multi-specific antibodies of the present disclosure may inhibit binding of a CD47-ligand or CD47 binding partner to CD47, including e.g., where ligand/binding partner binding is reduced by 5% or more, including e.g., 10% or more, 15% or more, 20% or more, 25% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70% or more, 80% or more, or 90% or more, as compared to binding by CD47 in the absence of the subject multi-specific antibody.

Multi-specific antibodies of the present disclosure are at least bispecific for MDR1 and CD47, where the configuration of the antibody may vary. The term "antibody" refers to a protein comprising one or more (e.g., one or two) heavy chain variable regions (VH) and/or one or more (e.g., one or two) light chain variable regions (VL), or subfragments thereof capable of binding an epitope. With regard to the herein described multi-specific antibodies, such antibodies are capable of binding at least two different epitopes present on two different target proteins. The number of different target proteins, and thus different epitopes, bound by the subject multi-specific antibodies may vary and may be two (i.e., bispecific), three (tri-specific), four, or greater.

In some embodiments, multi-specific antibodies of the present disclosure may include a common light chain. As used herein, the term "common light chain" will generally refer to the use, and incorporation, of two copies of the same light chain into the multi-specific antibody. Put another way, a light chain, in the assembled multi-specific antibody, will associate with the MDR1-specific heavy chain and a second copy of the same light chain will associate with the CD47-specific heavy chain.

The VH and VL regions can be further subdivided into regions of hypervariability, termed "complementarity determining regions (CDR)", interspersed with regions that are more conserved, termed "framework regions (FR)". The extent of the FR and CDRs has been precisely defined (see, Kabat, et al. (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242;

Chothia et al. (1987) *J. Mol. Biol.* 196: 901-917). A VH can comprise three CDRs and four FRs arranged from N-terminus to C-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Similarly, a VL can comprise three CDRs and four FRs arranged from N-terminus to C-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The VH or VL chain of an antibody can further include all or part of a heavy or light chain constant region, to thereby form a heavy or light immunoglobulin chain, respectively. In one embodiment, the antibody is a tetramer of two heavy and two light chains, wherein the heavy and light chains are interconnected by, for example, disulphide bonds. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. The light chain constant region is comprised of one domain, CL. The variable regions of the heavy and light chains comprise binding regions that interact with antigen. The constant regions of the antibodies typically mediate the binding of the antibody to host tissues and factors, including various cells of the immune system and the first component of the complement system. The term "antibody" includes immunoglobulins of types IgA, IgG, IgE, IgD, IgM and subtypes thereof. In some embodiments, a subject antibody is an IgG isotype.

As used herein the term "immunoglobulin" refers to a protein including one or more polypeptides substantially encoded by immunoglobulin genes. The recognized human immunoglobulin genes include the kappa, lambda, alpha (IgA1 and IgA2), gamma (IgG1, IgG2, IgG3, IgG4), delta, epsilon and mu constant region genes; and numerous immunoglobulin variable region genes. Full-length immunoglobulin light chains (about 25 kD or 214 amino acids) are encoded by a variable region gene at the N-terminus (about 110 amino acids) and a kappa or lambda constant region at the C-terminus. Full-length immunoglobulin heavy chains (about 50 kD or 446 amino acids) are encoded by a variable region gene at the N-terminus (about 116 amino acids) and one of the other aforementioned constant region genes at the C-terminus, e.g. gamma (encoding about 330 amino acids). In some embodiments, a subject antibody comprises full-length immunoglobulin heavy chain and a full-length immunoglobulin light chain.

In some embodiments, a subject antibody does not comprise a full-length immunoglobulin heavy chain and a full-length immunoglobulin light chain, and instead comprises antigen-binding fragments of one or more full-length immunoglobulin heavy chains and/or one or more antigen-binding fragments of a full-length immunoglobulin light chain. In some embodiments, the antigen-binding fragments are contained on separate polypeptide chains; in other embodiments, the antigen-binding fragments are contained within a single polypeptide chain.

The term "antigen-binding fragment" refers to one or more fragments of a full-length antibody that are capable of specifically binding to MDR1 or CD47 as described above. Examples of binding fragments include (i) a Fab fragment (a monovalent fragment including,

e.g., consisting of, the VL, VH, CL and CH1 domains; (ii) a F(ab')₂ fragment (a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region); (iii) a Fd fragment (including, e.g., consisting of, the VH and CH1 domains); (iv) a Fv fragment (including, e.g., consisting of, the VH and VL domains of a single arm of an antibody); (v) a dAb fragment (including, e.g., consisting of, the VH domain); (vi) an isolated CDR; (vii) a single chain Fv (scFv) (including, e.g., consisting of, the VH and VL domains of a single arm of an antibody joined by a synthetic linker using recombinant means such that the VH and VL domains pair to form a monovalent molecule); (viii) diabodies (including, e.g., consisting of, two scFvs in which the VH and VL domains are joined such that they do not pair to form a monovalent molecule; the VH of each one of the scFv pairs with the VL domain of the other scFv to form a bivalent molecule).

In some embodiments, a subject antibody is a recombinant or modified antibody, e.g., a chimeric, humanized, deimmunized or an *in vitro* generated antibody. The term "recombinant" or "modified" antibody as used herein is intended to include all antibodies that are prepared, expressed, created, or isolated by recombinant means, such as (i) antibodies expressed using a recombinant expression vector transfected into a host cell; (ii) antibodies isolated from a recombinant, combinatorial antibody library; (iii) antibodies isolated from an animal (e.g. a mouse) that is transgenic for human immunoglobulin genes; or (iv) antibodies prepared, expressed, created, or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant antibodies include humanized, CDR grafted, chimeric, deimmunized, and *in vitro* generated antibodies; and can optionally include constant regions derived from human germline immunoglobulin sequences.

Modified antibodies may include modified domains, including where any antibody domain may be modified from a naturally occurring form. In some embodiments, a modified antibody may include a modified heavy chain, including a modified Fc domain, including a modified CH2 and/or modified CH3 domain. In some instances, modified Fc domains may employ electrostatic steering effects, including but not limited to e.g., through the use of the procedures described in Gunasekeran et al, (2010) Journal of Biological Chemistry 285, 19637-19646; the disclosure of which is incorporated herein by reference in its entirety. In some instances, a bispecific antibody is assembled through charge pair substitutions at the CH3 domain, including but not limited to e.g., where one heavy chain is modified to contain K392D and K409D substitutions and the other heavy chain is modified to contained E356K and D399K substitutions. Charge pair substituted chains may preferentially form a heterodimer with one another. The numbering of the amino acid substitutions is per EU numbering system for HCs.

In some instances, an antibody of the present disclosure includes charge pair substitutions. In some instances, an antibody of the present disclosure does not include charge pair substitutions. In some instances, an alternative means of promoting preferential heterodimer formation of desired chains may be employed.

In some instances, a modified heavy chain may include a knob-into-hole modification. “Knobs-into-holes” amino acid modification is a rational design strategy in antibody engineering, used for heterodimerization of the heavy chains, in the production of multi-specific antibodies, including bispecific IgG antibodies. For example, in incorporating the knobs-into-holes strategy into a bispecific antibody made from two monoclonal antibodies of different specificities, amino acid changes are engineered in order to create a “knob” on the CH3 of the heavy chain of monoclonal antibody 1 (mAb1) and a “hole” on the CH3 of the heavy chain of monoclonal antibody 2 (mAb2). The knob may be represented by a large amino acid, such as e.g., a tyrosine (Y), whereas the hole may be represented by small amino acid, such as a threonine (T). For example, a knobs-into-holes pair modification may be created a T22Y substitution in a first CH3 domain and Y86T substitution in the partner CH3 domain. Examples of knobs-into-holes modifications are described in Carter, J. Immunol. Methods, 248(1-2):7-15 (2001); Ridgway, J. B. et al. Protein Eng. 9(7):617-2 (1996); and Merchant, A. M. et al. Nat. Biotechnol. 16(7):677-81 (1998); the disclosures of which are incorporated herein in their entirety. In antibodies generated from paired knob-into-hole modified domains the bispecific heterodimer will generally represent the major fraction.

As summarized above, multi-specific antibodies of the present disclosure will include a MDR1-binding domain and CD47-binding domain. Such domains may vary, including the epitopes bound by the domains, the variable region arrangement and sequences, etc.

A subject MDR1-binding domain specifically binds one or more epitopes of MDR1. Thus, the epitope is a MDR1 epitope. The size of a MDR1 epitope bound by a MDR1-binding domain may vary, including where the MDR1 epitope is formed by a polypeptide having a contiguous stretch of a MDR1 sequence that may range from 4 aa or less to 12 aa or more, including but not limited to e.g., 4 aa, 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, 12 aa, 4 aa to 10 aa, 5 aa to 10 aa, 6 aa to 10 aa, 4 aa to 8 aa, 5 aa to 8 aa, 6 aa to 8 aa, etc.

In some embodiments, the MDR1 epitope can be formed by a polypeptide having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of a MDR1 sequence, including but not limited to e.g., the human MDR1 sequence:

MDLEGDRNGGAKKKNFFKLNNKSEKDKKEKKPTVSVFSMFRYSNWLDKLYMVVGTLAAIIH
GAGLPLMMLVFGEMTDIFANAGNLEDLMSNITNRSNDINDTGFFMNLEEDMTRYAYYYSGIG
AGVLVAAYIQVSFWCLAAGRQIHKIRKQFFHAIMRQEIGWFDVHVDVGELNTRLTDDVSKINE

GIGDKIGMFFQSMATFFTGFIVGFTRGWKLT LVILAISPVLGLSAAVWAKILSSFTDKELLAYA
 KAGAVAEVLAIAIRTVIAFGGQKKELEERYNKNLEEA KRIGIKKAITANISIGAAFLLIYASYALAF
 WYGTTLVLSGEYSIGQVLT VFFSVLIGAFSVGQASPSIEAFANARGAAYEIFKIIDNKPSIDSY
 SKSGHKPDNIKGNLEFRNVHFSYPSRKEVKILKGLNLKVQSGQTVALVGN SGCGKSTTVQL
 MQRLYDPTEGMVSVDGQDIRTINVRLREIIGVVSQEPVLFATTIAENIRYGRENVMTMDEIEK
 AVKEANAYDFIMKLPHKFDTLVGERGAQLSGGQKQRIAIARALVRNPKILLLDEATSALDTES
 EAVVQVALDKARKGRTTIVIAHRLSTVRNADVIAGFDDGVIVEKGNHDELMKEKGIYFKLVMT
 QTAGNEVELENAADESKSEIDALEMSSNDSRSSLRKRSTRRSVRGSQAQDRKLSTKEALD
 ESIPPVSWFRIMKLNLTWPYFVVGVC AIIINGGLQPAFAIIFSKIIGVFTRIDDPETKRQNSNL
 FSLLFLALGIISFITFFLQGFTFGKAGEILTKRLRYMVFRSMLRQDVSWFDDPKNTTGALTTRL
 ANDAAQVKGAIGSRLAVITQNIANLGTGIIISFIYGWQLTLLLLAIVPIIAIAGVVEMKMLSGQAL
 KDKKELEGGSGKIA TEAIENFRTTVVSLTQE QKFEHMYA QSLQVPYRNSLRKAHIFGITFSFTQA
 MMYFSYAGCFRFGAYLVAHKLMSFEDVLLVFS AVVFGAMAVGQVSSFAPDYAKAKISAHHI
 MIEKTPLIDSYSTEGLMPNTLEGNTFGEVVFNYPTRPDIPVLQGLSLEVKKGQTLALVGSS
 GCGKSTVVQLLERFYDPLAGKVLLDGKEIKRLNVQWLR AHLGIVSQEPILFDCSIAENIAYGD
 NSRVVSQEEIVRAAKEANI HAFIESLPNKYSTKVGDKGTQLSGGQKQRIAIARALVRQPHILL
 LDEATSALDTESEKVVQEALDKAREGRTCIVIAHRLSTIQNADLIVVFQNGRVKEHGTHQQLL
 AQKGIYFSMVS VQAGTKRQ; or a rodent MDR1 sequence, such as e.g., the mouse MDR1
 sequence:

MEFEENLKGRADKNFSKMGKKSKEKKEKPAVG VFGMFRYADWLDKLCMILGT LAIIHG
 TLLPLLMLVFGNMTDSFTKAEASILPSITNQSGPNSTLIISNSSLEEEMAIYAYYYTGIGAGVLI
 VAYIQVSLWCLAAGRQIHKIRQKFFHAIMNQEIGWFDVHDVGELNTRLTDDVSKINDGIGDKI
 GMFFQSITTFLAGFIIGFISGWKLT LVILAVSPLIGLSSALWAKVLTSTFTNKELQAYAKAGAVA
 EEVLAIAIRTVIAFGGQKKELEERYNKNLEEA KNVGIKKAITASISIGIAYLLVYASYALAFWYGT
 LVLSNEYSIGEVLT VFFSILLGTFSIGH LAPNIEAFANARGAAFEIFKIIDNEPSIDSFSTKGYP
 DSIMGNLEFKNVHFNYP SRSEVQILKGLNLKV KSGQTVALVGN SGCGKSTTVQLMQRLYD
 PLEGVVSIDGQDIRTINVRLREIIGVVSQEPVLFATTIAENIRYGRENVMTMDEIEKAVKEANAY
 DFIMKLPHQFDTLVGERGAQLSGGQKQRIAIARALVRNPKILLLDEATSALDTESEAVVQAAL
 DKAREGRTTIVIAHRLSTVRNADVIAGFDGGVIVEQGNHDELMREKGIYFKLVMTQTRGNEI
 EPGNNAYGSQSDTDASELTSEESKSPLIRRSIYRSVHRKQDQERRLSMKEAVDEDVPLVSF
 WRILNLNLSEWPYLLVGVLCAVINGC IQPVFAIVFSRIVGVFSRDDD HETKRQNCNLSLFFL
 VMGLISFVTYFFQGFTFGKAGEILTKRVRYMVFKSMLRQDISWFDDHKNSTGSLTTRLASDA
 SSVKGAMGARLAVVTQNVANLGTGVILSLVYGWQLTLLL VVIPLIVLGGIEMKLLSGQALKD
 KKQLEISGKIA TEAIENFRTIVSLTREQKFETMYA QSLQVPYRNAMKKAHVFGITFSFTQAMM
 YFSYAACFRFGAYLVAQQLMTFENVMLVFS AVVFGAMAAGNTSSFAPDYAKAKVSASHIIRI
 IEKTPEIDSYSTEGLKPTLLEGNVKFNGVQFNYPTRPNIPVLQGLSLEVKKGQTLALVGSSG
 CGKSTVVQLLERFYDPMAGSVFLDGKEIKQLNVQWLR AHLGIVSQEPILFDCSIAENIAYGD

NSRAVSHEEIVRAAKEANIHQFIDSLPKYNTVRVGDKGTQLSGGQKQRIAIARALVRQPHILL
LDEATSALDTESEKVVQEALDKAREGRTCIVIAHRLSTIQNADLIVVIENGKVKEHGTHQQLL
AQKGIYFSMVQAGAKRS, or a non-human primate sequence, such as e.g., the Pan
troglodytes (Chimpanzee) sequence:

MDLEGDRNGGAKKKNFFKLNNKSEKDKKEKKPTVSVFSMFRYSNWLDKLYMVVGTAAIIH
GAGLPLMMLLVFGEMTDIFANAGNLEDLMSNITNRSDINDTGFFMNLEEDMTRYAYYYSGIG
AGVLVAAAIQVSFWCLAAGRQIHKIRKQFFHAIMRQEIGWFDVHDVGELNTRLTDDVSKINE
GIGDKIGMFFQSMATFFTGFIVGFTRGWKLTLLVLAISPVLGLSAAVWAKILSSFTDKELLAYA
KAGAVAEVLAARTVIAFGGQKKELEERYNKNLEEAKRIGIKKAITANISIGAAFLLIYASYALAF
WYGTTLVLSGEYSIGQVLTVFFSVLIGAFSVGQASPSIEAFANARGAAEYIFKIIDNKPSIDSY
SKSGHKPDNIKGNLEFRNVHFSYPSRKQVKILKGLNLKVQSGQTVALVGNSSGCGKSTTVQL
MQRLYDPTGEMVSVVDGQDIRTINVRFLREIIGVVSQEPVLFATTIAENIRYGRENVTMDEIEK
AVKEANAYDFIMKLPHKFDTLVGERGAQLSGGQKQRIAIARALVRNPKILLLDEATSALDTESE
EAVVQVALDKARKGRTTIVIAHRLSTVRNADVIAGFDDGVIVEKGNHDELMKEKGIYFKLVMT
QTAGNEVELENAADESKSEIDALEMSSNDSRSSLIRKRSTRRSVRGSQAQDRKLSTKEALD
ESIPPVSWFRIMKLNLTWPYFVVGVFCAIINGGLQPAFAIIFSKIIGVFTRIDDPETKRQNSNL
FSLFLVLGIISFITFFLQGFTFGKAGEILTKRLRYMVFRSMLRQDVSWFDDPKNTTGALTTRL
ANDAAQVKGAIGSRLAVITQNIANLGTGIIISFIYGWQLTLLLLAIVPIIAIAGVEMKMLSGQAL
KDKKELEGAGKIASEAIENFRTTVVSLTQEQKFEHMYAQSLQVPYRNSLRKAHIFGITFSFTQA
MMYFSYAGCFRFGAYLVAHKLMSFEDVLLVFSVAVFGAMAVGQVSSFAPDYAKAKISAAHII
MIEKTPLIDSYSTEGLTPNTLEGNVTFGEVVFNYPTRPDIPVLQGLSLEVKKGQTLALVGSS
GCGKSTVVQLLERFYDPLAGKVLLDGKEIKRLNVQWLRRAHLGIVSQEPILFDCSIAENIAYGD
NSRVVSQEEIVRAAKEANIHAFIESLPNKYSTRVGDKGTQLSGGQKQRIAIARALVRQPHILL
LDEATSALDTESEKVVQEALDKAREGRTCIVIAHRLSTIQNADLIVVFQNGRVKEHGTHQQLL
AQKGIYFSMVSQAGTKRQ, or the *Macaca fascicularis* (Crab-eating macaque) sequence:

MDLEGDRNGGAEKKNFFKLNNKSKDKKERKPTVSVFSMFRYSNWLDKLYMVVGTAAIIH
GAGLPLMMLLVFGDMTDTFANAGNLGDLGALLFNNTNSSNITDTPVMNLEEDMTRYAYYY
SGIGAGVLVAAAIQVSFWCLAAGRQIHKIRKQFFHAIMRQEIGWFDVHDVGELNTRLTDDVS
KINEGIGDKIGMFFQSMATFFTGFIVGFTRGWKLTLLVLAISPVLGLSAAVWAKILSSFTDKEL
LAYAKAGAVAEVLAARTVIAFGGQKKELEERYNKNLEEAKRIGIKKAITANISIGAAFLLIYASY
ALAFWYGTTLVLSKEYSIGQVLTVFFSVLIGAFSVGQASPSIEAFANARGAAFEYIFKIIDNKPSI
DSYSKSGHKPDNIKGNLEFRNVHFSYPSRKEVKILKGLNLKVQSGQTVALVGNSSGCGKSTT
VQLMQRLYDPTGEMVSVVDGQDIRTINVRFLREIIGVVSQEPVLFATTIAENIRYGREDVTMDE
IEKAVKEANAYDFIMKLPHKFDTLVGERGAQLSGGQKQRIAIARALVRNPKILLLDEATSALD
TESEAVVQVALDKARKGRTTIVIAHRLSTVRNADVIAGFDDGVIVEKGNHDELMKEKGIYFKL
VTMQTAGNEIELENAADESKSEIDTLEMSSHDSGSSSLIRKRSTRRSVRGSQGGQDRKLSTKE
ALDESIPPVSWFRIMKLNLTWPYFVVGVFCAIINGGLQPAFAVIFSKIIGIFTRNDDAETKRQ

NSNLFSLFLVLGIVSFITFFLQGFTFGKAGEILTKRLRYMVFRSMLRQDVSWFDDPKNTTGA
 LTTRLANDAAQVKGAIGSRLAIITQNIANLGTGIIISLIYGWQLTLLLLAIVPIIAIAGVVEMKMLS
 GQALKDKKELEGAGKIAIEFRTVVSLTQEQKFEHMYDQSLQVPYRNSLRKAHIFGITF
 SFTQAMMYFSYAGCFRFGAYLVAHSLMSFEDVLLVFSVAVFGAMAVGQVSSFAPDYAKAK
 VSAAHIIIEKTPLIDSYSTEGLKPNTLEGNVTFNEVVFNYPTRLDIPVLQGLSLEVKKGQTLA
 LVGSSGCGKSTVVQLLERFYDPLAGKVLLDGKEIKQLNVQWLRHLGIVSQEPILFDCSISE
 NIAYGDNRSRVVSQEEIVRAAKEANIHAFIESLPNKYSTRVGDKGTQLSGGQKQRIAIARALVR
 QPHILLDEATSALDTESEKVVQEALDKAREGRTCIVIAHRLSTIQNADLIVVFQNGRVKEHG
 THQQLLAQKGIYFSMVSQAGAKRQ, or the like.

A subject MDR1-binding domain exhibits high affinity binding to MDR1. For example, a subject MDR1-binding domain binds to MDR1 with an affinity of at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, at least about 10^{-10} M, at least about 10^{-11} M, or at least about 10^{-12} M, or greater than 10^{-12} M. A subject MDR1-binding domain binds to an epitope present on MDR1 with an affinity of from about 10^{-7} M to about 10^{-8} M, from about 10^{-8} M to about 10^{-9} M, from about 10^{-9} M to about 10^{-10} M, from about 10^{-10} M to about 10^{-11} M, or from about 10^{-11} M to about 10^{-12} M, or greater than 10^{-12} M.

A subject MDR1-binding domain exhibits substantially no binding to any epitopes formed by amino acids within other related, but sequence dissimilar, proteins such as related but sequence dissimilar EPs. Any binding of a subject MDR1-binding domain to an epitope formed by amino acids within a related, but sequence dissimilar, protein is generally non-specific binding of a substantially lower affinity than the specific binding of the MDR1-binding domain to the epitope on MDR1. A substantially lower affinity is generally at least a two fold, three fold, five fold, 10 fold, 50 fold, 100 fold, 500 fold, or 1000 fold lower affinity.

A subject MDR1-binding domain can reduce transport of molecules through a MDR1 transporter. For example, a subject MDR1-binding domain can reduce transport by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the degree of transport in the absence of the MDR1-binding domain.

In some embodiments, a subject antibody comprises: a variable domain comprising :
 a) a heavy chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to or includes the heavy chain CDR1 region of RYTMS; ii. a CDR2 region that is identical in amino acid sequence to or includes the heavy chain CDR2 region of TISSGGGNTYYPDSVKG; and iii. a CDR3 region that is identical in amino acid sequence to or includes the heavy chain CDR3 region of YGAGDAWFAY. These CDR1-3 regions are those present in the VH chain of the 15D3 antibody, having the following sequence:
 EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNTYY

PDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGLTVTSA
or a humanized version of this VH chain having the following sequence:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GG

X²TYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGLTV
TVSS, where X² is N, Q or S. These CDRs are based on the Kabat nomenclature.

In some embodiments, a subject antibody comprises a heavy chain variable region comprising one, two, or three of the heavy chain CDRs having an amino acid sequence selected from one or more of CDR1 (RYTMS), CDR2 (TISSGGG X²TYYPDSVKG, where X² is N, Q or S), and CDR3 (YGAGDAWFAY); and FR regions that are mammalian sequences, including e.g., rodent, non-human primate, and human sequences (e.g., encoded by the respective heavy chain FR-encoding sequences). For example, in some embodiments, a subject antibody comprises a heavy chain variable region that comprises, in order from N-terminus to C-terminus: a human heavy chain FR1; a CDR1 comprising the amino acid sequence RYTMS; a human heavy chain FR2; a CDR2 comprising the amino acid sequence TISSGGGNTYYYPDSVKG; a human heavy chain FR3; a CDR3 comprising the amino acid sequence YGAGDAWFAY; and a human heavy chain FR4.

A subject antibody can comprise a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, including 100%, identical to the sequence:

EVKVVESGGVLVRPGLSLKLSAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNTYY
PDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGLTVTVSS.

A subject antibody can comprise a heavy chain variable region comprising one, two, or three of the heavy chain complementarity determining regions (CDRs) having an amino acid sequence selected from one or more of CDR1 (RYTMS), CDR2 (TISSGGGNTYYYPDSVKG), and CDR3 (YGAGDAWFAY). In certain aspects, the VH chain is humanized to include human framework regions and the humanized VH chain may include an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, including 100%, identical to the sequence:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGX²TY
YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGLTVTVS
S, where X² is N, Q or S.

A subject CD47-binding domain specifically binds one or more epitopes of CD47. Thus, the epitope is a CD47 epitope. The size of a CD47 epitope bound by a CD47-binding domain may vary, including where the CD47 epitope is formed by a polypeptide having a contiguous stretch of a CD47 sequence that may range from 4 aa or less to 12 aa or more, including but

not limited to e.g., 4 aa, 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, 10 aa, 11 aa, 12 aa, 4 aa to 10 aa, 5 aa to 10 aa, 6 aa to 10 aa, 4 aa to 8 aa, 5 aa to 8 aa, 6 aa to 8 aa, etc.

In some embodiments, the CD47 epitope can be formed by a polypeptide having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to a contiguous stretch of a CD47 sequence, including but not limited to e.g., the human CD47 sequence:

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFK
GRDIYTFD GALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTREG
ETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLVITVIVI
VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIALVIQVIAYILAVVGLSLCI
AACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMMND

E; or a rodent CD47 sequence, such as e.g., the mouse CD47 sequence:

MWPLAAALLLGSACCGSAQLLFSNVNSIEFTSCNETVVIPCIVRNVEAQSTEEMFVKWKLNK
SYIFIYDGNKNSTTTDQNFTSAKISVSDLINGIASLKMDKRDAMVGNYTCEVTELSREGKTVI
ELKNRTVSWFSPNEKILIVIFPILAILLFWGKFGILTLYKSSHTNKRIILLVAGLVLTIVVVGAI
LLIPGEKPVKNASGLGLIVISTGILILLQYNVFMATFGMTSFTIAILITQVLGYVLALVGLCLCIMA
CEPVHGPLLISGLGIIALAELLGLVYMKFVASNQRTIQPPRNR, or a non-human primate

sequence, such as e.g., the *Pongo abelii* (Sumatran orangutan) sequence:

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFK
GRDIYTFD GALNKSTVPTDFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTREG
ETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLIITVIVI
VGAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLNSFVIALVIQVIAYILAVVGLSLC
IAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMMND

E, or the *Macaca mulatta* (Rhesus macaque) sequence:

MWPLVAALLLGSACCGSAQLLFNKTKSVEFTFCNDTVVIPCFVTNMEAQNTTEVYVKWKFK
GRDIYTFD GALNKSTAPANFSSAKIEVSQLLKGDASLKMDKSDAVSHTGNYTCEVTELTRE
GETIIELKYRVVSWFSPNENILIVIFPIFAILLFWGQFGIKTLKYRSGGMDEKTIALLVAGLMITVI
VIVGAILFVPGEYSLKNATGLGLIVTSTGILILLHYYVFSTAIGLTSFVIALVIQVIAYILAVVGLSL
CIAACIPMHGPLLISGLSILALAQLLGLVYMKFVASNQKTIQPPRKAVEEPLNAFKESKGMMN

DE, or the like.

A subject CD47-binding domain exhibits high affinity binding to CD47. For example, a subject CD47-binding domain binds to CD47 with an affinity of at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, at least about 10^{-10} M, at least about 10^{-11} M, or at least about 10^{-12} M, or greater than 10^{-12} M. A subject CD47-binding domain binds to an epitope present on CD47 with an affinity of from about 10^{-7} M to about 10^{-8} M, from about 10^{-8} M to

about 10^{-9} M, from about 10^{-9} M to about 10^{-10} M, from about 10^{-10} M to about 10^{-11} M, or from about 10^{-11} M to about 10^{-12} M, or greater than 10^{-12} M.

A subject CD47-binding domain exhibits substantially no binding to any epitopes formed by amino acids within other related, but sequence dissimilar, proteins such as related but sequence dissimilar immune checkpoint markers. Any binding of a subject CD47-binding domain to an epitope formed by amino acids within a related, but sequence dissimilar, protein is generally non-specific binding of a substantially lower affinity than the specific binding of the CD47-binding domain to the epitope on CD47. A substantially lower affinity is generally at least a two fold, three fold, five fold, 10 fold, 50 fold, 100 fold, 500 fold, or 1000 fold lower affinity.

A subject CD47-binding domain can reduce the binding of CD47-binding-partners to CD47, including e.g., thrombospondin-1 (TSP-1), signal-regulatory protein alpha (SIRP α) and integrins (e.g., integrin α v β 3). For example, a subject CD47-binding domain can reduce CD47-binding-partner binding by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the degree of binding in the absence of the CD47-binding domain.

In some embodiments, a subject antibody comprises: a variable domain comprising: a) a heavy chain variable domain comprising: i. a CDR1 region that is identical in amino acid sequence to or includes the heavy chain CDR1 region of NYNMH; ii. a CDR2 region that is identical in amino acid sequence to or includes the heavy chain CDR2 region of TIYPGNDDTSYNQKFKD; and iii. a CDR3 region that is identical in amino acid sequence to or includes the heavy chain CDR3 region of GGYRAMDY. These CDR1-3 regions are those present in the VH chain of the 5F9 antibody, having the following sequence: QVQLVQSGAEVKKPGASVKVSCKASGYFTNYSNMHWVRQAPGQRLEWMGTIYPGNDDT SYNQKFKDRTITADTSASTAYMELSSLRSEDVAVYYCARGGYRAMDYWGQGTLVTVSS. These CDRs are based on the Kabat nomenclature.

A subject antibody can comprise a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, including 100%, identical to the sequence: QVQLVQSGAEVKKPGASVKVSCKASGYFTNYSNMHWVRQAPGQRLEWMGTIYPGNDDT SYNQKFKDRTITADTSASTAYMELSSLRSEDVAVYYCARGGYRAMDYWGQGTLVTVSS. A subject antibody can comprise a heavy chain variable region comprising one, two, or three of the heavy chain complementarity determining regions (CDRs) having an amino acid sequence selected from one or more of CDR1 (NYNMH), CDR2 (TIYPGNDDTSYNQKFKD), and CDR3 (GGYRAMDY).

In some embodiments, a subject antibody comprises a heavy chain variable region comprising one, two, or three of the heavy chain CDRs having an amino acid sequence selected from one or more of CDR1 (NYNMH), CDR2 (TIYPGNDDTSYNQKFKD), and CDR3 (GGYRAMDY); and FR regions that are mammalian, e.g., rodent, non-human primate, or human, sequences (e.g., encoded by the respective heavy chain FR-encoding sequences). For example, in some embodiments, a subject antibody comprises a heavy chain variable region that comprises, in order from N-terminus to C-terminus: a human heavy chain FR1; a CDR1 comprising the amino acid sequence NYNMH; a human heavy chain FR2; a CDR2 comprising the amino acid sequence TIYPGNDDTSYNQKFKD; a human heavy chain FR3; a CDR3 comprising the amino acid sequence GGYRAMDY; and a human heavy chain FR4.

A subject antibody can comprise a light chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more, including 100%, identical to the sequence:

DVLTMTQTPVSLSVSLGDAQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKISNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGGTKLEIK or a humanized version of this sequence, having the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGX¹TYLEWYQQRPGQPPRLIYKISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGGGGTKLEIK, where X¹ is N, Q or S.

A subject antibody can comprise a light chain variable region comprising light chain CDRs having the amino acid sequence set forth in CDR1 (RSSQSIVHSTG X¹TYLEW), where X¹ is N, Q or S, CDR2 (KISNRFSG), and CDR3 (FQASHFPRTF) or CDR1 (RSSQSLLHSDGFDYLNW), CDR2 (ALSNRASG) and CDR3 (MZALQAPITF). In some instances, such light chain variable region may be employed in a common light chain of the subject multi-specific antibody.

In some embodiments, a subject antibody comprises a light chain variable region comprising light chain CDRs having the amino acid sequence set forth in CDR1 (RSSQSIVHSTG X¹TYLEW), where X¹ is N, Q or S, CDR2 (KISNRFSG), and CDR3 (FQASHFPRTF) or CDR1 (RSSQSLLHSDGFDYLNW), CDR2 (ALSNRASG) and CDR3 (MZALQAPITF); and FR regions that are mammalian, including e.g., rodent, non-human primate, or human, sequences (e.g., encoded by the respective light chain FR-encoding sequences). For example, in some embodiments, a subject antibody comprises a light chain variable region that comprises, in order from N-terminus to C-terminus: a human light chain FR1; a CDR1 comprising the amino acid sequence RSSQSIVHSTG X¹TYLEW where X¹ is N, Q or S; a human light chain FR2; a CDR2 comprising the amino acid sequence KISNRFSG; a human light chain FR3; a CDR3 comprising the amino acid sequence set forth in FQASHFPRTF; and a human light chain FR4.

In some embodiments, a subject antibody comprises: optionally a heavy chain FR1 region; a CDR1 comprising the amino acid sequence RYTMS; a heavy chain FR2 region; a CDR2 comprising the amino acid sequence TISSGGG(N/S/Q)TYYPDSVKG; a heavy chain FR3 region; a CDR3 comprising the amino acid sequence YGAGDAWFAY; and a heavy chain FR4 region; and optionally a heavy chain FR1 region; a CDR1 comprising the amino acid sequence NYNMH; a heavy chain FR2 region; a CDR2 comprising the amino acid sequence TIYPGNDDTSYNQKFKD; a heavy chain FR3 region; a CDR3 comprising the amino acid sequence GGYRAMDY; and a heavy chain FR4 region. In some of these embodiments, each of the FR regions is mammalian FR region, including e.g., a human FR region.

In some embodiments, a subject antibody comprises: the heavy chain sequences as described in the preceding paragraph and further: a light chain FR1 region; a CDR1 comprising the amino acid sequence RSSQSIVHSTGNTYLEW or RSSQSLLHSDGFDYLNW; a light chain FR2 region; a CDR2 comprising the amino acid sequence KISNRFSG or ALSNRASG; a light chain FR3 region; a CDR3 comprising the amino acid sequence FQASHFPRTF or MZALQAPITF; optionally a light chain FR4 region.

In some embodiments, a subject antibody comprises an anti-MDR1 heavy chain sequence that includes a charge-to-charge swap (KK) modification having the sequence: EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNTYY PDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGLTVTVSS ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSVVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKELTKN QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLKSDGSFFLYSKLTVDKSRWQQGN VFSCSVMHEALHNHYTQKSLSLSPG, an anti-CD47 heavy chain sequence that includes a charge-to-charge swap (DD) modification having the sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYFTNRYNMHWVRQAPGQRLEWMGTIYPGNDDT SYNQKFKDRVTITADTSASTAYMELSSLRSEDATVYYCARGGYRAMDYWGQGLTVTVSSA STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YLSLSVVVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRKELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLKSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPG, and an anti-MDR1 light chain sequence having the sequence:

DVLTMTQTPVSLSVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKISNRFSG VPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIKRTVAAPSVFIFP

PSDEQLKSGTASVCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSTL
 TSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC. In some instances, the subject
 antibody may include an alternative heterodimeric Fc pairing strategy.

The charge to charge swap modification refers to substitutions where one heavy chain is modified to contain K392D and K409D substitutions and the other heavy chain is modified to contained E356K and D399K substitutions. Charge pair substituted chains favor formation of heterodimer with one another. The numbering of the amino acid substitutions is per EU numbering system for Ig HCs.

Monospecific Bivalent Antibodies and Bispecific Antibodies

Also provided herein are antibodies that may be monospecific bivalent antibodies or bispecific antibodies derived therefrom.

In certain aspects, an antibody of the present disclosure comprises a variable light (VL) chain comprising light chain CDRs (LCDRs) of a VL chain having the sequence: DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKISNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK, where X¹ is N, Q or S; and a variable heavy (VH) chain comprising heavy chain CDRs (HCDRs) of a VH chain having the sequence: EVKVVESGGVLRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGX²TYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGTLLTVSS, where X² is N, Q or S.

In certain aspects, the VL chain comprises LCDRs of the VL chain having the sequence: DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKISNRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK; and the VH chain comprises HCDRs of the VH chain having the sequence: EVKVVESGGVLRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNTYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGTLLTVSS, or the VL chain comprises LCDRs of the VL chain having the sequence: DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPPRLIYKISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK; and the VH chain comprises HCDRs of the VH chain having the sequence: EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGQTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGTLLTVSS, or the VL chain comprises LCDRs of the VL chain having the sequence: DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPGQPPRLIYKISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK; and the VH

chain comprises LCDRs of the VH chain having the sequence:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGSTY
YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGLTVTS
S.

In certain aspects, the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE, RSSQSIVHSTGQTYLE, or RSSQSIVHSTGSTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT.

In certain aspects, the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG, TISSGGGQTYYPDSVKG, or TISSGGGSTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY.

In certain aspects, the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY.

In certain aspects, the LCDR1 comprises the sequence: RSSQSIVHSTGQTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY.

In certain aspects, the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGSTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY.

In certain aspects, the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY, or the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGSTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY.

In certain aspects, the LCDR1 comprises the sequence: RSSQSIVHSTGQTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the

sequence: TISSGGGNTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY.

In certain aspects, the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY.

In certain aspects, the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY.

In certain aspects, the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY.

In certain aspects, the antibody is a monospecific bivalent antibody that specifically binds to MDR-1. In certain aspects, the monospecific bivalent antibody that specifically binds to MDR-1 comprises a humanized VL chain having an amino acid sequence at least 80% identical (e.g., at least 85% identical, at least 90% identical, at least 95% identical, or a 100% identical) to the VL chain having the amino acid sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGNTYLEWYQQRPGQPPRLLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGGGTKLEIK;

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPPRLLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK; or

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPGQPPRLLIYKIS
RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK.

In certain aspects, the amino acid residues that differ from the VL chain sequence or the VH chain sequence are located in the framework regions.

In certain aspects, the monospecific bivalent antibody that specifically binds to MDR-1 comprises a humanized VH chain having an amino acid sequence at least 80% identical (e.g., at least 85% identical, at least 90% identical, at least 95% identical, or a 100% identical) to the VH chain having the amino acid sequence:

EVQLVESGGV VVQPGGSLRLS CAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGNTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LTVSS;

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGQTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LVTVSS; or

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGSTYYPDVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LVTVSS.

In certain aspects, the antibody is a bi-specific antibody comprising the VL chain as a common light chain.

In certain aspects, the bispecific antibody comprises an MDR-1 binding domain and a tumor associated antigen (TAA) binding domain wherein each of the MDR-1 binding domain and the TAA binding domain comprises the LCDR1-3 of the VL chain.

In certain aspects, the TAA is CD47 and wherein the TAA binding domain comprises HCDRs of the VH chain having the sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYSNMHWVRQAPGQRLEWMGTIYPGNDDT
SYNQKFKDRVTITADTSASTAYMELSSLRSEDVAVYYCARGGYRAMDYWGQGT LVTVSS.

In certain aspects, the VH chain of the CD47 binding domain comprises the HCDR1 comprising the sequence: NYNMH, the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD, and the HCDR3 comprising the sequence: GGYRAMDY.

In certain aspects, an antibody of the present disclosure comprises a variable light (VL) chain comprising light chain CDRs (LCDRs) of a VL chain having the sequence:

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK, wherein
X¹ is N, Q or S; and a variable heavy (VH) chain comprising heavy chain CDRs (HCDRs) of a VH chain having the sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYSNMHWVRQAPGQRLEWMGTIYPGNDDT
SYNQKFKDRVTITADTSASTAYMELSSLRSEDVAVYYCARGGYRAMDYWGQGT LVTVSS.

In certain aspects, the VH chain comprises the HCDR1 comprising the sequence: NYNMH, the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD, and the HCDR3 comprising the sequence: GGYRAMDY.

In certain aspects, the antibody is a monospecific bivalent antibody that specifically binds to CD47. In other aspects, the antibody is a bispecific antibody that binds to CD47 and MRD-1.

In certain aspects, a monospecific bivalent antibody that specifically binds to MDR-1 and a monospecific bivalent antibody that specifically binds to CD47 as described herein may be used in a method for treating cancer in a subject, the method may involve co-administering the two antibodies to the subject in an amount effective for treating the cancer.

In certain aspects, the method may further involve administering a chemotherapeutic agent to the subject.

Regions and/or chains of the subject antibodies may or may not be joined by one or more linker regions. Where present, the linker region can be from about 5 amino acids to about 50 amino acids in length, e.g., from about 5 aa to about 10 aa, from about 10 aa to about 15 aa, from about 15 aa to about 20 aa, from about 20 aa to about 25 aa, from about 25 aa to about 30 aa, from about 30 aa to about 35 aa, from about 35 aa to about 40 aa, from about 40 aa to about 45 aa, or from about 45 aa to about 50 aa in length.

Linkers suitable for use in a subject antibody include "flexible linkers". If present, the linker molecules are generally of sufficient length to permit some flexible movement between linked regions. The linker molecules are generally about 6-50 atoms long. The linker molecules may also be, for example, aryl acetylene, ethylene glycol oligomers containing 2-10 monomer units, diamines, diacids, amino acids, or combinations thereof. Other linker molecules which can bind to polypeptides may be used in light of this disclosure.

Suitable linkers can be readily selected and can be of any of a suitable of different lengths, such as from 1 amino acid (e.g., Gly) to 20 amino acids, from 2 amino acids to 15 amino acids, from 3 amino acids to 12 amino acids, including 4 amino acids to 10 amino acids, 5 amino acids to 9 amino acids, 6 amino acids to 8 amino acids, or 7 amino acids to 8 amino acids, and may be 1, 2, 3, 4, 5, 6, or 7 amino acids.

Exemplary flexible linkers include glycine polymers (G)_n, glycine-serine polymers (including, for example, (GS)_n, GSGGS_n (SEQ ID NO: //) and GGGS_n (SEQ ID NO: //), where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. Glycine and glycine-serine polymers are of interest since both of these amino acids are relatively unstructured, and therefore may serve as a neutral tether between components. Glycine polymers are of particular interest since glycine accesses significantly more phi-psi space than even alanine, and is much less restricted than residues with longer side chains (see Scheraga, *Rev. Computational Chem.* 11173-142 (1992)). Exemplary flexible linkers include, but are not limited to GGSG (SEQ ID NO: //), GGSGG (SEQ ID NO: //), GSGSG (SEQ ID NO: //), GSGGG (SEQ ID NO: //), GGGSG (SEQ ID NO: //), GSSSG (SEQ ID NO: //), and the like. The ordinarily skilled artisan will recognize that design of a peptide conjugated to any elements described above can include linkers that are all or partially flexible, such that the linker can include a flexible linker as well as one or more portions that confer less flexible structure.

In some embodiments, a subject antibody is "humanized." The term "humanized antibody" refers to an antibody comprising at least one chain comprising variable region framework residues substantially from a human antibody chain (referred to as the acceptor

immunoglobulin or antibody) and at least one CDR substantially from a non-human antibody (such as e.g., a rodent (e.g., mouse antibody), non-human primate, etc.), (referred to as the donor immunoglobulin or antibody). See, Queen et al., Proc. Natl. Acad. Sci. USA 86:10029 10033 (1989), U.S. Pat. No. 5,530,101, U.S. Pat. No. 5,585,089, U.S. Pat. No. 5,693,761, WO 90/07861, and U.S. Pat. No. 5,225,539. The constant region(s), if present, can also be substantially or entirely from a human immunoglobulin. In some embodiments, a subject antibody comprises one or more MDR1 CDRs and one or more CD47 CDRs and one or more FR regions from a human antibody. Methods of making humanized antibodies are known in the art. See, e.g., U.S. Patent No. 7,256,273.

The substitution of mouse CDRs into a human variable domain framework can result in retention of their correct spatial orientation where, e.g., the human variable domain framework adopts the same or similar conformation to the mouse variable framework from which the CDRs originated. This can be achieved by obtaining the human variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine variable framework domains from which the CDRs were derived. The heavy and light chain variable framework regions can be derived from the same or different human antibody sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies or can be consensus sequences of several human antibodies. See Kettleborough et al., Protein Engineering 4:773 (1991); Kolbinger et al., Protein Engineering 6:971 (1993).

Having identified the complementarity determining regions of the murine donor immunoglobulin and appropriate human acceptor immunoglobulins, the next step is to determine which, if any, residues from these components should be substituted to optimize the properties of the resulting humanized antibody. In general, substitution of human amino acid residues with murine should be minimized, because introduction of murine residues increases the risk of the antibody eliciting a human-anti-mouse-antibody (HAMA) response in humans. Art-recognized methods of determining immune response can be performed to monitor a HAMA response in a particular patient or during clinical trials. Patients administered humanized antibodies can be given an immunogenicity assessment at the beginning and throughout the administration of said therapy. The HAMA response is measured, for example, by detecting antibodies to the humanized therapeutic reagent, in serum samples from the patient using a method known to one in the art, including surface plasmon resonance technology (BIAcore) and/or solid-phase ELISA analysis. In many embodiments, a subject humanized antibody does not substantially elicit a HAMA response in a human subject.

Certain amino acids from the human variable region framework residues are selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. The unnatural juxtaposition of murine CDR regions with human variable framework

region can result in unnatural conformational restraints, which, unless corrected by substitution of certain amino acid residues, lead to loss of binding affinity.

The selection of amino acid residues for substitution can be determined, in part, by computer modeling. Computer hardware and software for producing three-dimensional images of immunoglobulin molecules are known in the art. In general, molecular models are produced starting from solved structures for immunoglobulin chains or domains thereof. The chains to be modeled are compared for amino acid sequence similarity with chains or domains of solved three-dimensional structures, and the chains or domains showing the greatest sequence similarity is/are selected as starting points for construction of the molecular model. Chains or domains sharing at least 50% sequence identity are selected for modeling, and preferably those sharing at least 60%, 70%, 80%, 90% sequence identity or more are selected for modeling. The solved starting structures are modified to allow for differences between the actual amino acids in the immunoglobulin chains or domains being modeled, and those in the starting structure. The modified structures are then assembled into a composite immunoglobulin. Finally, the model is refined by energy minimization and by verifying that all atoms are within appropriate distances from one another and that bond lengths and angles are within chemically acceptable limits.

CDR and framework regions are as defined by Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md., 1987 and 1991). An alternative structural definition has been proposed by Chothia et al., *J. Mol. Biol.* 196:901 (1987); *Nature* 342:878 (1989); and *J. Mol. Biol.* 186:651 (1989) (collectively referred to as "Chothia"). When framework residues, as defined by Kabat, supra, constitute structural loop residues as defined by Chothia, supra, the amino acids present in the mouse antibody may be selected for substitution into the humanized antibody. Residues which are "adjacent to a CDR region" include amino acid residues in positions immediately adjacent to one or more of the CDRs in the primary sequence of the humanized immunoglobulin chain, for example, in positions immediately adjacent to a CDR as defined by Kabat, or a CDR as defined by Chothia (See e.g., Chothia and Lesk *JMB* 196:901 (1987)). These amino acids are particularly likely to interact with the amino acids in the CDRs and, if chosen from the acceptor, to distort the donor CDRs and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit et al., *Science*, 233:747 (1986)) and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

In some embodiments, a subject antibody comprises scFv multimers. For example, in some embodiments, a subject antibody is an scFv dimer (e.g., comprises two tandem scFv (scFv₂)), an scFv trimer (e.g., comprises three tandem scFv (scFv₃)), an scFv tetramer (e.g., comprises four tandem scFv (scFv₄)), or is a multimer of more than four scFv (e.g., in tandem).

The scFv monomers can be linked in tandem via linkers of from about 2 amino acids to about 10 amino acids in length, e.g., 2 aa, 3 aa, 4 aa, 5 aa, 6 aa, 7 aa, 8 aa, 9 aa, or 10 aa in length. Suitable linkers include, e.g., (Gly)_x, where x is an integer from 2 to 10. Other suitable linkers are those discussed above. In some embodiments, each of the scFv monomers in a subject scFV multimer is humanized, as described above.

In some embodiments, a subject antibody comprises a constant region of an immunoglobulin (e.g., an Fc region). The Fc region, if present, can be a human Fc region. If constant regions are present, the antibody can contain both light chain and heavy chain constant regions. Suitable heavy chain constant region include CH1, hinge, CH2, CH3, and CH4 regions. The antibodies described herein include antibodies having all types of constant regions, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. An example of a suitable heavy chain Fc region is a human isotype IgG1 Fc. Light chain constant regions can be lambda or kappa. A subject antibody (e.g., a subject humanized antibody) can comprise sequences from more than one class or isotype. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab' F(ab')₂, and Fv, or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer.

In some embodiments, a subject antibody comprises a free thiol (-SH) group at the carboxyl terminus, where the free thiol group can be used to attach the antibody to a second polypeptide (e.g., another antibody, including a subject antibody), a scaffold, a carrier, etc.

A subject antibody can be covalently linked to a second moiety (e.g., a lipid, a polypeptide other than a subject antibody, a synthetic polymer, a carbohydrate, and the like) using for example, glutaraldehyde, a homobifunctional cross-linker, or a heterobifunctional cross-linker. Glutaraldehyde cross-links polypeptides via their amino moieties. Homobifunctional cross-linkers (e.g., a homobifunctional imidoester, a homobifunctional N-hydroxysuccinimidyl (NHS) ester, or a homobifunctional sulfhydryl reactive cross-linker) contain two or more identical reactive moieties and can be used in a one-step reaction procedure in which the cross-linker is added to a solution containing a mixture of the polypeptides to be linked. Homobifunctional NHS ester and imido esters cross-link amine containing polypeptides. In a mild alkaline pH, imido esters react only with primary amines to form imidoamides, and overall charge of the cross-linked polypeptides is not affected. Homobifunctional sulfhydryl reactive cross-linkers includes bismaleimidhexane (BMH), 1,5-difluoro-2,4-dinitrobenzene (DFDNB), and 1,4-di-(3',2'-pyridyldithio) propinoamido butane (DPDPB).

COMPOSITIONS AND FORMULATIONS

The present disclosure provides a composition comprising a subject antibody. A subject antibody composition can comprise, in addition to a subject antibody, one or more of: a salt, e.g., NaCl, MgCl₂, KCl, MgSO₄, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; glycerol; and the like.

Compositions of the present disclosure also include pharmaceutical compositions that include a multi-specific antibody described herein. In general, a formulation comprises an effective amount of the subject antibody. An "effective amount" means a dosage sufficient to produce a desired result, e.g., reduction in a cancer of a subject, reduction in the growth rate of a cancer in a subject, amelioration of a symptom of cancer, and the like. Generally, the desired result is at least a reduction in a symptom of a cancer, reduction in the growth of a cancer, reduction in the size of a cancer, etc., as compared to a control. A subject antibody can be delivered, or be formulated, in such a manner as to avoid the blood-brain barrier. In some instances, an antibody may include a delivery enhancer, including where such enhancers may facilitate crossing of the blood-brain barrier, increased permeability, e.g., allowing for efficient transdermal delivery, and the like. Useful delivery enhancers include but are not limited to e.g., cereport, regadenoson, borneol, puerarin, propylene glycols, oleic acid, azone, N-methyl pyrrolidone, Tween 80, limonene, lipid-based nanoparticles (NPs), liposomes, niosomes, transfersomes, ethosomes, dendrimers, micellar NPs, polymeric nanostructures, metallic nanostructures, magnetic nanostructures, recombinant human hyaluronidase, and the like.

In the subject methods, a subject antibody can be administered to the host using any convenient means capable of resulting in the desired therapeutic effect or diagnostic effect. Thus, the agent can be incorporated into a variety of formulations for therapeutic administration. More particularly, a subject antibody can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols.

In pharmaceutical dosage forms, a subject antibody can be administered in conjunction with a pharmaceutically acceptable excipient, or they may also be used alone or in appropriate association, as well as in combination, with other pharmaceutically active

compounds. The following methods and excipients are merely exemplary and are in no way limiting.

For oral preparations, a subject antibody can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents. In some instances, oral delivery of antibodies may be enhanced through complexation of the antibody with an appropriate hydrogel.

A subject antibody can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

Pharmaceutical compositions comprising a subject antibody are prepared by mixing the antibody having the desired degree of purity with optional physiologically acceptable carriers, excipients, stabilizers, surfactants, buffers and/or tonicity agents. Acceptable carriers, excipients and/or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid, glutathione, cysteine, methionine and citric acid; preservatives (such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride, or combinations thereof); amino acids such as arginine, glycine, ornithine, lysine, histidine, glutamic acid, aspartic acid, isoleucine, leucine, alanine, phenylalanine, tyrosine, tryptophan, methionine, serine, proline and combinations thereof; monosaccharides, disaccharides and other carbohydrates; low molecular weight (less than about 10 residues) polypeptides; proteins, such as gelatin or serum albumin; chelating agents such as EDTA; sugars such as trehalose, sucrose, lactose, glucose, mannose, maltose, galactose, fructose, sorbose, raffinose, glucosamine, N-methylglucosamine, galactosamine, and neuraminic acid; and/or non-ionic surfactants such as Tween, Brij Pluronics, Triton-X, or polyethylene glycol (PEG).

The pharmaceutical composition may be in a liquid form, a lyophilized form or a liquid form reconstituted from a lyophilized form, wherein the lyophilized preparation is to be reconstituted with a sterile solution prior to administration. The standard procedure for reconstituting a lyophilized composition is to add back a volume of pure water (typically equivalent to the volume removed during lyophilization); however solutions comprising

antibacterial agents may be used for the production of pharmaceutical compositions for parenteral administration; see also Chen (1992) Drug Dev Ind Pharm 18, 1311-54.

Exemplary antibody concentrations in a subject pharmaceutical composition may range from about 1 mg/mL to about 200 mg/mL or from about 50 mg/mL to about 200 mg/mL, or from about 150 mg/mL to about 200 mg/mL.

An aqueous formulation of the antibody may be prepared in a pH-buffered solution, e.g., at pH ranging from about 4.0 to about 7.0, or from about 5.0 to about 6.0, or alternatively about 5.5. Examples of buffers that are suitable for a pH within this range include phosphate-, histidine-, citrate-, succinate-, acetate-buffers and other organic acid buffers. The buffer concentration can be from about 1 mM to about 100 mM, or from about 5 mM to about 50 mM, depending, e.g., on the buffer and the desired tonicity of the formulation.

A tonicity agent may be included in the antibody formulation to modulate the tonicity of the formulation. Exemplary tonicity agents include sodium chloride, potassium chloride, glycerin and any component from the group of amino acids, sugars as well as combinations thereof. In some embodiments, the aqueous formulation is isotonic, although hypertonic or hypotonic solutions may be suitable. The term "isotonic" denotes a solution having the same tonicity as some other solution with which it is compared, such as physiological salt solution or serum. Tonicity agents may be used in an amount of about 5 mM to about 350 mM, e.g., in an amount of 100 mM to 350 mM.

A surfactant may also be added to the antibody formulation to reduce aggregation of the formulated antibody and/or minimize the formation of particulates in the formulation and/or reduce adsorption. Exemplary surfactants include polyoxyethylensorbitan fatty acid esters (Tween), polyoxyethylene alkyl ethers (Brij), alkylphenylpolyoxyethylene ethers (Triton-X), polyoxyethylene-polyoxypropylene copolymer (Poloxamer, Pluronic), and sodium dodecyl sulfate (SDS). Examples of suitable polyoxyethylenesorbitan-fatty acid esters are polysorbate 20, (sold under the trademark Tween 20™) and polysorbate 80 (sold under the trademark Tween 80™). Examples of suitable polyethylene-polypropylene copolymers are those sold under the names Pluronic® F68 or Poloxamer 188™. Examples of suitable Polyoxyethylene alkyl ethers are those sold under the trademark Brij™. Exemplary concentrations of surfactant may range from about 0.001% to about 1% w/v.

A lyoprotectant may also be added in order to protect the labile active ingredient (e.g. a protein) against destabilizing conditions during the lyophilization process. For example, known lyoprotectants include sugars (including glucose and sucrose); polyols (including mannitol, sorbitol and glycerol); and amino acids (including alanine, glycine and glutamic acid). Lyoprotectants can be included in an amount of about 10 mM to 500 mM.

In some embodiments, a subject formulation includes a subject antibody, and one or more of the above-identified agents (e.g., a surfactant, a buffer, a stabilizer, a tonicity agent)

and is essentially free of one or more preservatives, such as ethanol, benzyl alcohol, phenol, m-cresol, p-chlor-m-cresol, methyl or propyl parabens, benzalkonium chloride, and combinations thereof. In other embodiments, a preservative is included in the formulation, e.g., at concentrations ranging from about 0.001 to about 2% (w/v).

For example, a subject formulation can be a liquid or lyophilized formulation suitable for parenteral administration, and can comprise: about 1 mg/mL to about 200 mg/mL of a subject antibody; about 0.001 % to about 1 % of at least one surfactant; about 1 mM to about 100 mM of a buffer; optionally about 10 mM to about 500 mM of a stabilizer; and about 5 mM to about 305 mM of a tonicity agent; and has a pH of about 4.0 to about 7.0.

As another example, a subject parenteral formulation is a liquid or lyophilized formulation comprising: about 1 mg/mL to about 200 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM Sucrose; and has a pH of 5.5.

As another example, a subject parenteral formulation comprises a lyophilized formulation comprising: 1) 15 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM sucrose; and has a pH of 5.5; or 2) 75 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM sucrose; and has a pH of 5.5; or 3) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM Sucrose; and has a pH of 5.5; or 4) 75 mg/mL of a subject antibody; 0.04% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5; or 6) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5.

As another example, a subject parenteral formulation is a liquid formulation comprising: 1) 7.5 mg/mL of a subject antibody; 0.022% Tween 20 w/v; 120 mM L-histidine; and 250 125 mM sucrose; and has a pH of 5.5; or 2) 37.5 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 10 mM L-histidine; and 125 mM sucrose; and has a pH of 5.5; or 3) 37.5 mg/mL of a subject antibody; 0.01% Tween 20 w/v; 10 mM L-histidine; and 125 mM sucrose; and has a pH of 5.5; or 4) 37.5 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 10 mM L-histidine; 125 mM trehalose; and has a pH of 5.5; or 5) 37.5 mg/mL of a subject antibody; 0.01% Tween 20 w/v; 10 mM L-histidine; and 125 mM trehalose; and has a pH of 5.5; or 6) 5 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5; or 7) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM mannitol; and has a pH of 5.5; or 8) 75 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L histidine; and 140 mM sodium chloride; and has a pH of 5.5; or 9) 150 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM trehalose; and has a pH of 5.5; or 10) 150 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 250 mM mannitol; and has a pH of 5.5; or 11) 150 mg/mL of a subject antibody; 0.02% Tween 20 w/v; 20 mM L-histidine; and 140 mM sodium chloride; and has a pH of 5.5;

or 12) 10 mg/mL of a subject antibody; 0.01% Tween 20 w/v; 20 mM L-histidine; and 40 mM sodium chloride; and has a pH of 5.5.

A subject antibody can be utilized in aerosol formulation to be administered via inhalation. A subject antibody can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

Furthermore, a subject antibody can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. A subject antibody can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more inhibitors. Similarly, unit dosage forms for injection or intravenous administration may comprise a subject antibody in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and animal subjects, each unit containing a predetermined quantity of compounds of the present invention calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle. The specifications for a subject antibody may depend on the particular antibody employed and the effect to be achieved, and the pharmacodynamics associated with each antibody in the host.

Other modes of administration will also find use with the subject invention. For instance, a subject antibody can be formulated in suppositories and, in some cases, aerosol and intranasal compositions. For suppositories, the vehicle composition will include traditional binders and carriers such as, polyalkylene glycols, or triglycerides. Such suppositories may be formed from mixtures containing the active ingredient in the range of about 0.5% to about 10% (w/w), e.g., about 1% to about 2%.

Intranasal formulations will usually include vehicles that neither cause irritation to the nasal mucosa nor significantly disturb ciliary function. Diluents such as water, aqueous saline or other known substances can be employed with the subject invention. The nasal formulations may also contain preservatives such as, but not limited to, chlorobutanol and benzalkonium chloride. A surfactant may be present to enhance absorption of the subject proteins by the nasal mucosa.

A subject antibody can be administered as an injectable formulation. Typically, injectable compositions are prepared as liquid solutions or suspensions; solid forms suitable

for solution in, or suspension in, liquid vehicles prior to injection may also be prepared. The preparation may also be emulsified or the antibody encapsulated in liposome vehicles.

Suitable excipient vehicles are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, if desired, the vehicle may contain minor amounts of auxiliary substances such as wetting or emulsifying agents or pH buffering agents. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in the art. See, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania, 17th edition, 1985. The composition or formulation to be administered will, in any event, contain a quantity of a subject antibody adequate to achieve the desired state in the subject being treated.

The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

In some embodiments, a subject antibody is formulated in a controlled release formulation. Sustained-release preparations may be prepared using methods well known in the art. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody in which the matrices are in the form of shaped articles, e.g. films or microcapsules. Examples of sustained-release matrices include polyesters, copolymers of L-glutamic acid and ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, hydrogels, polylactides, degradable lactic acid-glycolic acid copolymers and poly-D-(-)-3-hydroxybutyric acid. Possible loss of biological activity and possible changes in immunogenicity of antibodies comprised in sustained-release preparations may be prevented by using appropriate additives, by controlling moisture content and by developing specific polymer matrix compositions.

Controlled release within the scope of this invention can be taken to mean any one of a number of extended release dosage forms. The following terms may be considered to be substantially equivalent to controlled release, for the purposes of the present invention: continuous release, controlled release, delayed release, depot, gradual release, long-term release, programmed release, prolonged release, proportionate release, protracted release, repository, retard, slow release, spaced release, sustained release, time coat, timed release, delayed action, extended action, layered-time action, long acting, prolonged action, repeated action, slowing acting, sustained action, sustained-action medications, and extended release. Further discussions of these terms may be found in Lesczek Krowczynski, Extended-Release Dosage Forms, 1987 (CRC Press, Inc.).

Dosages

A suitable dosage can be determined by an attending physician or other qualified medical personnel, based on various clinical factors. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex of the patient, time, and route of administration, general health, and other drugs being administered concurrently. A subject antibody may be administered in amounts between 1 ng/kg body weight and 20 mg/kg body weight per dose, e.g. between 0.1 mg/kg body weight to 10 mg/kg body weight, e.g. between 0.5 mg/kg body weight to 5 mg/kg body weight; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. If the regimen is a continuous infusion, it can also be in the range of 1 µg to 10 mg per kilogram of body weight per minute.

Those of skill will readily appreciate that dose levels can vary as a function of the specific antibody, the severity of the symptoms and the susceptibility of the subject to side effects. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means.

Routes of administration

A subject antibody is administered to an individual using any available method and route suitable for drug delivery, including *in vivo* and *ex vivo* methods, as well as systemic and localized routes of administration.

Conventional and pharmaceutically acceptable routes of administration include intranasal, intramuscular, intratracheal, subcutaneous, intradermal, topical application, intravenous, intraarterial, rectal, nasal, oral, and other enteral and parenteral routes of administration. Routes of administration may be combined, if desired, or adjusted depending upon the antibody and/or the desired effect. A subject antibody composition can be administered in a single dose or in multiple doses. In some embodiments, a subject antibody composition is administered orally. In some embodiments, a subject antibody composition is administered via an inhalational route. In some embodiments, a subject antibody composition is administered intranasally. In some embodiments, a subject antibody composition is administered locally. In some embodiments, a subject antibody composition is administered intracranially. In some embodiments, a subject antibody composition is administered intravenously.

The agent can be administered to a host using any available conventional methods and routes suitable for delivery of conventional drugs, including systemic or localized routes. In general, routes of administration contemplated by the invention include, but are not necessarily limited to, enteral, parenteral, or inhalational routes.

Parenteral routes of administration other than inhalation administration include, but are not necessarily limited to, topical, transdermal, subcutaneous, intramuscular, intraorbital, intracapsular, intraspinal, intrasternal, and intravenous routes, *i.e.*, any route of administration other than through the alimentary canal. Parenteral administration can be carried to effect systemic or local delivery of a subject antibody. Where systemic delivery is desired, administration typically involves invasive or systemically absorbed topical or mucosal administration of pharmaceutical preparations.

A subject antibody can also be delivered to the subject by enteral administration. Enteral routes of administration include, but are not necessarily limited to, oral and rectal (*e.g.*, using a suppository) delivery.

By treatment is meant at least an amelioration of the symptoms associated with the pathological condition afflicting the host, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, *e.g.* symptom, associated with the pathological condition being treated, such as cancer and/or the growth of a cancer and pain associated therewith. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, *e.g.* prevented from happening, or stopped, *e.g.* terminated, such that the host no longer suffers from the pathological condition, or at least the symptoms that characterize the pathological condition.

A variety of hosts (wherein the term “host” is used interchangeably herein with the terms “subject,” “individual,” and “patient”) are treatable according to the subject methods. Generally, such hosts are “mammals” or “mammalian,” where these terms are used broadly to describe organisms which are within the class mammalia, including the orders carnivore (*e.g.*, dogs and cats), rodentia (*e.g.*, mice, guinea pigs, and rats), and primates (*e.g.*, humans, chimpanzees, and monkeys). In some embodiments, the hosts will be humans.

Kits with unit doses of a subject antibody, *e.g.* in oral or injectable doses, are provided. In some embodiments, in addition to the containers containing the unit doses will be an informational package insert describing the use and attendant benefits of the antibody in treating pathological condition of interest.

NUCLEIC ACIDS

The present disclosure provides nucleic acids comprising nucleotide sequences encoding a subject antibody. A nucleotide sequence encoding a subject antibody can be operably linked to one or more regulatory elements, such as a promoter and enhancer, that allow expression of the nucleotide sequence in the intended target cells (*e.g.*, a cell that is genetically modified to synthesize and/or secrete the encoded antibody).

Suitable promoter and enhancer elements are known in the art. For expression in a bacterial cell, suitable promoters include, but are not limited to, *lacI*, *lacZ*, T3, T7, *gpt*, *lambda*

P and trc. For expression in a eukaryotic cell, suitable promoters include, but are not limited to, light and/or heavy chain immunoglobulin gene promoter and enhancer elements; cytomegalovirus immediate early promoter; herpes simplex virus thymidine kinase promoter; early and late SV40 promoters; promoter present in long terminal repeats from a retrovirus; mouse metallothionein-I promoter; and various art-known tissue specific promoters.

A nucleotide sequence encoding a subject antibody can be present in an expression vector and/or a cloning vector. Where a subject antibody comprises two or more separate polypeptides, nucleotide sequences encoding the two polypeptides can be cloned in the same or separate vectors. Separate polypeptides may be expressed from a single nucleic acid or single vector using various strategies, such as separate promoters, one or more internal ribosomal entry sites (IRES), one or more self-cleaving sequences (e.g., 2A cleavage sequences, e.g., P2A, T2A, E2A, and F2A), combinations thereof, and the like. An expression vector can include a selectable marker, an origin of replication, and other features that provide for replication and/or maintenance of the vector.

Large numbers of suitable vectors and promoters are known to those of skill in the art; many are commercially available for generating a subject recombinant construct. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene, La Jolla, Calif., USA); pTrc99A, pKK223-3, pKK233-3, pDR540, and pRIT5 (Pharmacia, Uppsala, Sweden). Eukaryotic: pWLneo, pSV2cat, pOG44, PXR1, pSG (Stratagene) pSVK3, pBPV, pMSG and pSVL (Pharmacia).

Expression vectors generally have convenient restriction sites located near the promoter sequence to provide for the insertion of nucleic acid sequences encoding heterologous proteins. A selectable marker operative in the expression host may be present. Suitable expression vectors include, but are not limited to, viral vectors (e.g. viral vectors based on vaccinia virus; poliovirus; adenovirus (see, e.g., Li et al., Invest Ophthalmol Vis Sci 35:2543 2549, 1994; Borrás et al., Gene Ther 6:515 524, 1999; Li and Davidson, PNAS 92:7700 7704, 1995; Sakamoto et al., H Gene Ther 5:1088 1097, 1999; WO 94/12649, WO 93/03769; WO 93/19191; WO 94/28938; WO 95/11984 and WO 95/00655); adeno-associated virus (see, e.g., Ali et al., Hum Gene Ther 9:81 86, 1998, Flannery et al., PNAS 94:6916 6921, 1997; Bennett et al., Invest Ophthalmol Vis Sci 38:2857 2863, 1997; Jomary et al., Gene Ther 4:683 690, 1997, Rolling et al., Hum Gene Ther 10:641 648, 1999; Ali et al., Hum Mol Genet 5:591 594, 1996; Srivastava in WO 93/09239, Samulski et al., J. Vir. (1989) 63:3822-3828; Mendelson et al., Virol. (1988) 166:154-165; and Flotte et al., PNAS (1993) 90:10613-10617); SV40; herpes simplex virus; human immunodeficiency virus (see, e.g., Miyoshi et al., PNAS 94:10319 23, 1997; Takahashi et al., J Virol 73:7812 7816, 1999); a retroviral vector (e.g., Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as

Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus); and the like.

As noted above, a subject nucleic acid comprises a nucleotide sequence encoding a subject multi-specific antibody. A subject nucleic acid can comprise a nucleotide sequence encoding heavy- and light-chain CDRs, including MDR1 CDRs and CD47 CDRs. In some embodiments, a subject nucleic acid comprises a nucleotide sequence encoding heavy- and/or light-chain MDR1 CDRs, where the CDR-encoding sequences are interspersed with FR-encoding nucleotide sequences. In some embodiments, a subject nucleic acid comprises a nucleotide sequence encoding heavy- and/or light-chain CD47 CDRs, where the CDR-encoding sequences are interspersed with FR-encoding nucleotide sequences. In some embodiments, the FR-encoding nucleotide sequences are human FR-encoding nucleotide sequences.

In some embodiments, a subject nucleic acid comprises a nucleotide sequence encoding an amino acid sequence, wherein the amino acid sequence has at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% sequence identity, to the following amino acid sequence: EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNTYY PDSVKGRFTVSRDNAMSSLYLQMSSLRSEDTALYYCARYGAGDAWFAYWGQGLTVTVSS.

In some embodiments, a subject nucleic acid comprises a nucleotide sequence encoding an amino acid sequence, wherein the amino acid sequence has at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% sequence identity, to the following amino acid sequence: QVQLVQSGAEVKKPGASVKVSCKASGYFTFTNYNMHWVRQAPGQRLEWMGTIYPGNDDT SYNQKFKDRVTITADTSASTAYMELSSLRSEDTAVYYCARGGYRAMDYWGQGLTVTVSS.

In some embodiments, a subject nucleic acid comprises a nucleotide sequence encoding an amino acid sequence, wherein the amino acid sequence has at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100% sequence identity, to the following amino acid sequence: DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKISNRFSG VPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK.

Nucleic acids, e.g., as described herein, may, in some instances, be introduced into a cell, e.g., by contacting the cell with the nucleic acid. Cells with introduced nucleic acids will generally be referred to herein as genetically modified cells. Various methods of nucleic acid delivery may be employed including but not limited to e.g., naked nucleic acid delivery, viral delivery, chemical transfection, biolistics, and the like.

CELLS

The present disclosure provides isolated genetically modified cells (e.g., *in vitro* cells, *ex vivo* cells, cultured cells, etc.) that are genetically modified with a subject nucleic acid. In some embodiments, a subject isolated genetically modified cell can produce a subject antibody. In some instances, a genetically modified cell can deliver an antibody, e.g., to a subject in need thereof. In some instances, a genetically modified cell may be used in the production, screening, and/or discovery of multi-specific antibodies. Genetically modified cells may also, in some instances, include cells where endogenous gene expression has been reduced, e.g., inhibited, knocked-down, etc., or abolished, e.g., knocked-out. Genetically modified cells may also, in some instances, include cells where expression of a gene has been enhanced, e.g., the expression of an endogenous gene is increased or the expression of a heterologous gene is increased.

Suitable cells include eukaryotic cells, such as a mammalian cell, an insect cell, a yeast cell; and prokaryotic cells, such as a bacterial cell. Introduction of a subject nucleic acid into the host cell can be effected, for example by calcium phosphate precipitation, DEAE dextran mediated transfection, liposome-mediated transfection, electroporation, or other known method.

Suitable mammalian cells include primary cells and immortalized cell lines. Suitable mammalian cell lines include human cell lines, non-human primate cell lines, rodent (e.g., mouse, rat) cell lines, and the like. Suitable mammalian cell lines include, but are not limited to, HeLa cells (e.g., American Type Culture Collection (ATCC) No. CCL-2), CHO cells (e.g., ATCC Nos. CRL9618, CCL61, CRL9096), 293 cells (e.g., ATCC No. CRL-1573), Vero cells, NIH 3T3 cells (e.g., ATCC No. CRL-1658), Huh-7 cells, BHK cells (e.g., ATCC No. CCL10), PC12 cells (ATCC No. CRL1721), COS cells, COS-7 cells (ATCC No. CRL1651), RAT1 cells, mouse L cells (ATCC No. CCL1.3), human embryonic kidney (HEK) cells (ATCC No. CRL1573), HLHepG2 cells, and the like.

In some instances, useful mammalian cells may include cells derived from a mammalian tissue or organ. In some instances, cells employed are kidney cells, including e.g., kidney cells of an established kidney cell line, such as HEK 293T cells.

Suitable yeast cells or fungi or algae cells include, but are not limited to, *Pichia pastoris*, *Pichia finlandica*, *Pichia trehalophila*, *Pichia koclamae*, *Pichia membranaefaciens*, *Pichia opuntiae*, *Pichia thermotolerans*, *Pichia salictaria*, *Pichia guercuum*, *Pichia pijperi*, *Pichia stiptis*, *Pichia methanolica*, *Pichia sp.*, *Saccharomyces cerevisiae*, *Saccharomyces sp.*, *Hansenula polymorpha*, *Kluyveromyces sp.*, *Kluyveromyces lactis*, *Candida albicans*, *Aspergillus nidulans*, *Aspergillus niger*, *Aspergillus oryzae*, *Trichoderma reesei*, *Chrysosporium lucknowense*, *Fusarium sp.*, *Fusarium gramineum*, *Fusarium venenatum*, *Neurospora crassa*, *Chlamydomonas reinhardtii*, and the like.

Suitable prokaryotic cells include, but are not limited to, any of a variety of laboratory strains of *Escherichia coli*, *Lactobacillus* sp., *Salmonella* sp., *Shigella* sp., and the like. See, e.g., Carrier et al. (1992) *J. Immunol.* 148:1176-1181; U.S. Patent No. 6,447,784; and Sizemore et al. (1995) *Science* 270:299-302. Examples of *Salmonella* strains which can be employed in the present invention include, but are not limited to, *Salmonella typhi* and *S. typhimurium*. Suitable *Shigella* strains include, but are not limited to, *Shigella flexneri*, *Shigella sonnei*, and *Shigella dysenteriae*. Typically, the laboratory strain is one that is non-pathogenic. Non-limiting examples of other suitable bacteria include, but are not limited to, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Pseudomonas mevalonii*, *Rhodobacter sphaeroides*, *Rhodobacter capsulatus*, *Rhodospirillum rubrum*, *Rhodococcus* sp., and the like. In some embodiments, the host cell is *Escherichia coli*.

In some instances, cells of the present disclosure may be immune cells. As used herein, the term "immune cells" generally includes white blood cells (leukocytes) which are derived from hematopoietic stem cells (HSC) produced in the bone marrow. "Immune cells" includes, e.g., lymphocytes (T cells, B cells, natural killer (NK) cells) and myeloid-derived cells (neutrophil, eosinophil, basophil, monocyte, macrophage, dendritic cells). "T cell" includes all types of immune cells expressing CD3 including T-helper cells (CD4+ cells), cytotoxic T-cells (CD8+ cells), T-regulatory cells (Treg) and gamma-delta T cells. A "cytotoxic cell" includes CD8+ T cells, natural-killer (NK) cells, and neutrophils, which cells are capable of mediating cytotoxicity responses.

In some instances, useful cells expressing a multi-specific antibody of the present disclosure may include producer T cells. Nonlimiting examples of producer T cells include those described in Tsai & Davila *Oncoimmunology*. (2016) 5(5): e1122158; the disclosure of which is incorporated herein by reference in its entirety. Producer T cells engineered to include nucleic acid sequence encoding a multi-specific antibody of the present disclosure may, in some instances, be employed to deliver the antibody to a subject in need thereof.

Cells of the present disclosure also include cells genetically modified to change and/or amend expression of one or more of MDR1 and CD47 in the cell. Such modified cells are useful for various purposes including assaying the binding of multi-specific antibodies, including but not limited to those produced according to the description and methods provided herein. In some instances, MDR1 may be knocked out or knocked down in a subject cell line. In some instances, CD47 may be knocked out or knocked down in a subject cell line. In some instances, MDR1 may be constitutively or inducibly overexpressed in a subject cell line. In some instances, CD47 may be constitutively or inducibly overexpressed in a subject cell line. In some instances, both MDR1 and CD47 may be knocked down, knocked out, or constitutively or inducibly overexpressed in a subject cell line. Any convenient and appropriate method for

knockdown, knockout and/or overexpression may be employed. Introduced nucleic acid may be stably integrated or present transiently.

In some embodiments, cells of the present disclosure include a genetically modified human cell line that expresses CD47 and includes an exogenous nucleic acid comprising a sequence encoding MDR1 for overexpression of MDR1. In such cells CD47 expression may be endogenous or exogenously derived (i.e., introduced) and MDR1 expression may be stable or transient. In some instances, cells lines of the present disclosure, that express CD47, may be configured to produce a genetically modified human cell expressing CD47 and stably overexpressing MDR1.

Cells and cell lines of the present disclosure may be cultured, including e.g., through use of culture methods described herein. In some instances, a cell, into which nucleic acid have been introduced to genetically modify the cell, may be cultured to produce a cell line. Useful cells lines may include but are not limited to e.g., genetically modified cell lines, including human cell lines, expressing CD47 and stably over expressing MDR1.

Cells of the present disclosure, and cell lines thereof, may be employed in various methods of the disclosure, e.g., as test samples, controls, and the like. For example, in some instances cells in which MDR1 and/or CD47 have been knocked out and/or knocked down may be employed as reference cells, e.g., to which the binding of a multi-specific antibody of the present disclosure may be compared. Other useful reference cells include but are not limited to e.g., non-cancerous cells, as well as normal cells and cells expressing normal levels of various proteins, including normal level of MDR1 and/or CD47.

METHODS

As summarized above, methods of the present disclosure include methods of contacting a cell with an antibody of the present disclosure, methods of treating a subject according to a method that involves administering to the subject an antibody of the present disclosure, methods of making elements described in the instant application, including e.g., multi-specific antibodies, compositions and formulations, nucleic acids, expression vectors, cells, and the like.

As summarized above, methods of the present disclosure include contacting a cancer cell with the multi-specific antibody of the present disclosure, e.g., to facilitate and/or enhance killing of the cancer cell. In some instances, killing of the cancer cell is mediated by an immune response or immune cell acting upon the cancer cell as a result of opsonization of the cancer cell by bispecific targeting when the two targets are co-expressed on the cancer cell. In some instances, killing of the cancer cell is mediated by an immune response or immune cell acting upon the cancer cell, e.g., as a result of masking or antagonizing of a CD47 epitope present on the surface of the cancer cell by the multi-specific antibody. In some instances, killing of

the cancer cell is mediated by inhibition of cellular efflux of the cancer cell, e.g., as a result of MDR1 antagonism on the cancer cell by the multi-specific antibody. In some instances, the cell contacted with the multi-specific antibody may be a multidrug resistant cancer cell. Methods that involve contacting a cancer cell with a multi-specific antibody of the present disclosure may or may not include contacting the cancer cell with an additional therapy or active agent, including e.g., a chemotherapeutic, an immunotherapy, radiation therapy, or the like.

Contacting a cancer cell with a multi-specific antibody of the present disclosure will generally enhance the killing of the cancer cell, e.g., as compared to the level of killing of the cancer cell in the absence of the multi-specific antibody. In some instances, where an additional active agent is employed, enhanced killing of the cancer cell may be seen as compared to the level of killing observed using the additional active agent alone. The amount of enhancement of cancer cell killing attributable to the multi-specific antibody will vary and may range from at least a 5% increase in cancer cell killing to at least 90% or more, including but not limited to e.g., at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, etc. Such increases may be compared to contacting with one or more additional active agents alone.

Enhanced killing of a cancer cell may be assessed by a variety of means including but not limited to e.g., observational studies, in vitro cell-based cytotoxicity assays, flow cytometry, cell viability labeling (e.g., using one or more cell viability stains), and the like.

Treatment Methods

The present disclosure provides methods of treating a cancer, the methods generally involving administering to an individual in need thereof (e.g., an individual having a cancer) an effective amount of a subject multi-specific antibody, alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents. Administration of a multi-specific antibody of the present disclosure may be performed by any convenient and appropriate route of delivery.

Aspects of the present disclosure include the bispecific antibody molecule according to the preceding section of the specification for use in a method of treating cancer in a subject, the method comprising administering the antibody to the subject. The method comprises administering the antibody in combination with at least one additional active agent, wherein the at least one additional active agent comprises a chemotherapeutic agent, an inhibitor of a multidrug resistance transporter, an immunotherapy agent, or a combination thereof. In certain aspects, the at least one additional active agent is a chemotherapeutic agent, optionally wherein the chemotherapeutic agent is a taxol, a vinca alkaloid, or an anthracycline. *Some* chemotherapeutic agents that are substrates for MDR1 pump include paclitaxel, Colchicine, Verapamil, Vinblastine, Topotecan, Doxorubicin, Daunorubicin, Etoposide, and Nilotinib.

Also disclosed herein is a chemotherapy agent for use in a method of treating cancer in a subject, the method comprising administering the chemotherapy agent in combination with the antibody described herein to the subject, optionally wherein the chemotherapy agent is a taxol, a vinca alkaloid, or an anthracycline.

Accordingly, administration includes but is not limited to e.g., delivery of the antibody by injection, delivery of the antibody by infusion, delivery of a nucleic acid or expression vector encoding the multi-specific antibody, delivery of the antibody by administering to the subject a cell that expresses and secretes the multi-specific antibody, and the like. Administration of an agent, a nucleic acid encoding an agent, a cell expressing an agent, etc. may include contacting with the agent, contacting with the nucleic acid, contacting with the cell, etc.

In some embodiments, an effective amount of a subject multi-specific antibody is an amount that, when administered alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to reduce an adverse symptom of cancer by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or more, compared to the severity of the adverse symptom in the absence of treatment with the antibody.

In some embodiments, an effective amount of a subject multi-specific antibody is an amount that, when administered alone (e.g., in monotherapy) or in combination (e.g., in combination therapy) with one or more additional therapeutic agents, in one or more doses, is effective to improve the cancer (i.e., slow the growth of the cancer, stop the growth of the cancer, reverse the growth of the cancer, kill cancer cells (including tumor cells, or the like) in the individual being treated. For example, an effective amount of a subject antibody can reduce a cancer growth rate or reduce a cancer size in an individual by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 40%, at least about 50%, or more, compared to in the absence of treatment with the multi-specific antibody.

In some instances, a subject may be treated systemically, including with the subject multi-specific antibody, with or without one or more additional reagents. By “systemic treatment”, as used herein, is meant a treatment that is not directed solely to target a specific tumor (such as e.g., a primary tumor or a defined secondary tumor) or a specific cancer containing tissue (such as e.g., the liver in the case of liver cancer, the blood in the case of a blood cancer, etc.). Systemic treatments will generally be directed to the subject's body as a whole and may include but are not limited to e.g., systemic radiation therapy, systemic chemotherapy, systemic immunotherapy, combinations thereof and the like.

In some instances, a subject may be treated locally, including with the subject multi-specific antibody, with or without one or more additional reagents. By “local treatment”, as used herein, is meant a treatment that is specifically directed to the location of a tumor (such as e.g., a primary tumor or a defined secondary tumor) or specifically directed to a cancer containing tissue (such as e.g., the liver in the case of liver cancer, the blood in the case of a blood cancer, etc.). In some instances, local treatment may also be administered in such a way as to affect the environment surrounding a tumor, such as tissue surrounding the tumor, such as tissue immediately adjacent to the tumor. Local treatment will generally not affect or not be targeted to tissues distant from the site of cancer including the site of a tumor, such as a primary tumor. Useful local treatments that may be administered in addition to or in combination with a subject multi-specific antibody, e.g., include but are not limited to surgery, local radiation therapy, local cryotherapy, local laser therapy, local topical therapy, combinations thereof, and the like.

In some embodiments, a subject treatment method involves administering a subject multi-specific antibody and one or more additional therapeutic agents. Suitable additional therapeutic agents include, but are not limited to, chemotherapeutic agents, radiation therapy reagents, immunotherapy reagents, other antibody or multi-specific antibody agents, and the like. Additional therapies that may be administered to a subject before, during or after a subject administering a multi-specific antibody of the present disclosure will vary depending on numerous factors including e.g., the type of cancer, the subject's medical history, general state of health and/or any co-morbidities, and the like. Useful cancer therapies include but are not limited to e.g., radiation therapy, chemotherapy, immunotherapy, and the like.

Radiation therapy includes, but is not limited to, x-rays or gamma rays that are delivered from either an externally applied source such as a beam, or by implantation of small radioactive sources.

Suitable antibodies for use in cancer treatment include, but are not limited to, naked antibodies, e.g., trastuzumab (Herceptin), bevacizumab (Avastin™), cetuximab (Erbix™), panitumumab (Vectibix™), Ipilimumab (Yervoy™), rituximab (Rituxan), alemtuzumab (Lemtrada™), Ofatumumab (Arzerra™), Oregovomab (OvaRex™), Lambrolizumab (MK-3475), pertuzumab (Perjeta™), ranibizumab (Lucentis™) etc., and conjugated antibodies, e.g., gemtuzumab ozogamicin (Mylortarg™), Brentuximab vedotin (Adcetris™), 90Y-labelled ibritumomab tiuxetan (Zevalin™), 131I-labelled tositumoma (Bexxar™), etc. Suitable antibodies for use in cancer treatment also include, but are not limited to, antibodies raised against tumor-associated antigens. Such antigens include, but are not limited to, CD20, CD30, CD33, CD52, EpCAM, CEA, gpA33, Mucins, TAG-72, CAIX, PSMA, Folate-binding protein, Gangliosides (e.g., GD2, GD3, GM2, etc.), Le y, VEGF, VEGFR, Integrin alpha-V-beta-3,

Integrin alpha-5-beta-1, EGFR, ERBB2, ERBB3, MET, IGF1R, EPHA3, TRAILR1, TRAILR2, RANKL, FAP, Tenascin, etc.

Conventional cancer therapies also include targeted therapies for cancer including but not limited to e.g., Ado-trastuzumab emtansine (Kadcyla) targeting HER2 (ERBB2/neu) (approved for use in Breast cancer); Afatinib (Gilotrif) targeting EGFR (HER1/ERBB1), HER2 (ERBB2/neu) (approved for use in Non-small cell lung cancer); Aldesleukin (Proleukin) targeting (approved for use in Renal cell carcinoma, Melanoma); Alectinib (Alecensa) targeting ALK (approved for use in Non-small cell lung cancer); Alemtuzumab (Campath) targeting CD52 (approved for use in B-cell chronic lymphocytic leukemia); Atezolizumab (Tecentriq) targeting PD-L1 (approved for use in Urothelial carcinoma, Non-small cell lung cancer); Avelumab (Bavencio) targeting PD-L1 (approved for use in Merkel cell carcinoma); Axitinib (Inlyta) targeting KIT, PDGFR β , VEGFR1/2/3 (approved for use in Renal cell carcinoma); Belimumab (Benlysta) targeting BAFF (approved for use in Lupus erythematosus); Belinostat (Beleodaq) targeting HDAC (approved for use in Peripheral T-cell lymphoma); Bevacizumab (Avastin) targeting VEGF ligand (approved for use in Cervical cancer, Colorectal cancer, Fallopian tube cancer, Glioblastoma, Non-small cell lung cancer, Ovarian cancer, Peritoneal cancer, Renal cell carcinoma); Blinatumomab (Blinicyto) targeting CD19/CD3 (approved for use in Acute lymphoblastic leukemia (precursor B-cell)); Bortezomib (Velcade) targeting Proteasome (approved for use in Multiple myeloma, Mantle cell lymphoma); Bosutinib (Bosulif) targeting ABL (approved for use in Chronic myelogenous leukemia); Brentuximab vedotin (Adcetris) targeting CD30 (approved for use in Hodgkin lymphoma, Anaplastic large cell lymphoma); Brigatinib (Alunbrig) targeting ALK (approved for use in Non-small cell lung cancer (ALK+)); Cabozantinib (Cabometyx, Cometriq) targeting FLT3, KIT, MET, RET, VEGFR2 (approved for use in Medullary thyroid cancer, Renal cell carcinoma); Carfilzomib (Kyprolis) targeting Proteasome (approved for use in Multiple myeloma); Ceritinib (Zykadia) targeting ALK (approved for use in Non-small cell lung cancer); Cetuximab (Erbix) targeting EGFR (HER1/ERBB1) (approved for use in Colorectal cancer, Squamous cell cancer of the head and neck); Cobimetinib (Cotellic) targeting MEK (approved for use in Melanoma); Crizotinib (Xalkori) targeting ALK, MET, ROS1 (approved for use in Non-small cell lung cancer); Dabrafenib (Tafinlar) targeting BRAF (approved for use in Melanoma, Non-small cell lung cancer); Daratumumab (Darzalex) targeting CD38 (approved for use in Multiple myeloma); Dasatinib (Sprycel) targeting ABL (approved for use in Chronic myelogenous leukemia, Acute lymphoblastic leukemia); Denosumab (Xgeva) targeting RANKL (approved for use in Giant cell tumor of the bone); Dinutuximab (Unituxin) targeting B4GALNT1 (GD2) (approved for use in Pediatric neuroblastoma); Durvalumab (Imfinzi) targeting PD-L1 (approved for use in Urothelial carcinoma); Elotuzumab (Empliciti) targeting SLAMF7 (CS1/CD319/CRACC) (approved for use in Multiple myeloma); Enasidenib (Idhifa)

targeting IDH2 (approved for use in Acute myeloid leukemia); Erlotinib (Tarceva) targeting EGFR (HER1/ERBB1) (approved for use in Non-small cell lung cancer, Pancreatic cancer); Everolimus (Afinitor) targeting mTOR (approved for use in Pancreatic, gastrointestinal, or lung origin neuroendocrine tumor, Renal cell carcinoma, Nonresectable subependymal giant cell astrocytoma, Breast cancer); Gefitinib (Iressa) targeting EGFR (HER1/ERBB1) (approved for use in Non-small cell lung cancer); Ibritumomab tiuxetan (Zevalin) targeting CD20 (approved for use in Non-Hodgkin's lymphoma); Ibrutinib (Imbruvica) targeting BTK (approved for use in Mantle cell lymphoma, Chronic lymphocytic leukemia, Waldenstrom's macroglobulinemia); Idelalisib (Zydelig) targeting PI3K δ (approved for use in Chronic lymphocytic leukemia, Follicular B-cell non-Hodgkin lymphoma, Small lymphocytic lymphoma); Imatinib (Gleevec) targeting KIT, PDGFR, ABL (approved for use in GI stromal tumor (KIT+), Dermatofibrosarcoma protuberans, Multiple hematologic malignancies); Ipilimumab (Yervoy) targeting CTLA-4 (approved for use in Melanoma); Ixazomib (Ninlaro) targeting Proteasome (approved for use in Multiple Myeloma); Lapatinib (Tykerb) targeting HER2 (ERBB2/neu), EGFR (HER1/ERBB1) (approved for use in Breast cancer (HER2+)); Lenvatinib (Lenvima) targeting VEGFR2 (approved for use in Renal cell carcinoma, Thyroid cancer); Midostaurin (Rydapt) targeting FLT3 (approved for use in acute myeloid leukemia (FLT3+)); Necitumumab (Portrazza) targeting EGFR (HER1/ERBB1) (approved for use in Squamous non-small cell lung cancer); Neratinib (Nerlynx) targeting HER2 (ERBB2/neu) (approved for use in Breast cancer); Nilotinib (Tasigna) targeting ABL (approved for use in Chronic myelogenous leukemia); Niraparib (Zejula) targeting PARP (approved for use in Ovarian cancer, Fallopian tube cancer, Peritoneal cancer); Nivolumab (Opdivo) targeting PD-1 (approved for use in Colorectal cancer, Head and neck squamous cell carcinoma, Hodgkin lymphoma, Melanoma, Non-small cell lung cancer, Renal cell carcinoma, Urothelial carcinoma); Obinutuzumab (Gazyva) targeting CD20 (approved for use in Chronic lymphocytic leukemia, Follicular lymphoma); Ofatumumab (Arzerra, HuMax-CD20) targeting CD20 (approved for use in Chronic lymphocytic leukemia); Olaparib (Lynparza) targeting PARP (approved for use in Ovarian cancer); Olaratumab (Lartruvo) targeting PDGFR α (approved for use in Soft tissue sarcoma); Osimertinib (Tagrisso) targeting EGFR (approved for use in Non-small cell lung cancer); Palbociclib (Ibrance) targeting CDK4, CDK6 (approved for use in Breast cancer); Panitumumab (Vectibix) targeting EGFR (HER1/ERBB1) (approved for use in Colorectal cancer); Panobinostat (Farydak) targeting HDAC (approved for use in Multiple myeloma); Pazopanib (Votrient) targeting VEGFR, PDGFR, KIT (approved for use in Renal cell carcinoma); Pembrolizumab (Keytruda) targeting PD-1 (approved for use in Classical Hodgkin lymphoma, Melanoma, Non-small cell lung cancer (PD-L1+), Head and neck squamous cell carcinoma, Solid tumors (MSI-H)); Pertuzumab (Perjeta) targeting HER2 (ERBB2/neu) (approved for use in Breast cancer (HER2+)); Ponatinib (Iclusig) targeting ABL, FGFR1-3,

FLT3, VEGFR2 (approved for use in Chronic myelogenous leukemia, Acute lymphoblastic leukemia); Ramucirumab (Cyramza) targeting VEGFR2 (approved for use in Colorectal cancer, Gastric cancer or Gastroesophageal junction (GEJ) adenocarcinoma, Non-small cell lung cancer); Regorafenib (Stivarga) targeting KIT, PDGFR β , RAF, RET, VEGFR1/2/3 (approved for use in Colorectal cancer, Gastrointestinal stromal tumors, Hepatocellular carcinoma); Ribociclib (Kisqali) targeting CDK4, CDK6 (approved for use in Breast cancer (HR+, HER2-)); Rituximab (Rituxan, Mabthera) targeting CD20 (approved for use in Non-Hodgkin's lymphoma, Chronic lymphocytic leukemia, Rheumatoid arthritis, Granulomatosis with polyangiitis); Rituximab/hyaluronidase human (Rituxan Hycela) targeting CD20 (approved for use in Chronic lymphocytic leukemia, Diffuse large B-cell lymphoma, Follicular lymphoma); Romidepsin (Istodax) targeting HDAC (approved for use in Cutaneous T-cell lymphoma, Peripheral T-cell lymphoma); Rucaparib (Rubraca) targeting PARP (approved for use in Ovarian cancer); Ruxolitinib (Jakafi) targeting JAK1/2 (approved for use in Myelofibrosis); Siltuximab (Sylvant) targeting IL-6 (approved for use in Multicentric Castleman's disease); Sipuleucel-T (Provenge) targeting (approved for use in Prostate cancer); Sonidegib (Odomzo) targeting Smoothed (approved for use in Basal cell carcinoma); Sorafenib (Nexavar) targeting VEGFR, PDGFR, KIT, RAF (approved for use in Hepatocellular carcinoma, Renal cell carcinoma, Thyroid carcinoma); Temsirolimus (Torisel) targeting mTOR (approved for use in Renal cell carcinoma); Tositumomab (Bexxar) targeting CD20 (approved for use in Non-Hodgkin's lymphoma); Trametinib (Mekinist) targeting MEK (approved for use in Melanoma, Non-small cell lung cancer); Trastuzumab (Herceptin) targeting HER2 (ERBB2/neu) (approved for use in Breast cancer (HER2+), Gastric cancer (HER2+)); Vandetanib (Caprelsa) targeting EGFR (HER1/ERBB1), RET, VEGFR2 (approved for use in Medullary thyroid cancer); Vemurafenib (Zelboraf) targeting BRAF (approved for use in Melanoma); Venetoclax (Venclexta) targeting BCL2 (approved for use in Chronic lymphocytic leukemia); Vismodegib (Erivedge) targeting PTCH, Smoothed (approved for use in Basal cell carcinoma); Vorinostat (Zolinza) targeting HDAC (approved for use in Cutaneous T-cell lymphoma); Ziv-aflibercept (Zaltrap) targeting PIGF, VEGFA/B (approved for use in Colorectal cancer); and the like.

Biological response modifiers suitable for use in connection with the methods of the present disclosure include, but are not limited to, (1) inhibitors of tyrosine kinase (RTK) activity; (2) inhibitors of serine/threonine kinase activity; (3) tumor-associated antigen antagonists, such as antibodies that bind specifically to a tumor antigen; (4) apoptosis receptor agonists; (5) interleukin-2; (6) interferon- α ; (7) interferon- γ ; (8) colony-stimulating factors; (9) inhibitors of angiogenesis; and (10) antagonists of tumor necrosis factor.

Chemotherapeutic agents are non-peptidic (i.e., non-proteinaceous) compounds that reduce proliferation of cancer cells, and encompass cytotoxic agents and cytostatic agents.

Non-limiting examples of chemotherapeutic agents include alkylating agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones.

Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (CytosanTM), melphalan (L-sarcolysin), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, uracil mustard, chlormethine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.

Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, 10-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatine, and gemcitabine.

Suitable natural products and their derivatives, (e.g., vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (Taxol[®]), docetaxel (Taxotere[®]), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, e.g. vincristine, vinblastine, vinorelbine, vindesine, etc.; podophyllotoxins, e.g. etoposide, teniposide, etc.; antibiotics, e.g. anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, etc.; phenoxizone biscyclopeptides, e.g. dactinomycin; basic glycopeptides, e.g. bleomycin; anthraquinone glycosides, e.g. plicamycin (mithramycin); anthracenediones, e.g. mitoxantrone; azirinopyrrolo indolediones, e.g. mitomycin; macrocyclic immunosuppressants, e.g. cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, etc.; and the like.

Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrozole, letrozole, capecitabine, reloxafine, cyclophosphamide, ifosamide, and droloxafine.

Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (e.g., NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (Taxol[®]), Taxol[®] derivatives, docetaxel (Taxotere[®]), thiocolchicine (NSC 361792), trityl cysterin, vinblastine sulfate, vincristine sulfate, natural and synthetic epothilones including but not limited to, eopthilone A, eopthilone B, discodermolide; estramustine, nocodazole, and the like.

Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, e.g. prednisone, dexamethasone,

etc.; estrogens and progestins, e.g. hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; etc.; and adrenocortical suppressants, e.g. aminoglutethimide; 17 α -ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyltestosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, Flutamide (Drogenil), Toremifene (Fareston), and Zoladex. Estrogens stimulate proliferation and differentiation, therefore compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids may inhibit T cell proliferation.

Other chemotherapeutic agents include metal complexes, e.g. cisplatin (cis-DDP), carboplatin, etc.; ureas, e.g. hydroxyurea; and hydrazines, e.g. N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur; etc. Other anti-proliferative agents of interest include immunosuppressants, e.g. mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); Iressa® (ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)quinazoline); etc.

"Taxanes" include paclitaxel, as well as any active taxane derivative or pro-drug. "Paclitaxel" (which should be understood herein to include analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL™, TAXOTERE™ (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3'-N-desbenzoyl-3'-N-t-butoxycarbonyl analogs of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076; U.S. Pat. Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; and EP 590,267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from *Taxus brevifolia*; or T-1912 from *Taxus yunnanensis*).

Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (e.g., Taxotere™ docetaxel, as noted above) and paclitaxel conjugates (e.g., paclitaxel-PEG, paclitaxel-dextran, paclitaxel-xylose, or paclitaxel-albumin).

Also included within the term "taxane" are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but not limited to, galactose and mannose derivatives described in International Patent Application No. WO 99/18113; piperazino and other derivatives described in WO 99/14209; taxane derivatives described in WO 99/09021, WO 98/22451, and U.S. Patent No. 5,869,680; 6-thio derivatives described in WO 98/28288; sulfenamide derivatives described in U.S. Patent No. 5,821,263; and taxol derivative described in U.S. Patent No. 5,415,869. It further includes

prodrugs of paclitaxel including, but not limited to, those described in WO 98/58927; WO 98/13059; and U.S. Patent No. 5,824,701.

Useful immunotherapies include: anti-PD-1/PD-L1 immunotherapies, and/or other immunotherapy targets, such as e.g., immune check point markers, such as CTLA-4, LAG-3 and TIM-3, that may be targeted in treatment methods. Anti-PD-1/PD-L1 immunotherapies which include but are not limited to e.g., those therapies that include administering to a subject an effective amount of one or more anti-PD-1/PD-L1 therapeutic antagonists where such antagonists include but are not limited to e.g., OPDIVO® (nivolumab), KEYTRUDA® (pembrolizumab), Tecentriq™ (atezolizumab), durvalumab (MEDI4736), avelumab (MSB0010718C), BMS-936559 (MDX-1105), CA-170, BMS-202, BMS-8, BMS-37, BMS-242 and the like.

CTLA-4, also known as CD152, binds to CD80 and CD86. Antibodies against CTLA-4 have been approved for treating some cancer types. The co-inhibitory effect of CTLA-4 with other immunotherapies make CTLA-4 a good candidate for use in combination with other immunotherapies to treat certain cancers. TIM-3 may also be targeted for immunotherapy for several cancer types.

LAG-3 is in clinical trials for treating cancers. Anti-LAG-3 immunotherapies include those that employ antagonist LAG-3 antibodies that can both activate T effector cells (by downregulating the LAG-3 inhibiting signal into pre-activated LAG-3+ cells) and inhibit induced (i.e. antigen-specific) Treg suppressive activity. Useful LAG-3 antagonistic antibodies include relatlimab (BMS-986016; developed by Bristol-Myers Squibb), IMP701 (developed by Immutep), TSR-033 (anti-LAG-3 mAb; developed by TESARO, Inc.), and the like.

Immunotherapies also include T cell-based immunotherapies such as e.g., adoptive cell therapy (ACT) and chimeric antigen receptor (CAR) T cell therapies. For example, a subject may be administered a population of CAR T cells engineered to target an antigen expressed by the subject's cancer. A T cell-based therapy may involve, in some instances, obtaining a cellular sample from a subject, such as a blood sample or a tumor biopsy, and culturing immune cells from the sample ex vivo, with or without genetic modification of the cultured immune cells. As an example, immune cells may be obtained from a subject, cultured ex vivo and modified with a CAR specific for an antigen expressed by the cancer to produce a population of CAR T cells. Then, the CAR T cells may be reintroduced into the subject to target the cancer. T cell-based immunotherapies may be configured in various ways, e.g., by targeting various antigens, by collecting/culturing various cell types, etc., depending on a particular cancer to be treated. In addition, T cell-based immunotherapies may be administered systemically, e.g., by intravenous injection, or locally, e.g., by infusion (e.g., intraperitoneal infusion, pleural catheter infusion, etc.), direct injection, and the like.

In some instances, a method of treatment described herein may include administering to a subject one or more inhibitors of a multidrug resistance transporter, including but not limited to e.g., a multidrug resistance transporter other than MDR1. Useful inhibitors of multidrug resistance transporters include e.g., tyrosine kinase inhibitors, natural products, microRNAs, and small molecule inhibitors. Inhibitors of multidrug resistance transporters include ABC transporter inhibitors. A summary of such MDR modulators or reverts is provided in Choi (2005), *Cancer Cell Int*, 5:30; the disclosure of which is incorporated by reference herein in its entirety.

Individuals suitable for treatment using a method of the present disclosure include an individual having a cancer; an individual diagnosed as having a cancer; an individual being treated for a cancer with chemotherapy, radiation therapy, antibody therapy, surgery, etc.); an individual who has been treated for a cancer (e.g., with one or more of chemotherapy, radiation therapy, antibody therapy, surgery, etc.), and who has failed to respond to the treatment; an individual who has been treated for a cancer (e.g., with one or more of chemotherapy, radiation therapy, antibody therapy, surgery, etc.), and who initially responded to the treatment but who subsequently relapsed, i.e., the cancer recurred.

The methods of the present disclosure may be employed to target and treat a variety of cancers, including e.g., primary cancer, secondary cancers, re-growing cancers, recurrent cancers, refractory cancers and the like. For example, in some instances, the methods of the present disclosure may be employed as an initial treatment of a primary cancer identified in a subject. In some instances, the methods of the present disclosure may be employed as a non-primary (e.g., secondary or later) treatment, e.g., in a subject with a cancer that is refractory to a prior treatment, in a subject with a cancer that is re-growing following a prior treatment, in a subject with a mixed response to a prior treatment (e.g., a positive response to at least one tumor in the subject and a negative or neutral response to at least a second tumor in the subject), and the like.

In some instances, the methods of the present disclosure may be employed to treat a subject with a drug resistant cancer, such as a multi-drug resistant cancer. Multidrug resistance (MDR) is the mechanism by which many cancers develop resistance to chemotherapy drugs, resulting in minimal cell death and the expansion of drug-resistant tumors. MDR cancers may involve one or more resistance mechanisms including but not limited to e.g., increased expression of efflux pumps, decreased absorption of drug, inhibition of cell death or apoptosis, modulating drug metabolism, and the like. In some instances, the methods of the present disclosure may prevent, reverse or circumvent MDR.

In some instances, methods of the present disclosure may include treating a subject having a cancer that is resistant to a first agent with an effective amount of a subject multi-specific antibody described herein in combination with a second agent that is different from

the first agent. For example, in some instances, cancer of a subject may be resistant to a first chemotherapeutic and the subject may be treated by administering an effective amount of a subject multi-specific antibody as described herein in combination with a second chemotherapeutic that is different from the first. Various combinations of first and second chemotherapeutics may be employed depending on e.g., the type of cancer to be treated, the likelihood of developing resistance, etc.

Numerous cancers are known to develop drug resistance. For this and other reasons the methods of the present disclosure may find use in treating various cancers including but not limited to, e.g., Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Adrenocortical Carcinoma, AIDS-Related Cancers (e.g., Kaposi Sarcoma, Lymphoma, etc.), Anal Cancer, Appendix Cancer, Astrocytomas, Atypical Teratoid/Rhabdoid Tumor, Basal Cell Carcinoma, Bile Duct Cancer (Extrahepatic), Bladder Cancer, Bone Cancer (e.g., Ewing Sarcoma, Osteosarcoma and Malignant Fibrous Histiocytoma, etc.), Brain Stem Glioma, Brain Tumors (e.g., Astrocytomas, Central Nervous System Embryonal Tumors, Central Nervous System Germ Cell Tumors, Craniopharyngioma, Ependymoma, etc.), Breast Cancer (e.g., female breast cancer, male breast cancer, childhood breast cancer, etc.), Bronchial Tumors, Burkitt Lymphoma, Carcinoid Tumor (e.g., Childhood, Gastrointestinal, etc.), Carcinoma of Unknown Primary, Cardiac (Heart) Tumors, Central Nervous System (e.g., Atypical Teratoid/Rhabdoid Tumor, Embryonal Tumors, Germ Cell Tumor, Lymphoma, etc.), Cervical Cancer, Childhood Cancers, Chordoma, Chronic Lymphocytic Leukemia (CLL), Chronic Myelogenous Leukemia (CML), Chronic Myeloproliferative Neoplasms, Colon Cancer, Colorectal Cancer, Craniopharyngioma, Cutaneous T-Cell Lymphoma, Duct (e.g., Bile Duct, Extrahepatic, etc.), Ductal Carcinoma In Situ (DCIS), Embryonal Tumors, Endometrial Cancer, Ependymoma, Esophageal Cancer, Esthesioneuroblastoma, Ewing Sarcoma, Extracranial Germ Cell Tumor, Extragenital Germ Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer (e.g., Intraocular Melanoma, Retinoblastoma, etc.), Fibrous Histiocytoma of Bone (e.g., Malignant, Osteosarcoma, etc.), Gallbladder Cancer, Gastric (Stomach) Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Stromal Tumors (GIST), Germ Cell Tumor (e.g., Extracranial, Extragenital, Ovarian, Testicular, etc.), Gestational Trophoblastic Disease, Glioma, Hairy Cell Leukemia, Head and Neck Cancer, Heart Cancer, Hepatocellular (Liver) Cancer, Histiocytosis (e.g., Langerhans Cell, etc.), Hodgkin Lymphoma, Hypopharyngeal Cancer, Intraocular Melanoma, Islet Cell Tumors (e.g., Pancreatic Neuroendocrine Tumors, etc.), Kaposi Sarcoma, Kidney Cancer (e.g., Renal Cell, Wilms Tumor, Childhood Kidney Tumors, etc.), Langerhans Cell Histiocytosis, Laryngeal Cancer, Leukemia (e.g., Acute Lymphoblastic (ALL), Acute Myeloid (AML), Chronic Lymphocytic (CLL), Chronic Myelogenous (CML), Hairy Cell, etc.), Lip and Oral Cavity Cancer, Liver Cancer (Primary), Lobular Carcinoma In Situ (LCIS), Lung Cancer (e.g., Non-Small Cell, Small

Cell, etc.), Lymphoma (e.g., AIDS-Related, Burkitt, Cutaneous T-Cell, Hodgkin, Non-Hodgkin, Primary Central Nervous System (CNS), etc.), Macroglobulinemia (e.g., Waldenström, etc.), Male Breast Cancer, Malignant Fibrous Histiocytoma of Bone and Osteosarcoma, Melanoma, Merkel Cell Carcinoma, Mesothelioma, Metastatic Squamous Neck Cancer with Occult Primary, Midline Tract Carcinoma Involving NUT Gene, Mouth Cancer, Multiple Endocrine Neoplasia Syndromes, Multiple Myeloma/Plasma Cell Neoplasm, Mycosis Fungoides, Myelodysplastic Syndromes, Myelodysplastic/Myeloproliferative Neoplasms, Myelogenous Leukemia (e.g., Chronic (CML), etc.), Myeloid Leukemia (e.g., Acute (AML), etc.), Myeloproliferative Neoplasms (e.g., Chronic, etc.), Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin Lymphoma, Non-Small Cell Lung Cancer, Oral Cancer, Oral Cavity Cancer (e.g., Lip, etc.), Oropharyngeal Cancer, Osteosarcoma and Malignant Fibrous Histiocytoma of Bone, Ovarian Cancer (e.g., Epithelial, Germ Cell Tumor, Low Malignant Potential Tumor, etc.), Pancreatic Cancer, Pancreatic Neuroendocrine Tumors (Islet Cell Tumors), Papillomatosis, Paraganglioma, Paranasal Sinus and Nasal Cavity Cancer, Parathyroid Cancer, Penile Cancer, Pharyngeal Cancer, Pheochromocytoma, Pituitary Tumor, Pleuropulmonary Blastoma, Primary Central Nervous System (CNS) Lymphoma, Prostate Cancer, Rectal Cancer, Renal Cell (Kidney) Cancer, Renal Pelvis and Ureter, Transitional Cell Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoma (e.g., Ewing, Kaposi, Osteosarcoma, Rhabdomyosarcoma, Soft Tissue, Uterine, etc.), Sézary Syndrome, Skin Cancer (e.g., Childhood, Melanoma, Merkel Cell Carcinoma, Nonmelanoma, etc.), Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Cell Carcinoma, Squamous Neck Cancer (e.g., with Occult Primary, Metastatic, etc.), Stomach (Gastric) Cancer, T-Cell Lymphoma, Testicular Cancer, Throat Cancer, Thymoma and Thymic Carcinoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Ureter and Renal Pelvis Cancer, Urethral Cancer, Uterine Cancer (e.g., Endometrial, etc.), Uterine Sarcoma, Vaginal Cancer, Vulvar Cancer, Waldenström Macroglobulinemia, Wilms Tumor, and the like.

The methods of treating described herein may, in some instances, be performed in a subject that has previously undergone one or more conventional treatments. For example, in the case of oncology, the methods described herein may, in some instances, be performed following a conventional cancer therapy including but not limited to e.g., conventional chemotherapy, conventional radiation therapy, conventional immunotherapy, surgery, etc. In some instances, the methods described herein may be used when a subject has not responded to or is refractory to a conventional therapy. In some instances, the methods described herein may be used when a subject has responded to a conventional therapy.

In some instances, the method of the present disclosure may be employed to target, treat or clear a subject for minimal residual disease (MRD) remaining after a prior cancer

therapy. Targeting, treating and/or clearance of MRD may be pursued using the instant methods whether the MRD is or has been determined to be refractory to the prior treatment or not. In some instances, a method of the present disclosure may be employed to target, treat and/or clear a subject of MRD following a determination that the MRD is refractory to a prior treatment or one or more available treatment options other than those employing the herein described multi-specific antibodies.

In some instances, the instant methods may be employed prophylactically for surveillance. For example, a subject in need thereof may be administered a treatment involving one or more of the herein described multi-specific antibodies when the subject does not have detectable disease but is at risk of developing a recurrent cancer, including e.g., a drug resistant cancer. In some instances, a prophylactic approach may be employed when a subject is at particularly high risk of developing a primary cancer that would be predicted to be drug resistant or expected to become drug resistant. In some instances, a prophylactic approach may be employed when a subject has been previously treated for a cancer and is at risk of reoccurrence or development of drug resistance.

In some instances, methods of the present disclosure may involve analyzing a cancer for expression of one or more markers or therapeutic targets. For example, in some instances, methods may involve analyzing a sample of a cancer from a subject to determine whether the cancer expresses MDR1 above a predetermined threshold, a TAA (e.g., CD47, PD-L1, or EGFR) above a predetermined threshold, or both.

In some instances, whether a subject is treated with a multi-specific antibody of the present disclosure may depend on the results of the TAA and/or MDR1 testing. For example, in some instances, if a cancer expresses the TAA at or above a predetermined threshold then the subject may be treated with a multi-specific antibody of the present disclosure, and if the cancer expresses the TAA below the predetermined threshold then the subject may not be treated with the multi-specific antibody, e.g., the subject may be treated with a conventional therapy for the relevant cancer without the subject multi-specific antibody. In some instances, if a cancer expresses MDR1 at or above a predetermined threshold then the subject may be treated with a multi-specific antibody of the present disclosure, and if the cancer expresses MDR1 below the predetermined threshold then the subject may not be treated with the multi-specific antibody, e.g., the subject may be treated with a conventional therapy for the relevant cancer without the subject multi-specific antibody. In some instances, if a cancer expresses both the TAA and MDR1 at or above predetermined thresholds then the subject may be treated with a multi-specific antibody of the present disclosure, and if the cancer expresses the TAA and MDR1 below the predetermined thresholds then the subject may not be treated with the multi-specific antibody, e.g., the subject may be treated with a conventional therapy for the relevant cancer without the subject multi-specific antibody.

Any convenient assay may be employed for analyzing MDR1 and/or TAA levels, including but not limited to e.g., flow cytometry, nucleic acid-based assays (e.g., amplification, sequencing, etc.), cell cytometry, immunohistochemistry, and the like. Any convenient biological sample may be employed, including but not limited to e.g., cancer biopsy samples. Useful predetermined thresholds for assessing expression of one or more markers and/or targets may be determined by any convenient and appropriate method, including comparison of the measured level of expression to a corresponding control. For example, in some instances, a useful predetermined threshold for the level of MDR1 and/or TAA assayed in a sample may correspond to a level of MDR1 and/or TAA as measured in a reference cell, such as a healthy/normal cell. The TAA may be CD47, PD-L1, or EGFR.

Methods of Making

As summarized above, methods of the present disclosure also include methods or making and/or identifying multi-specific antibodies as described herein. A subject antibody can be produced by any known method, e.g., conventional synthetic methods for protein synthesis; recombinant DNA methods; etc.

Where a subject antibody is a single chain polypeptide, it can be synthesized using standard chemical peptide synthesis techniques. Where a polypeptide is chemically synthesized, the synthesis may proceed via liquid-phase or solid-phase. Solid phase polypeptide synthesis (SPPS), in which the C-terminal amino acid of the sequence is attached to an insoluble support followed by sequential addition of the remaining amino acids in the sequence, is an example of a suitable method for the chemical synthesis of a subject antibody. Various forms of SPPS, such as Fmoc and Boc, are available for synthesizing a subject antibody. Techniques for solid phase synthesis are described by Barany and Merrifield, *Solid-Phase Peptide Synthesis*; pp. 3-284 in *The Peptides: Analysis, Synthesis, Biology*. Vol. 2: *Special Methods in Peptide Synthesis, Part A.*, Merrifield, et al. J. Am. Chem. Soc., 85: 2149-2156 (1963); Stewart et al., *Solid Phase Peptide Synthesis*, 2nd ed. Pierce Chem. Co., Rockford, Ill. (1984); and Ganesan A. 2006 *Mini Rev. Med Chem.* 6:3-10 and Camarero JA et al. 2005 *Protein Pept Lett.* 12:723-8. Briefly, small insoluble, porous beads are treated with functional units on which peptide chains are built. After repeated cycling of coupling/deprotection, the free N-terminal amine of a solid-phase attached is coupled to a single N-protected amino acid unit. This unit is then deprotected, revealing a new N-terminal amine to which a further amino acid may be attached. The peptide remains immobilized on the solid-phase and undergoes a filtration process before being cleaved off.

Standard recombinant methods can be used for production of a subject antibody. For example, nucleic acids encoding light and heavy chain variable regions, optionally linked to constant regions, are inserted into expression vectors. The light and heavy chains can be cloned in the same or different expression vectors. The DNA segments encoding

immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides. Expression control sequences include, but are not limited to, promoters (e.g., naturally-associated or heterologous promoters), signal sequences, enhancer elements, and transcription termination sequences. The expression control sequences can be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells (e.g., COS or CHO cells). Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the antibodies.

Because of the degeneracy of the code, a variety of nucleic acid sequences can encode each immunoglobulin amino acid sequence. The desired nucleic acid sequences can be produced by de novo solid-phase DNA synthesis or by polymerase chain reaction (PCR) mutagenesis of an earlier prepared variant of the desired polynucleotide. Oligonucleotide-mediated mutagenesis is an example of a suitable method for preparing substitution, deletion and insertion variants of target polypeptide DNA. See Adelman et al., DNA 2:183 (1983). Briefly, the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a single-stranded DNA template. After hybridization, a DNA polymerase is used to synthesize an entire second complementary strand of the template that incorporates the oligonucleotide primer, and encodes the selected alteration in the target polypeptide DNA.

Suitable expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers (e.g., ampicillin-resistance, hygromycin-resistance, tetracycline resistance, kanamycin resistance or neomycin resistance) to permit detection of those cells transformed with the desired DNA sequences.

Escherichia coli is an example of a prokaryotic host cell that can be used for cloning a subject antibody-encoding polynucleotide. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*, *Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (e.g., an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, are also useful for expression. *Saccharomyces* (e.g., *S. cerevisiae*) and *Pichia* are examples of suitable yeast host cells, with suitable vectors having expression control sequences (e.g., promoters), an origin of replication, termination

sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

In addition to microorganisms, mammalian cells (e.g., mammalian cells grown in *in vitro* cell culture) can also be used to express and produce the polypeptides of the present invention (e.g., polynucleotides encoding immunoglobulins or fragments thereof). See Winnacker, *From Genes to Clones*, VCH Publishers, N.Y., N.Y. (1987). Suitable mammalian host cells include CHO cell lines, various Cos cell lines, HeLa cells, myeloma cell lines, and transformed B-cells or hybridomas. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (Queen et al., *Immunol. Rev.* 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Examples of suitable expression control sequences are promoters derived from immunoglobulin genes, SV40, adenovirus, bovine papilloma virus, cytomegalovirus and the like. See Co et al., *J. Immunol.* 148:1149 (1992).

Once synthesized (either chemically or recombinantly), the whole antibodies, their dimers, individual light and heavy chains, or other forms of a subject antibody (e.g., scFv, etc.) can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, high performance liquid chromatography (HPLC) purification, gel electrophoresis, and the like (see generally Scopes, *Protein Purification* (Springer-Verlag, N.Y., (1982)). A subject antibody can be substantially pure, e.g., at least about 80% to 85% pure, at least about 85% to 90% pure, at least about 90% to 95% pure, or 98% to 99%, or more, pure, e.g., free from contaminants such as cell debris, macromolecules other than a subject antibody, etc.

In some embodiments, method of generating a multi-specific antibody of the present disclosure may include producing candidate antibodies and screening for activity. Such methods may generate a multi-specific antibody that specifically binds a cell expressing both MDR1 and CD47 through the use of a series of steps. Steps of such methods may include: producing a multi-specific antibody or a plurality of antibodies that each include or are expected to include a MDR1-binding domain and a CD47-binding domain; contacting a first test cell expressing MDR1 and CD47 with the multi-specific antibody or plurality of antibodies; contacting a second cell expressing either MDR1 or CD47 with the multi-specific antibody or plurality of antibodies; comparing the binding of the multi-specific antibody, or the antibodies of the plurality, to the first cell with the binding of the multi-specific antibody to the second cell to determine a binding-specificity ratio; and identifying the multi-specific antibody, or one or more of the antibodies of the plurality, as specific for the cell expressing both MDR1 and CD47

when the ratio is above a predetermined threshold. Where such a threshold for comparative binding is employed, the threshold may vary and may range from 1.5:1 or more, including but not limited to e.g., 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 20:1, 50:1, 100:1, etc.

Various cells may be used in such methods, including but not limited to e.g., the cells described herein. In some instances, the binding of the antibody to both MDR1-only expressing cells and CD47-only expressing cells may be performed. For example, in some instances, the method may include, relative to the steps describe above, where the second cell expresses MDR1 and not CD47 and the method further comprises contacting a third cell expressing CD47 but not MDR1 with the multi-specific antibody.

In some instances, such methods may employ one or more controls, including but not limited to e.g., control cells, control reagents, and the like. Useful control cells include those that have a known expression or known lack of expression of one or more relevant genes or proteins. Useful control reagents may include but are not limited to e.g., control antibodies such as but not limited to e.g., monospecific antibodies to known targets. For example, in some instances, such methods of the present disclosure may further include contacting the first cell, the second cell, and/or the third cell with a control antibody selected from: a monospecific anti-MDR1 antibody and a monospecific anti-CD47 antibody. Depending on the particular method used, various other or additional controls, as appropriate, may be employed.

KITS

Aspects of the present disclosure also include kits. The kits may include, e.g., any combination of the multi-specific antibodies, reagents, compositions, formulations, cells, nucleic acids, expression vectors, or the like, described herein. A subject kit can include one or more of: a subject multi-specific antibody, a nucleic acid encoding the same, or a cell comprising a subject multi-specific nucleic acid. Kits may be configured for various purposes, including e.g., treatment kits (e.g., where a kit may include a multi-specific antibody and e.g., one or more additional active agents, such as a chemotherapeutic), kits for producing antibodies, kits for screening antibodies, and the like.

Optional components of the kit will vary and may, e.g., include: a buffer; a protease inhibitor; etc. Where a subject kit comprises a subject nucleic acid, the nucleic acid may also have restrictions sites, multiple cloning sites, primer sites, etc. The various components of the kit may be present in separate containers or certain compatible components may be pre-combined into a single container, as desired.

In addition to above-mentioned components, a subject kit can include instructions for using the components of the kit to practice a subject method. The instructions for practicing a subject method are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the

instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging) etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. compact disc-read only memory (CD-ROM), digital versatile disk (DVD), diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

EXEMPLARY NON-LIMITING ASPECTS OF THE DISCLOSURE

Aspects, including embodiments, of the present subject matter described above may be beneficial alone or in combination, with one or more other aspects or embodiments. Without limiting the foregoing description, certain non-limiting aspects of the disclosure are provided below. As will be apparent to those of ordinary skill in the art upon reading this disclosure, each of the individually numbered aspects may be used or combined with any of the preceding or following individually numbered aspects. This is intended to provide support for all such combinations of aspects and is not limited to combinations of aspects explicitly provided below. It will be apparent to one of ordinary skill in the art that various changes and modifications can be made without departing from the spirit or scope of the invention.

1. A bispecific antibody molecule that binds multidrug resistance protein 1 (MDR1) and a tumor associated antigen (TAA), the antibody molecule comprising two identical variable light (VL) chains, a first variable heavy (VH) chain, and a second VH chain,

wherein the VL chains each comprise an antigen-binding site for MDR1, the first VH chain comprises an antigen-binding site for MDR1, and the second VH chain comprises an antigen-binding site for the TAA, and wherein the second VH chain binds the TAA when paired with one of the VL chains,

wherein the bispecific antibody binds to cancer cells expressing both MDR1 and the TAA while showing reduced binding to non-cancer cells expressing MDR1 and/or the TAA.

2. The bispecific antibody molecule according to aspect 1, wherein the antigen-binding site of the two VL chains comprises light chain CDRs 1-3 (LCDRs 1-3) of a VL chain having the sequence:

DVLMQTQTPVSLSVSLGDQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGGTKLEIK (SEQ ID
NO:1), wherein X¹ is N, Q or S.

3. The bispecific antibody molecule according to aspect 2, wherein the two VL chains comprise LCDRs 1-3 of the VL chain having the sequence:

(i)

DVLMQTQTPVSLSVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKISNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGGTKLEIK (SEQ ID NO:2);

(ii)

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPPRLIIYKISNRFSG
VPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK (SEQ ID NO:3);

or

(iii)

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPGQPPRLIIYKISN
RFGVDPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK (SEQ ID
NO:4).

4. The bispecific antibody molecule according to aspect 2 or 3, wherein:

(i) the LCDR1 comprises the sequence: RSSQSIVHSTGX¹TYLE (SEQ ID NO:5);

(ii) the LCDR2 comprises the sequence: KISNRF (SEQ ID NO:6); and

(iii) the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7);

wherein X¹ is N, Q or S.

5. The bispecific antibody molecules of any one of the preceding aspects, wherein the two VL chains are humanized.

6. The bispecific antibody molecule according to any one of aspects 2 to 5, wherein the two VL chains comprise the sequence or an amino acid at least 90% identical to the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGNTYLEWYQQRPGQPPRLIIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGGGGTKLEIK (SEQ ID
NO:8);

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPPRLIIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK (SEQ ID
NO:3); or

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPGQPPRLLIYKISN
RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGKLEIK (SEQ ID
NO:4).

7. The bispecific antibody molecule according to aspect 6, wherein the two VL chains comprise the sequence or a sequence at least 90% identical to the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGNTYLEWYQQRPGQPPRLLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGGGTKLEIK (SEQ ID
NO:8).

8. The bispecific antibody molecule according to any one of the preceding aspects, wherein the antigen binding site of first VH chain comprises heavy chain CDRs 1-3 (HCDRs 1-3) of a VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGG
GX²TYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGT
LTVSS (SEQ ID NO:9), wherein X² is N, Q or S.

9. The bispecific antibody molecule according to any one of the preceding aspects, wherein the antigen binding site of the first VH chain comprises HCDRs 1-3 of the VH chain having the sequence:

(i)

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNTYY
PDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGT LTVSS
(SEQ ID NO:10), or

(ii)

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGQTY
YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGT LTVS
S (SEQ ID NO:11), or

(iii)

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGSTY
YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGT LTVS
S (SEQ ID NO:12).

10. The bispecific antibody molecule according to aspect 8 or 9, wherein:

(i) the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13);

- (ii) the HCDR2 comprises the sequence: TISSGGG X²TYYPDSVKG (SEQ ID NO:14);
and
(iii) the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15);
wherein X² is N, Q or S.

11. The bispecific antibody molecule according to any one of the preceding aspects, wherein the first and/or second VH chain is humanized.

12. The bispecific antibody molecule according to any one of aspects 8 to 11, wherein the first VH chain comprises the amino acid sequence or an amino acid at least 90% identical to the amino acid sequence:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGNTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LVTVSS (SEQ ID NO:16);

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGQTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LVTVSS (SEQ ID NO:11); or

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGSTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LVTVSS (SEQ ID NO:12).

13. The bispecific antibody molecule according to any one of aspects 2-12, wherein:

- (i) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:26),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG (SEQ ID NO:29), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
(ii) the LCDR1 comprises the sequence: RSSQSIVHSTGQTYLE (SEQ ID NO:27),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG (SEQ ID NO:30), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
(iii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE (SEQ ID NO:28),

the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGSTYYPDSVKG (SEQ ID NO:31), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
(iv) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:26),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGGQTYYPDSVKG (SEQ ID NO:30), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
(v) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:26),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGSTYYPDSVKG (SEQ ID NO:31), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
(vi) the LCDR1 comprises the sequence: RSSQSIVHSTGQTYLE (SEQ ID NO:27),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGGNTYYPDSVKG (SEQ ID NO:29), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
(vii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE (SEQ ID NO:28),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGGNTYYPDSVKG (SEQ ID NO:29), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
(viii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE (SEQ ID NO:28),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGGQTYYPDSVKG (SEQ ID NO:30), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
(ix) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:26),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and

the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
 the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
 the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG (SEQ ID NO:30), and
 the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15).

14. The bispecific antibody molecule according to any one of the preceding aspects, wherein the second VH chain is derived from a monospecific antibody molecule which binds the TAA, and wherein the affinity of the bispecific antibody molecule for the TAA when paired with one of the light chains is at least 2-fold lower than the affinity of the monospecific antibody molecule for the TAA from which the VH chain is derived.

15. The bispecific antibody molecule according to any one of the preceding aspects, wherein the TAA is CD47.

16. The bispecific antibody molecule according to aspect 15, wherein the antigen-binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
 NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSED TAVYYCARGGYRAMDYWGQGT LVT
 VSS (SEQ ID NO:17).

17. The bispecific antibody molecule according to aspect 15 or 16, wherein the second VH chain comprises the HCDR1 comprising the sequence: NYNMH (SEQ ID NO:18), the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD (SEQ ID NO:19), and the HCDR3 comprising the sequence: GGYRAMDY (SEQ ID NO:20).

18. The bispecific antibody molecule according to any one of aspects 15 to 16, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
 NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSED TAVYYCARGGYRAMDYWGQGT LVT
 VSS (SEQ ID NO:17),

EVQLVESGGGLVQP GGSRLS CAASGYTFTNYNMHWVRQAPGKGLEWMGTIYPG
 NDDTSYNQKFKDRVTISRDN SKNTLYLQMNSLRAED TAVYYCARGGYRAMDYWGQGT LVT
 TVSS (SEQ ID NO:21),

EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYNMHWVRQMPGKGLEWMGTIYPG
 NDDTSYNQKFKDQVTISADKSISTAYLQWSSLKASDTAMYYCARGGYRAMDYWGQGTTVT
 VSS (SEQ ID NO:22), or

QVQLVQSGSELKKPGASVKVSCASGYTFTNYNMHWVRQAPGQGLEWMGTIYPG
 NDDTSYNQKFKDRFVFSLDTSVSTAYLQISSLKAEDEVYYCARGGYRAMDYWGQGTTVT
 VSS (SEQ ID NO:23).

19. The bispecific antibody molecule according to any one of aspects 1-14, wherein the TAA is PD-L1.

20. The bispecific antibody molecule according to aspect 19, wherein the antigen-binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPY
 GGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTL
 VTVSS (SEQ ID NO:32).

21. The bispecific antibody molecule according to aspect 19 or 20, wherein the antigen-binding site of the second VH chain comprises the HCDR1 comprising the sequence: DSWIH (SEQ ID NO:33), the HCDR2 comprising the sequence: WISPYGGSTYYADSVKG (SEQ ID NO:34), and the HCDR3 comprising the sequence: RHWPGGFDY (SEQ ID NO:35).

22. The bispecific antibody molecule according to any one of aspects 19 to 21, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTY
 YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLTVTVSS
 (SEQ ID NO:32).

23. The bispecific antibody molecule according to any one of aspects 1-14, wherein the TAA is EGFR.

24. The bispecific antibody molecule according to aspect 23, wherein the antigen-binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYSGSTDY
 NPSLKSRTVMSVDTSKNQFSLKVNSTAADEVYYCARVSIFGVGTFDYWGQGTLTVTVSS
 (SEQ ID NO:36).

25. The bispecific antibody molecule according to aspect 23 or 24, wherein the antigen-binding site of the second VH chain comprises the HCDR1 comprising the sequence:

SGDYYWS (SEQ ID NO:37), the HCDR2 comprising the sequence: YIYSGSTDYNPSLKS (SEQ ID NO:38), and the HCDR3 comprising the sequence: VSIFGVGTFDY (SEQ ID NO:39).

26. The bispecific antibody molecule according to any one of aspects 24 to 25, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYS
GSTDYNPSLKSRTMSVDTSKNQFSLKVNSTAAADTAVYYCARVSIFGVGTFDYWGQGT
LTVSS (SEQ ID NO:36).

27. The bispecific antibody molecule according to aspect 23, wherein the antigen-binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSG
NTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT
LTVSA (SEQ ID NO:40).

28. The bispecific antibody molecule according to aspect 26 or 27, wherein the second VH chain comprises the HCDR1 comprising the sequence: NYGVH (SEQ ID NO:41), the HCDR2 comprising the sequence: VIWSSGNTDYNTPFTS (SEQ ID NO:42), and the HCDR3 comprising the sequence: ALTYDYEFAY (SEQ ID NO:43).

29. The bispecific antibody molecule according to any one of aspects 26 to 28, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSG
NTDYNTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT
LTVSA (SEQ ID NO:40).

30. The bispecific antibody molecule according to aspect 1, wherein the antigen-binding site of the two VL chains comprises light chain CDRs 1-3 (LCDRs 1-3) of a VL chain having the sequence:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHFPRTFGGGTRLEIK (SEQ ID NO:25).

31. The bispecific antibody molecule according to aspect 30, wherein:

- (i) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:44);
- (ii) the LCDR2 comprises the sequence: KISRLEA (SEQ ID NO:45); and
- (iii) the LCDR3 comprises the sequence: FQGSHFPRT (SEQ ID NO:46).

32. The bispecific antibody molecule according to aspect 30 or 31, wherein the VL chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

DVLMQTQTPSLPVS LGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGV PDRFSGSGSGTDFTLKISRLEAEDLG VYYCFQGSHPRTFGGGTRLEIK (SEQ ID
NO:25).

33. The bispecific antibody molecule according to any one of aspects 30-32,
wherein the antigen binding site of first VH chain comprises heavy chain CDRs 1-3 (HCDRs
1-3) of a VH chain having the sequence:

EVILVESGGGLVKPGGSLKLSCAASGFTFSSYTMSWVRQTPEKRLEWVATISSGGG
NTYYPDSVKGRFTISRDNANKNNLYLQMSSLRSED TALYYCARYRYEAWFASWGQGTLVT
VSA.

34. The bispecific antibody molecule according to aspect 33, wherein:

- (i) the HCDR1 comprises the sequence: SYTMS;
- (ii) the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG; and
- (iii) the HCDR3 comprises the sequence: YYRYEAWFAS.

35. The bispecific antibody molecule according to any one of aspects 30-34,
wherein the first VH chain comprises the amino acid sequence or an amino acid at least 90%
identical to the amino acid sequence:

EVILVESGGGLVKPGGSLKLSCAASGFTFSSYTMSWVRQTPEKRLEWVATISSGGG
NTYYPDSVKGRFTISRDNANKNNLYLQMSSLRSED TALYYCARYRYEAWFASWGQGTLVT
VSA.

36. The bispecific antibody molecule according to any one of aspects 30-32,
wherein the antigen binding site of first VH chain comprises heavy chain CDRs 1-3 (HCDRs
1-3) of a VH chain having the sequence:

EVKVVESGGVLVRP GGS LKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGX²TY
YPDSVKGRFTVSRDNAMSSLYLQMSSLRSED TALYYCARYGAGDAWFAYWGQGTLVTVS
S (SEQ ID NO:9), wherein X² is N, Q or S.

37. The bispecific antibody molecule according to aspect 36, wherein:

- (i) the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13);
- (ii) the HCDR2 comprises the sequence: TISSGGG X²TYYPDSVKG (SEQ ID NO:14);

and

- (iii) the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15);

wherein X² is N, Q or S.

38. The bispecific antibody molecule according to any one of aspects 36-37,
wherein the first VH chain comprises the amino acid sequence or an amino acid at least 90%
identical to the amino acid sequence:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGNTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LVTVSS (SEQ ID NO:16);

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGQTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LVTVSS (SEQ ID NO:11); or

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGSTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LVTVSS (SEQ ID NO:12).

39. The bispecific antibody molecule according to any one of aspects 1-7, wherein the antigen binding site of first VH chain comprises heavy chain CDRs 1-3 (HCDRs 1-3) of a VH chain having the sequence:

EVILVESGGGLVKPGGSLKLSAASGFTFSSYTMSWVRQTPEKRLEWVATISSGGG
NTYYPDSVKGRFTISRDNKNNLYLQMSSLRSEDTALYYCARYYRYEAWFASWGQGT
LVT VSA.

40. The bispecific antibody molecule according to aspect 39, wherein:

- (i) the HCDR1 comprises the sequence: SYTMS;
- (ii) the HCDR2 comprises the sequence: TISSGGNTYYPDSVKG; and
- (iii) the HCDR3 comprises the sequence: YRYEAWFAS.

41. The bispecific antibody molecule according to any one of aspects 39-40, wherein the first VH chain comprises the sequence or an amino acid at least 90% identical to the sequence:

EVILVESGGGLVKPGGSLKLSAASGFTFSSYTMSWVRQTPEKRLEWVATISSGGG
NTYYPDSVKGRFTISRDNKNNLYLQMSSLRSEDTALYYCARYYRYEAWFASWGQGT
LVT VSA.

42. The bispecific antibody molecule according to any one of aspects 30-41, wherein the second VH chain is derived from a monospecific antibody molecule which binds the TAA, and wherein the affinity of the bispecific antibody molecule for the TAA when paired with one of the light chains is at least 2-fold lower than the affinity of the monospecific antibody molecule for the TAA from which the VH chain is derived.

43. The bispecific antibody molecule according to any one of aspects 30-42, wherein the TAA is CD47.

44. The bispecific antibody molecule according to aspect 43, wherein the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDTAVYYCARGGYRAMDYWGQGTLVT
VSS (SEQ ID NO:17).

45. The bispecific antibody molecule according to aspect 44, wherein the second VH chain comprises the HCDR1 comprising the sequence: NYNMH (SEQ ID NO:18), the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD (SEQ ID NO:19), and the HCDR3 comprising the sequence: GGYRAMDY (SEQ ID NO:20).

46. The bispecific antibody molecule according to aspect 44 or 45, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDTAVYYCARGGYRAMDYWGQGTLVT
VSS (SEQ ID NO:17),

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYNMHWVRQAPGKGLEWMGTIYPG
NDDTSYNQKFKDRVTISRDN SKNTLYLQMNSLRAEDTAVYYCARGGYRAMDYWGQGTLV
TVSS (SEQ ID NO:21),

EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYNMHWVRQMPGKGLEWMGTIYPG
NDDTSYNQKFKDQVTISADKSISTAYLQWSSLKASDTAMYCARGGYRAMDYWGQGTTVT
VSS (SEQ ID NO:22), or

QVQLVQSGSELKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQGLEWMGTIYPG
NDDTSYNQKFKDRFVFSLDTSVSTAYLQISSLKAEDTAVYYCARGGYRAMDYWGQGTTVT
VSS (SEQ ID NO:23).

47. The bispecific antibody molecule according to any one of aspects 30-42, wherein the TAA is PD-L1.

48. The bispecific antibody molecule according to aspect 47, wherein the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTY YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS (SEQ ID NO:32).

49. The bispecific antibody molecule according to aspect 47 or 48, wherein the second VH chain comprises the HCDR1 comprising the sequence: DSWIH (SEQ ID NO:33), the HCDR2 comprising the sequence: WISPYGGSTYYADSVKG (SEQ ID NO:34), and the HCDR3 comprising the sequence: RHWPGGFDY (SEQ ID NO:35).

50. The bispecific antibody molecule according to any one of aspects 48 to 49, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAVISPY
GGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGGQTL
VTVSS (SEQ ID NO:32).

51. The bispecific antibody molecule according to any one of aspects 30-42, wherein the TAA is EGFR.

52. The bispecific antibody molecule according to aspect 51, wherein the antigen binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYSGSTDY
NPSLKSRVTMSVDTSKNQFSLKVNSVTAADTAVYYCARVSIFGVGTFDYWGQGLTVTVSS
(SEQ ID NO:36).

53. The bispecific antibody molecule according to aspect 52, wherein the second VH chain comprises the HCDR1 comprising the sequence: SGDYYWS (SEQ ID NO:37), the HCDR2 comprising the sequence: YIYYSGSTDYNPSLKS (SEQ ID NO:38), and the HCDR3 comprising the sequence: VSIFGVGTFDY (SEQ ID NO:39).

54. The bispecific antibody molecule according to any one of aspects 52 to 53, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYSGSTDY
NPSLKSRVTMSVDTSKNQFSLKVNSVTAADTAVYYCARVSIFGVGTFDYWGQGLTVTVSS
(SEQ ID NO:36).

55. The bispecific antibody molecule according to aspect 51, wherein the antigen binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSSGGNTDY
NTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTVSA
(SEQ ID NO:40).

56. The bispecific antibody molecule according to aspect 52, wherein the antigen binding site of the second VH chain comprises the HCDR1 comprising the sequence: NYGVH (SEQ ID NO:41), the HCDR2 comprising the sequence: VIWSSGGNTDYNTPFTS (SEQ ID NO:42), and the HCDR3 comprising the sequence: ALTYDYEFAY (SEQ ID NO:43).

57. The bispecific antibody molecule according to any one of aspects 52 to 53, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:
QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVWSSGGNTDY
NTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTVSA
(SEQ ID NO:40).

58. The bispecific antibody molecule according to any one of the preceding aspects, wherein the antibody has greater than twice the affinity for a cell expressing both MDR1 and the TAA as compared to a cell expressing either MDR1 or the TAA.

59. The bispecific antibody molecule according to any one of the preceding aspects, wherein the antibody is capable of increasing sensitivity of a cancer cell to treatment with a chemotherapeutic agent, wherein the half maximal inhibitory concentration (IC₅₀) of the chemotherapeutic agent when co-administered with the antibody is at least 2 times lower than the IC₅₀ of the chemotherapeutic agent when co-administered with the anti-MDR1 antibody 15D3 comprising a VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGT
LTVSA (SEQ ID NO:24); and

a VL chain having the sequence:

DVLMQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVIYCFQGSHFPRTFGGGTRLEIK (SEQ ID
NO:25), wherein optionally the IC₅₀ is measured in vitro.

60. The bispecific antibody molecule according to aspect 59, wherein the cancer cell is a NALM6 ADR cell and optionally wherein the chemotherapeutic agent comprises paclitaxel, Colchicine, Verapamil, Vinblastine, Topotecan, Doxorubicin, Daunorubicin, Etoposide, or Nilotinib.

61. The bispecific antibody molecule according to any one of the preceding aspects, wherein the antibody, when bound to a cell expressing a MDR1, inhibits efflux by the MDR1.

62. The bispecific antibody molecule according to any one of the preceding aspects, wherein the antibody binds to MDR1 with at least 2-fold lower affinity than the anti-MDR1 antibody 15D3 comprising a VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGT
LTVSA (SEQ ID NO:24); and

a VL chain having the sequence:

DVLMQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVIYCFQGSHFPRTFGGGTRLEIK (SEQ ID
NO:25).

63. The bispecific antibody molecule according to any one of the preceding aspects, wherein the antibody comprises a Fc domain that has been modified to reduce or abrogate binding of the antibody to one or more Fcγ receptors.

64. The bispecific antibody molecule according to any one of the preceding aspects for use in a method of treating cancer in a subject, the method comprising administering the antibody to the subject.

65. The bispecific antibody molecule for use according to aspect 64, wherein the method comprises administering the antibody in combination with at least one additional active agent, wherein the at least one additional active agent comprises a chemotherapeutic agent, an inhibitor of a multidrug resistance transporter, an immunotherapy agent, or a combination thereof.

66. The bispecific antibody molecule for use according to aspect 65, wherein the at least one additional active agent is a chemotherapeutic agent, optionally wherein the chemotherapeutic agent is a taxol, a vinca alkaloid, or an anthracycline.

67. A chemotherapy agent for use in a method of treating cancer in a subject, the method comprising administering the chemotherapy agent in combination with the antibody according to any one of aspects 1 to 63 to the subject, optionally wherein the chemotherapy agent is a taxol, a vinca alkaloid, or an anthracycline.

68. The bispecific antibody molecule for use according to aspects 67, wherein the subject being treated has a cancer that has been determined to be resistant to the chemotherapeutic agent.

69. A method of treating a subject for a cancer, the method comprising administering to the subject a therapeutically effective amount of the bispecific antibody molecule according to any of aspects 1 to 63.

70. The method according to aspect 69, wherein the method comprises administering the bispecific antibody molecule in combination with at least one additional active agent, wherein the at least one additional active agent comprises a chemotherapeutic agent, an inhibitor of a multidrug resistance transporter, an immunotherapy agent, or a combination thereof.

71. The method according to aspect 70, wherein the at least one additional active agent is a chemotherapeutic agent, optionally wherein the chemotherapeutic agent is a taxol, a vinca alkaloid, or an anthracycline.

72. The method according to aspect 71, wherein the subject being treated has a cancer that has been determined to be resistant to treatment with the chemotherapeutic agent, wherein optionally the chemotherapeutic agent comprises paclitaxel, Colchicine, Verapamil, Vinblastine, Topotecan, Doxorubicin, Daunorubicin, Etoposide, or Nilotinib.

73. An antibody comprising:

a variable light (VL) chain comprising light chain CDRs (LCDRs) of a VL chain having the sequence:

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK,

wherein X¹ is N, Q or S; and

a variable heavy (VH) chain comprising heavy chain CDRs (HCDRs) of a VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGG
GX²TYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGT
LVTVSS,

wherein X² is N, Q or S; or

an antibody comprising:

a variable light (VL) chain comprising light chain CDRs (LCDRs) of a VL chain having the sequence:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHPRTFGGGTRLEIK (SEQ ID
NO:25); and

a variable heavy (VH) chain comprising heavy chain CDRs (HCDRs) of a VH chain having the sequence:

EVILVESGGGLVKPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGG
NTYYPDSVKGRFTISRDNANKNNLYLQMSSLRSEDALYYCARYRYEAWFASWGQGT
LVT VSA.

74. The antibody according to aspect 73, wherein:

the VL chain comprises LCDRs 1-3 of the VL chain having the sequence:

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK; and

the VH chain comprises HCDRs 1-3 of the VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGT
LVT VSS, or

the VL chain comprises LCDRs 1-3 of the VL chain having the sequence:

DIVMTQTPLSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPRLIIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK; and

the VH chain comprises HCDRs 1-3 of the VH chain having the sequence:

EVQLVESGGVTVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGQTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGT
LVT VSS, or

the VL chain comprises LCDRs 1-3 of the VL chain having the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPGQPPRLLIYKISN
RFSGVPDRFSGSGAGTDFTLKISRVEAEDVGYYCFQASHFPRTFGQGKLEIK; and

the VH chain comprises LCDRs 1-3 of the VH chain having the sequence:

EVQLVESGGVTVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGSTYYPDSPVKGFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LTVSS.

75. The antibody according to aspect 74, wherein:

the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE,
RSSQSIVHSTGQTYLE, or RSSQSIVHSTGSTYLE,

the LCDR2 comprises the sequence: KISNRFS, and

the LCDR3 comprises the sequence: FQASHFPRT.

76. The antibody according to aspect 75, wherein:

(i) the HCDR1 comprises the sequence: RYTMS;

(ii) the HCDR2 comprises the sequence: TISSGGG X²TYYPDSVKG; and

(iii) the HCDR3 comprises the sequence: YGAGDAWFAY;

wherein X² is N, Q or S.

77. The antibody according to any one of aspects 74-75, wherein:

- (i) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE,
the LCDR2 comprises the sequence: KISNRFS, and
the LCDR3 comprises the sequence: FQASHFPRT; and
the HCDR1 comprises the sequence: RYTMS,
the HCDR2 comprises the sequence: TISSGGGNTYYPDSPVKG, and
the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (ii) the LCDR1 comprises the sequence: RSSQSIVHSTGQTYLE,
the LCDR2 comprises the sequence: KISNRFS, and
the LCDR3 comprises the sequence: FQASHFPRT; and
the HCDR1 comprises the sequence: RYTMS,
the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and
the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (iii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE,
the LCDR2 comprises the sequence: KISNRFS, and
the LCDR3 comprises the sequence: FQASHFPRT; and
the HCDR1 comprises the sequence: RYTMS,
the HCDR2 comprises the sequence: TISSGGGSTYYPDSPVKG, and
the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (iv) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE,
the LCDR2 comprises the sequence: KISNRFS, and

- the LCDR3 comprises the sequence: FQASHFPRT; and
the HCDR1 comprises the sequence: RYTMS,
the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and
the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (v) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE,
the LCDR2 comprises the sequence: KISNRFS, and
the LCDR3 comprises the sequence: FQASHFPRT; and
the HCDR1 comprises the sequence: RYTMS,
the HCDR2 comprises the sequence: TISSGGGSTYYPDSVKG, and
the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (vi) the LCDR1 comprises the sequence: RSSQSIVHSTGQTYLE,
the LCDR2 comprises the sequence: KISNRFS, and
the LCDR3 comprises the sequence: FQASHFPRT; and
the HCDR1 comprises the sequence: RYTMS,
the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG, and
the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (vii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE,
the LCDR2 comprises the sequence: KISNRFS, and
the LCDR3 comprises the sequence: FQASHFPRT; and
the HCDR1 comprises the sequence: RYTMS,
the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG, and
the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (viii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE,
the LCDR2 comprises the sequence: KISNRFS, and
the LCDR3 comprises the sequence: FQASHFPRT; and
the HCDR1 comprises the sequence: RYTMS,
the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and
the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (ix) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE,
the LCDR2 comprises the sequence: KISNRFS, and
the LCDR3 comprises the sequence: FQASHFPRT; and
the HCDR1 comprises the sequence: RYTMS,
the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and
the HCDR3 comprises the sequence: YGAGDAWFAY.

78. The antibody according to any one of aspects 73-77, wherein the VL chain of the antibody comprises the amino acid sequence or comprises an amino acid sequence at least 90% identical to the amino acid sequence:

DVLMTQTTPVSLSVSLGDQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK, and

wherein the VH chain of the antibody comprises the amino acid sequence or
comprises an amino acid sequence at least 90% identical to the amino acid sequence:
EVKVVESGGVLVRPGGSLKLSAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGX²TY
YPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGLTVTS
S.

79. The antibody according to aspect 73, wherein the an antibody comprises:
a variable light (VL) chain comprising light chain CDRs 1-3 (LCDRs) of a VL chain
having the sequence:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHPRTFGGGTRLEIK (SEQ ID
NO:25); and

a variable heavy (VH) chain comprising heavy chain CDRs 1-3 (HCDRs) of a VH
chain having the sequence:

EVILVESGGGLVKPGGSLKLSAASGFTFSYTMWVRQTPEKRLEWVATISSGGG
NTYYPDSVKGRFTISRDNANKNNLYLQMSSLRSEDALYYCARYRYEAWFASWGQGLTVT
VSA.

80. The antibody according to aspect 79, wherein:

- (i) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:44);
- (ii) the LCDR2 comprises the sequence: KISRLEA (SEQ ID NO:45); and
- (iii) the LCDR3 comprises the sequence: FQGSHPRT (SEQ ID NO:46); and
- (i) the HCDR1 comprises the sequence: SYTMS;
- (ii) the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG; and
- (iii) the HCDR3 comprises the sequence: YYRYEAWFAS.

81. The antibody according to aspect 79 or 80, wherein the VL chain of the
antibody comprises the amino acid sequence or comprises an amino acid sequence at least
90% identical to the amino acid sequence:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVSNRFSG
VPDRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHPRTFGGGTRLEIK (SEQ ID NO:25);

and wherein the VH chain of the antibody comprises the amino acid sequence or comprises
an amino acid sequence at least 90% identical to the amino acid sequence:

EVILVESGGGLVKPGGSLKLSAASGFTFSYTMWVRQTPEKRLEWVATISSGGGNTYYP
DSVKGRFTISRDNANKNNLYLQMSSLRSEDALYYCARYRYEAWFASWGQGLTVTVSA.

82. The antibody according to any one of aspects 73-81, wherein the antibody is
a monospecific bivalent antibody that specifically binds to MDR-1.

83. The antibody according to any one of aspects 73-81, wherein the antibody is a bi-specific antibody comprising the VL chain as a common light chain.

84. The antibody according to aspect 83, wherein the bispecific antibody comprises an MDR-1 binding domain and a tumor associated antigen (TAA) binding domain wherein each of the MDR-1 binding domain and the TAA binding domain comprises the LCDRs 1-3 of the VL chain.

85. The antibody according to aspect 84, wherein the TAA is CD47 and wherein the TAA binding domain comprises HCDRs 1-3 of the VH chain having the sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVITADTSASTAYMELSSLRSEDTAVYYCARGGYRAMDYWGQGLTVT
VSS.

86. The antibody according to aspect 85, wherein the VH chain of the CD47 binding domain comprises the HCDR1 comprising the sequence: NYNMH, the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD, and the HCDR3 comprising the sequence: GGYRAMDY.

87. The antibody according to aspect 84, wherein the TAA is PD-L1 and wherein the PD-L1 binding domain comprises HCDRs 1-3 of the VH chain having the sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHVVRQAPGKGLEWVAVWISPYGGSTY
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGLTVTVSS
(SEQ ID NO:32).

88. The antibody according to aspect 87, wherein the VH chain of the PD-L1 binding domain comprises the HCDR1 comprising the sequence: DSWIH (SEQ ID NO:33), the HCDR2 comprising the sequence: WISPYGGSTYYADSVKG (SEQ ID NO:34), and the HCDR3 comprising the sequence: RHWPGGFDY (SEQ ID NO:35).

89. The antibody according to aspect 84, wherein the TAA is EGFR and wherein the EGFR binding domain comprises HCDRs of the VH chain having the sequence:
QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYSGSTDY
NPSLKSRTMSVDTSKNQFSLKVNSTAAADTAVYYCARVSIFGVGTFDYWGQGLTVTVSS
(SEQ ID NO:36).

90. The antibody according to aspect 89, wherein the VH chain of the EGFR binding domain comprises the HCDR1 comprising the sequence: SGDYYWS (SEQ ID NO:37), the HCDR2 comprising the sequence: YIYYSGSTDYNPSLKS (SEQ ID NO:38), and the HCDR3 comprising the sequence: VSIFGVGTFDY (SEQ ID NO:39).

91. The antibody according to aspect 84, wherein the TAA is EGFR and wherein the EGFR binding domain comprises HCDRs of the VH chain having the sequence:
QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGGNTDY

NTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVSA (SEQ ID NO:40).

92. The antibody according to aspect 91, wherein the VH chain of the EGFR binding domain comprises the HCDR1 comprising the sequence: NYGVH (SEQ ID NO:41), the HCDR2 comprising the sequence: VIWSSGNTDYNTPTFS (SEQ ID NO:42), and the HCDR3 comprising the sequence: ALTYDYEFAY (SEQ ID NO:43).

93. An antibody comprising:

a variable light (VL) chain comprising light chain CDRs (LCDRs) of a VL chain having the sequence:

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK,

wherein X¹ is N, Q or S; or

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHPRTFGGGTRLEIK (SEQ ID NO:25); and

a variable heavy (VH) chain comprising heavy chain CDRs (HCDRs) of a VH chain having the sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVITADTSASTAYMELSSLRSEDTAVYYCARGGYRAMDYWGQGLTVT
VSS; or

EVQLVESGGGLVQPGLSLRSLCAASGFTFSDSWIHWVRQAPGKGLEWVAVISPY
GGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGLT
VTVSS (SEQ ID NO:32); or

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYS
GSTDYNPSLKSRTMSVDTSKNQFSLKNSVTAADTAVYYCARVSIFGVGTFDYWGQGLT
VTVSS (SEQ ID NO:36); or

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSG
NTDYNTPTFSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVT
VSA (SEQ ID NO:40).

94. The antibody according to aspect 93, wherein the VL chain comprises the (i) the LCDR1 comprises the sequence: RSSQSIVHSTGX¹TYLE (SEQ ID NO:5); (ii) the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6); and (iii) the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7), wherein X¹ is N, Q or S and wherein the VH chain comprises:

(i) the HCDR1 comprising the sequence: NYNMH, the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD, and the HCDR3 comprising the sequence: GGYRAMDY; or

- (ii) the HCDR1 comprising the sequence: DSWIH (SEQ ID NO:33), the HCDR2 comprising the sequence: WISPYGGSTYYADSVKG (SEQ ID NO:34), and the HCDR3 comprising the sequence: RHWPGGFDY (SEQ ID NO:35); or
- (iii) the HCDR1 comprising the sequence: SGDYYWS (SEQ ID NO:37), the HCDR2 comprising the sequence: YIYYSGSTDYNPSLKS (SEQ ID NO:38), and the HCDR3 comprising the sequence: VSIFGVGTDFY (SEQ ID NO:39); or
- (iv) the HCDR1 comprising the sequence: NYGVH (SEQ ID NO:41), the HCDR2 comprising the sequence: VIWSGGNTDYNTPTS (SEQ ID NO:42), and the HCDR3 comprising the sequence: ALTYDYEFAY (SEQ ID NO:43).

95. The antibody according to aspect 93, wherein the VL chain comprises the (i) the LCDR1 comprising the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:44); (ii) the LCDR2 the the sequence: KISRLEA (SEQ ID NO:45); and (iii) the LCDR3 the sequence: FQGSHFPT (SEQ ID NO:46) and wherein the VH chain comprises:

the HCDR1 comprising the sequence: NYNMH, the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD, and the HCDR3 comprising the sequence: GGYRAMDY; or

the HCDR1 comprising the sequence: DSWIH (SEQ ID NO:33), the HCDR2 comprising the sequence: WISPYGGSTYYADSVKG (SEQ ID NO:34), and the HCDR3 comprising the sequence: RHWPGGFDY (SEQ ID NO:35); or

the HCDR1 comprising the sequence: SGDYYWS (SEQ ID NO:37), the HCDR2 comprising the sequence: YIYYSGSTDYNPSLKS (SEQ ID NO:38), and the HCDR3 comprising the sequence: VSIFGVGTDFY (SEQ ID NO:39); or

the HCDR1 comprising the sequence: NYGVH (SEQ ID NO:41), the HCDR2 comprising the sequence: VIWSGGNTDYNTPTS (SEQ ID NO:42), and the HCDR3 comprising the sequence: ALTYDYEFAY (SEQ ID NO:43).

96. The antibody according to any one of aspects 93-95, wherein the antibody is a bispecific antibody.

97. The antibody according to any one of aspects 1-96, wherein the antibody is a humanized antibody or a chimeric antibody comprising human Fc domain.

98. The antibody according to aspect 97, comprising immunoglobulin G1 (IgG1) Fc domain.

99. The antibody according to any one of aspects 1-98, wherein a first VH chain is fused to a first Fc domain and a second VH chain is fused to a second Fc domain.

100. The antibody according to aspect 99, wherein the Fc domains comprise modified CH3 domains that preferentially form heterodimers comprising the first and second VH chains.

101. The antibody according to aspect 100, wherein the first and second Fc domains are human immunoglobulin G1 (IgG1) Fc domains.

102. A pharmaceutical composition comprising:
the antibody of any of the preceding aspects; and
a pharmaceutically acceptable excipient.

103. The pharmaceutical composition according to aspect 102, further comprising at least one additional active agent.

104. The pharmaceutical composition according to aspect 103, wherein the at least one additional active agent comprises a chemotherapy agent.

105. The pharmaceutical composition according to aspect 104, wherein the chemotherapy agent is a taxol, a vinca alkaloid, or an anthracycline.

106. The pharmaceutical composition according to any of aspects 103 to 105, wherein the at least one additional active agent comprises an inhibitor of a multidrug resistance transporter.

107. The pharmaceutical composition according to aspect 103, wherein the at least one additional active agent comprises an immunotherapy agent.

108. One or more nucleic acids comprising one or more sequences encoding the antibody according to any of the preceding aspects.

109. The one or more nucleic acid according to aspect 108, wherein the one or more sequences are operably linked to a promoter.

110. One or more recombinant expression vectors comprising the one or more nucleic acids according to aspect 108 or 109.

111. A mammalian cell genetically modified with the one or more recombinant expression vectors according to aspect 110.

112. The cell according to aspect 111, wherein the cell is an immune cell.

113. A kit comprising:
the antibody or a nucleic acid encoding the antibody, according to any of aspects 1 to 63 and 73-101; and
at least one additional active agent.

114. The kit according to aspect 113, wherein the at least one additional active agent comprises a chemotherapy agent, an inhibitor of a multidrug resistance transporter, an immunotherapy agent, or a combination thereof.

115. A method of killing a cancer cell, the method comprising contacting the cancer cell with the antibody according to any of aspects 1 to 63 and 73-101.

116. The method according to aspect 115, comprising administering at least one additional active agent.

117. The method according to aspect 116, wherein the at least one additional active agent comprises a chemotherapy agent.

118. The method according to aspect 116 or 117, wherein the method increases the killing of the cancer cell by at least 5% as compared to contacting with the at least one additional active agent alone.

119. The method according to any of aspects 115 to 118, wherein the cancer cell is a drug resistant cancer cell.

120. A method of treating a subject for a cancer, the method comprising administering to the subject the antibody according to any of aspects 1 to 63 and 73-101 or the pharmaceutical composition according to any of aspects 102-107.

121. The method according to aspect 120, wherein the subject has been treated previously for the cancer.

122. The method according to aspect 120 or 121, wherein the cancer is drug resistant or multidrug resistant.

123. The method according to aspect 122, wherein the cancer is resistant to a chemotherapeutic agent.

124. The method according to aspect 122, wherein the cancer is resistant to an immunotherapy agent.

125. The method according to any of aspects 120 to 122, wherein the cancer is resistant to an inhibitor of a multidrug resistance transporter.

126. The method according to any of aspects 120 to 125, further comprising administering at least one additional active agent to the subject.

127. The method according to aspect 126, wherein the at least one additional active agent comprises a chemotherapy agent.

128. The method according to aspect 127, wherein the chemotherapy agent is a taxol, a vinca alkaloid, or an anthracycline.

129. The method according to any of aspects 126 to 128, wherein the at least one additional active agent comprises an inhibitor of a multidrug resistance transporter.

130. The method according to any of aspects 126 to 128, wherein the at least one additional active agent comprises an immunotherapy agent.

131. The method according to any of aspects 126 to 128, wherein the method increases the effectiveness of the at least one additional active agent as compared to treatment with the at least one additional active agent alone.

132. The method according to aspect 131, wherein the increased effectiveness comprises an at least 5% increase in cancer cell killing.

133. The method according to any of aspects 120 to 132, further comprising analyzing a sample of the cancer to determine whether the cancer expresses MDR1 above a

predetermined threshold, a tumor associated antigen (TAA) above a predetermined threshold, or both, wherein optionally the TAA comprises CD47.

134. The method according to aspect 133, wherein the predetermined threshold corresponds to a level of MDR1 and/or TAA expressed by a reference cell.

135. The method according to aspect 134, wherein MDR1 and/or TAA has been knocked-out of or knocked-down in the reference cell.

136. The method according to aspect 134 or 135, wherein the reference cell is a non-cancerous cell.

137. The method according to aspect 134, wherein the non-cancerous cell expresses a normal level of MDR1 and/or TAA.

138. The method according to any of aspects 120 to 137, wherein if the cancer expresses MDR1 and TAA at or above the predetermined thresholds then the subject is administered the multi-specific antibody, and if the cancer expresses MDR1 or TAA below the predetermined thresholds then the subject is treated with a conventional therapy without administering the multi-specific antibody.

139. A method of generating a multi-specific antibody that specifically binds a cell expressing both multidrug resistance protein 1 (MDR1) and a tumor associated antigen (TAA), wherein optionally the TAA comprises CD47, PD-L1, or EGFR, the method comprising:

producing a multi-specific antibody comprising a MDR1-binding domain and a TAA-binding domain;

contacting a first cell expressing MDR1 and TAA with the multi-specific antibody;

contacting a second cell expressing either MDR1 or TAA with the multi-specific antibody;

comparing the binding of the multi-specific antibody to the first cell with the binding of the multi-specific antibody to the second cell to determine a binding-specificity ratio; and

identifying the multi-specific antibody as specific for the cell expressing both MDR1 and TAA when the ratio is above a predetermined threshold.

140. The method according to aspect 139, wherein the predetermined threshold is greater than 2:1.

141. The method according to aspect 139 or 140, wherein the second cell expresses MDR1 and not the TAA and the method further comprises contacting a third cell expressing the TAA but not MDR1 with the multi-specific antibody.

142. The method according to any of aspects 139 or 140, wherein the method further comprises contacting the first cell, the second cell, and/or the third cell with a control antibody selected from: a monospecific anti-MDR1 antibody and a monospecific anti-TAA antibody.

143. A genetically modified human cell line, the cell line expressing a tumor associated antigen (TAA), wherein optionally the TAA comprises leukocyte surface antigen

CD47 and comprising an exogenous nucleic acid comprising a sequence encoding a multidrug resistance protein 1 (MDR1) for overexpression of MDR1.

144. The cell line according to aspect 143, wherein the cell line is a kidney cell line.

145. The cell line according to aspect 144, wherein the cell line is a HEK 293T cell line.

146. A method of making the cell line according to any of aspects 143 to 145, the method comprising contacting a human cell that expresses the TAA with the exogenous nucleic acid under conditions sufficient to introduce the exogenous nucleic acid into the cell to produce a genetically modified human cell expressing the TAA and stably overexpressing MDR1; and

culturing the genetically modified human cell under conditions sufficient to produce a genetically modified human cell line expressing the TAA and stably overexpressing MDR1.

The following example(s) is/are offered by way of illustration and not by way of limitation.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with
5 a complete disclosure and description of how to make and use the present invention, and are not
intended to limit the scope of what the inventors regard as their invention nor are they intended
to represent that the experiments below are all or the only experiments performed. Efforts have
been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.)
but some experimental errors and deviations should be accounted for. Unless indicated
10 otherwise, parts are parts by weight, molecular weight is weight average molecular weight,
temperature is in degrees Centigrade, and pressure is at or near atmospheric.

General methods in molecular and cellular biochemistry can be found in such standard
textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook et al., HaRBor
Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel et al. eds., John
15 Wiley & Sons 1999); Protein Methods (Bollag et al., John Wiley & Sons 1996); Nonviral Vectors
for Gene Therapy (Wagner et al. eds., Academic Press 1999); Viral Vectors (Kaplift & Loewy eds.,
Academic Press 1995); Immunology Methods Manual (I. Lefkovits ed., Academic Press 1997);
and Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, John
Wiley & Sons 1998), the disclosures of which are incorporated herein by reference. Reagents,
20 cloning vectors, cells, and kits for methods referred to in, or related to, this disclosure are available
from commercial vendors such as BioRad, Agilent Technologies, Thermo Fisher Scientific,
Sigma-Aldrich, New England Biolabs (NEB), Takara Bio USA, Inc., and the like, as well as
repositories such as e.g., Addgene, Inc., American Type Culture Collection (ATCC), and the like.

25 *Example 1: Generation of Bispecific mAbs that bind specifically to, and re-sensitize, cells
simultaneously expressing Pgp (MDR1) and CD47, but not cells having reduced or absent
expression of either protein*

This example demonstrates the development of molecules based on antibody formats that
can effectively blockade efflux by binding to extracellular domains of EPs in a selective manner
30 while also specifically targeting antigens expressed by cancer cells. EP blockade results in the
re-sensitization and killing of cells that are or have become resistant to chemotherapeutic agents.
In this example bispecific antibody molecules have been constructed that bind the extracellular
domain (ECD) of an EP, blockading efflux while also targeting a cancer immune checkpoint

protein. Application of the bispecific antibody described below re-sensitizes cells to chemotherapeutic agents in a selective manner when the EP is co-expressed with the targeted immune checkpoint protein.

Materials and Methods

Cell Lines and Cell Viability Experiments

HEK 293T, MCF-7, N6ADR and SKNF7 cell lines expressing Pgp were obtained from the American Type Culture Collection. N6/ADR cells is also referred to as NALM6/ADR cells. All cell lines and lines derived from them were maintained in RPMI 1640 or DMEM supplemented with up to 10% fetal bovine serum (Sigma), nonessential amino acids, and 2 mmol/L L-glutamine at 37 °C and 5% CO₂ in a humidified incubator (unless otherwise indicated). Cells were used as supplied or were modified to overexpress (Ox) Pgp or were subject to having Pgp expression knocked down (KD) with lentiviral-mediated short hairpin RNA or knock out (KO) of the functional CD47 gene by CRISPR/Cas-mediated knock out technology essentially as described (Cong, L. et al. (2013) *Science* 339, 819–823). For vincristine and paclitaxel IC₅₀ determination, cells were plated in normal growth medium and allowed to adhere overnight. Paclitaxel or vincristine (Sigma) were added in a dilution series and any modulators were added within ranges of 0 to 500µM/L. Cell viability was measured 72 h later using the Celltiter-Glo Luminescent Cell Viability Assay (Promega). The concentration of drug resulting in 50% inhibition of cell viability (IC₅₀) was calculated from a multi-parameter curve analysis (GraphPad Prism software GraphPad Software, Inc.) and was determined from a minimum of 2 repeats. Cell lines that did not show 50% reduction in cell viability in response to drug and / or modulator treatment in the majority of experiments conducted were considered to not have reached an IC₅₀ by definition and are listed as having an IC₅₀ of >1000 nmol/L for paclitaxel or the drug / modulator combination under study.

Recombinant cell lines with stable expression of the described monoclonal antibodies (mAbs) were also produced.

Recombinant DNA Techniques

Standard methods were used to manipulate DNA as described in Sambrook, J. et al., *Molecular cloning: A laboratory manual*; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989. Reagents were used according to the manufacturer's instructions. General information regarding the nucleotide sequences of human immunoglobulins light and heavy chains is given in: Kabat, E. A. et al., (1991) *Sequences of Proteins of Immunological Interest*, 5th ed., NIH Publication No. 91-3242 Amino acids of antibody chains were numbered and referred to according to Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest*, 5th ed., Public Health Service, National Institutes of Health, Bethesda, Md., (1991).

DNA Sequence Determination

DNA sequences were determined by double strand sequencing.

DNA and Protein Sequence Analysis and Sequence Data Management

The Vector NTI (ThermoFisher) software package was used for sequence mapping,
5 analysis, annotation and illustration.

Cell Culture Techniques and Antibody Production

Standard cell culture techniques are used as described in Current Protocols in Cell Biology (2000), Bonifacino, J. S., Dasso, M., Harford, J. B., Lippincott-Schwartz, J. and Yamada, K. M. (eds.), John Wiley & Sons, Inc.

10 293 & CHO cells were used for transient production of mAbs, Fab'2s, Fabs and bispecific mAbs. Different antibody constructs were expressed using polymer-based co-transfection of Expi293 cells (A14527, ThermoFisher). Cells were grown in suspension with the mammalian expression vectors following the manufacturer's recommendations.

For preparation of bispecific constructs, cells were transfected with the corresponding
15 expression vectors in a 1:1:4 ratio (heavy chain KK: heavy chain DD: light chain). For standard antibody expression a 1:2 ratio (heavy chain: light chain) was used.

Six days after transfection the cells were harvested by centrifugation. In detail, 1 µg of total encoding DNA per 1ml of transfected culture was diluted into of Opti-MEM® medium (Life Technologies), and incubated with Expifectamine reagent (Life Technologies) in the same
20 medium for 20 min. The mixture was then added into the Expi293® cells growing in suspension in Expi293® Expression medium (Life Technologies) at 2.5 million cells/ml at 37° C with and overlay of 8% of CO₂ in air. After 6 days, the medium containing the antibody construct was harvested by centrifugation.

Reagent Cell Lines Used To Test: Binding, Efflux Blockade, Cell Sensitization To 25 Chemotherapeutics

Human embryonic kidney (HEK) cell line HEK 293FT (Life Technologies) was maintained in Dulbecco's modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (HyClone), 2 mM GlutaMAX (Life Technologies), 100 U/mL penicillin, and 100 g/mL streptomycin at 37° C with 5% CO₂ incubation.

30 293T cells were transiently transfected with the Human P Glycoprotein Tagged ORF Clone in pLenti-C-Myc-DDK-P2A-Puro plasmid using the optimized PEIPro™ transfection protocol (Polyplus). DNA and JetPEI® were respectively diluted in culture media before being gently mixed for approximately 10min. This mixing led to the formation of a transfection complex, which was

directly added to the cell culture. Efflux blockade was measure using the Multidrug Resistance Direct Dye Efflux Assay (Chemicon) following the manufacturer's protocol.

Sources of Target Sequences, Antibody Sequences and Specific Anti-Target Antibody Sequences

P Glycoprotein (Pgp), also known as Multidrug resistance protein 1 (MDR1), (gene ABCB1) (NM_000927) Human Tagged ORF Clone in pLenti-C-Myc-DDK-P2A-Puro was obtained from Origene, anti CD47 antibody (CC2C6, Seiffert M, *et al.* (1999) *Blood* 94:3633) from Biolegend, anti-ABCB1 JSB-1 (MAB4120) from Millipore.

Generation of a Stable ABCB1 Overexpressing (Ox) Cell Lines

In order to characterize both binding and *in vitro* efficacy, a cell line that stably over-expressed ABCB1 was developed. Adherent 293T naïve cells obtained from American Type Culture Collection (ATCC) were utilized. As characterized by flow cytometry using a commercially available ABCB1 antibody (Biolegend, clone 4E3.16), this cell line expresses ABCB1 endogenously at a low to moderate degree on the cell surface. 293T naïve cells were transfected with ABCB1 using Polyplus PEIpro reagent. Three days after transfection, cells were put under selection using a Hygromycin B solution (Millipore Sigma). Fourteen days after continuous Hygromycin B selection 293T cells were evaluated for ABCB1 cell surface expression. To ensure non-transfected cells would not expand in future cultures, a bulk sort using fluorescent activated cell sorting (FACS) of ABCB1 positive 293T cells was performed using a FACSArial (BD Biosciences). The bulk sorted 293T ABCB1 over-expressing cells were expanded and ABCB1 over-expression was subsequently re-confirmed. Similar methods were used to generate ABCB1 over-expressing cells from 293T-CD47 Knock-Out cells generated as described herein.

Generation of a Stable ABCB1 KD 293T Cell Line

In order to characterize both binding and *in vitro* efficacy a cell line that had a stable knockdown of ABCB1 expression was developed. Lentivirus was produced in 293T naïve cells by transfection with a R8.74 helper plasmid, a VSVG envelope plasmid, and a GE Dharmacon GIPZ lentiviral vector containing a shRNA for ABCB1. Harvested lentivirus was then used to transduce adherent 293T naïve cells. Three days post transduction, the 293T transduced cells were evaluated for ABCB1 cell surface expression by flow cytometry (Biolegend, clone 4E3.16). As compared to 293T naïve cells, which express ABCB1 endogenously at a low level, the transduced 293T cells had no ABCB1 expression. In addition to this, the GIPZ lentiviral vector contains GFP. All transduced 293T cells were GFP+, indicating, in conjunction with the decrement in expression, that the transduction was successful. A lack of ABCB1 expression was re-confirmed by flow cytometry in subsequent passages.

Generation of KO Cell Lines

To construct ABCB1, and CD47 gene knockout HEK293 cell line, HEK293 host cells were first cultured in DMEM (Dulbecco's Modified Eagle's Medium, Gibco, Grand Island, N.Y., USA) supplemented with 10% (v/v) FBS, and glutamine via adhesion culture. Cells were cultivated at 37°C with 5% CO₂ at saturated humidity.

The design of the gRNAs was performed using the online CHOPCHOP web tool for selecting target sites for CRISPR/Cas9, CRISPR/Cpf1 or TALEN-directed mutagenesis. (see Kornel Labun et al., (2016). *Nucleic Acids Research*; and Tessa G. Montague et al., (2014) *Nucleic Acids Res.* 42:W401-W407). All designed gRNAs were chemically synthesized (ThermoFisher).

Transfection of 293T cells was performed by lipid-based transfection using CRISPRMax reagent (ThermoFisher) according to the manufacturer's protocol. Briefly, one day prior to transfection, adherent cells were plated onto 96-well plates at 0.2×10^5 cells per well. On the day of transfection, a solution of GeneArt Platinum Cas9 protein, gRNA and transfecting reagent was added to cells. 72 h post-transfection, cell culture was continued for 2 weeks in a 96 well plate format after the selection of single cells by a limiting dilution method. Subsequently, the picked clones were passaged to 24-well plates and tested by genotype confirmation using Guide-it kit (Takara) according to the manufacturer's protocol. The genomic region surrounding a CRISPR target site for each gene was PCR amplified to determine whether gene editing resulted in indels on one allele (monoallelic) or both alleles (biallelic) in singly isolated clones. The expression of the protein of interest on the clones with mutations in both alleles were tested by FACS.

Construction of the Human-Mouse Sequences of the Molecules as Tested (human Fc, mouse Fvs)

Expression Vectors: For the generation of the antibody expression vectors, the variable regions of heavy and light chain DNA sequences were subcloned in frame with either the human IgG1 constant heavy chain or the human IgG1 kappa constant light chain pre-inserted into the respective generic recipient expression vector optimized for expression in mammalian cell lines. The genes to be expressed were cloned into the pCI-neo Mammalian Expression Vector (Promega) that uses the full-length human cytomegalovirus (CMV) immediate-early promoter for high level gene expression. The two antibody chains were cloned into two different vectors.

The N-terminal signal sequences from mouse IgG heavy chain and kappa light chain were used for the secreted expression of the heavy and light chain, respectively. The signal peptide was cleaved during expression, leaving intact N-terminus. In the Fab constructs, the C-terminus of the CH1 IgG1 constant region was fused with a 6× His tag to facilitate purification.

For the generation of bispecific antibody vectors, the IgG1 derived bispecific molecules include at least of two antigen binding moieties capable of binding specifically to two distinct targets: Pgp (ABCB1) and CD47. The antigen binding moieties are Fab fragments composed of a heavy and a light chain, each including a variable and a constant region. A common light chain was identified that was able to pair and effect acceptable binding both as Fab anti-Pgp (aPgp) and Fab anti-CD47 (aCD47); its use enabled the avoidance of LC mispairing (see e.g., U.S. Patent No 8,765,412, the disclosure of which is incorporated herein by reference in its entirety). Bispecific constructs were made based on electrostatic steering effects, (see e.g., Gunasekeran et al, (2010) Journal of Biological Chemistry 285, 19637-19646; the disclosure of which is incorporated herein by reference in its entirety). Briefly, the polypeptide chains or half antibodies against the targets are assembled as a bispecific antibody through charge pair substitutions at the CH3 domain: one heavy chain contained K392D and K409D substitutions and the other contained E356K and D399K substitutions.

FIG. 2 provides a diagram of a bispecific mAb that includes sections containing sequences from: A, 15D3; B, 5F9, both on a human IgG1Fc together with C, a common light chain kappa sequence derived from MRK16.

Monoclonal antibody 15D3 (see e.g., U.S. Patent 5,959,084; the disclosure of which is incorporated herein by reference in its entirety) and MRK16 (Iwahashi et al., Cancer Research 53, 1993; the disclosure of which is incorporated herein by reference in its entirety) monoclonal antibodies raised previously against Pgp were cloned as recombinant engineered antibodies into a Human IgG1/Kappa expression vector.

Variable heavy and light chain fragments from mouse hybridoma sequences are available and were cloned into the same background of leader sequence and constant region.

Anti CD47 (5F9; see e.g., U.S. Patent No. 9,017,675; the disclosure of which is incorporated herein by reference in its entirety) antibody variable heavy and light fragments were cloned into the same background of leader sequence and constant region in two separate vectors.

Numerous combinations of these and other heavy and light chain fragments were generated and tested. The majority of the tested constructs did not generate acceptable binding or activity. Examples of antibodies constructed and tested, as well as their binding and cell killing properties, are provided in FIG. 8.

FIG. 8 provides comparisons of binding and enhanced cell killing by different bispecific antibodies (with various humanized or chimerized heavy or light chain combinations). Specifically, column 1 ("Ab") of FIG. 8 provides the various bispecific formats tested, e.g., in row 1: 15D3 IgG1 DD/5F9 IgG1 KK/MRK16 indicates: i) 15D3 heavy chain sequence (chimerized in a human IgG1

framework) with the DD CH3 mutations described herein, ii) 5F9 heavy chain sequence with the KK CH3 mutations as described herein, and iii) MRK16 light chain (chimerized in a human IgG Kappa framework). All the light chains shown were in the human Kappa format. Binding (FIG. 8, col. 2-3; "Kd (nM)" and "Bmax") and killing (FIG. 8, col. 3; "Killing") were determined as follows:

5 "Kd (nM)", binding by ELISA (solid phase with Fc-tagged CD47 as capture layer on the plate); "Bmax", Bmax binding to the plates in described for "Kd (nM)"; "Killing", enhanced 293 naïve cell-killing (sensitization) in the presence of paclitaxel titration ($20\mu\text{M} - 10^{-8} \mu\text{M}$ range); "-" = zero, "±" = 0 to 0.5 Log shift (at 50% cell kill curve), "+" = 1 to 2 Log shift, "++" ≥ 2 Log shift. Columns 5-8 ("Binding (293)", "Binding (293 KO CD47)", Binding (293 KO CD47, OX ABCB1)", and "Binding

10 (293 OX ABCB1)") provide FACS mean fluorescence intensities of antibodies binding to the indicated cell lines compared with non-binding control antibodies: "-" = ~zero, "±" = 0 to ~0.5 Log shift, "+" = ~1 Log shift, "++" = 1 - >2 Log shift (see FIG. 3A for examples of non-binding controls and binding antibodies).

Of note are those antibodies constructed and tested that showed unacceptable binding

15 characteristics or inefficient killing of target cells that express both MDR1 and CD47, particularly as compared to the lead candidate.

For example, as shown in FIG. 8, the antibody containing 15D3 and 5F9 heavy chain charge-to-charge swapped Fc regions and a common MRK16 derived light chain ("15D3 IgG1 DD/5F9 IgG1 KK/MRK16") was tested showed desirable binding characteristics and produced

20 enhanced killing (++) of target cells expressing both MDR1 and CD47. This antibody was thus selected as the lead candidate. In comparison, when the common light chain of the lead candidate (i.e., "MRK16") was substituted with alternative common light chains, such as "UIC2" (see e.g., "15D3 IgG1 DD/5F9 IgG1 KK/UIC2") or "9F11" (see e.g., "15D3 IgG1 KK/5F9 IgG1 DD/9F11") light chains, essentially no specific killing of cells that express both CD47 and MDR1 was

25 observed. In addition, substituting alternative anti-MDR1 heavy chains, such as "MM4.17 2" or "UIC2" heavy chains, for the "15D3" heavy chain also resulted in significantly reduced specific killing of cells expressing both CD47 and MDR1 (see e.g., FIG. 8, e.g., "MM4.17 2 IgG1 DD/5F9 IgG1 KK/MRK16" and "UIC2 IgG1 KK/5F9 IgG1 DD/MRK16").

The effect of swapping the employed light chain (i.e., "LC shuffle") on antibody binding

30 cells expressing the antigens was evaluated by ELISA and FACS binding assays. In testing LC shuffling with a CD47-specific heavy chain, the modified anti-CD47 heavy chain (5F9) was combined with a M89, 15D3, MRK16 or UIC2 light chain and binding was assessed. Results showing the effect of LC shuffle on the bispecific antibody binding of various anti-MDR1 molecules to CD47-Fc coated plates by ELISA are provided in Table 2:

Table 2:

	5F9/5F9	5F9/M89	5F9/15D3	5F9/MRK16	5F9/UIC2
Bmax	2.782	4.22	2.957	2.939	3.076
Kd (nM)	0.001968	1510	0.2571	0.1695	6.297

Of note in the above table is the 100-fold decrease in affinity seen in the combination of 5F9/MRK16 as compared to the un-swapped 5F9 control. Antibodies having a Kd for CD47 similar to the 5F9/MRK16 combination are preferred as they provide the requisite degree of specificity when used in a bispecific format such that the bispecific antibody has lower binding to normal cells and increased specificity for cancer cells that express CD47 at a higher level than a normal cell.

The results of LC shuffle testing, showing the effects of combining the 15D3 heavy chain with UIC2, MRK16 or 5F9 light chains on binding to CD47 as measured by ELISA, are provided in Table 3:

Table 3:

	15D3/15D3	15D3/UIC2	15D3/MRK16	15D3/5F9
Bmax	3.202	3.449	3.935	3.095
Kd (nM)	10.9	168.6	229.1	251.9

Of note in the above table is the surprising affinity of 15D3 to CD47 which was decreased ~20-fold after the combination of 15D3 HC with the MRK16 LC compared to the un-swapped 15D3 control.

The candidate construct having a combination of 15D3 and 5F9 heavy chains with a common MRK16 light chain has acceptable binding and target antagonizing characteristics. In comparison, other tested heavy and light chain combinations showed unacceptable binding and/or target cell killing results when tested. See Fig. 8.

Purification of mAbs

To purify antibody formats containing the Fc, 10 µl of MabSelect™ SuRe™ (GE Healthcare) per 1 ml of supernatant were added to the harvested medium and kept stirring at 4°C overnight. The next day, the protein A resin was applied in a 24 well filter plate using a vacuum

manifold unit (Pall Lifesciences, USA). The resin was washed with PBS and the antibody eluted in 50 mM phosphate pH 3 and neutralized with 10x PBS pH 13.

Histidine-tagged Fabs were purified following the same procedure using Ni Sepharose 6 Fast Flow histidine-tagged protein purification resin (GE Healthcare). The beads were washed with PBS followed by washes with 25 mM phosphate buffer pH 7.4, 150 mM NaCl supplemented with 20 mM of imidazole. The complex was eluted with 2 volumes of 25 mM phosphate buffer pH 7.4, 150 mM NaCl supplemented with 500 mM of imidazole. Finally, purified Fab was buffer-exchanged into PBS.

Sequences

Pgp/MDR1 has the following amino acid sequence:

MDLEGDRNGGAKKKNFFKLNNKSEKDKKEKKPTVSVFSMFRYSNWLDKLYMVVGTLAAIIHGA
GLPLMMLVFGEMTDIFANAGNLEDLMSNITNRSDINDTGFFMNLEEDMTRYAYYYSGIGAGVLV
AAYIQVSFWCLAAGRQIHKIRKQFFHAIMRQEIGWFDVHDVGELNTRLTDDVSKINEGIGDKIGM
FFQSMATFFTGFIVGFTRGWKLTLLVLAISPVLGLSAAVWAKILSSFTDKELLAYAKAGAVAEVL
AAIRTVIAFGGQKKELERYNKNLEEAKRIGIKKAITANISIGAAFLLIYASYALAFWYGTTLVLSGEY
SIGQVLTVFFSVLIGAFSVGQASPSIEAFANARGAAYEIFKIIDNKPSIDSYSKSGHKPDNIKGNLE
FRNVHFSYPSRKEVKILKGLNLKVQSGQTVALVGNSGCGKSTTVQLMQRLYDPTEGMVSVDG
QDIRTINVRFLREIIGVVSQEPVLFATTIAENIRYGRENVMTDEIEKAVKEANAYDFIMKLPKHFDT
LVGERGAQLSGGQKQRIAIARALVRNPKILLDEATSALDTESEAVVQVALDKARKGRTTIVIAH
RLSTVRNADVIAGFDDGVIVEKGNHDELMKEKGIYFKLVTMQTAGNEVELENAADESKSEIDAL
EMSSNDSRSSLIRKRSTRRSVRGSQAQDRKLSTKEALDESIPPVSFWRIMRLNLTEWPYFVVG
VFCAIINGGLQPAFAIIFSKIIGVFTRIDDPETKRQNSNLFSLFLALGIISFITFFLQGFTFGKAGEIL
TKRLRYMVFRSMLRQDVSWFDDPKNTTGALTTRLANDAAQVKGAIGSRLAVITQNIANLGTGIII
SFIYGWQLTLLLLAIVPPIAAGVVEMKMLSGQALKDKKELEGSGKIAIEAENFRTTVVSLTQEQKF
EHMYAQLQVPYRNSLRKAHIFGITFSFTQAMMYFSYAGCFRFGAYLVAHKLMSFEDVLLVFSA
VVFGAMAVGQVSSFAPDYAKAKISAAHIIMIEKTPLIDSYSTEGLMPNTLEGNVTFGEVVFNYPT
RPDIPVLQGLSLEVKKGQTLALVGSSGCGKSTVVQLLERFYDPLAGKVLLDGKEIKRLNVQWLR
AHLGIVSQEPILFDCSIAENIAYGDNSRVVSQEEIVRAATEANIHAIESLPNKYSTKVGDKGTQL
SGGQKQRIAIARALVRQPHILLDEATSALDTESEKVVQEALDKAREGRTCIVIAHRLSTIQNADLI
VVFQNGRVKEHGTHQQLLAQKGIYFSMVSQAGTKRQ.

Nucleic acid sequences encoding Pgp/MDR1 are available: P Glycoprotein (ABCB1) (NM_000927) Human genomic DNA Homo sapiens ATP binding cassette subfamily B member 1 (ABCB1), Ref Seq Gene on chromosome 7 (NG_011513 gen)

Nucleic acid sequences encoding CD47 are available: Homo sapiens CD47 molecule (CD47), transcript variant 1, mRNA NCBI Reference Sequence: NM_001777.3

Anti CD47 5F9 antibody variable heavy chain sequence is as follows:

QVQLVQSGAEVKKPGASVKVSCASGYTFTNYNMHWVRQAPGQRLEWMGTIYPGNDTSYN
5 QKFKDRVTITADTSASTAYMELSSLRSEDVAVYYCARGGYRAMDYWGQGTLVTVSS.

Anti-CD47 5F9 antibody variable light chain sequence is as follows:

DIVMTQSPLSLPVTPGEPASISCRSSQSIVYSNGNTYLGWYLQKPGQSPQLLIYKVSNRFSGVP
DRFSGSGSGTDFTLKISRVEADVGVYYCFQGSHVPYTFGGGTKLEIK.

Anti-MDR1 MRK16 antibody variable heavy chain sequence is as follows:

10 EVILVESGGGLVKPGGSLKLSCAASGFTFSSYTMSWVRQTPEKRLEWVATISSGGGNTYYPDS
VKGRFTISRDNANKNNLYLQMSSLRSEDALYYCARYRYEAWFASWGQGLTVTVSA.

The sequence of the MRK16 antibody variable light chain is as follows:

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKISNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK.

15 Antibody 15D3 sequences are available from U.S. Patent No.5849877, wherein the antibody 15D3 Heavy chain sequence is as follows:

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNTYYPD
SVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGLTVTVSA, and
the antibody 15D3 Light chain sequence is as follows:

20 DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVP
DRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHPRTFGGGTRLEIK.

The sequence of the anti-MDR1 UIC2 antibody light chain employed as described herein is as follows:

DVVMQTTPRSLPVSLGDQASISCRSSQSLLHSNGNTYLHWYLQKPGQSPKLLIYKVSNRFSGV
25 PDRFSGSGSGTDFTLKISRVEAEDLGVYFCSQSTHIPPWTFGGGTKLDIK.

The sequence of the anti-MDR1 UIC2 antibody heavy chain employed as described herein is as follows:

AVQLQQSGPELVKTGASVKISCKASGYFSNYYIHWVKQSHGKSLEWIGFISCYNGATFYNQK
FKGKATFTVDTSSSTAYMKFNSLTFEDSAVYYCARLPQFGNFYPMDYWGQGSVTVSS.

30 The sequence of the anti-MDR1 9F11 antibody light chain employed as described herein is as follows:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHRTGNTYLEWYLQKPGQSPKLLIYKVSNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHPYTFGGGTKLEIK.

The sequence of the anti-MDR1 9F11 antibody heavy chain employed as described herein is as follows:

EVKLVESGGGLVKFGGSLKLSCAASGFTLSSYYMSWVRQSPEKRLELVAVINSNGGSTYYPD
VKGRFTISRDNANTLYLQMSSLKSEDTALYYCARPFYYSNSPFAYWGQGTLVTVSS.

5 The sequence of the anti-MDR1 MM4.17 antibody heavy chain employed as described herein is as follows:

QVQLQESGGDLVKPGGSLKLSCAASGFTFSRYGMSWVRQTPDKRLEWVATISSGGSYTYFPD
SVKGRFTISRDNANTLYLQVSSLKSEDTAMYYCARPAEFRGYSWFAYWGQGTTVTVSS.

10 The sequence of the anti-MDR1 M89 antibody light chain employed as described herein is as follows:

EIVLTQSPATLSLSPGERATLSCRASQSVGGSYLAWYQQKPGQAPRLLIYGASRRATGIPARFS
GSGSGTDFTLTISSLQPEDFASYFCQQTNTFPLTFGGGTKVEIK.

The sequence of the anti-MDR1 M89 antibody heavy chain employed as described herein is as follows:

15 QVQLVQSGGGVVPGRSLRLSCAASGFTFSSYGMHWVRQDPGKGLMWVSSISTDGSATKYA
DSVKGRFTISRDNANTVSLQMNSLRAEDTAVYYCVGGFLGWVGQGTTLTVTVSS.

Results

20 *Biophysical analytical test for purified mAbs (GXII instrument, reduced and non-reduced protein)*

Purity and monomer content of the final protein preparation was determined by high-throughput analysis on a Caliper LabChip GXII using Protein Express LabChip Kit (Perkin -Elmer) as described by the manufacturer. The chip was automatically primed on the instrument with polymer solution containing 0.2% SDS and fluorescent staining dye. The de-stain channels were
25 filled with polymer solution free of SDS and dye. Briefly, proteins in reducing and not reducing conditions were prepared by mixing a small volume (2-5 μ L) of sample with the caliper sample buffer with or without DDT. The samples were denatured at 75°C for 5 minutes, centrifuged at 2000g for 3 minutes, and then run. Electropherograms were generated by LabChip GXII Touch software (Perkin Elmer).

30 *Detection of CD47 and ABCB1 specific binding*

Binding specificity of the mAbs, Fab and Bispecific IgG1 targeting CD47 was tested by ELISA using human CD47-Fc fusion protein (R&D systems). Briefly, microtiter plates were coated with 50 μ l purified human CD47-Fc fusion protein at 0.5 μ g/ml in PBS, and then blocked with 100 μ l of 0.4% BSA in PBS. Dilutions of the different antibody formats were added in 1/3 sequential

dilutions to each well and incubated for 1 hour at room temperature. The 5F9 known anti-CD47 antibody was used as a positive control, and human IgG1 was used as an isotype control. Plates were subsequently washed three times with PBS/Tween and then incubated with HRP-conjugated donkey anti-human constant specific secondary reagent for 1 hour at room
5 temperature. After washing, plates were developed with HRP substrate. The reaction was stopped with 2M H₂SO₄, and OD was measured at 520 nM.

Binding specificity of the mAbs, Fab and Bispecific IgG1 was tested by FACS using 293T cell lines naturally expressing CD47, 293T naïve cells overexpressing human ABCB1 target, 293T naïve cells having ABCB1 knocked down using Lentiviral RNA vectors, CD47 knock out 293T
10 cells and CD47 knock out 293T cells overexpressing human ABCB1. Briefly, the different cell lines were incubated with various amounts of mAbs or bispecific mAbs, or a human IgG1 isotype control antibody on ice for 1 hr. The cells were washed three times with FACS buffer (PBS containing 0.5% BSA). Alexa647 labeled goat anti-human antibody was added as a secondary antibody, and the samples were incubated on ice for another 1 hour. Samples were washed and
15 analyzed using a BD FACS Canto (BD Biosciences).

This example demonstrates that construction of a bispecific hetero-bivalent antibody molecule, one arm of which binds to the efflux pump MDR1/Pgp and the other arm of which binds to the cancer checkpoint protein CD47. When both targets are simultaneously present on the surface of a cell, the bispecific antibody binds to the cell with relatively high affinity / avidity. In
20 comparison, if either MDR1/Pgp or CD47 are absent, or substantially reduced, the binding of the bispecific antibody is significantly reduced or undetectable. (Fig 4).

The bispecific antibody built, illustrated in FIG. 2, contains one arm (arm A) that binds to and antagonizes a transporter protein, (the efflux pump Pgp), and renders the cells more sensitive to chemotherapeutic agents. At the same time the other arm (arm B) binds to an “immuno-don’t
25 eat me” signal, (CD47), on the cell surface which, together with the IgG1 Fc, permits a more robust immune response against the cell.

FIG. 3A-3F provide illustrations of FACS binding of the various reagent cell lines used to test candidate molecules. Some of these reagent cell lines were engineered, as described in the Methods and Materials section, with knock-down, knock-out or overexpression of target proteins
30 to enable a precise and clear understanding of the targets involved; their presence or absence and the susceptibility of the cells to various antineoplastic agents. Specifically, FIG. 3A shows binding to naïve 293T cells, FIG. 3B shows binding to 293T CD47-KO (Knock-out) cells, FIG. 3C shows binding to 293T Pgp-KD (Knock-down) cells, FIG. 3D shows binding to 293T Pgp-ox (‘over-

expressors'), FIG. 3E shows binding to N6ADR (NALM6 Adriamycin resistant) cells, and FIG. 3F shows binding to 293T CD47-KO+Pgp-ox cells.

In FIG. 4A-4B, the binding of the bispecific antibody is shown by FACS analysis against a variety of cell lines. The first scan (left panel in FIG. 4A and FIG. 4B) illustrates binding of the antibody to cells co-expressing both targets, (Pgp and CD47). The next two scans (middle and right panels in FIG. 4A and FIG. 4B) illustrate the absence or significantly diminished binding when either Pgp (referred to as "KBP1", right panel) or CD47 (referred to as "KT14", middle panel) is knocked down (KD) or knocked out (KO) of the cell line. FIG. 4A-4B further show binding with titrated antibody concentrations of 600 nM, 200 nM, 66 nM, 7.4 nM and 2 nM, with the signal measured from isotype and no antibody control also provided. The respective antibody concentrations are indicated in the left panel of FIG. 4A and FIG. 4B, and the measured signal from these different concentrations are generally overlapping in the middle and right panels of FIG. 4A and 4B. FIG. 4A and FIG. 4B represent results obtained from two different batches of antibody.

FIG. 5 and FIG. 6A-6C illustrate the enhanced killing of cells, by the antineoplastic agent paclitaxel, when cells are bound by the 15D3.aCD47:MRK16 bispecific antibody (highlighted with arrows). The data provided in FIG. 5 shows that the sensitivity of naïve 293T cells and N6ADR cells to the chemotherapeutic was enhanced by the 15D3.aCD47:MRK16 bispecific antibody, e.g., as compared to treatment with chemotherapy only ("Chemo only") or chemotherapy in combination with the other antibodies (15D3:MRK16 Fab; a15D3:MRK16 antibody; aCD47:MRK16 antibody; aCD47:MRK16 Fab; and 15D3:9F11 Fab).

FIG. 6A provides form-fitted plots of the data provided in FIG. 5, produced using GraphPad Prism software, GraphPad Software, Inc. FIG. 6B provides a re-representation of the data shown in FIG. 5 and FIG. 6A, showing a side-by-side comparison of the raw data (left) with the fitted data (right) for the naïve 293T cells. FIG. 6C provides a re-representation of the data shown in FIG. 5 and FIG. 6A, showing a side-by-side comparison of the raw data (left) with the fitted data (right) for the N6ADR cells. Collectively these data illustrate enhanced susceptibility and/or a drug sensitizing effect of two different drug resistant cell lines (293T and N6ADR) that co-express the targets Pgp and CD47 to a chemotherapeutic when in the presence of the 15D3.aCD47:MRK16 bispecific antibody. In comparison, a variety of other tested antibodies directed to the same targets have no significant sensitization effects.

FIG. 7 shows enhanced in vitro killing of naïve 293T in the presence of 15D3.aCD47:MRK16 bispecific antibody (25 nM) and the chemotherapeutic vincristine, e.g., as compared to cells treated with vincristine alone. These data demonstrate that the drug sensitizing

effect is not limited to treatment with paclitaxel and that the 15D3.aCD47:MRK16 bispecific antibody broadly enhances drug sensitivity when used in combination with multiple different agents.

This combination of specific and concomitant targeting revealed in these results demonstrates enhanced therapeutic effects with antineoplastic agents against cancer cells, including those that may be chemo-resistant, while sparing normal tissue from these antagonistic effects. At the same time, the binding to and potentially antagonizing or masking the second protein, e.g. the “don’t eat me” signal in this example, is confined to cancer cells co-overexpressing this target together with the transporter protein (efflux pump) thereby enabling simultaneous, specific and beneficial targeting of this second protein.

Thus, specificity is achieved by preferential binding when the combination of targets is displayed on the cell surface. This binding by itself may focus an immune response against the cells (e.g., by incorporation of an IgG1 Fc into the bispecific molecule) but there are also added benefits where the bispecific molecule counteracts activity of one or both of the pair of target proteins involved in the specific binding - as is the case demonstrated in this example. Put another way, in addition to the desirable degree of tissue (tumor) specificity that the bispecific antibody provides, the antibodies of this disclosure also provide the benefits of concurrently targeting two different cellular mechanisms whereby the result may be additive, synergistic or otherwise advantageous. The effects of this “orthogonal targeting” by the antibody molecule may be further enhanced by tuning or adapting the Fc region of the antibody to enhance or reduce a concomitant immune response towards the bound molecules or cells.

Accordingly, this approach has many potential applications in the realms of cancer therapy where drug resistance, multi-drug resistance, immuno-masking or other combinations of targetable cell surface proteins are involved, (including e.g., TK activators, signaling proteins, aberrantly over-expressed proteins, etc.), particularly if one of the involved proteins is a transporter protein. Furthermore, where more than two different targets may be simultaneously co-overexpressed on cancer cells, this approach is applicable with the use of appropriate tri-valent, or other multi-valent, molecules that enable specific and active targeting of such cells.

In many cases, relative to normal cells, co-overexpression of certain proteins occurs on cancer cells, particularly cancer cells that are multi-drug resistant. The bispecific molecule described herein binds a cell strongly when two different target proteins (i.e., the transporter protein and the second protein) are present but less strongly when the concentration of one or the other of this pair of proteins is absent or reduced, e.g. as seen on normal cells. When bound, the bispecific antibody modulates viability, activity or susceptibility of the bound cell by

antagonizing, activating or masking (or a combination of these effects) the surface proteins targeted.

In an advantageous format the two distinct Heavy Chains, each binding to separate targets, are brought together using standard methods (to form a hetero-bivalent pair). These distinct Heavy Chains may be employed in some embodiments in combination with an appropriate single Light Chain that permits binding of either HC to its cognate target separately but also permits each arm to bind their respective targets when they are combined into the resulting bispecific antibody format. If both targets are present simultaneously on a cell surface (rather than only one or the other) the binding of the bispecific antibody is significantly enhanced thus resulting in tissue specificity.

In this instance, the bispecific antibody may bind strongly and specifically to a cancer cell displaying the described combination of targets while antagonizing one or both of these targets, and/or inhibiting their normal functions, to render the cell more susceptible or sensitized, e.g., to further chemotherapeutic agents and/or the subject's immune system.

Example 2: Reduced Affinity Monospecific Bivalent anti-MDR1 antibody and anti-CD47 antibody anti-cancer activity and comparison to the bispecific antibody

This example demonstrates that reduced affinity anti-MDR1 antibody and anti-CD47 antibody while ineffective in treating cancer when administered alone, do reduce tumor volume when co-administered. The bispecific antibody (15D3 HC:MRK16 LC::5F9 HC:MRK16 LC) was more efficacious than the co-administration of the anti-MDR1 antibody and anti-CD47 antibody in the absence of a chemotherapeutic agent. 15D3 HC:MRK16 LC::5F9 HC:MRK16 LC is also referred to herein as 15D3 HC :: MRK16 LC::5F9 HC.

The anti-MDR1 antibody included two heavy chains of the 15D3 antibody and two light chains of the MRK16 antibody. This antibody is referred to as 15D3/MRK16 monoclonal in Fig. 9. The anti-CD47 antibody included two heavy chains of the 5F9 antibody and two light chains of the MRK16 antibody. This antibody is referred to as KT14/MRK16 monoclonal in Fig. 9. The bispecific antibody included 15D3 HC:MRK16 LC :: 5F9 HC:MRK16 LC and is referred to as KbisP1.1 in Fig. 9.

Fig. 9 shows the effect of administration of the indicated antibodies on the volume of tumors containing multidrug resistant N6ADR cells. N6ADR cells were injected into a cohort of 6 mice per test group prior to administration of the indicated antibodies. The antibodies were administered once palpable tumors reached a size of about 100-150mm³ (about 7 days after injection of tumor cells). Antibodies were administered on day 0, day 4, day 7, and day 11. Tumor

volumes were measured and are plotted. Panel A: 15D3/MRK16 monoclonal anti-MDR1 antibody. Panel B: KT14/MRK16 monoclonal anti-CD47 antibody. Panel C: Co-administration of 15D3/MRK16 monoclonal anti-MDR1 antibody and KT14/MRK16 monoclonal anti-CD47 antibody. Panel D: KbisP1.1 antibody.

5 The indicated antibodies were administered with or without the chemotherapeutic agent paclitaxel. Antibody was dosed about 1 - 4 hrs prior to Paclitaxel.

 The 15D3/MRK16 monoclonal anti-MDR1 antibody and the KT14/MRK16 monoclonal anti-CD47 antibody alone showed no significant effect on tumor volume. The 15D3/MRK16 monoclonal anti-MDR1 antibody rendered the chemoresistant N6ADR cells sensitive to paclitaxel.
10 The chemosensitization effect was specific to 15D3/MRK16 monoclonal anti-MDR1 antibody and was not observed for the KT14/MRK16 monoclonal anti-CD47 antibody. See Panels A and B.

 The co-administration of the 15D3/MRK16 monoclonal anti-MDR1 antibody and the KT14/MRK16 monoclonal anti-CD47 antibody reduced tumor volume to an extent similar to seen when 15D3/MRK16 monoclonal anti-MDR1 antibody and paclitaxel were co-administered. The
15 efficacy of co-administration of the 15D3/MRK16 monoclonal anti-MDR1 antibody and the KT14/MRK16 monoclonal anti-CD47 antibody against the tumor was further increased when paclitaxel was also administered. These data show that the co-administration of the 15D3/MRK16 monoclonal anti-MDR1 antibody and the KT14/MRK16 monoclonal anti-CD47 antibody can be used in the presence or absence of a chemotherapeutic agent to treat cancer. See Panel C.

20 Panel D shows that the bispecific antibody is more efficacious than co-administration of the 15D3/MRK16 monoclonal anti-MDR1 antibody and the KT14/MRK16 monoclonal anti-CD47 antibody.

 The materials and methods for this example are similar to those described for Example 1.

25 *Example 3: The bispecific antibody is efficacious against N6ADR cell-based xenografts*

Materials:

 Cells: NALM6 (ATCC) and NALM6 ADR (ATCC), B cell precursor lymphoblastic leukemia cell lines used in this study. NALM6 ADR is a multidrug resistant cell line derived from NALM6 cells.

30 Mice: Sixty-five 5-6-weeks-old female SCID-Biege mice (Charles River) were used in this study.

 Reagents: BisP1.1 (15D3 heavy chain/KT14 heavy chain/MRK16 light chain), Human Isotype IgG1 (Bioxcell), Paclitaxel (Sigma), Valspodar (Sigma).

Methods:

Cell culture: Both NALM6 and NALM6 ADR cells were maintained in RPMI medium supplemented with 10% FBS and 1% penicillin and 1% streptomycin at 37°C, 5% CO₂. Cell lines used were authentic and confirmed to be mycoplasma negative.

Inoculation-2 X 10⁶ cells diluted in PBS:Matrigel (1:1) were subcutaneously injected using a 27G insulin syringe into fifty anesthetized 5–6-week-old female SCID-Biege mice under sterile conditions.

Dosing-

NALM6 subcutaneous tumors: Mice were randomized into three groups of five mice each- (i) Control vehicle, (ii) 5 mg/kg and (iii) 20 mg/kg of paclitaxel.

NALM6 ADR subcutaneous tumors: Mice were randomized into nine groups of five mice each-(i) Control isotype IgG1 15 mg/kg, (ii) Control isotype IgG1 15mg/kg and 5 mg/kg of paclitaxel, (iii) Control isotype IgG1 15mg/kg and 20 mg/kg of paclitaxel, (iv) BisP1.1 15 mg/kg, (v) BisP1.1 15 mg/kg and 5 mg/kg of paclitaxel, (vi) BisP1.1 15 mg/kg and 20 mg/kg of paclitaxel (vii) Valspodar 20 mg/kg (viii) Valspodar 20 mg/kg and 5 mg/kg of paclitaxel and (ix) Valspodar 20 mg/kg and 5 mg/kg of paclitaxel. Antibody, Valspodar and paclitaxel were dosed intraperitoneally twice a week for four consecutive weeks. Antibody/valsopodar were injected at least 4 hours prior to paclitaxel injection. Paclitaxel preparation: Since paclitaxel is poorly soluble, a master stock of 50 mg/ml was prepared in absolute ethanol:Kolliphor (1:1). Prior to injection the stock was diluted to recommended doses of 5 mg/kg or 20 mg/kg with PBS maintaining a ratio of (1:16).

Measurements: Tumor measurements were done thrice a week using a calibrated Vernier Caliper and tumor volume calculated as per the formula $\frac{1}{2} \times L \times S \times S$ where L is the long axis and s is the short axis of the tumor. Body weights were recorded before treatment started and were continuously monitored throughout the study. Animal survival was evaluated from the first day of treatment until death. To avoid suffering, mice were euthanized when turning moribund according to the above-mentioned predefined criteria: rapid weight loss, loss of ability to ambulate, labored respiration, or inability to drink or feed.

Statistics: Tumor volume and body weights were compared between different experimental groups using exact one-way analysis of variance (ANOVA). Kaplan–Meier survival curves were compared between different treatment groups using the log-rank (Mantel–Cox) test. Statistical analyses were performed using Graph Pad Prism (GraphPad, San Diego, CA, USA) software. The level of significance was set at $p < 0.05$ for all analyses.

Results

NALM6 cells demonstrated increased sensitivity to paclitaxel compared to the multidrug resistant NALM6ADR cells. NALM6 cells demonstrated significant ($p < 0.0001$) tumor growth inhibition to paclitaxel at doses of 5 mg/kg and 20 mg/kg. In contrast NALM6ADR cells showed significantly less tumor growth inhibition at 5 mg/kg with modest inhibition at 20 mg/kg (Fig.11). This confirmed paclitaxel resistance in NALM6ADR cells in vivo (Fig.10 and Fig.11).

Further in NALM6ADR models injection of BisP1.1 at 15 mg/kg dose had a strong inhibition of tumor growth (Fig.11). Similarly, BisP1.1 at 15 mg/kg in combination with 5 mg/kg and 20 mg/kg of paclitaxel significantly reduced tumor growth (Fig.11). Consistently valspodar, a small molecule inhibitor of ABCB1, chemosensitized NALM6ADR tumors (Fig.12). However, in contrast to the BisP1.1 plus paclitaxel combination group, mice treated with a combination of valspodar and paclitaxel 20 mg/kg showed significant reduction in body weight (Fig.13 and Fig.14).

Mice treated with valspodar and paclitaxel 20 mg/kg had the lowest survival compared to control. Mice treated with BisP1.1 or BisP1.1 plus paclitaxel had prolonged survival compared to control or paclitaxel treated mice (Fig.15).

Conclusion: BisP1.1 significantly inhibited tumor growth of NALM6ADR cells both alone and in combination with paclitaxel. BisP1.1 did not induce any loss in mouse body weight. Mice treated with BisP1.1 and Bis1.1 plus paclitaxel combination demonstrated prolonged survival in mice compared to control and paclitaxel treated cohorts.

Example 4: The bispecific antibody is efficacious against multiple different cancer models

The BisP1.1 bispecific antibody was efficacious against two additional multidrug resistant (MDR) cancer cell lines in xenograft studies. The A2780ADR MDR cancer cell line expresses both CD47 and ABCB1. The HeyT30 MDR cancer cell line also expresses both CD47 and ABCB1 but at a lower level compared to A2780ADR cells (Figs. 16 and 17). Both the A2780ADR and the HeyT30 cell lines are of ovarian origin. PE-conjugated, commercially available anti-MDR1 and anti-CD47 reagent antibodies were used; for MDR1, Biolegend Cat. No. 348606 and, CD47, Biolegend Cat. No. 323108.

A2780ADR MDR cancer cell line and HeyT30 MDR cancer cell line were injected into mice according to protocols described in Example 3. Mice were treated with BisP1.1 bispecific antibody with or without paclitaxel. As seen in Fig. 18, BisP1.1 bispecific antibody is efficacious in reducing A2780ADR MDR cancer cell tumor volume with or without paclitaxel. BisP1.1 bispecific antibody is also efficacious in reducing HeyT30 MDR cancer cell tumor volume with paclitaxel when a lower

concentration of the antibody is used. At a higher concentration the BisP1.1 bispecific antibody was efficacious with and without paclitaxel (Fig. 19).

Example 5: KbisP1.1 bispecific antibody is more efficacious than anti-MDR1 15D3 antibody against MDR cells and chemo-sensitizes MDR cancer cells in a dose dependent manner

Tumors formed by injecting multidrug resistant N6ADR cells in mice were treated by administering the anti-MDR1 antibody 15D3, with or without paclitaxel or administering KbisP1.1 with or without paclitaxel.

As seen in Fig. 20, KbisP1.1 is more efficacious than the 15D3 antibody against in vivo N6ADR cell tumors.

Fig. 21 shows KbisP1.1 chemo-sensitization of multidrug resistant N6ADR tumor to Paclitaxel is dose-dependent, *in-vivo*.

Example 6: Significantly reduced binding of KbisP1.1 bispecific antibody to human and cynomolgus erythrocytes

KBisP1.1 antibody exhibits no significant binding to human and cynomolgus erythrocytes in contrast to the anti-CD47 5F9 antibody and is thus not expected to cause general cytotoxicity or be subject to an erythrocyte 'antigen sink'. Human and cynomolgus RBC binding data are shown in Figs. 22A and 22B, respectively. Figs. 22A and 22B also show that an anti-CD47 antibody comprising 5F9 HCs and MRK16 LCs ("KT14_MRK16") does not significantly bind to human and cynomolgus red blood cells (RBCs) in contrast to the anti-CD47 5F9 antibody ("KT14").

Subsequent testing of the KBisP1.1 antibody in cynomolgus monkeys showed that the antibody does not result in significant reduction in body weight, hemolytic anemia, and no increase in morbidity was associated with this antibody. Furthermore, the clearance of the KBisP1.1 appeared normal without an apparent 'antigen sink' effect being observed.

The cynomolgus monkeys were administered the KBisP1.1 antibody at a dose of 10 mg/kg. The half-life of the antibody was from 114-148 hrs.

Example 7: KbisP1.1 bispecific antibody cynomolgus monkey toxicity study

Fifteen cynomolgus monkeys (*macaca fascicularis*) were enrolled in a single dose study to evaluate KbisP1.1 toxicity. Monkeys were divided into 5 cohorts (n=3 animals/cohort):

Cohort	KBisP1.1 dose	paclitaxel dose
1	10 mg/kg	none
2	10 mg/kg	2 mg/kg
3	50 mg/kg	none
4	50 mg/kg	2 mg/kg
5	none	2 mg/kg

All animals received their full dose of antibody and/or paclitaxel. No animals exhibited any signs of infusion reaction. Post infusion of Paclitaxel there was the expected amount of minimal erythema at the infusion site. There was no erythema at the site of the infusion of the antibody alone.

KBisP1.1 was well tolerated in all cohorts with no changes in activity or appetite. Weight remained stable and detailed clinical examinations were normal.

A slight decrease in hematocrit in 11 of 15 animals was observed at 7 days post dosing which did not correlate with KbisP1.1 dose or having received paclitaxel. All other clinical chemistry and hematology finding were normal.

Example 8: KbisP1.1 bispecific antibody modified to reduce immunogenicity or modify ADCC

Materials and Methods

Cells: MES-SA/Dx5 cell line (Sigma, catalogue no 95051031) is a uterine sarcoma, derived from parent MES-SA cell line (Sigma, catalogue no 95051030), was used in this study. MES-SA/Dx5 cell line demonstrates ~100-fold more resistance to doxorubicin compared to its parent cells.

Mice: 5-6-week-old female nude mice (Charles River) were used in this study.

Human Isotype IgG1 (Bioxcell) served as control.

Cell culture- MES-SA/Dx5 cells were maintained in RPMI medium supplemented with 10% FBS and 1% penicillin and 1% streptomycin at 37°C, 5% CO₂. Treatment with 10E-7 M Adriamycin at least once a week. Cell lines used were authentic and confirmed to be mycoplasma negative.

Inoculation-0.5 X 10⁶ cells diluted in PBS:Matrigel (1:1) were subcutaneously injected using a 27G insulin syringe into thirty anesthetized 5–6-week-old female nude mice under sterile

conditions. All animal maintenance, handling, surveillance, and animal procedures were performed in accordance with and approval from the APLAC protocol.

Dosing-

MES-SA/Dx5 subcutaneous tumors: Once tumors reached 100-150 mm³, mice were
5 randomized into six groups of five mice each-(i) Control isotype IgG1 3 mg/kg, (ii) Control isotype
IgG1 3mg/kg and 20mg/kg of paclitaxel, (iii) KNJYBisP1.1 3 mg/kg, (iv) KNJYBisP1.1 3 mg/kg
and 20mg/kg of paclitaxel, (v) KNJYBisP1.1 LALAPG 3 mg/kg and (vi) KNJYBisP1.1 LALAPG 3
mg/kg and 20 mg/kg of paclitaxel. Antibody and paclitaxel were dosed intraperitoneally twice a
week for two consecutive weeks. Antibodies were injected at least 4 hours prior to paclitaxel
10 injection.

Paclitaxel preparation: Since paclitaxel is poorly soluble, a master stock of 50 mg/ml was
prepared in absolute ethanol:Kolliphor (1:1). Prior to injection the stock was diluted to
recommended doses of 20 mg/kg with PBS maintaining a ratio of (1:16).

Measurements-Tumor measurements were done thrice a week using a calibrated Vernier
15 Caliper and tumor volume calculated as per the formula $\frac{1}{2} \times L \times S \times S$ where L is the long axis and s
is the short axis of the tumor. Body weights were recorded before treatment started and were
continuously monitored throughout the study. Animal survival was evaluated from the first day of
treatment until death. Animals were euthanized when turning moribund according to the above-
mentioned predefined criteria rapid weight loss, loss of ability to ambulate, labored respiration, or
20 inability to drink or feed to avoid animal suffering.

KbisP1.1 antibody is also referred to herein as KNJYBisP1.1. The same protocols were
followed for KNJYBisP1.1 N297A antibody.

KbisP1.1 humanized antibody

25 KbisP1.1 antibody (also referred to herein as KNJYBisP1.1) was modified to reduce
immunogenicity by making chimeric and humanized versions of the KbisP1.1 antibody. KbisP1.1
chimeric antibody included the 15D3 variable heavy chain region fused to human IgG1 Fc region
with the charged pair substitutions K392D and K409D and 5F9 variable heavy chain region fused
to human IgG1 Fc region with the charged pair substitutions E356K and D399K.

30 In order to select human antibody framework regions (FR) to be used as templates for
CDR-grafting, the mouse 15D3 VH and MRK16 VL regions were compared with those of human
germline sequences. The FRs of the mouse 15D3 VH regions were found to have the highest
homology with human IGHV3 subgroup. The FRs of the mouse MRK16 VL region exhibited the
highest homology with human IGKV2 subgroup. To reduce potential posttranslational modification

and improve the stability of the antibodies, N56Q/N56S was introduced in the 15D3 CDR H2, and N30Q/N30S was introduced in the MRK16 CDR L1. Three versions of each humanized VH (15D3 Hz0, 15D3 Hz1, and 15D3 Hz2) and VL (MRK16 Hz0, MRK16 Hz1, and MRK16 Hz2) were designed. The variable region sequences are as follows:

5 15D3 Hz0:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGG
NTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGTLVTV
SS

15D3 Hz1:

10 EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGG
QTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGTLVTV
SS

15D3 Hz2:

15 EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGG
STYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGTLVTV
S

MRK16 Hz0:

20 DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGNTYLEWYQQRPQGPPRLIIYKISNRF
SGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGGGTKLEIK

MRK16 Hz1:

25 DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPQGPPRLIIYKISNRF
SGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK

MRK16 Hz2:

30 DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPQGPPRLIIYKISNRF
SGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK

Sequence alignments of each version are provided in Figs. 23-24.

As shown in Fig. 25, humanized antibody chemosensitized multidrug resistant MES-SA-DX5 cell-generated tumors in mice. As shown in Fig. 26, humanized antibody chemosensitized multidrug resistant A2780ADR cell-generated tumors in mice. In Fig. 26, the KBisP1.1 antibody is referred to as "KNJYBisP1.1" and the humanized KBisP1.1 antibody is referred to as "Humanized."

Humanized KBisP1.1 antibodies were characterized by binding titration and chemotoxicity assay.

Cell Binding Assays. Antibody binding to cells was evaluated by flow cytometry. 293T cells stably transfected to express human ABCB1 (293T_ABCB1_OX) were washed once in flow cytometry buffer (PBS + 2% FBS + 0.02% sodium azide), resuspended at 2×10^6 cells/mL in flow cytometry buffer, and dispensed into 96-well microtiter plates at 0.1 mL/well. Recombinant antibodies were added to cells at 5 ug/mL for initial binding confirmation, or serially diluted from 100 ug/mL in flow cytometry buffer. After incubating cells on ice for 30 min, cells were washed twice with flow cytometry buffer. Bound antibody was detected with PE-labeled F(ab')₂ fragment goat anti-human IgG (Jackson ImmunoResearch) and evaluated on an Attune NxT flow cytometer. EC50 is calculated to be the concentration of antibody that gives half maximal response.

Cytotoxicity Assays. The effect of antibodies on vincristine cytotoxicity was evaluated on N6/ADR, a doxorubicin-selected, B1-positive variant of the human acute lymphoblastic leukemia (ALL) cell line, NALM6. Cells were plated in 0.05 mL of Assay Media (RPMI-1640 +10% FBS) at 5000 cells/well in white flat bottom 96-well tissue culture plates. Vincristine was prepared at 2X final assay concentration by serial dilution from 200 uM in assay media containing test antibodies or control antibodies at 100 ug/mL (2X final concentration), or valspodar, a small molecule B1 inhibitor at 7 uM (2X final concentration). An equivalent volume (0.05 mL) of the vincristine/antibody mixture was added to the N6/ADR cells in 96-well plates. The plates were then incubated at 37° C, in 5% CO₂. After 72 hr plates were equilibrated to room temperature and cell viability assessed using Promega® CellTiter-Glo® Luminescent Cell Viability Assay according to the manufacturer's recommended protocol. Luminescence was measured on a Molecular Devices® FlexStation® 3 Multi-Mode Microplate Reader and data analyzed using GraphPad Prism 8.0 software. IC50 is the concentration of drug (vincristine or other chemotherapy cytotoxic agent) where the response (cell growth) is reduced by 50%.

Cell Binding of bispecific antibodies to 293T_ABCB1_OX cell is summarized in Table 4:

EC50 (nM)	Ab	HC1	HC2	LC
75.5	Ab1	15D3 IgG1 DD	KT14 IgG1 KK	MRK16 VL-Hz1
212.4	Ab2	15D3 IgG1 DD	KT14 IgG1 KK	MRK16 VL-Hz2
79.1	Ab3	15D3 IgG1 DD	KT14 IgG1 KK	MRK16 VL Hz0
101.1	Ab4	15D3 HC-Hz1 DD hIgG1	KT14 IgG1 KK	MRK16 VL Hz0
137.1	Ab5	15D3 HC-Hz1 DD hIgG1	KT14 IgG1 KK	MRK16 VL-Hz1

230.4	Ab6	15D3 HC-Hz1 DD hIgG1	KT14 IgG1 KK	MRK16 VL-Hz2
63.5	Ab7	15D3 HC-Hz2 DD hIgG1	KT14 IgG1 KK	MRK16 VL-Hz0
216.6	Ab8	15D3 HC-Hz2 DD hIgG1	KT14 IgG1 KK	MRK16 VL-Hz1
218.8	Ab9	15D3 HC-Hz2 DD hIgG1	KT14 IgG1 KK	MRK16 VL-Hz2
34.7	Ab10	15D3 IgG1 DD Hz0	KT14 IgG1 KK	MRK16 VL-Hz1
52.4	Ab11	15D3 IgG1 DD Hz0	KT14 IgG1 KK	MRK16 VL-Hz2
33.5	Ab12	15D3 IgG1 DD Hz0	KT14 IgG1 KK	MRK16 VL Hz0
14.6	15D3			
0.3	KT14			
40.3	KBisP1.1			

The effect of antibodies on vincristine cytotoxicity is summarized in Table 5:

Vincristine IC50 (nM)	Ab	HC1	HC2	LC
0.0020	Ab1	15D3 IgG1 DD	KT14 IgG1 KK	MRK16 VL-Hz1
0.0030	Ab2	15D3 IgG1 DD	KT14 IgG1 KK	MRK16 VL-Hz2
0.0003	Ab3	15D3 IgG1 DD	KT14 IgG1 KK	MRK16 VL Hz0
0.0024	Ab4	15D3 HC-Hz1 DD hIgG1	KT14 IgG1 KK	MRK16 VL Hz0
0.0589	Ab5	15D3 HC-Hz1 DD hIgG1	KT14 IgG1 KK	MRK16 VL-Hz1
0.0479	Ab6	15D3 HC-Hz1 DD hIgG1	KT14 IgG1 KK	MRK16 VL-Hz2
0.0076	Ab7	15D3 HC-Hz2 DD hIgG1	KT14 IgG1 KK	MRK16 VL-Hz0
0.5084	Ab8	15D3 HC-Hz2 DD hIgG1	KT14 IgG1 KK	MRK16 VL-Hz1
0.2417	Ab9	15D3 HC-Hz2 DD hIgG1	KT14 IgG1 KK	MRK16 VL-Hz2
0.0572	Ab10	15D3 IgG1 DD Hz0	KT14 IgG1 KK	MRK16 VL-Hz1
0.1074	Ab11	15D3 IgG1 DD Hz0	KT14 IgG1 KK	MRK16 VL-Hz2
0.0001	Ab12	15D3 IgG1 DD Hz0	KT14 IgG1 KK	MRK16 VL Hz0
0.4663	15D3			
0.7335	MRK16			
0.0461	KBisP1.1			
0.5429	Vincristine alone			
0.7869	Human IgG1			

5 **KbisP1.1 LALAPG Fc-muted antibody**

The IgG1 Fc region of the chimeric antibodies was modified to include the LALAPG effector function mutation (L234A, L235A, and P329G) or the N297A mutation to reduce antibody

dependent cellular cytotoxicity (ADCC). Such antibodies are referred herein as muted antibodies. The position of the substitutions are with reference to the amino acid sequence of human Ig G1.

As shown in Fig. 25 muted Fc LALAPG bispecific antibody chemosensitized multidrug resistant MES-SA-DX5 cell-generated tumors in mice. The data for muted Fc LALAPG bispecific antibody suggests that Fc effector functions of KNJYBisP1.1 are dispensable for chemosensitization of multidrug resistant tumors to paclitaxel. Fc effector functions of KNJYBisP1.1 appear to be partially responsible for anti-tumor effects of antibody alone in the absence of chemotherapy.

KbisP1.1 N297A Fc-muted antibody

The IgG1 Fc region of the chimeric antibodies was modified to include N297A mutation to reduce antibody dependent cellular cytotoxicity (ADCC). Such antibodies are referred herein as muted antibodies. The position of the substitution is with reference to the amino acid sequence of human Ig G1.

Fig. 27A shows that the KBisP1.1 bispecific antibody with N297A substitution (labeled as KNJY N297A in the figure) chemosensitizes multidrug resistant MES-SA/Dx5 cell tumors to paclitaxel in vivo. The Fc effector functions of KBisP1.1 appear to be dispensable for chemosensitization of multidrug resistant tumors to paclitaxel. The Fc effector functions of KBisP1.1 appear to be partially responsible for anti-tumor effects of antibody alone in the absence of chemotherapy.

KbisP1.1 N297A Fc-muted antibody was also tested against a different cancer cell line, the A2780ADR cell line.

Materials and Methods

Cells: A2780ADR cell line (Sigma, catalogue no 93112520) is an epithelial ovarian carcinoma, derived from parent A2780 cell line (Sigma, catalogue no 93112519), was used in this study.

Mice: 5-6-weeks-old female nude mice (Charles River) were used in this study. We accounted for 10% extra mice that did not undergo treatment.

Human Isotype IgG1 (Bioxcell) served as control.

Cell culture- A2780ADR cells were maintained in RPMI medium supplemented with 10% FBS and 1% penicillin and 1% streptomycin at 37°C, 5% CO₂. Treatment with 10E-7 M Adriamycin at least once a week. Cell lines used were authentic and confirmed to be mycoplasma negative.

Inoculation-0.5 X 10⁶ cells diluted in PBS:Matrigel (1:1) were subcutaneously injected using a 27G insulin syringe into thirty anesthetized 5–6-week-old female nude mice under sterile

conditions. All animal maintenance, handling, surveillance, and animal procedures were performed in accordance with and approval from the APLAC protocol.

Dosing-

A2780ADR subcutaneous tumors: Mice were randomized into six groups of five mice each-(i) Control isotype IgG1 3 mg/kg, (ii) Control isotype IgG1 3mg/kg and 20mg/kg of paclitaxel, (iii) KNJYBisP1.1 3 mg/kg, (iv) KNJYBisP1.1 3 mg/kg and 20mg/kg of paclitaxel, (v) KNJYBisP1.1 N297A 3 mg/kg and (vi) KNJYBisP1.1 N297A 3 mg/kg and 20 mg/kg of paclitaxel. Antibody and paclitaxel were dosed intraperitoneally twice a week for two consecutive weeks. Antibodies were injected at least 4 hours prior to paclitaxel injection. Paclitaxel preparation: Since paclitaxel is poorly soluble, a master stock of 50 mg/ml was prepared in absolute ethanol:Kolliphor (1:1). Prior to injection the stock was diluted to recommended doses of 20 mg/kg with PBS maintaining a ratio of (1:16).

Measurements-Tumor measurements were done thrice a week using a calibrated Vernier Caliper and tumor volume calculated as per the formula $\frac{1}{2} \times L \times S \times S$ where L is the long axis and s is the short axis of the tumor. Body weights were recorded before treatment started and were continuously monitored throughout the study. Animal survival was evaluated from the first day of treatment until death. Animals were euthanized when turning moribund according to the above-mentioned predefined criteria rapid weight loss, loss of ability to ambulate, labored respiration, or inability to drink or feed to avoid animal suffering.

Fig. 27B shows that KBisP1.1 N297A antibody (labeled as N297A in the figure) chemosensitizes multidrug resistant A2780ADR cells to paclitaxel in vivo. In this model the Fc effector functions of KBisP1.1 are dispensable for chemosensitization of multidrug resistant tumors to paclitaxel. The Fc effector functions of KBisP1.1 are partially responsible for anti-tumor effects of antibody alone in the absence of chemotherapy.

Example 9: KbisP1.1 bispecific antibody compared to monospecific antibodies binding to the same targets

The anti-tumor effects of KNJYBisP1.1 antibody in comparison to the anti-MDR1 monoclonal antibody, 15D3 and the anti-CD47 KT14 monoclonal antibody was assessed using an A2780ADR and a MES-SA/Dx5 in vivo multidrug resistance model.

Materials and Methods

Cell lines and tumor models are as described in Example 7.

Dosing-

A2780ADR subcutaneous tumors: Once tumors reached 100-150 mm³, mice were randomized into ten groups of five mice each-(i) Control isotype IgG1 3 mg/kg, (ii) Control isotype IgG1 3mg/kg and 20mg/kg of paclitaxel, (iii) KNJYBisP1.1 3 mg/kg, (iv) KNJYBisP1.1 3 mg/kg and 20mg/kg of paclitaxel, (v) 15D3 hybridoma monoclonal Ab 3 mg/kg, (vi) 15D3 hybridoma 3 mg/kg and 20 mg/kg of paclitaxel, (vii) KT14/KT14 monoclonal Ab 3 mg/kg, (viii) KT14/KT14 monoclonal Ab 3 mg/kg and 20 mg/kg of paclitaxel, (ix) 15D3 hybridoma + KT14/KT14, 1.5 mg each and (x) 15D3 hybridoma + KT14/KT14, 1.5 mg each and 20 mg/kg of paclitaxel. Antibodies and paclitaxel were dosed intraperitoneally twice a week for two consecutive weeks. Antibody were injected at least 4 hours prior to paclitaxel injection. Paclitaxel preparation: Since paclitaxel is poorly soluble, a master stock of 50 mg/ml was prepared in absolute ethanol:Kolliphor (1:1). Prior to injection the stock was diluted to recommended doses of 20 mg/kg with PBS maintaining a ratio of (1:16).

MES-SA/Dx5 subcutaneous tumors: Once tumors reached 100-150 mm³, mice were randomized into ten groups of five mice each-(i) Control isotype IgG1 3 mg/kg, (ii) Control isotype IgG1 3mg/kg and 20mg/kg of paclitaxel, (iii) KNJYBisP1.1 3 mg/kg, (iv) KNJYBisP1.1 3 mg/kg and 20mg/kg of paclitaxel, (v) 15D3 hybridoma monoclonal Ab 3 mg/kg, (vi) 15D3 hybridoma 3 mg/kg and 20 mg/kg of paclitaxel, (vii) KT14 monoclonal Ab 3 mg/kg, (viii) KT14 monoclonal Ab 3 mg/kg and 20 mg/kg of paclitaxel, (ix) 15D3 hybridoma + KT14, 1.5 mg each and (x) 15D3 hybridoma + KT14, 1.5 mg each and 20 mg/kg of paclitaxel. Antibody and paclitaxel were dosed intraperitoneally twice a week for two consecutive weeks. Antibodies were injected at least 4 hours prior to paclitaxel injection. Paclitaxel preparation: Since paclitaxel is poorly soluble, a master stock of 50 mg/ml was prepared in absolute ethanol:Kolliphor (1:1). Prior to injection the stock was diluted to recommended doses of 20 mg/kg with PBS maintaining a ratio of (1:16).

Fig. 28 shows that the KBisP1.1 antibody chemosensitizes multidrug resistant A2780ADR cells to paclitaxel in vivo. The chemosensitizing effect of KBisP1.1 was stronger compared to either the anti-MDR1 15D3 hybridoma antibody or anti-CD47 KT14 monoclonal antibody. The chemosensitizing effect of KNJYBisP1.1 was comparable with co-injections of 15D3 hybridoma + KT14 monoclonal antibodies. Although both the 15D3 hybridoma and the KT14 monoclonal antibodies demonstrate strong binding to their respective targets ex vivo KNJYBisP1.1 is more efficacious than either the 15D3 or KT14 monoclonal antibodies.

As shown in Fig. 29, the KBisP1.1 antibody chemosensitizes multidrug resistant MES-SA/Dx5 cells to paclitaxel in vivo. The chemosensitizing effect of KBisP1.1 was stronger than either the 15D3 hybridoma or KT14 monoclonal antibodies. Combined with paclitaxel the

chemosensitizing effect of the single KBisP1.1 bispecific antibody was comparable to the co-injection combination of the 15D3 hybridoma + KT14 monoclonal antibodies.

In Figs. 28-29, KBisP1.1 antibody=KNJYBisP1.1; 15D3 hybridoma=anti-MDR1 monoclonal antibody 15D3; KT14/KT14=anti-CD47 monoclonal antibody 5F9.

5 The effect of KBis P1.1 treatment as a single bispecific antibody plus paclitaxel is consistent with *in vitro* results shown in Fig. 6B indicating that an efficacious paclitaxel payload accumulates in otherwise resistant tumors that co-express high concentrations of the two targets. The KBis P1.1 antibody does not show significant binding to cells not expressing both target antigens (see Figs. 4A) and will therefore provide more specificity in targeting cancer cells that
10 express both target antigens as compared to the individual monoclonal antibodies.

Example 10: Anti-tumor effects of KBisP1.1 in patient-derived breast cancer model CTG-2616 in vivo

Materials and Methods

15 Cells: CTG-2616 is a ER⁺/Her2⁺ primary breast cancer, derived from a patient presenting with recurrent ductal adenocarcinoma of the breast.

Mice: 5-6-weeks-old athymic Nude-Foxn1^{nu} female nude mice (Envigo) were used in this study.

Inoculation- Snap-Frozen pieces of patient-derived tumors were thawed, cut into 5x5x5
20 mm and were subcutaneously injected into anesthetized 5–6-week-old female nude mice under sterile conditions. For tumor implantation procedure, a 1-1.5 cm incision was made on the implantation site and forceps were used to implant the tumor fragment. When sufficient stock animals reached 1000-1500 mm³, tumors were harvested for re-implantation into pre-study animals. Pre-study animals were implanted unilaterally on the left flank with tumor fragments
25 harvested from stock animals. Each animal was implanted from a specific passage lot and documented. Pre-study tumor volumes were recorded for each experiment beginning seven to ten days after implantation. When tumors reached an average tumor volume of 150- 300mm³, animals were matched by tumor volume into treatment or control groups to be used for dosing and dosing initiated on Day 0. All surgical procedures were conducted under sterile conditions.

30 Dosing-

CTG-2616 subcutaneous tumors: Once tumors were in the range of 150-300 mm³, mice were randomized into three groups of three mice each-(i) Control isotype IgG1 (Bioxcell) 6 mg/kg + vehicle, (ii) Control isotype IgG1 6 mg/kg and 5 mg/kg of paclitaxel and (iii) KNJYBisP1.1 6 mg/kg and 5mg/kg of paclitaxel. Antibody and paclitaxel were dosed intraperitoneally twice a week

for four consecutive weeks. Antibodies were injected at least 4 hours prior to paclitaxel injection. Paclitaxel preparation: Prior to injection the stock was diluted to recommended doses of 5 mg/kg with PBS.

Measurement of tumor volume was carried out as described in the previous examples.

Fig. 30 shows that KBisP1.1 bispecific antibody chemosensitizes paclitaxel resistant CTG-2616 patient-derived breast tumors and resulted in tumor growth inhibition in vivo. KNJYBisP1.1 did not induce any change in body weight in CTG-2616 bearing animals. Thus, the KNJYBisP1.1 bispecific antibody, in combination with paclitaxel, is efficacious in chemosensitizing paclitaxel-resistant Her2⁺ CTG-2616 patient-derived breast tumors in vivo.

Example 11: Generation of Bispecific mAbs that bind PD-L1 and MDR-1

Strategy used for generation of the KBis1.1 antibody described in Examples 1-8 was utilized for generating a bispecific antibody that simultaneously binds MDR-1 and PD-L1. This antibody is referred to herein as KBis2.1. A common variable light (VL) chain from the MRK16 antibody with an antigen-binding site for MDR1, a first variable heavy (VH) chain from the 15D3 antibody with an antigen-binding site for MDR1 and a second VH chain with an antigen-binding site for PD-L1 was utilized to make the bispecific antibody. The MRK16 VL chain and the 15D3 VH chain are the same as that used for the KBis1.1 antibody. The VH chain of the anti-PD-L1 antibody atezolizumab served as the second VH chain with an antigen-binding site for PD-L1. This antibody is referred to as: 15D3 HC :: PDL1 HC :: MRK16 LC. A variant of this antibody where the humanized versions of the 15D3 HC and MRK16 LC was used was also constructed. This antibody may be referred to as: 15D3 HC Hz:: PDL1 HC :: MRK16 LC Hz. The 15D3 Hz0 VH chain and the MRK16 Hz0 VL chain was used. The atezolizumab VH chain when paired with MRK16 VL chain retains binding to PD-L1 as measured by ELISA (data not shown). ELISA was carried out in the manner described for CD47 in Example 1. In addition, as expected from the data from the KBisP1.1 antibody, the 15D3 HC :: MRK16 LC arm of the KBisP2.1 antibody retained binding to MDR1 as measured by FACS using 293 T cells overexpressing MDR1 (data not shown). FACS measurement for MDR1 binding was performed as described in Example 1. This data is summarized in Fig. 31.

The use of the common light chain not only increases the yield of the correctly paired antibody but also decreases the affinity of each arm of the antibody for its target as compared to the originally paired VH-VL antibody, e.g., as in the 15D3 monoclonal antibody and the anti-PD-L1 antibody atezolizumab. This decreased affinity increases the specificity of the KBis2.1 antibody for cancer cells that express at least one of MDR1 and PD-L1 at a high level as compared to

normal cells that express only one of the targets at relatively low levels or both targets at a low level.

The sequences for the 15D3 VH chain and the MRK16 VL chain in the KBis2.1 antibody is in the preceding examples. Both the chimeric and humanized versions of the 15D3 heavy chain and MRK16 light chain were used. The heavy chain of atezolizumab has the following sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWRQAPGKGLEWVAVWISPYGG
STYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEAL
HNHYTQKSLSLSPGK.

The Fc region of the heavy chain was mutated to introduce charge pair substitutions "KK" to encourage pairing with the 15D3 heavy chain with "DD" substitutions. DD substitutions refer to K392D and K409D substitutions, KK substitutions refer to E356K and D399K substitutions. The numbering of the amino acid substitutions is per EU numbering system for HCs.

The HCDRs 1-3 of the anti-PDL1 antibody atezolizumab defined as per Kabat nomenclature are as follows:

HCDR1: DSWIH
HCDR2: WISPYGGSTYYADSVKG
HCDR3: RHWPGGFDY

While the light chain of the anti-PDL1 antibody atezolizumab was not used in the bispecific antibody, the sequence is provided below for sake of completeness:

DIQMTQSPSSLSASVGDRVTITCRASQDVSTAVAWYQQKPGKAPKLLIYSASFLYSGVP
SRFSGSGSGTDFTLTISSLQPEDFATYYCQQYLYHPATFGQGTKVEIKRTVAAPSVFIFPPSDE
QLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADY
EKHKVYACEVTHQGLSSPVTKSFNRGEC

The LCDRs1-3 defined as per Kabat nomenclature are as follows:

LCDR1: RASQDVSTAVA
LCDR2: SASFLYS
LCDR3: QQYLYHPAT

The EC50 for the anti-PDL1 antibody atezolizumab as measured using 293T cells overexpressing PDL1 is 0.29 nM.

Example 12: Generation of Bispecific mAbs that bind EGFR and MDR-1**Anti-MDR1 Anti-EGFR Bispecific Antibody Version 1**

Strategy used for generation of the KBis1.1 antibody described in Examples set forth above was utilized for generating a bispecific antibody that simultaneously binds MDR-1 and EGFR.

A common variable light (VL) chain from the MRK16 antibody with an antigen-binding site for MDR1, a first variable heavy (VH) chain from the 15D3 antibody with an antigen-binding site for MDR1, and a second VH chain with an antigen-binding site for EGFR was utilized to make the bispecific antibodies. The MRK16 VL chain and the 15D3 VH chain are the same as that described in this application. The VH chain of the anti-EGFR antibody cetuximab or necitumumab served as the second VH chain with an antigen-binding site for EGFR.

The K_d for cetuximab measured by ELISA as described herein ranges from 0.05 nM-0.1nM. The EC₅₀ for necitumumab measured by FACS using 293T cells overexpressing EGFR is 0.044nM.

The bispecific antibodies included the following combination of the heavy and light chains:

- i. 15D3 DD HC :: cetuximab KK HC :: MRK16 LC
- ii. 15D3 DD HC :: necitumumab KK HC :: MRK16 LC

The abbreviations "DD" and "KK" refer to the charged pair substitutions.

The 15D3 DD HC :: cetuximab KK HC :: MRK16 LC antibody retained binding to MDR1 (as measured by FACS using 293T cell overexpressing MDR1) and to EGFR (as measured by ELISA).

The 15D3 DD HC :: necitumumab KK HC :: MRK16 LC antibody retained binding to MDR1 (as measured by FACS using 293T cell overexpressing MDR1) and to EGFR (as measured by FACS using 293T cell overexpressing EGFR). This antibody did not show significant binding in ELISA assay.

Additional combination of VH and VL chains were also tested. The VH chain of the anti-MDR1 antibody UIC2 was used instead of 15D3 to generate the bispecific antibody:

UIC2 DD HC:: cetuximab KK HC :: MRK16 LC. UIC2 VH sequence is provided in the detailed description of the application. However, this antibody did not show significant binding to MDR1.

These data are summarized in Fig. 31.

Anti-MDR1 Anti-EGFR Bispecific Antibody Version 2

The VH chain of the anti-MDR1 MRK16 antibody and the VL chain of the anti-MDR1 antibody 15D3 were tested in combination with the VH region of the anti-EGFR antibody cetuximab to generate the antibody - MRK16 DD HC :: cetuximab KK HC :: 15D3 LC. This antibody also retained binding to MDR1 (as measured by FACS using 293T cell overexpressing MDR1) and to EGFR (as measured by ELISA).

Anti-MDR1 Anti-EGFR Bispecific Antibody Version 3

Bispecific antibodies that retained VH and VL regions from the same anti-MDR1 antibody and combined with the cetuximab VH region were generated:

- i. MRK16 DD HC :: cetuximab KK HC :: MRK16 LC
- ii. 15D3 DD HC :: cetuximab KK HC :: 15D3 LC

As summarized in Fig. 31, the 15D3 DD HC :: cetuximab KK HC :: 15D3 LC antibody binds to both MDR-1 and EGFR. The MRK16 DD HC :: cetuximab KK HC :: MRK16 LC also binds to both MDR-1 and EGFR, although binding to EGFR with a lower affinity compared to the 15D3 DD HC :: cetuximab KK HC :: 15D3 LC antibody.

UIC2 HC DD::EGFR HC KK::15D3 LC antibody was also constructed. Similar to the observation when MRK16 LC was combined with the UIC HC, this antibody also failed to bind to MDR1. See Fig. 31.

The sequences for the 15D3 VH/VL chain and the MRK16 VL/VH chain in the bispecific antibody is in the detailed description of the application. The amino acid sequences of the heavy and light chain regions of cetuximab and necitumumab are provided below.

Cetuximab Heavy chain

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGGNT
DYNTPFSTRLSINKDNSKSKQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTSAA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTV
LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGF
YPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN
HYTQKSLSLSPGK

HCDR1: NYGVH

HCDR2: VIWSSGGNTDYNTPFTS

HCDR3: ALTYDYEFAY

Cetuximab Light chain

DILLTQSPVILSVSPGERVSFSCRASQSIGTNIHWYQQRTNGSPRLLIKAYASESISGIPSR
FSGSGSGTDFTLSINSVESEDIADYYCQQNNNWPTTFGAGTKLELKRTVAAPSVFIFPPSDEQL
5 KSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEK
HKVYACEVTHQGLSSPVTKSFNRGEC

LCDR1: RASQSIGTNIH

LCDR2: YASESIS

LCDR3: QQNNNWPTT

Necitumumab Heavy chain

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYSGS
TDYNPSLKSRTMSVDTSKNQFSLKVNSTAAADTAVYYCARVSIFGVGTFDYWGQGLVTVSS
ASTKGPSVLPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
15 SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLT
VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK
GFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVMHEAL
HNHYTQKSLSLSPGK

HCDR1: SGDYYWS

HCDR2: YIYYSGSTDYNPSLKS

HCDR3: VSIFGVGTFDY

Necitumumab Light chain

EIVMTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRATGIP
ARFSGSGSGTDFTLTISSELPEDFAVYYCHQYGSTPLTFGGGTCAEIKRTVAAPSVFIFPPSDEQ
LKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC

LCDR1: RASQSVSSYLA

LCDR2: DASNRAT

LCDR3: HQYGSTPLT

Fig. 31 summarized binding of the indicated antibodies to MDR1 (measured by FACs) or to a TAA (measured by ELISA or FACS). aCD47=anti-CD47; aEGFR=anti-EGFR; aPDL1=anti-PDL1.

“+” in Figure 31 indicates that the EC50 ranged from 5 nM-700 nM. “±” in Figure 31 indicates that the EC50 ranged from 701 nM-900 nM. “-” in Figure 31 indicates that the EC50 was > 1000 nM.

Figs. 32A-32C show MFI measurement for the listed antibodies:

Human IgG1 antibody (“hlgG”),

anti-EGFR antibody necitumumab (“KT3-2”),

anti-EGFR anti-MDR1 bispecific antibodies:

15D3 KK HC :: cetuximab DD HC :: 15D3 LC (“KT3QDD 15D3KK 15D3”);

MRK16 KK HC :: cetuximab DD HC :: MRK16 LC (“KT3QDD MRK16KK MRK16”);

15D3 DD HC :: cetuximab KK HC :: MRK16 LC (“KT3QKK 15D3DD MRK16”);

15D3 DD HC :: necitumumab KK HC :: MRK16 LC (“KT3-2KK 15D3DD 1MRK16”)

Fig. 32A shows that each of the bispecific antibodies bound to 293T cells overexpressing MDR1. The anti-EGFR antibody necitumumab indicated in the graph as KT3-2 did not show significant binding to these cells, similar to the negative control human IgG1 antibody.

Fig. 32B shows that each of the bispecific antibodies bound to 293T cells overexpressing EGFR with an affinity significantly less than the anti-EGFR antibody necitumumab “KT3-2”. Fig. 32C shows the binding data for the bispecific antibodies shown in Fig. 32B with a Y-axis showing MFI in range of ≤ 400 . The 293T cells do not express EGFR (see Fig. 32A). The 293T cells overexpressing EGFR express EGFR to a detectable level however, the EGFR expression is relatively low which accounts for the low affinity of the bispecific antibodies for these cells. In contrast, the expression level of MDR1 in 293T cells overexpressing MDR1 is quite high which accounts for the binding of the bispecific antibodies even in absence of expression of EGFR.

A summary of the EC50 measured for the bispecific antibodies is provided below in Tables 6-7. In these 293 cell-based FACS assays the EC50 is the concentration of antibody that generates half of the maximum response (OD450):

Table 6: Anti-MDR1 Anti-EGFR Bispecific Ab binding to 293T-B10X cells

Ab	EC50(nM)
15D3DD: necitumumab KK:MRK16	33.1
15D3DD: cetuximab KK:MRK16	53

15D3KK: cetuximab DD:15D3	76.8
MRK16KK: cetuximab DD:MRK16	144.7
15D3	25.1
MRK16	58.8
hlgG1	NB

Table 7: Anti-MDR1 Anti-EGFR Bispecific Ab binding to 293T-EGFR OX cells

Ab	EC50(nM)
15D3DD: necitumumab KK:MRK16	110.2
15D3DD: cetuximab KK:MRK16	85.3
15D3KK: cetuximab DD:15D3	ND
MRK16KK: cetuximab DD:MRK16	112.1
necitumumab	0.13
15D3	ND
MRK16	ND
hlgG1	NB

NB=No Binding

ND=Not Done

5

Example 13: Generation of bispecific anti-MDR1 anti-CD47 antibody with humanized 5F9 VH region

In order to select human antibody frameworks (FR) to be used as templates for CDR-grafting, the mouse 5F9 VH region was compared with those of human germline sequences. The FRs from human VH5-51 and VH7-4 and VH3-23 were ultimately selected as the starting point for designing humanized 5F9 VH.

Next, humanized 15D3 HC :: 5F9 HC :: MRK16 LC bispecific constructs were produced and examined by flow cytometry analysis with 293T cells and 293T-CD47 KO cells. Both 5F9.huVH5 and 5F9.huVH3 HCs in pair with humanized MRK16 LC can bind to CD47 (data not shown).

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it is readily apparent to those of ordinary

skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Accordingly, the preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. Moreover, nothing disclosed herein is intended to be dedicated to the public regardless of whether such disclosure is explicitly recited in the claims.

The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims. In the claims, 35 U.S.C. §112(f) or 35 U.S.C. §112(6) is expressly defined as being invoked for a limitation in the claim only when the exact phrase "means for" or the exact phrase "step for" is recited at the beginning of such limitation in the claim; if such exact phrase is not used in a limitation in the claim, then 35 U.S.C. § 112 (f) or 35 U.S.C. §112(6) is not invoked.

CLAIMSWHAT IS CLAIMED IS:

1. A bispecific antibody molecule that binds multidrug resistance protein 1 (MDR1) and a tumor associated antigen (TAA), the antibody molecule comprising two identical variable light (VL) chains, a first variable heavy (VH) chain, and a second VH chain,

wherein the VL chains each comprise an antigen-binding site for MDR1, the first VH chain comprises an antigen-binding site for MDR1, and the second VH chain comprises an antigen-binding site for the TAA, and wherein the second VH chain binds the TAA when paired with one of the VL chains,

wherein the bispecific antibody binds to cancer cells expressing both MDR1 and the TAA while showing reduced binding to non-cancer cells expressing MDR1 and/or the TAA.

2. The bispecific antibody molecule according to claim 1, wherein the antigen-binding site of the two VL chains comprises light chain CDRs 1-3 (LCDRs 1-3) of a VL chain having the sequence:

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK (SEQ ID NO:1), wherein X¹ is N, Q or S.

3. The bispecific antibody molecule according to claim 2, wherein the two VL chains comprise LCDRs 1-3 of the VL chain having the sequence:

(i)

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKISNRFSGVP
DRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK (SEQ ID NO:2);

(ii)

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPPRLIYKISNRFSG
VPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK (SEQ ID NO:3);

or

(iii)

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPGQPPRLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK (SEQ ID NO:4).

4. The bispecific antibody molecule according to claim 2 or 3, wherein:
- (i) the LCDR1 comprises the sequence: RSSQSIVHSTGX¹TYLE (SEQ ID NO:5);
 - (ii) the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6); and
 - (iii) the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7);
- wherein X¹ is N, Q or S.

5. The bispecific antibody molecules of any one of the preceding claims, wherein the two VL chains are humanized.

6. The bispecific antibody molecule according to any one of claims 2 to 5, wherein the two VL chains comprise the sequence or an amino acid at least 90% identical to the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGNTYLEWYQQRPGQPPRLLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGGGTKLEIK (SEQ ID
NO:8);

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPPRLLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK (SEQ ID
NO:3); or

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPGQPPRLLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK (SEQ ID
NO:4).

7. The bispecific antibody molecule according to claim 6, wherein the two VL chains comprise the sequence or a sequence at least 90% identical to the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGNTYLEWYQQRPGQPPRLLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGGGTKLEIK (SEQ ID
NO:8).

8. The bispecific antibody molecule according to any one of the preceding claims, wherein the antigen binding site of first VH chain comprises heavy chain CDRs 1-3 (HCDRs 1-3) of a VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGG
GX²TYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGT
LVTVSS (SEQ ID NO:9), wherein X² is N, Q or S.

9. The bispecific antibody molecule according to any one of the preceding claims, wherein the antigen binding site of the first VH chain comprises HCDRs 1-3 of the VH chain having the sequence:

(i)

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGNTYY
PDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGT LVTVSS
(SEQ ID NO:10), or

(ii)

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGQTY
YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGT LTVS
S (SEQ ID NO:11), or

(iii)

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSGGGSTY
YPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGT LTVS
S (SEQ ID NO:12).

10. The bispecific antibody molecule according to claim 8 or 9, wherein:

(i) the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13);

(ii) the HCDR2 comprises the sequence: TISSGGG X²TYYPDSVKG (SEQ ID NO:14);

and

(iii) the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15);

wherein X² is N, Q or S.

11. The bispecific antibody molecule according to any one of the preceding claims, wherein the first and/or second VH chain is humanized.

12. The bispecific antibody molecule according to any one of claims 8 to 11, wherein the first VH chain comprises the amino acid sequence or an amino acid at least 90% identical to the amino acid sequence:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGNTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQG
TLVTVSS (SEQ ID NO:16);

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGQTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQG
TLVTVSS (SEQ ID NO:11); or

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGSTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEDTALYYCARYGAGDAWFAYWGQGT
LTVTVSS (SEQ ID NO:12).

13. The bispecific antibody molecule according to any one of claims 2-12, wherein:
 - (i) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:26), the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13), the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG (SEQ ID NO:29), and the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
 - (ii) the LCDR1 comprises the sequence: RSSQSIVHSTGQTYLE (SEQ ID NO:27), the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13), the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG (SEQ ID NO:30), and the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
 - (iii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE (SEQ ID NO:28), the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13), the HCDR2 comprises the sequence: TISSGGGSTYYPDSVKG (SEQ ID NO:31), and the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or
 - (iv) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:26), the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13), the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG (SEQ ID NO:30), and

the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or

(v) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:26),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGSTYYPDSVKG (SEQ ID NO:31), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or

(vi) the LCDR1 comprises the sequence: RSSQSIVHSTGQTYLE (SEQ ID NO:27),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG (SEQ ID NO:29), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or

(vii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE (SEQ ID NO:28),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG (SEQ ID NO:29), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or

(viii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE (SEQ ID NO:28),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG (SEQ ID NO:30), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15), or

(ix) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:26),
the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6), and
the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7); and
the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13),
the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG (SEQ ID NO:30), and
the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15).

14. The bispecific antibody molecule according to any one of the preceding claims, wherein the second VH chain is derived from a monospecific antibody molecule which binds

the TAA, and wherein the affinity of the bispecific antibody molecule for the TAA when paired with one of the light chains is at least 2-fold lower than the affinity of the monospecific antibody molecule for the TAA from which the VH chain is derived.

15. The bispecific antibody molecule according to any one of the preceding claims, wherein the TAA is CD47.

16. The bispecific antibody molecule according to claim 15, wherein the antigen-binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDVAVYYCARGGYRAMDYWGQGTLLV
VSS (SEQ ID NO:17).

17. The bispecific antibody molecule according to claim 15 or 16, wherein the second VH chain comprises the HCDR1 comprising the sequence: NYNMH (SEQ ID NO:18), the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD (SEQ ID NO:19), and the HCDR3 comprising the sequence: GGYRAMDY (SEQ ID NO:20).

18. The bispecific antibody molecule according to any one of claims 15 to 16, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDVAVYYCARGGYRAMDYWGQGTLLV
VSS (SEQ ID NO:17),

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYNMHWVRQAPGKGLEWMGTIYPG
NDDTSYNQKFKDRVTISRDN SKNTLYLQMNSLRAEDVAVYYCARGGYRAMDYWGQGTLLV
TVSS (SEQ ID NO:21),

EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYNMHWVRQMPGKGLEWMGTIYPG
NDDTSYNQKFKDQVTISADKSISTAYLQWSSLKASDTAMYYCARGGYRAMDYWGQGTTV
TVSS (SEQ ID NO:22), or

QVQLVQSGSELKKPGASVKVSCASGYTFTNYNMHWVRQAPGQGLEWMGTIYPG
NDDTSYNQKFKDRFVFLDTSVSTAYLQISLKAEDTAVYYCARGGYRAMDYWGQGTTVT
VSS (SEQ ID NO:23).

19. The bispecific antibody molecule according to any one of claims 1-14, wherein the TAA is PD-L1.

20. The bispecific antibody molecule according to claim 19, wherein the antigen-binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAVISPY
GGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTL
VTVSS (SEQ ID NO:32).

21. The bispecific antibody molecule according to claim 19 or 20, wherein the antigen-binding site of the second VH chain comprises the HCDR1 comprising the sequence: DSWIH (SEQ ID NO:33), the HCDR2 comprising the sequence: WISPYGGSTYYADSVKG (SEQ ID NO:34), and the HCDR3 comprising the sequence: RHWPGGFDY (SEQ ID NO:35).

22. The bispecific antibody molecule according to any one of claims 19 to 21, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:
EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAVISPYGGSTY
YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS
(SEQ ID NO:32).

23. The bispecific antibody molecule according to any one of claims 1-14, wherein the TAA is EGFR.

24. The bispecific antibody molecule according to claim 23, wherein the antigen-binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYSGSTD
YNPSLKSRTMSVDTSKNQFSLKVNSVTAADTAVYYCARVSIFGVGTFDYWGQGTLVTVS
S (SEQ ID NO:36).

25. The bispecific antibody molecule according to claim 23 or 24, wherein the antigen-binding site of the second VH chain comprises the HCDR1 comprising the sequence: SGDYYWS (SEQ ID NO:37), the HCDR2 comprising the sequence: YIYYSGSTDYNPSLKS (SEQ ID NO:38), and the HCDR3 comprising the sequence: VSIFGVGTFDY (SEQ ID NO:39).

26. The bispecific antibody molecule according to any one of claims 24 to 25, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYSGSTDYNPSLKSRTMSVDTSKNQFSLKVNSVTAADTAVYYCARVSIFGVGTFDYWGQGTLVTVSS (SEQ ID NO:36).

27. The bispecific antibody molecule according to claim 23, wherein the antigen-binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG
GNTDYNTPTFSTRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT
LVSA (SEQ ID NO:40).

28. The bispecific antibody molecule according to claim 26 or 27, wherein the second VH chain comprises the HCDR1 comprising the sequence: NYGVH (SEQ ID NO:41), the HCDR2 comprising the sequence: VIWSGGNTDYNTPTFS (SEQ ID NO:42), and the HCDR3 comprising the sequence: ALTYDYEFAY (SEQ ID NO:43).

29. The bispecific antibody molecule according to any one of claims 26 to 28, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG
GNTDYNTPTFSTRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGT
LVSA (SEQ ID NO:40).

30. The bispecific antibody molecule according to claim 1, wherein the antigen-binding site of the two VL chains comprises light chain CDRs 1-3 (LCDRs 1-3) of a VL chain having the sequence:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVIYCFQGSHFPRTFGGGTRLEIK (SEQ ID NO:25).

31. The bispecific antibody molecule according to claim 30, wherein:

- (i) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:44);
- (ii) the LCDR2 comprises the sequence: KISRLEA (SEQ ID NO:45); and
- (iii) the LCDR3 comprises the sequence: FQGSHFPRT (SEQ ID NO:46).

32. The bispecific antibody molecule according to claim 30 or 31, wherein the VL chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVIYCFQGSHFPRTFGGGTRLEIK (SEQ ID NO:25).

33. The bispecific antibody molecule according to any one of claims 30-32, wherein the antigen binding site of first VH chain comprises heavy chain CDRs 1-3 (HCDRs 1-3) of a VH chain having the sequence:

EVILVESGGGLVKPGGSLKLSCAASGFTFSSYTMSWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTISRDNANKNNLYLQMSSLRSEDALYYCARYYRYEAWFASWGQGTLV
TVSA.

34. The bispecific antibody molecule according to claim 33, wherein:

- (i) the HCDR1 comprises the sequence: SYTMS;
- (ii) the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG; and
- (iii) the HCDR3 comprises the sequence: YYRYEAWFAS.

35. The bispecific antibody molecule according to any one of claims 30-34, wherein the first VH chain comprises the amino acid sequence or an amino acid at least 90% identical to the amino acid sequence:

EVILVESGGGLVKPGGSLKLSCAASGFTFSYTM²SWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTISRDN²AKNNLYLQMSSLRSED²TALYYCARYYRYEAWFASWGQGT²LV
TVSA.

36. The bispecific antibody molecule according to any one of claims 30-32, wherein the antigen binding site of first VH chain comprises heavy chain CDRs 1-3 (HCDRs 1-3) of a VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGX²TY
YPDSVKGRFTVSRDNAMSSLYLQMSSLRSED²TALYYCARYGAGDAWFAYWGQGT²LVTV
S (SEQ ID NO:9), wherein X² is N, Q or S.

37. The bispecific antibody molecule according to claim 36, wherein:

(i) the HCDR1 comprises the sequence: RYTMS (SEQ ID NO:13);

(ii) the HCDR2 comprises the sequence: TISSGGG X²TYYPDSVKG (SEQ ID NO:14);

and

(iii) the HCDR3 comprises the sequence: YGAGDAWFAY (SEQ ID NO:15);

wherein X² is N, Q or S.

38. The bispecific antibody molecule according to any one of claims 36-37, wherein the first VH chain comprises the amino acid sequence or an amino acid at least 90% identical to the amino acid sequence:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGNTYYPDSVKGRFTVSRDNSKNSLYLQMNSLR²TEDTALYYCARYGAGDAWFAYWGQ
TLVTVSS (SEQ ID NO:16);

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGQTYYPDSVKGRFTVSRDNSKNSLYLQMNSLR²TEDTALYYCARYGAGDAWFAYWGQ
TLVTVSS (SEQ ID NO:11); or

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMSWVRQAPGKGLEWVATISSG
GGSTYYPDSVKGRFTVSRDNSKNSLYLQMNSLR²TEDTALYYCARYGAGDAWFAYWGQGT
LVTVSS (SEQ ID NO:12).

39. The bispecific antibody molecule according to any one of claims 1-7, wherein the antigen binding site of first VH chain comprises heavy chain CDRs 1-3 (HCDRs 1-3) of a VH chain having the sequence:

EVILVESGGGLVKPGGSLKLSCAASGFTFSSYTMSWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTISRDNANKNNLYLQMSSLRSEDALYYCARYYYRYEAWFASWGQGLTV
TVSA.

40. The bispecific antibody molecule according to claim 39, wherein:

- (i) the HCDR1 comprises the sequence: SYTMS;
- (ii) the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG; and
- (iii) the HCDR3 comprises the sequence: YYRYEAWFAS.

41. The bispecific antibody molecule according to any one of claims 39-40, wherein the first VH chain comprises the sequence or an amino acid at least 90% identical to the sequence:

EVILVESGGGLVKPGGSLKLSCAASGFTFSSYTMSWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTISRDNANKNNLYLQMSSLRSEDALYYCARYYYRYEAWFASWGQGLTV
TVSA.

42. The bispecific antibody molecule according to any one of claims 30-41, wherein the second VH chain is derived from a monospecific antibody molecule which binds the TAA, and wherein the affinity of the bispecific antibody molecule for the TAA when paired with one of the light chains is at least 2-fold lower than the affinity of the monospecific antibody molecule for the TAA from which the VH chain is derived.

43. The bispecific antibody molecule according to any one of claims 30-42, wherein the TAA is CD47.

44. The bispecific antibody molecule according to claim 43, wherein the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDALVYYCARGGYRAMDYWGQGLTV
VSS (SEQ ID NO:17).

45. The bispecific antibody molecule according to claim 44, wherein the second VH chain comprises the HCDR1 comprising the sequence: NYNMH (SEQ ID NO:18), the

HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD (SEQ ID NO:19), and the HCDR3 comprising the sequence: GGYRAMDY (SEQ ID NO:20).

46. The bispecific antibody molecule according to claim 44 or 45, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDVAVYYCARGGGYRAMDYWGQGTLVT
VSS (SEQ ID NO:17),

EVQLVESGGGLVQPGGSLRLSCAASGYTFTNYNMHWVRQAPGKGLEWMGTIYPG
NDDTSYNQKFKDRVTISRDN SKNTLYLQMNSLRAEDVAVYYCARGGGYRAMDYWGQGTLV
TVSS (SEQ ID NO:21),

EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYNMHWVRQMPGKGLEWMGTIYPG
NDDTSYNQKFKDQVTISADKSISTAYLQWSSLKASDTAMYYCARGGGYRAMDYWGQGTTV
TVSS (SEQ ID NO:22), or

QVQLVQSGSELKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQGLEWMGTIYPG
NDDTSYNQKFKDRFVFLDTSVSTAYLQISSLKAEDTAVYYCARGGGYRAMDYWGQGTTVT
VSS (SEQ ID NO:23).

47. The bispecific antibody molecule according to any one of claims 30-42, wherein the TAA is PD-L1.

48. The bispecific antibody molecule according to claim 47, wherein the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPYGGSTY YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS (SEQ ID NO:32).

49. The bispecific antibody molecule according to claim 47 or 48, wherein the second VH chain comprises the HCDR1 comprising the sequence: DSWIH (SEQ ID NO:33), the HCDR2 comprising the sequence: WISPYGGSTYYADSVKG (SEQ ID NO:34), and the HCDR3 comprising the sequence: RHWPGGFDY (SEQ ID NO:35).

50. The bispecific antibody molecule according to any one of claims 48 to 49, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAWISPY
GGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGT
LTVSS (SEQ ID NO:32).

51. The bispecific antibody molecule according to any one of claims 30-42, wherein the TAA is EGFR.

52. The bispecific antibody molecule according to claim 51, wherein the antigen binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYSGSTD
YNPSLKSRVTMSVDTSKNQFSLKVNSTAAADTAVYYCARVSIFGVGTFDYWGQGTLTSTVS
S (SEQ ID NO:36).

53. The bispecific antibody molecule according to claim 52, wherein the second VH chain comprises the HCDR1 comprising the sequence: SGDYYWS (SEQ ID NO:37), the HCDR2 comprising the sequence: YIYYSGSTDYNPSLKS (SEQ ID NO:38), and the HCDR3 comprising the sequence: VSIFGVGTFDY (SEQ ID NO:39).

54. The bispecific antibody molecule according to any one of claims 52 to 53, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYSGSTD
YNPSLKSRVTMSVDTSKNQFSLKVNSTAAADTAVYYCARVSIFGVGTFDYWGQGTLTSTVS
S (SEQ ID NO:36).

55. The bispecific antibody molecule according to claim 51, wherein the antigen binding site of the second VH chain comprises the HCDRs 1-3 of a VH chain comprising the amino acid sequence:

QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGGNTDY
NTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGTLTSTVSA
(SEQ ID NO:40).

56. The bispecific antibody molecule according to claim 52, wherein the antigen binding site of the second VH chain comprises the HCDR1 comprising the sequence: NYGVH (SEQ ID NO:41), the HCDR2 comprising the sequence: VIWSSGGNTDYNTPFTS (SEQ ID NO:42), and the HCDR3 comprising the sequence: ALTYDYEFAY (SEQ ID NO:43).

57. The bispecific antibody molecule according to any one of claims 52 to 53, wherein the second VH chain comprises the amino acid sequence or an amino acid sequence at least 90% identical to the amino acid sequence:

QVQLKQSGPGLVQPSQSLSTCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGGNTDY
NTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGTLTSTVSA
(SEQ ID NO:40).

58. The bispecific antibody molecule according to any one of the preceding claims, wherein the antibody has greater than twice the affinity for a cell expressing both MDR1 and the TAA as compared to a cell expressing either MDR1 or the TAA.

59. The bispecific antibody molecule according to any one of the preceding claims, wherein the antibody is capable of increasing sensitivity of a cancer cell to treatment with a chemotherapeutic agent, wherein the half maximal inhibitory concentration (IC₅₀) of the chemotherapeutic agent when co-administered with the antibody is at least 2 times lower than the IC₅₀ of the chemotherapeutic agent when co-administered with the anti-MDR1 antibody 15D3 comprising a VH chain having the sequence:

EVKVVESGGVLRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGTL
VTVSA (SEQ ID NO:24); and

a VL chain having the sequence:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHFPRTFGGGTRLEIK (SEQ ID
NO:25), wherein optionally the IC₅₀ is measured in vitro.

60. The bispecific antibody molecule according to claim 59, wherein the cancer cell is a NALM6 ADR cell and optionally wherein the chemotherapeutic agent comprises paclitaxel, Colchicine, Verapamil, Vinblastine, Topotecan, Doxorubicin, Daunorubicin, Etoposide, or Nilotinib.

61. The bispecific antibody molecule according to any one of the preceding claims, wherein the antibody, when bound to a cell expressing a MDR1, inhibits efflux by the MDR1.

62. The bispecific antibody molecule according to any one of the preceding claims, wherein the antibody binds to MDR1 with at least 2-fold lower affinity than the anti-MDR1 antibody 15D3 comprising a VH chain having the sequence:

EVKVVESGGVLRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGTL
VTVSA (SEQ ID NO:24); and

a VL chain having the sequence:

DVLMQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVIYCFQGSHFPRTFGGGTRLEIK (SEQ ID
NO:25).

63. The bispecific antibody molecule according to any one of the preceding claims, wherein the antibody comprises a Fc domain that has been modified to reduce or abrogate binding of the antibody to one or more Fcγ receptors.

64. The bispecific antibody molecule according to any one of the preceding claims for use in a method of treating cancer in a subject, the method comprising administering the antibody to the subject.

65. The bispecific antibody molecule for use according to claim 64, wherein the method comprises administering the antibody in combination with at least one additional active agent, wherein the at least one additional active agent comprises a chemotherapeutic agent, an inhibitor of a multidrug resistance transporter, an immunotherapy agent, or a combination thereof.

66. The bispecific antibody molecule for use according to claim 65, wherein the at least one additional active agent is a chemotherapeutic agent, optionally wherein the chemotherapeutic agent is a taxol, a vinca alkaloid, or an anthracycline.

67. A chemotherapy agent for use in a method of treating cancer in a subject, the method comprising administering the chemotherapy agent in combination with the antibody according to any one of claims 1 to 63 to the subject, optionally wherein the chemotherapy agent is a taxol, a vinca alkaloid, or an anthracycline.

68. The bispecific antibody molecule for use according to claims 67, wherein the subject being treated has a cancer that has been determined to be resistant to the chemotherapeutic agent.

69. A method of treating a subject for a cancer, the method comprising administering to the subject a therapeutically effective amount of the bispecific antibody molecule according to any of claims 1 to 63.

70. The method according to claim 69, wherein the method comprises administering the bispecific antibody molecule in combination with at least one additional active agent, wherein the at least one additional active agent comprises a chemotherapeutic agent, an inhibitor of a multidrug resistance transporter, an immunotherapy agent, or a combination thereof.

71. The method according to claim 70, wherein the at least one additional active agent is a chemotherapeutic agent, optionally wherein the chemotherapeutic agent is a taxol, a vinca alkaloid, or an anthracycline.

72. The method according to claim 71, wherein the subject being treated has a cancer that has been determined to be resistant to treatment with the chemotherapeutic agent, wherein optionally the chemotherapeutic agent comprises paclitaxel, Colchicine, Verapamil, Vinblastine, Topotecan, Doxorubicin, Daunorubicin, Etoposide, or Nilotinib.

73. An antibody comprising:
a variable light (VL) chain comprising light chain CDRs (LCDRs) of a VL chain having the sequence:

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK,

wherein X¹ is N, Q or S; and

a variable heavy (VH) chain comprising heavy chain CDRs (HCDRs) of a VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSAASGFTFSRYTMSWVRQTPEKRLEWVATISSGG
GX²TYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGT
LVTVSS,

wherein X² is N, Q or S; or

an antibody comprising:

a variable light (VL) chain comprising light chain CDRs (LCDRs) of a VL chain having the sequence:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHPRTFGGGTRLEIK (SEQ ID
NO:25); and

a variable heavy (VH) chain comprising heavy chain CDRs (HCDRs) of a VH chain having the sequence:

EVILVESGGGLVKPGGSLKLSCAASGFTFSYTMWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTISRDNANKNNLYLQMSSLRSEDALYYCARYYRYEAWFASWGQGLTV
TVSA.

74. The antibody according to claim 73, wherein:

the VL chain comprises LCDRs 1-3 of the VL chain having the sequence:

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK; and

the VH chain comprises HCDRs 1-3 of the VH chain having the sequence:

EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMWVRQTPEKRLEWVATISSGG
GNTYYPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGL
VTVSS, or

the VL chain comprises LCDRs 1-3 of the VL chain having the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGQTYLEWYQQRPGQPPRLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK; and

the VH chain comprises HCDRs 1-3 of the VH chain having the sequence:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMWVRQAPGKGLEWVATISSG
GGQTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQG
TLVTVSS, or

the VL chain comprises LCDRs 1-3 of the VL chain having the sequence:

DIVMTQTPLSSPVTLGQPASISCRSSQSIVHSTGSTYLEWYQQRPGQPPRLIYKIS
NRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCFQASHFPRTFGQGTKLEIK; and

the VH chain comprises LCDRs 1-3 of the VH chain having the sequence:

EVQLVESGGVVVQPGGSLRLSCAASGFTFSRYTMWVRQAPGKGLEWVATISSG
GGSTYYPDSVKGRFTVSRDNSKNSLYLQMNSLRTEALYYCARYGAGDAWFAYWGQGT
LTVTVSS.

75. The antibody according to claim 74, wherein:

the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE,
RSSQSIVHSTGQTYLE, or RSSQSIVHSTGSTYLE,

the LCDR2 comprises the sequence: KISNRF, and

the LCDR3 comprises the sequence: FQASHFPRT.

76. The antibody according to claim 75, wherein:

- (i) the HCDR1 comprises the sequence: RYTMS;
 - (ii) the HCDR2 comprises the sequence: TISSGGG X²TYYPDSVKG; and
 - (iii) the HCDR3 comprises the sequence: YGAGDAWFAY;
- wherein X² is N, Q or S.

77. The antibody according to any one of claims 74-75, wherein:

- (i) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (ii) the LCDR1 comprises the sequence: RSSQSIVHSTGQTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (iii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGSTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (iv) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE, the LCDR2 comprises the sequence: KISNRFS, and the LCDR3 comprises the sequence: FQASHFPRT; and the HCDR1 comprises the sequence: RYTMS, the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (v) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE, the LCDR2 comprises the sequence: KISNRFS, and

- the LCDR3 comprises the sequence: FQASHFPRT; and
 the HCDR1 comprises the sequence: RYTMS,
 the HCDR2 comprises the sequence: TISSGGGSTYYPDSVKG, and
 the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (vi) the LCDR1 comprises the sequence: RSSQSIVHSTGQTYLE,
 the LCDR2 comprises the sequence: KISNRFS, and
 the LCDR3 comprises the sequence: FQASHFPRT; and
 the HCDR1 comprises the sequence: RYTMS,
 the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG, and
 the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (vii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE,
 the LCDR2 comprises the sequence: KISNRFS, and
 the LCDR3 comprises the sequence: FQASHFPRT; and
 the HCDR1 comprises the sequence: RYTMS,
 the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG, and
 the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (viii) the LCDR1 comprises the sequence: RSSQSIVHSTGSTYLE,
 the LCDR2 comprises the sequence: KISNRFS, and
 the LCDR3 comprises the sequence: FQASHFPRT; and
 the HCDR1 comprises the sequence: RYTMS,
 the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and
 the HCDR3 comprises the sequence: YGAGDAWFAY, or
- (ix) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE,
 the LCDR2 comprises the sequence: KISNRFS, and
 the LCDR3 comprises the sequence: FQASHFPRT; and
 the HCDR1 comprises the sequence: RYTMS,
 the HCDR2 comprises the sequence: TISSGGGQTYYPDSVKG, and
 the HCDR3 comprises the sequence: YGAGDAWFAY.

78. The antibody according to any one of claims 73-77, wherein the VL chain of the antibody comprises the amino acid sequence or comprises an amino acid sequence at least 90% identical to the amino acid sequence:

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKIS
 NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGTKLEIK, and

wherein the VH chain of the antibody comprises the amino acid sequence or comprises an amino acid sequence at least 90% identical to the amino acid sequence: EVKVVESGGVLVRPGGSLKLSCAASGFTFSRYTMSWVRQTPEKRLEWVATISSGGGX²TY YPDSVKGRFTVSRDNAMSSLYLQMSSLRSEDALYYCARYGAGDAWFAYWGQGTLVTVS S.

79. The antibody according to claim 73, wherein the an antibody comprises: a variable light (VL) chain comprising light chain CDRs 1-3 (LCDRs) of a VL chain having the sequence:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS NRFGVPDRFSGSGSGTDFTLKISRLEAEDLGVIYCFQGSHFPRTFGGGTRLEIK (SEQ ID NO:25); and

a variable heavy (VH) chain comprising heavy chain CDRs 1-3 (HCDRs) of a VH chain having the sequence:

EVILVESGGGLVKPGGSLKLSCAASGFTFSYTMWVRQTPEKRLEWVATISSGG GNTYYPDSVKGRFTISRDNANKNNLYLQMSSLRSEDALYYCARYYYEAWFASWGQGTLV TVSA.

80. The antibody according to claim 79, wherein:

- (i) the LCDR1 comprises the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:44);
- (ii) the LCDR2 comprises the sequence: KISRLEA (SEQ ID NO:45); and
- (iii) the LCDR3 comprises the sequence: FQGSHFPRT (SEQ ID NO:46); and
- (i) the HCDR1 comprises the sequence: SYTMS;
- (ii) the HCDR2 comprises the sequence: TISSGGGNTYYPDSVKG; and
- (iii) the HCDR3 comprises the sequence: YYRYEAWFAS.

81. The antibody according to claim 79 or 80, wherein the VL chain of the antibody comprises the amino acid sequence or comprises an amino acid sequence at least 90% identical to the amino acid sequence:

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS NRFGS VPDRFSGSGSGTDFTLKISRLEAEDLGVIYCFQGSHFPRTFGGGTRLEIK (SEQ ID NO:25);

and wherein the VH chain of the antibody comprises the amino acid sequence or comprises an amino acid sequence at least 90% identical to the amino acid sequence:

EVILVESGGGLVKPGGSLKLSCAASGFTFSYTMWVRQTPEKRLEWVATISSGGGNTYY PDSVKGRFTISRDNANKNNLYLQMSSLRSEDALYYCARYYYEAWFASWGQGTLVTVSA.

82. The antibody according to any one of claims 73-81, wherein the antibody is a monospecific bivalent antibody that specifically binds to MDR-1.

83. The antibody according to any one of claims 73-81, wherein the antibody is a bi-specific antibody comprising the VL chain as a common light chain.

84. The antibody according to claim 83, wherein the bispecific antibody comprises an MDR-1 binding domain and a tumor associated antigen (TAA) binding domain wherein each of the MDR-1 binding domain and the TAA binding domain comprises the LCDRs 1-3 of the VL chain.

85. The antibody according to claim 84, wherein the TAA is CD47 and wherein the TAA binding domain comprises HCDRs 1-3 of the VH chain having the sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSED TAVYYCARGGYRAMDYWGQGTLVT
VSS.

86. The antibody according to claim 85, wherein the VH chain of the CD47 binding domain comprises the HCDR1 comprising the sequence: NYNMH, the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD, and the HCDR3 comprising the sequence: GGYRAMDY.

87. The antibody according to claim 84, wherein the TAA is PD-L1 and wherein the PD-L1 binding domain comprises HCDRs 1-3 of the VH chain having the sequence: EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAVISPYYGGSTY YADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGTLVTVSS (SEQ ID NO:32).

88. The antibody according to claim 87, wherein the VH chain of the PD-L1 binding domain comprises the HCDR1 comprising the sequence: DSWIH (SEQ ID NO:33), the HCDR2 comprising the sequence: WISPYGGSTYYADSVKG (SEQ ID NO:34), and the HCDR3 comprising the sequence: RHWPGGFDY (SEQ ID NO:35).

89. The antibody according to claim 84, wherein the TAA is EGFR and wherein the EGFR binding domain comprises HCDRs of the VH chain having the sequence: QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYYSGSTD YNPSLKSRVTMSVDTSKNQFSLKVN SVTAADTAVYYCARVSIFGVGTFDYWGQGTLVTVS S (SEQ ID NO:36).

90. The antibody according to claim 89, wherein the VH chain of the EGFR binding domain comprises the HCDR1 comprising the sequence: SGDYYWS (SEQ ID NO:37), the HCDR2 comprising the sequence: YIYYSGSTDYNPSLKS (SEQ ID NO:38), and the HCDR3 comprising the sequence: VSIFGVGTFDY (SEQ ID NO:39).

91. The antibody according to claim 84, wherein the TAA is EGFR and wherein the EGFR binding domain comprises HCDRs of the VH chain having the sequence: QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSSGNTDY NTPFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLTVTVSA (SEQ ID NO:40).

92. The antibody according to claim 91, wherein the VH chain of the EGFR binding domain comprises the HCDR1 comprising the sequence: NYGVH (SEQ ID NO:41), the HCDR2 comprising the sequence: VIWSSGNTDYNTPTFS (SEQ ID NO:42), and the HCDR3 comprising the sequence: ALTYDYEFAY (SEQ ID NO:43).

93. An antibody comprising:
a variable light (VL) chain comprising light chain CDRs (LCDRs) of a VL chain having the sequence:

DVLMTQTPVSLSVSLGDQASISCRSSQSIVHSTGX¹TYLEWYLQKPGQSPKLLIYKIS
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQASHFPRTFGGGGTKLEIK,

wherein X¹ is N, Q or S; or

DVLMTQTPLSLPVSLGDQASISCRSSQSIVHSTGNTYLEWYLQKPGQSPKLLIYKVS
NRFSGVPDRFSGSGSGTDFTLKISRLEAEDLGVYYCFQGSHPFRTFGGGTRLEIK (SEQ ID NO:25); and

a variable heavy (VH) chain comprising heavy chain CDRs (HCDRs) of a VH chain having the sequence:

QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYSNMHWVRQAPGQRLEWMGTIYPG
NDDTSYNQKFKDRVTITADTSASTAYMELSSLRSEDTAVYYCARGGYRAMDYWGQGLTVT
VSS; or

EVQLVESGGGLVQPGGSLRLSCAASGFTFSDSWIHWVRQAPGKGLEWVAVISPY
GGSTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCARRHWPGGFDYWGQGLT
VTVSS (SEQ ID NO:32); or

QVQLQESGPGLVKPSQTLSTCTVSGGSISSGDYYWSWIRQPPGKGLEWIGYIYY
SGSTDYNPSLKSRTMSVDTSKNQFSLKVNSTAAADTAVYYCARVSIFGVGTFDYWGQGLT
VTVSS (SEQ ID NO:36); or

QVQLKQSGPGLVQPSQSLITCTVSGFSLTNYGVHWVRQSPGKGLEWLGVIWSG
GNTDYNTPTFTSRLSINKDNSKSQVFFKMNSLQSNDAIYYCARALTYDYEFAYWGQGLV
TVSA (SEQ ID NO:40).

94. The antibody according to claim 93, wherein the VL chain comprises the (i) the LCDR1 comprises the sequence: RSSQSIVHSTGX¹TYLE (SEQ ID NO:5); (ii) the LCDR2 comprises the sequence: KISNRFS (SEQ ID NO:6); and (iii) the LCDR3 comprises the sequence: FQASHFPRT (SEQ ID NO:7), wherein X¹ is N, Q or S and wherein the VH chain comprises:

- (i) the HCDR1 comprising the sequence: NYNMH, the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD, and the HCDR3 comprising the sequence: GGYRAMDY; or
- (ii) the HCDR1 comprising the sequence: DSWIH (SEQ ID NO:33), the HCDR2 comprising the sequence: WISPYGGSTYYADSVKG (SEQ ID NO:34), and the HCDR3 comprising the sequence: RHWPGGFDY (SEQ ID NO:35); or
- (iii) the HCDR1 comprising the sequence: SGDYYS (SEQ ID NO:37), the HCDR2 comprising the sequence: YIYSGSTDYNPSLKS (SEQ ID NO:38), and the HCDR3 comprising the sequence: VSIFGVGTFDY (SEQ ID NO:39); or
- (iv) the HCDR1 comprising the sequence: NYGVH (SEQ ID NO:41), the HCDR2 comprising the sequence: VIWSGGNTDYNTPTFTS (SEQ ID NO:42), and the HCDR3 comprising the sequence: ALTYDYEFAY (SEQ ID NO:43).

95. The antibody according to claim 93, wherein the VL chain comprises the (i) the LCDR1 comprising the sequence: RSSQSIVHSTGNTYLE (SEQ ID NO:44); (ii) the LCDR2 the the sequence: KISRLEA (SEQ ID NO:45); and (iii) the LCDR3 the sequence: FQGSHPRT (SEQ ID NO:46) and wherein the VH chain comprises:

the HCDR1 comprising the sequence: NYNMH, the HCDR2 comprising the sequence: TIYPGNDDTSYNQKFKD, and the HCDR3 comprising the sequence: GGYRAMDY; or

the HCDR1 comprising the sequence: DSWIH (SEQ ID NO:33), the HCDR2 comprising the sequence: WISPYGGSTYYADSVKG (SEQ ID NO:34), and the HCDR3 comprising the sequence: RHWPGGFDY (SEQ ID NO:35); or

the HCDR1 comprising the sequence: SGDYYS (SEQ ID NO:37), the HCDR2 comprising the sequence: YIYSGSTDYNPSLKS (SEQ ID NO:38), and the HCDR3 comprising the sequence: VSIFGVGTFDY (SEQ ID NO:39); or

the HCDR1 comprising the sequence: NYGVH (SEQ ID NO:41), the HCDR2 comprising the sequence: VIWSSGGNTDYNTPFST (SEQ ID NO:42), and the HCDR3 comprising the sequence: ALTYDYEFAY (SEQ ID NO:43).

96. The antibody according to any one of claims 93-95, wherein the antibody is a bispecific antibody.

97. The antibody according to any one of claims 1-96, wherein the antibody is a humanized antibody or a chimeric antibody comprising human Fc domain.

98. The antibody according to claim 97, comprising immunoglobulin G1 (IgG1) Fc domain.

99. The antibody according to any one of claims 1-98, wherein a first VH chain is fused to a first Fc domain and a second VH chain is fused to a second Fc domain.

100. The antibody according to claim 99, wherein the Fc domains comprise modified CH3 domains that preferentially form heterodimers comprising the first and second VH chains.

101. The antibody according to claim 100, wherein the first and second Fc domains are human immunoglobulin G1 (IgG1) Fc domains.

102. A pharmaceutical composition comprising:
the antibody of any of the preceding claims; and
a pharmaceutically acceptable excipient.

103. The pharmaceutical composition according to claim 102, further comprising at least one additional active agent.

104. The pharmaceutical composition according to claim 103, wherein the at least one additional active agent comprises a chemotherapy agent.

105. The pharmaceutical composition according to claim 104, wherein the chemotherapy agent is a taxol, a vinca alkaloid, or an anthracycline.

106. The pharmaceutical composition according to any of claims 103 to 105, wherein the at least one additional active agent comprises an inhibitor of a multidrug resistance transporter.

107. The pharmaceutical composition according to claim 103, wherein the at least one additional active agent comprises an immunotherapy agent.

108. One or more nucleic acids comprising one or more sequences encoding the antibody according to any of the preceding claims.

109. The one or more nucleic acid according to claim 108, wherein the one or more sequences are operably linked to a promoter.

110. One or more recombinant expression vectors comprising the one or more nucleic acids according to claim 108 or 109.

111. A mammalian cell genetically modified with the one or more recombinant expression vectors according to claim 110.

112. The cell according to claim 111, wherein the cell is an immune cell.

113. A kit comprising:
the antibody or a nucleic acid encoding the antibody, according to any of claims 1 to 63 and 73-101; and
at least one additional active agent.

114. The kit according to claim 113, wherein the at least one additional active agent comprises a chemotherapy agent, an inhibitor of a multidrug resistance transporter, an immunotherapy agent, or a combination thereof.

115. A method of killing a cancer cell, the method comprising contacting the cancer cell with the antibody according to any of claims 1 to 63 and 73-101.

116. The method according to claim 115, comprising administering at least one additional active agent.

117. The method according to claim 116, wherein the at least one additional active agent comprises a chemotherapy agent.

118. The method according to claim 116 or 117, wherein the method increases the killing of the cancer cell by at least 5% as compared to contacting with the at least one additional active agent alone.

119. The method according to any of claims 115 to 118, wherein the cancer cell is a drug resistant cancer cell.

120. A method of treating a subject for a cancer, the method comprising administering to the subject the antibody according to any of claims 1 to 63 and 73-101 or the pharmaceutical composition according to any of claims 102-107.

121. The method according to claim 120, wherein the subject has been treated previously for the cancer.

122. The method according to claim 120 or 121, wherein the cancer is drug resistant or multidrug resistant.

123. The method according to claim 122, wherein the cancer is resistant to a chemotherapeutic agent.

124. The method according to claim 122, wherein the cancer is resistant to an immunotherapy agent.

125. The method according to any of claims 120 to 122, wherein the cancer is resistant to an inhibitor of a multidrug resistance transporter.

126. The method according to any of claims 120 to 125, further comprising administering at least one additional active agent to the subject.

127. The method according to claim 126, wherein the at least one additional active agent comprises a chemotherapy agent.

128. The method according to claim 127, wherein the chemotherapy agent is a taxol, a vinca alkaloid, or an anthracycline.

129. The method according to any of claims 126 to 128, wherein the at least one additional active agent comprises an inhibitor of a multidrug resistance transporter.

130. The method according to any of claims 126 to 128, wherein the at least one additional active agent comprises an immunotherapy agent.

131. The method according to any of claims 126 to 128, wherein the method increases the effectiveness of the at least one additional active agent as compared to treatment with the at least one additional active agent alone.

132. The method according to claim 131, wherein the increased effectiveness comprises an at least 5% increase in cancer cell killing.

133. The method according to any of claims 120 to 132, further comprising analyzing a sample of the cancer to determine whether the cancer expresses MDR1 above a predetermined threshold, a tumor associated antigen (TAA) above a predetermined threshold, or both, wherein optionally the TAA comprises CD47.

134. The method according to claim 133, wherein the predetermined threshold corresponds to a level of MDR1 and/or TAA expressed by a reference cell.

135. The method according to claim 134, wherein MDR1 and/or TAA has been knocked-out of or knocked-down in the reference cell.

136. The method according to claim 134 or 135, wherein the reference cell is a non-cancerous cell.

137. The method according to claim 134, wherein the non-cancerous cell expresses a normal level of MDR1 and/or TAA.

138. The method according to any of claims 120 to 137, wherein if the cancer expresses MDR1 and TAA at or above the predetermined thresholds then the subject is administered the multi-specific antibody, and if the cancer expresses MDR1 or TAA below the predetermined thresholds then the subject is treated with a conventional therapy without administering the multi-specific antibody.

139. A method of generating a multi-specific antibody that specifically binds a cell expressing both multidrug resistance protein 1 (MDR1) and a tumor associated antigen (TAA), wherein optionally the TAA comprises CD47, PD-L1, or EGFR, the method comprising:

producing a multi-specific antibody comprising a MDR1-binding domain and a TAA-binding domain;

contacting a first cell expressing MDR1 and TAA with the multi-specific antibody;

contacting a second cell expressing either MDR1 or TAA with the multi-specific antibody;

comparing the binding of the multi-specific antibody to the first cell with the binding of the multi-specific antibody to the second cell to determine a binding-specificity ratio; and

identifying the multi-specific antibody as specific for the cell expressing both MDR1 and TAA when the ratio is above a predetermined threshold.

140. The method according to claim 139, wherein the predetermined threshold is greater than 2:1.

141. The method according to claim 139 or 140, wherein the second cell expresses MDR1 and not the TAA and the method further comprises contacting a third cell expressing the TAA but not MDR1 with the multi-specific antibody.

142. The method according to any of claims 139 or 140, wherein the method further comprises contacting the first cell, the second cell, and/or the third cell with a control antibody selected from: a monospecific anti-MDR1 antibody and a monospecific anti-TAA antibody.

143. A genetically modified human cell line, the cell line expressing a tumor associate antigen (TAA), wherein optionally the TAA comprises leukocyte surface antigen CD47 and comprising an exogenous nucleic acid comprising a sequence encoding a multidrug resistance protein 1 (MDR1) for overexpression of MDR1.

144. The cell line according to claim 143, wherein the cell line is a kidney cell line.

145. The cell line according to claim 144, wherein the cell line is a HEK 293T cell line.

146. A method of making the cell line according to any of claims 143 to 145, the method comprising contacting a human cell that expresses the TAA with the exogenous nucleic acid under conditions sufficient to introduce the exogenous nucleic acid into the cell to produce a genetically modified human cell expressing the TAA and stably overexpressing MDR1; and

culturing the genetically modified human cell under conditions sufficient to produce a genetically modified human cell line expressing the TAA and stably overexpressing MDR1.

FIG. 1A

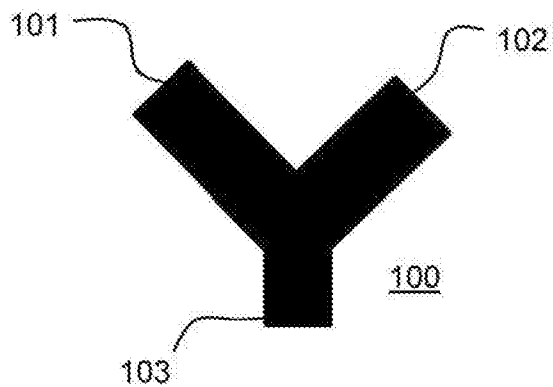


FIG. 1B

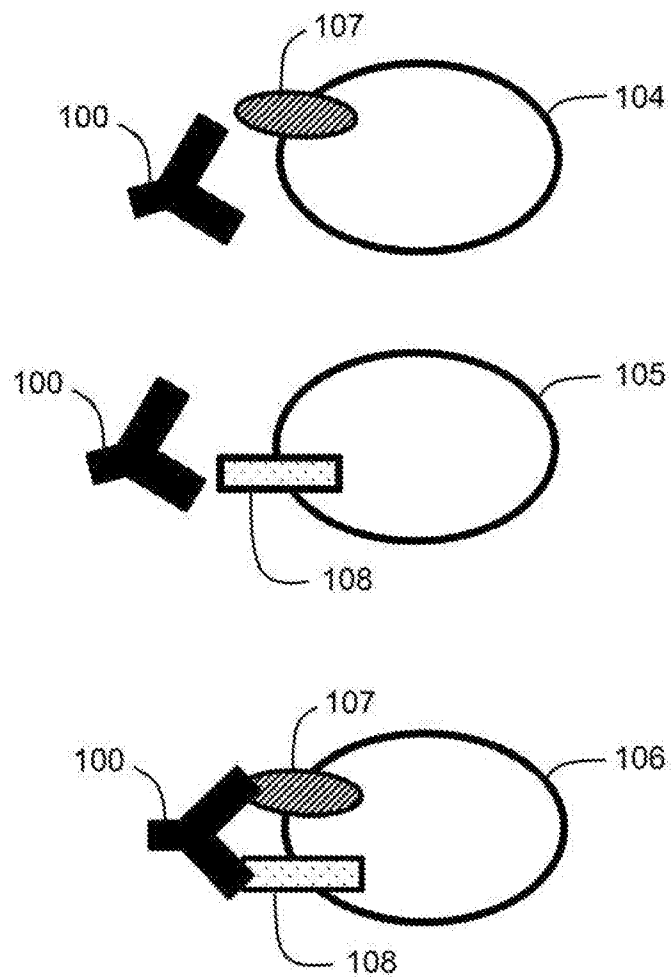


FIG. 2

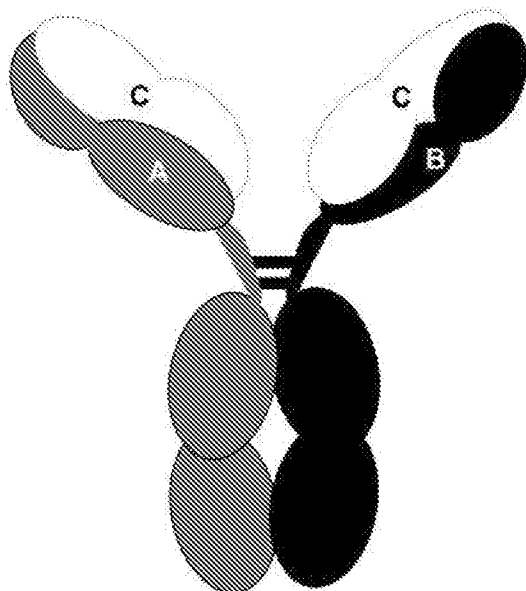


FIG. 3A

Cells: 293T naïve cells

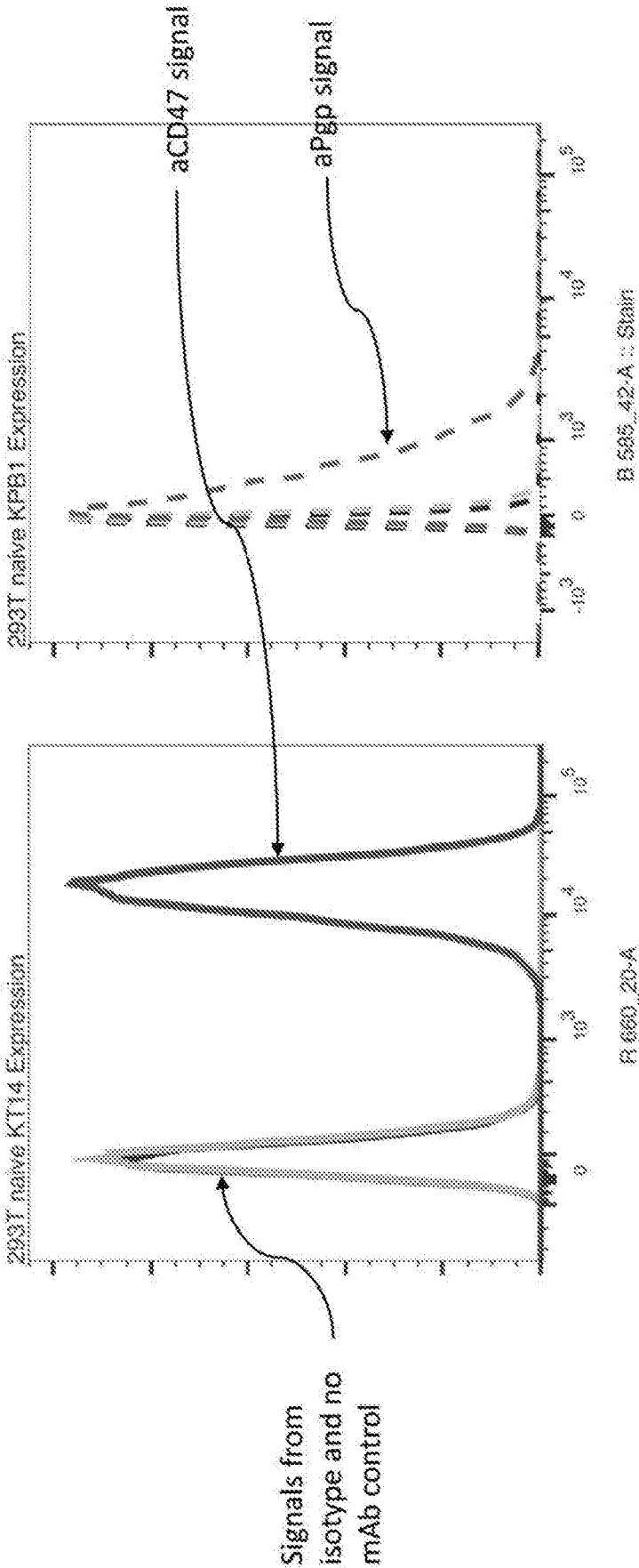


FIG. 3B

Cells: 293T CD47-KO
(Knock-out)

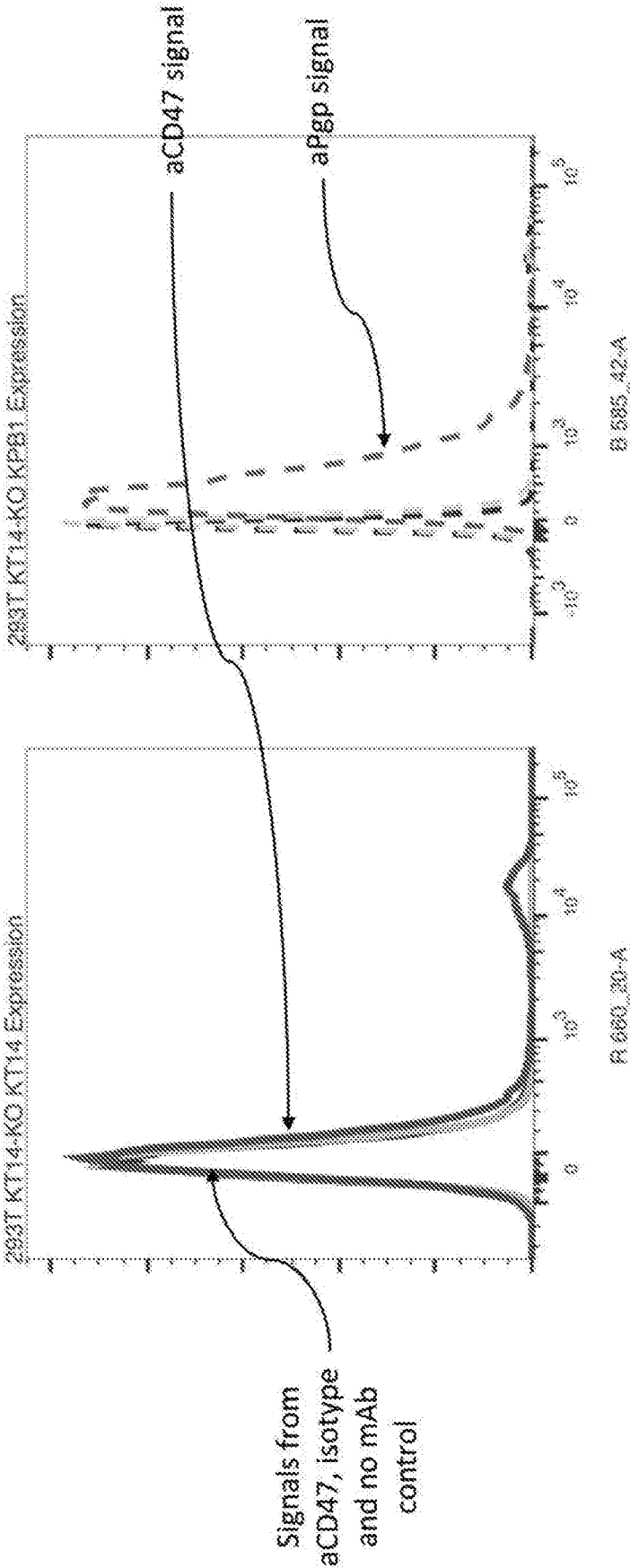


FIG. 3C

Cells: 293T Pgp-KD
(Knock-down)

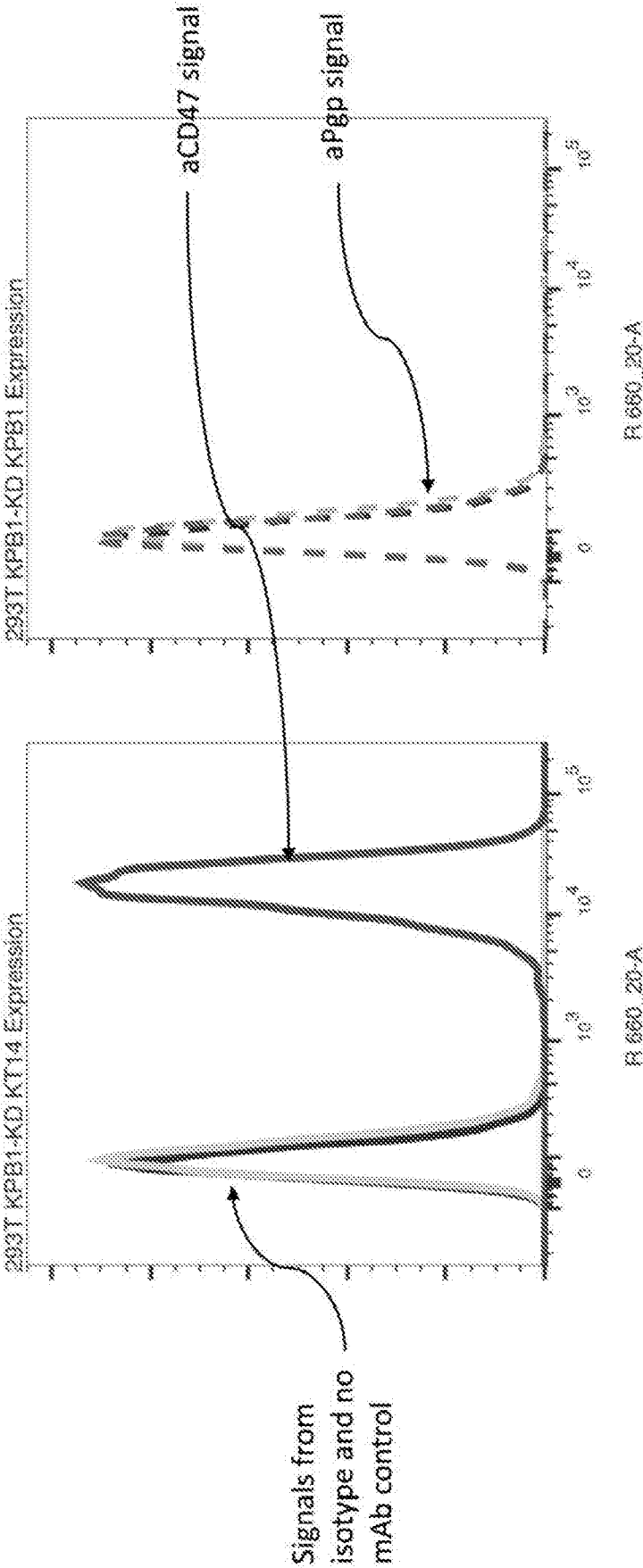


FIG. 3D

Cells: 293T Pgp-ox
(‘over-expressors’)

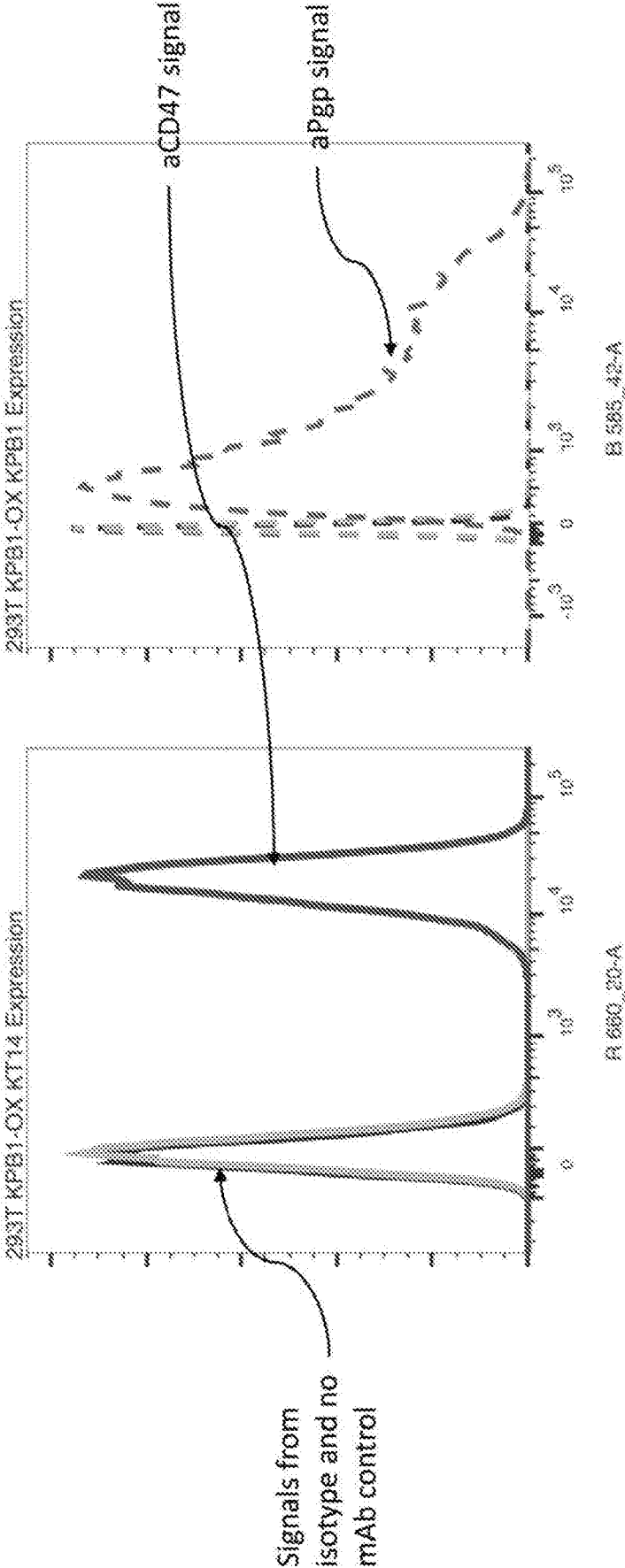


FIG. 3E

Cells: N6ADR (Adriamycin resistant)

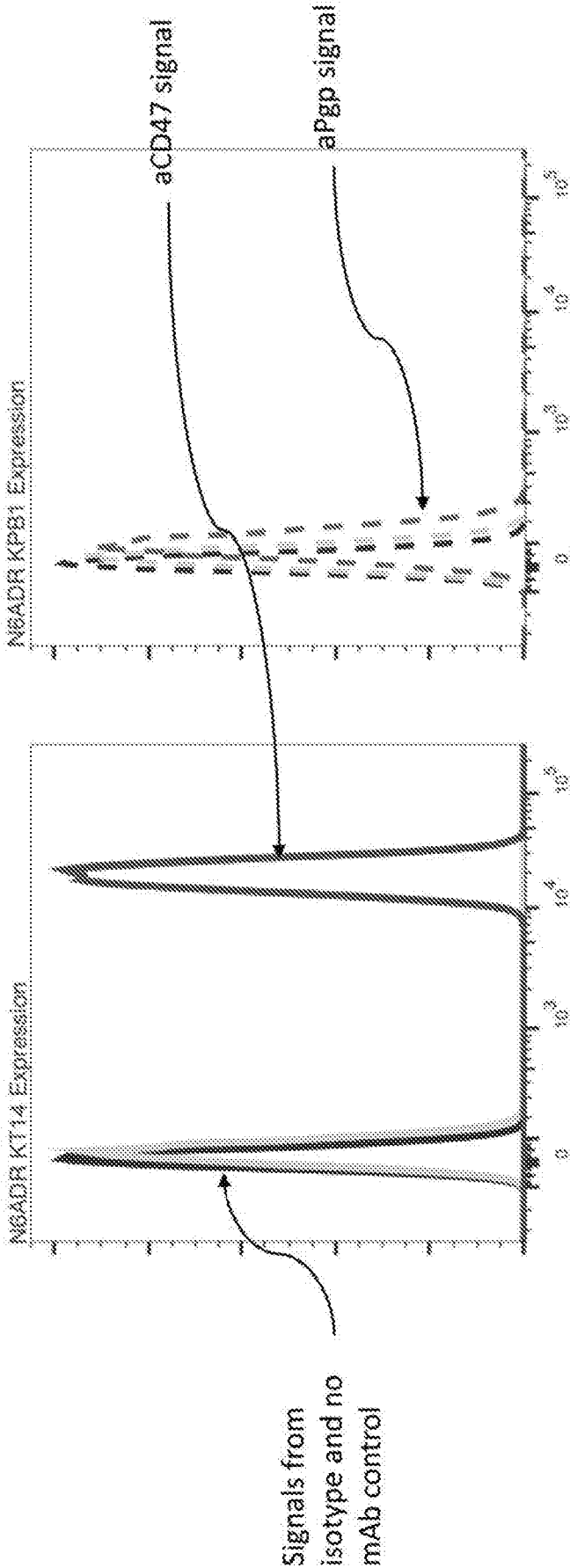


FIG. 3F

Cells: 293T CD47-KO+Pgpg-ox

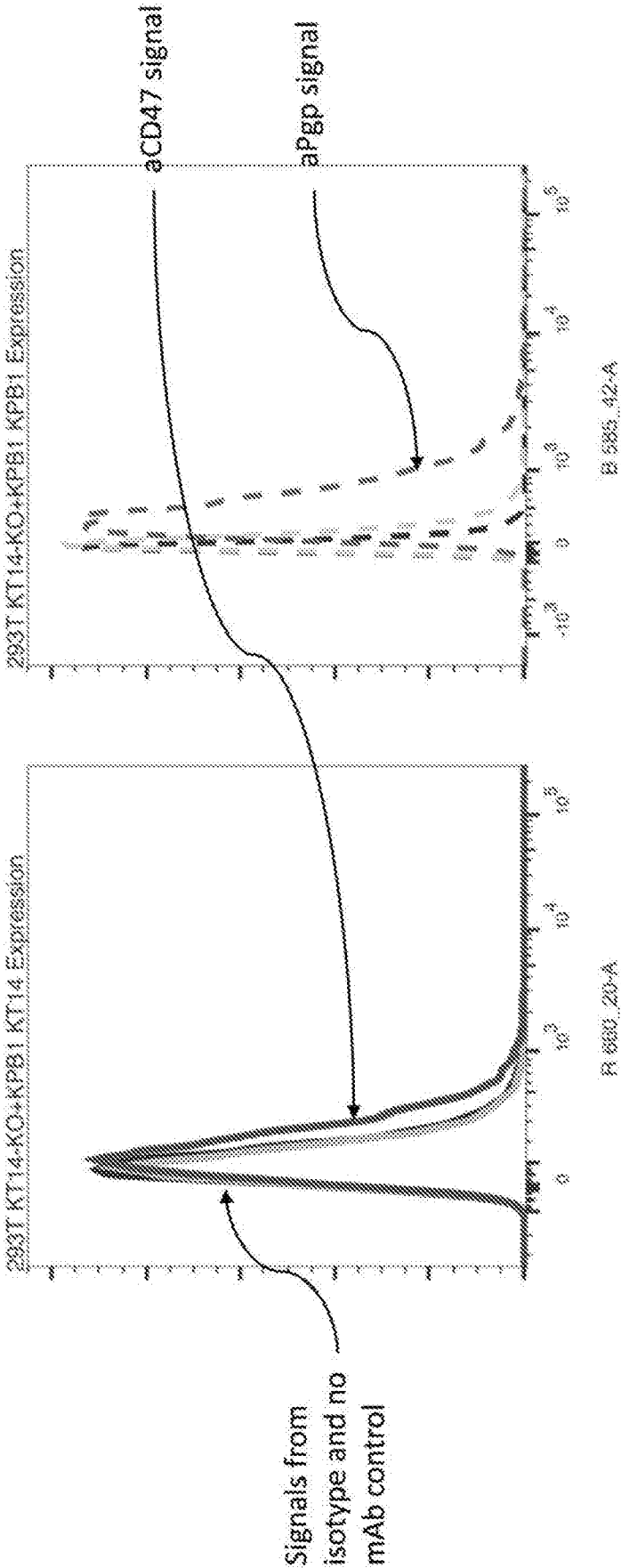


FIG. 4A

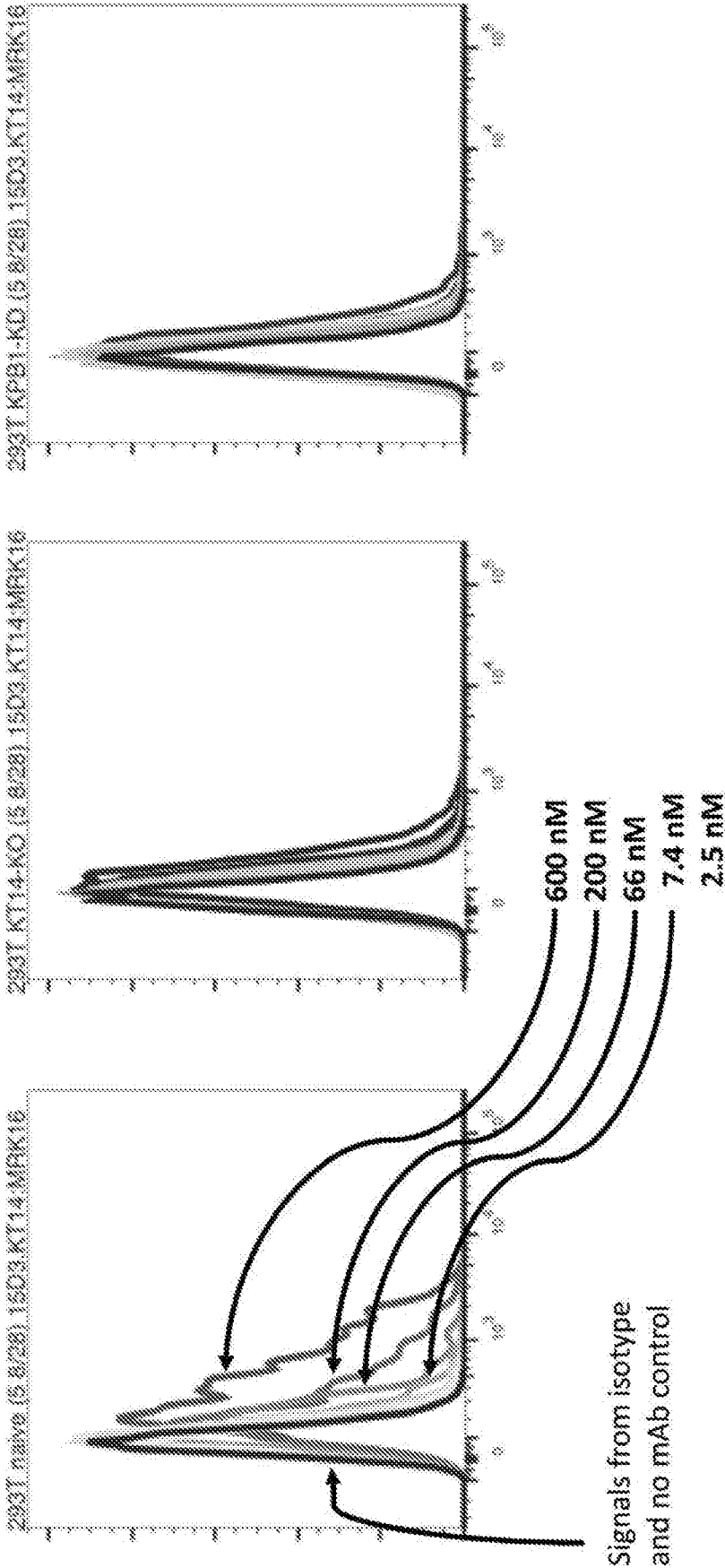


FIG. 4B

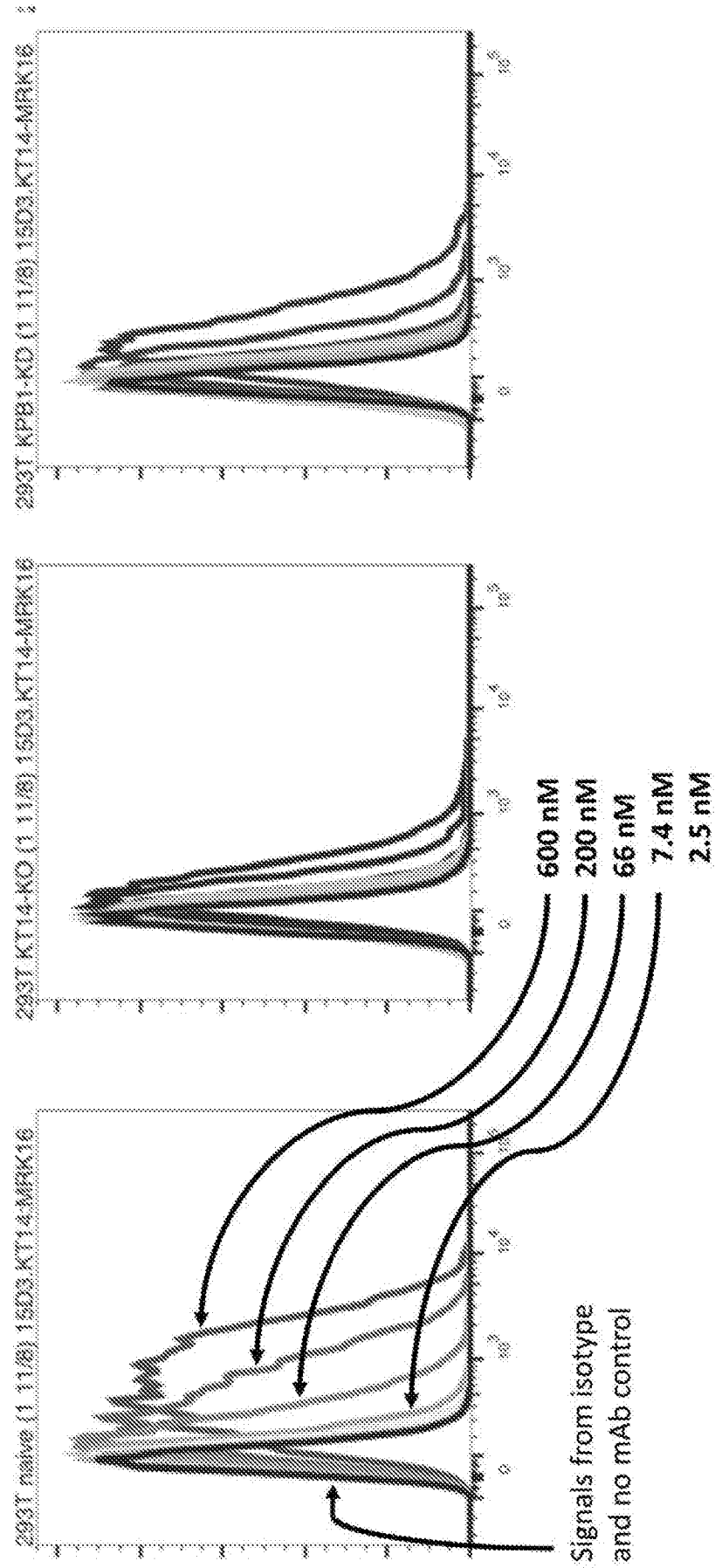


FIG. 5

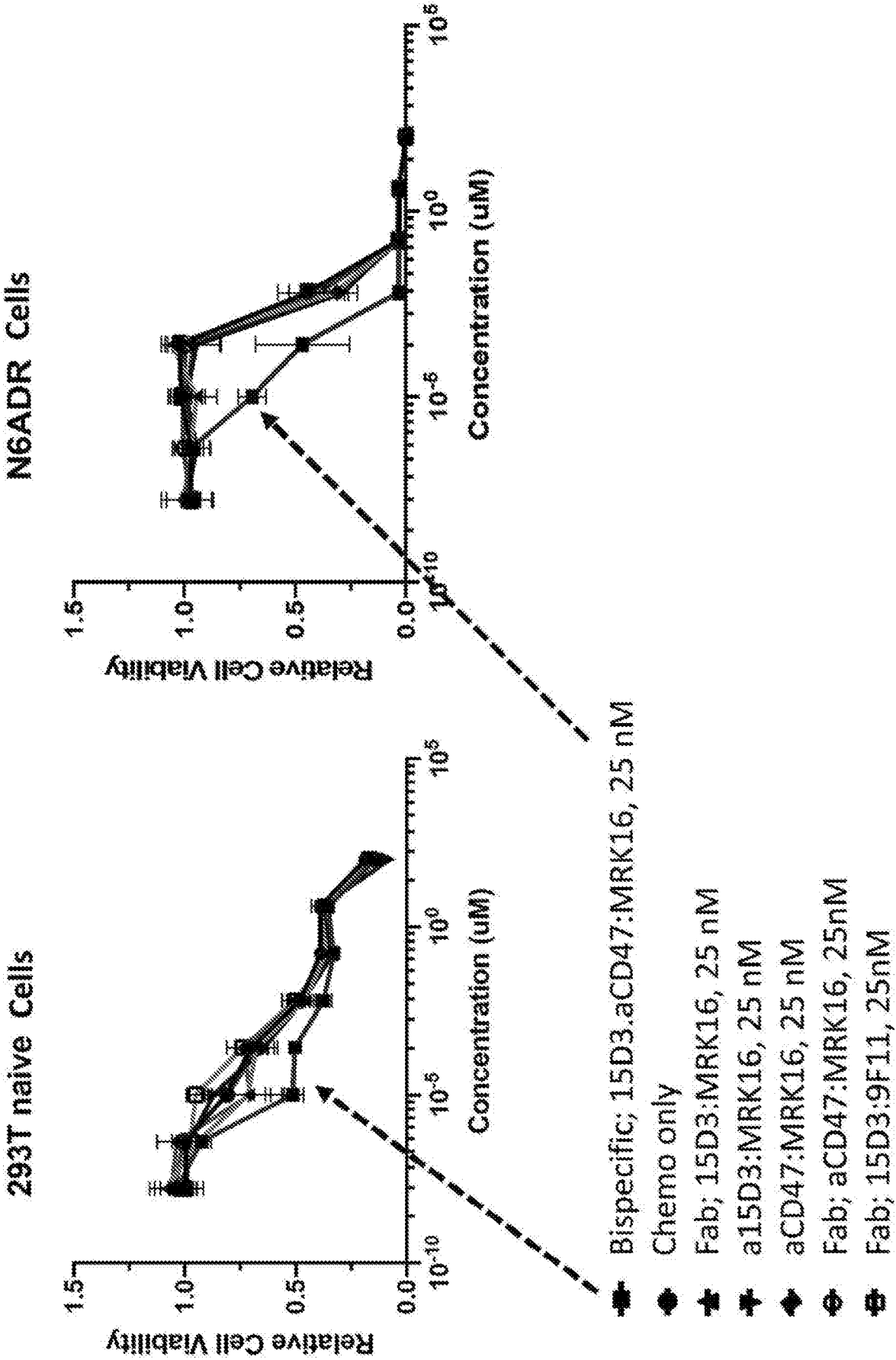


FIG. 6A

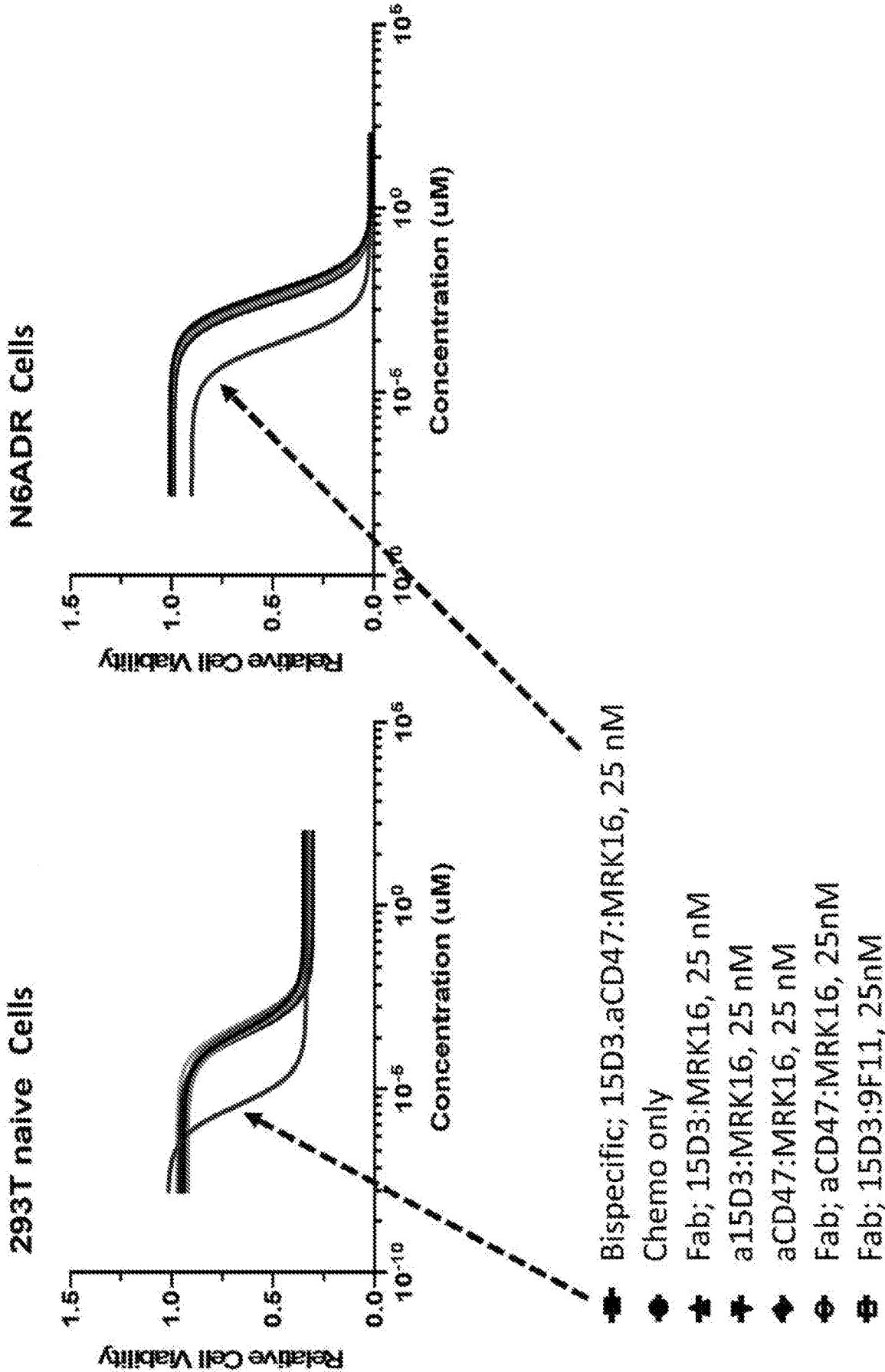


FIG. 6B

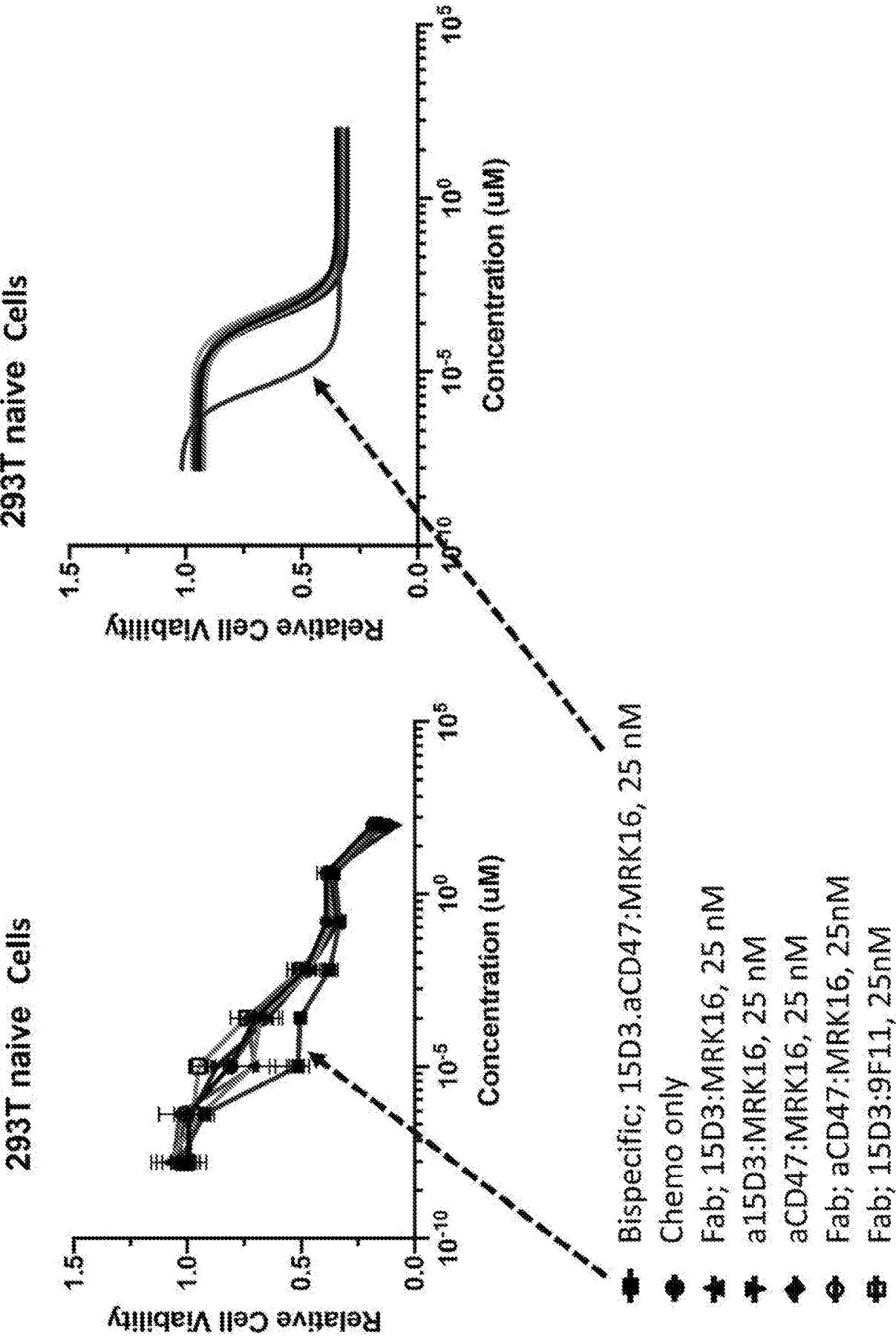


FIG. 6C

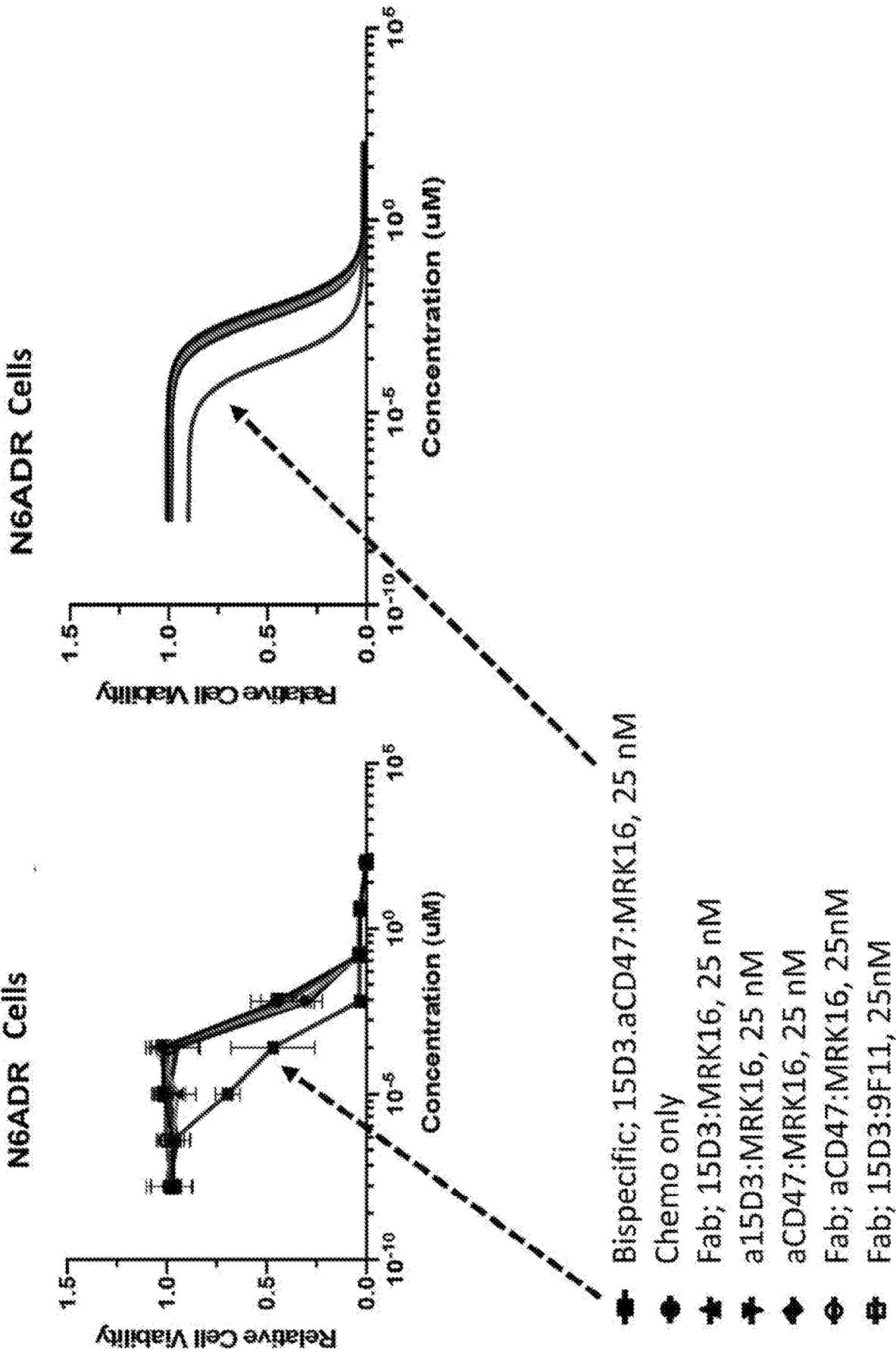


FIG. 7

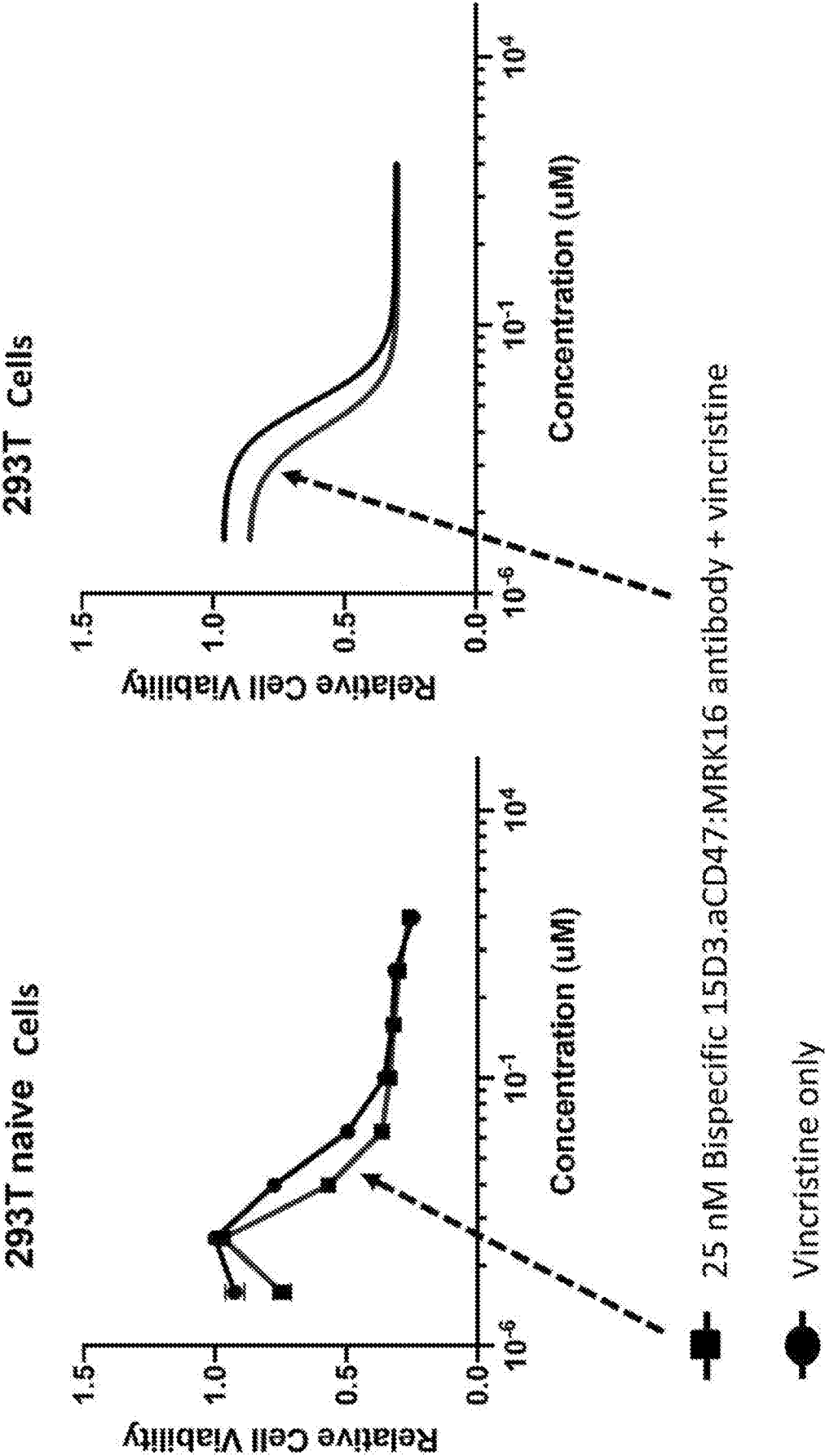


FIG. 8

Ab	Kd (nM)	Bmax	Killing	Binding (293)	Binding (293 KO CD47)	Binding (293 KO CD47, OX ABCB1)	Binding (293 OX ABCB1)
15D3 IgG1 DD/5F9 IgG1 KK/MRK16	144.8	1.327	++	+	-	++	+
15D3 IgG1 DD/5F9 IgG1 KK/UIC2	655.4	1.211	-	-	-	-	-
15D3 IgG1 KK/5F9 IgG1 DD/9F11	74.88	1.204	-	-	-	+	+
15D3 IgG1 KK/5F9 IgG1 DD/MRK16	200	1.097	+	+	-	++	+
15D3 IgG1 KK/5F9 IgG1 DD/UIC2	1619	1.969	-	-	-	-	-
MM4 17 2 IgG1 DD/5F9 IgG1 KK/9F11	44.64	1.347	±	+	-	++	+
MM4 17 2 IgG1 DD/5F9 IgG1 KK/MRK16	75.21	1.109	±	±	-	++	+
MRK16 IgG1 KK/5F9 IgG1 DD/MRK16	205.7	1.307	+	+	-	++	+
MRK16 IgG1 KK/5F9 IgG1 DD/UIC2	868.7	1.289	-	-	-	-	-
UIC2 IgG1 KK/5F9 IgG1 DD/9F11	6.261	1.119	-	++	+	+	±
UIC2 IgG1 KK/5F9 IgG1 DD/MRK16	53.26	1.099	-	±	-	-	-
UIC2 IgG1 KK/5F9 IgG1 DD/UIC2	494.5	1.049	-	±	-	-	+

FIG. 9

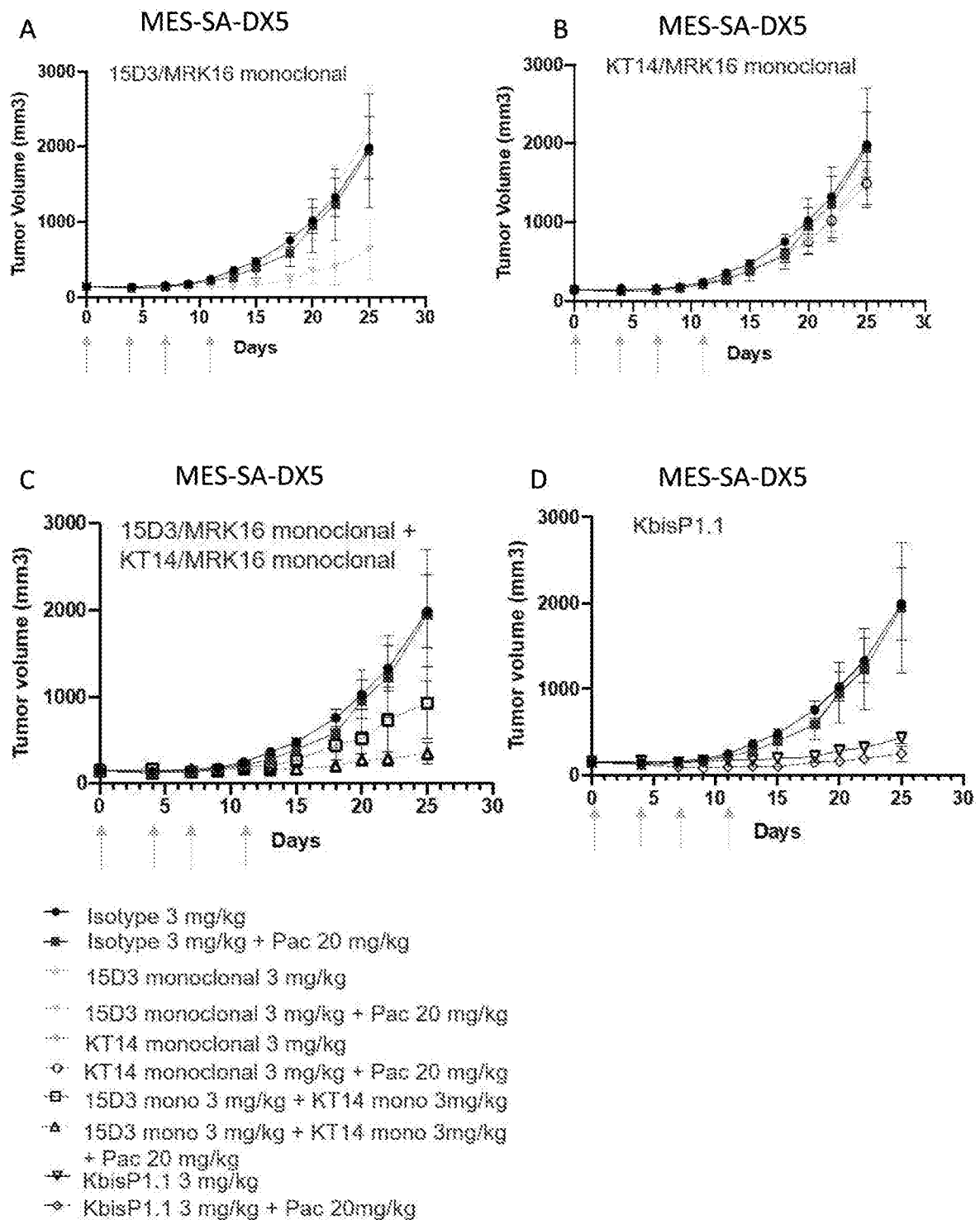


FIG. 10

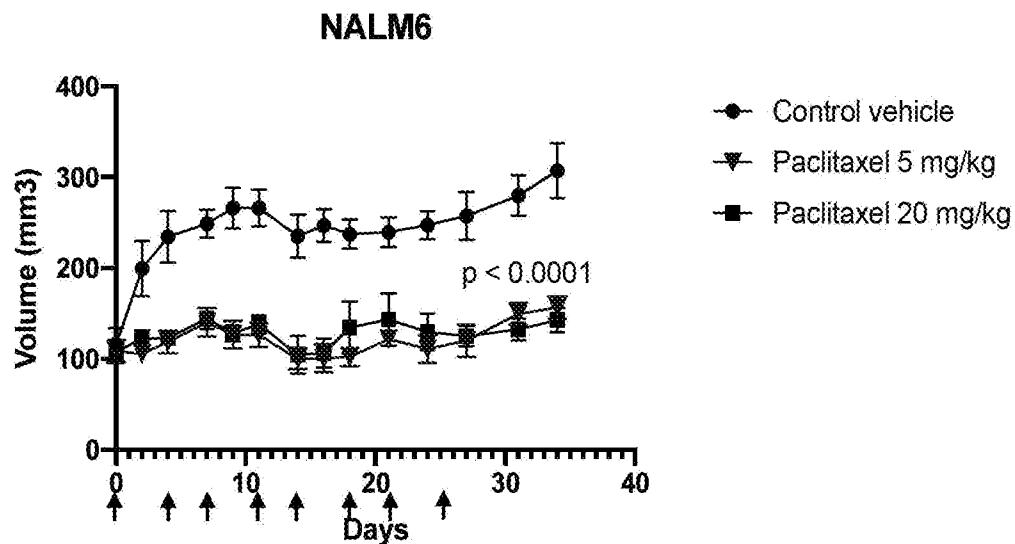


FIG. 11

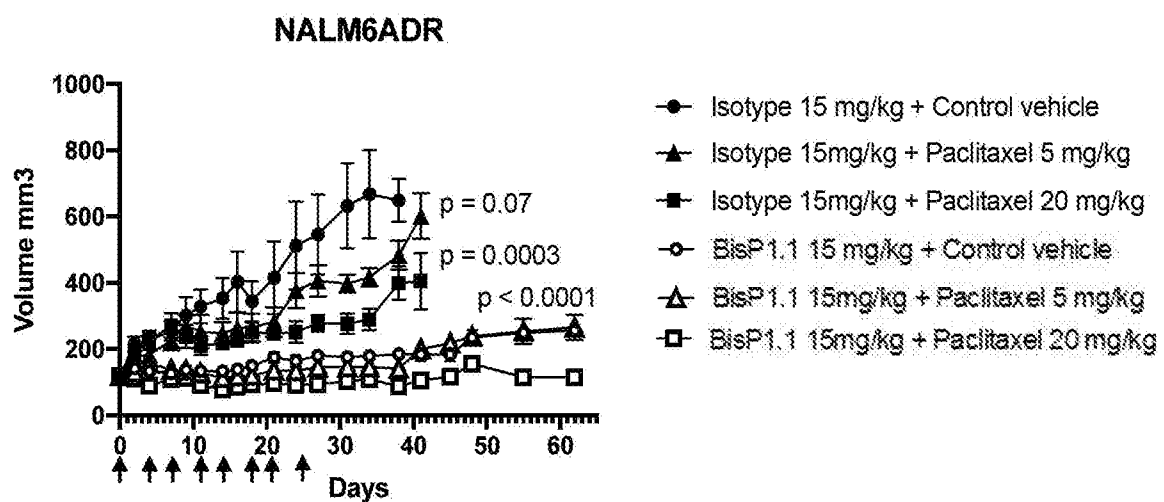


FIG. 12

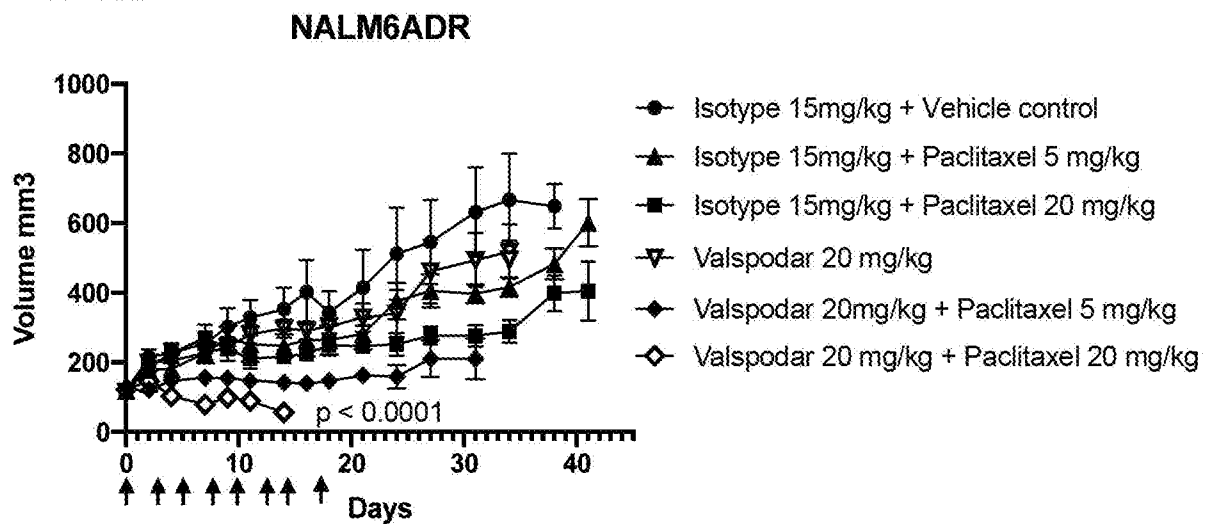


FIG. 13

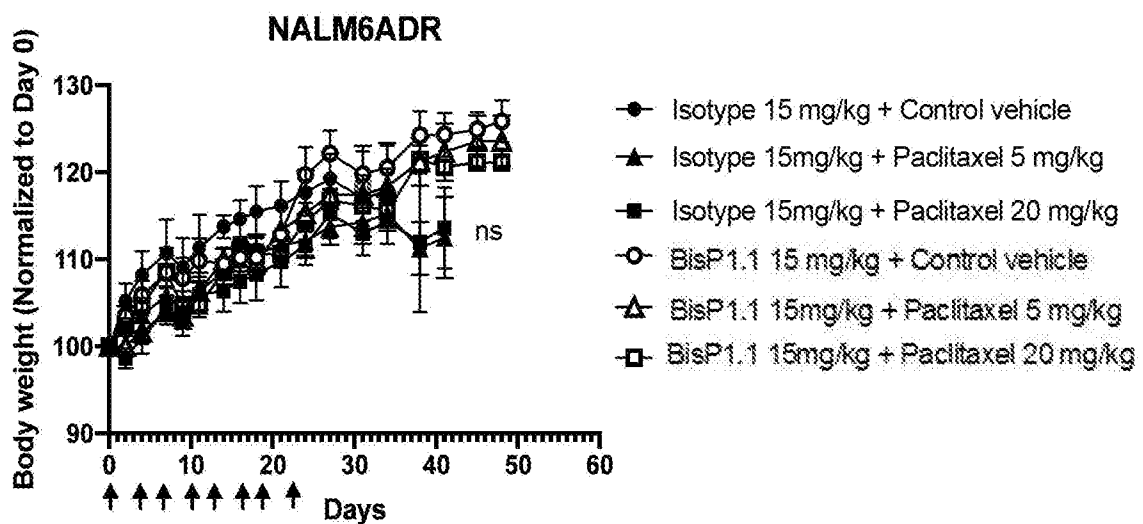


FIG. 14

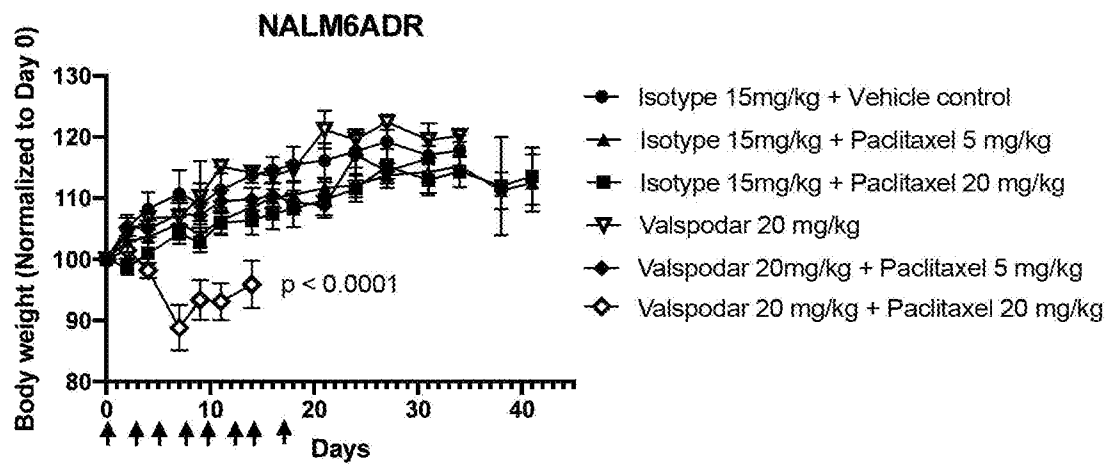


FIG. 15

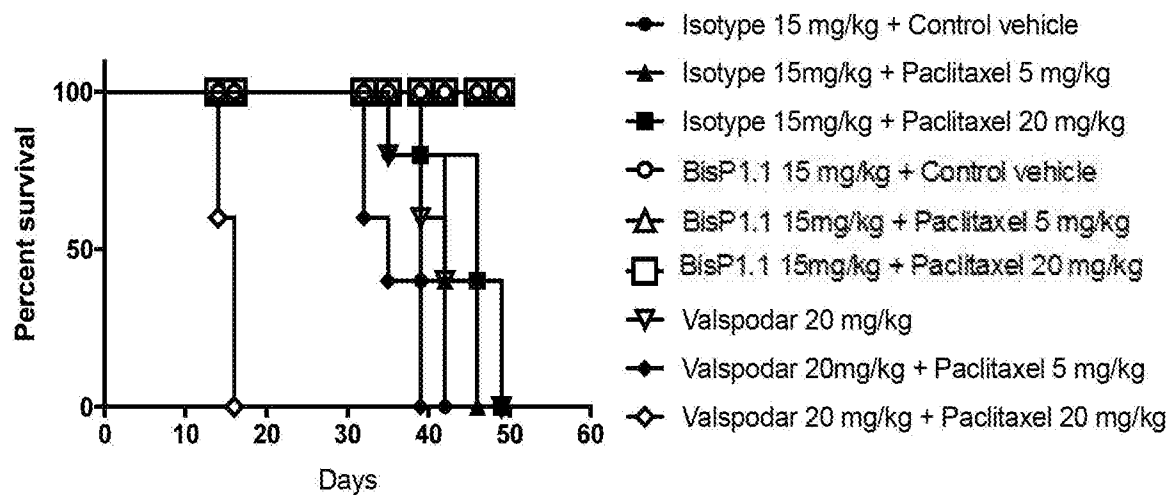


FIG. 16
A2780ADR

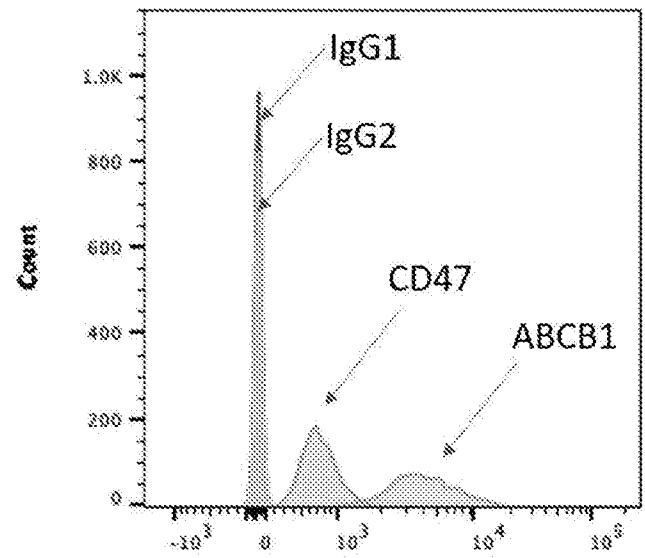
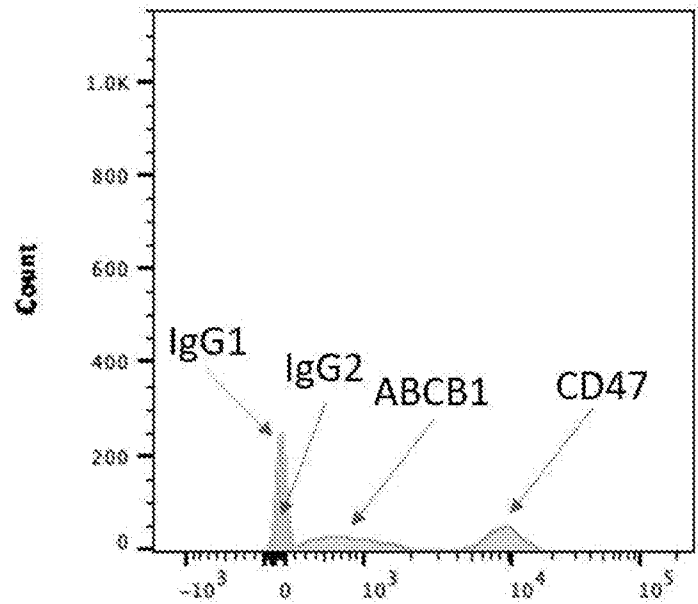


FIG. 17
HeyT30



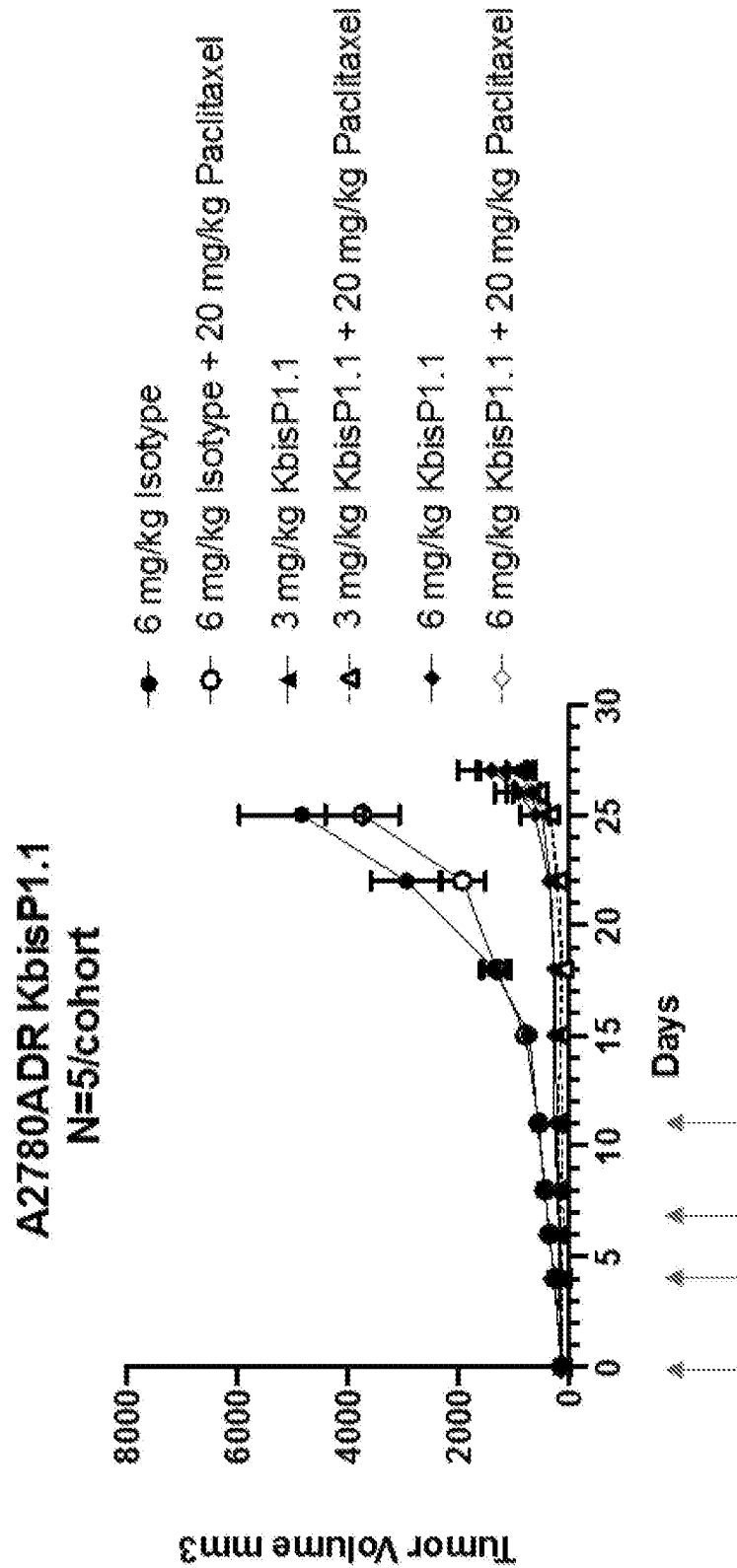


FIG. 18

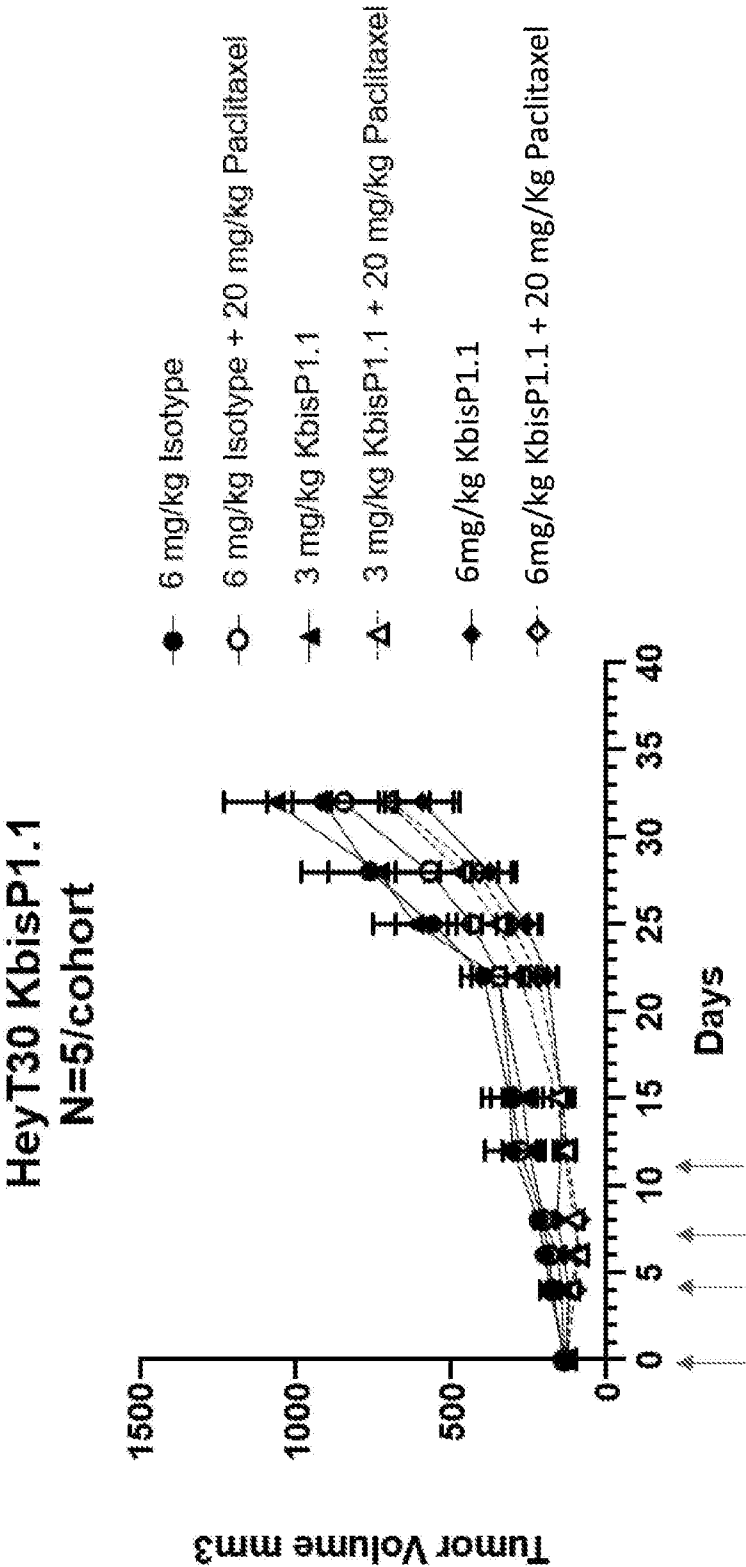


FIG. 19

FIG. 20

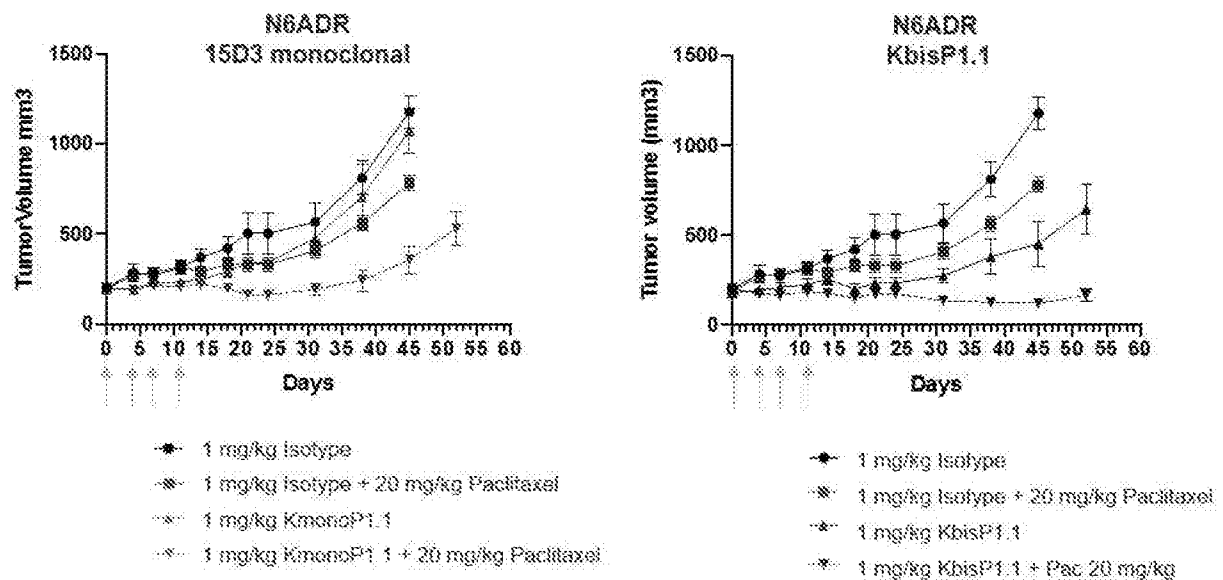
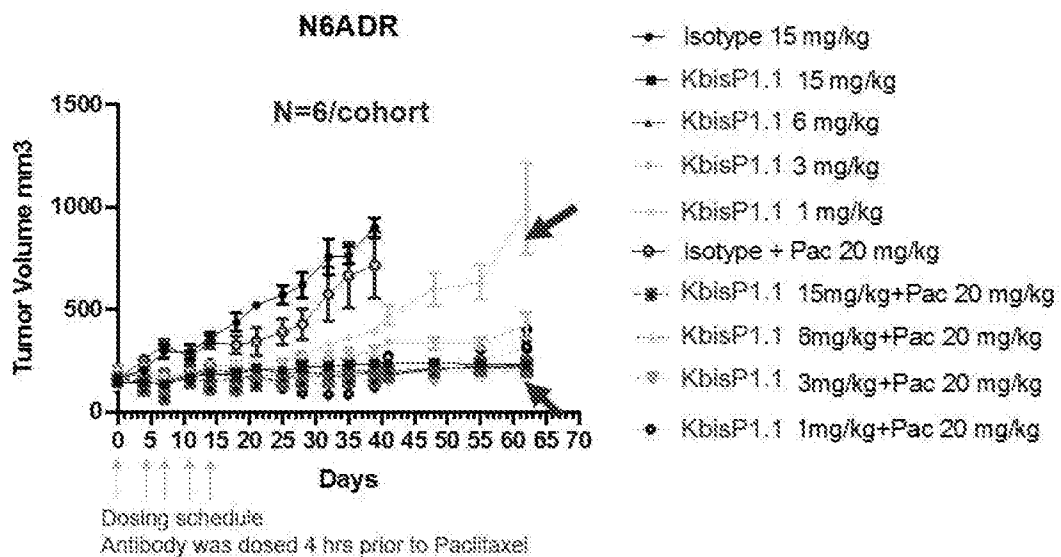


FIG. 21



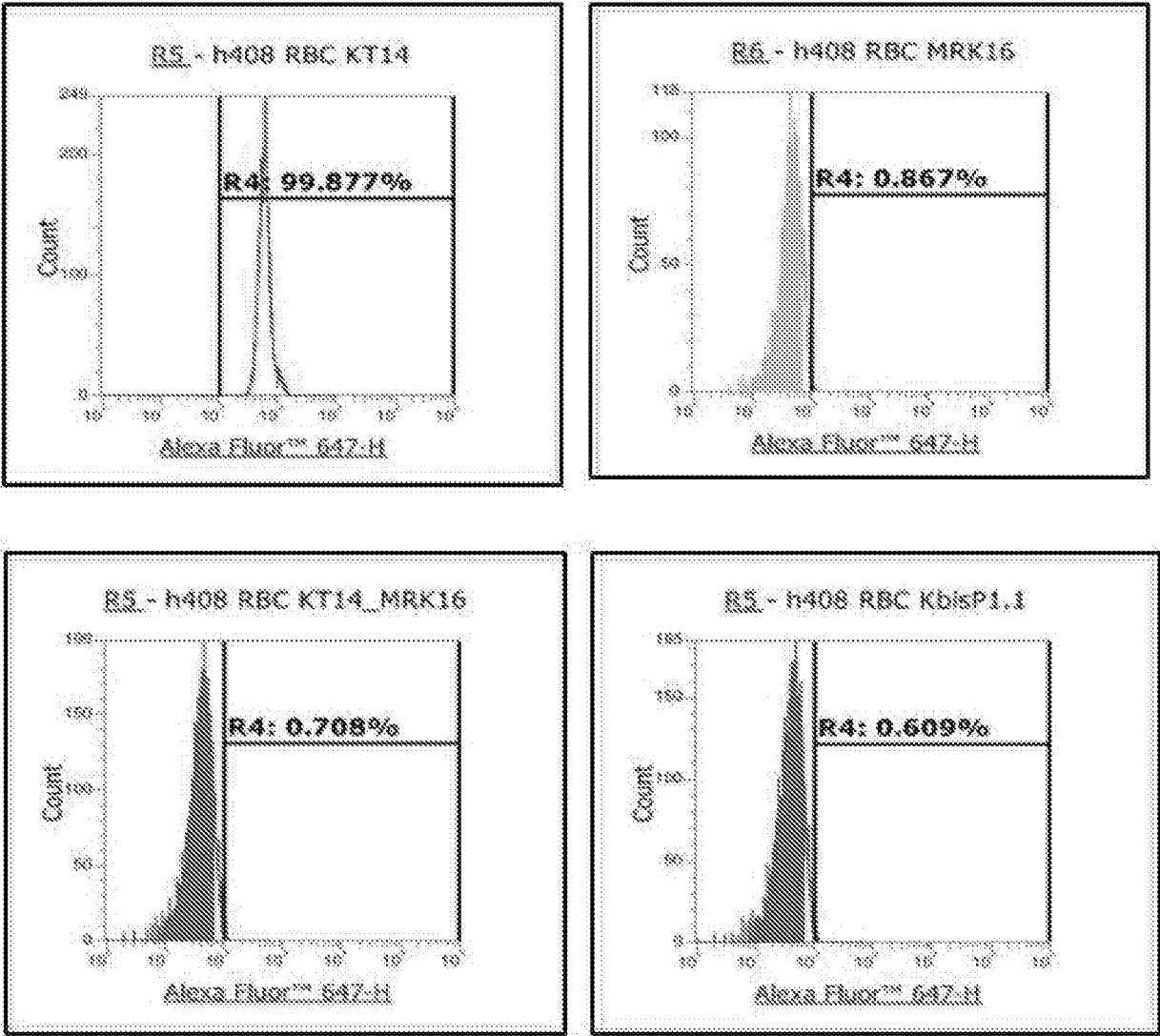


FIG. 22A

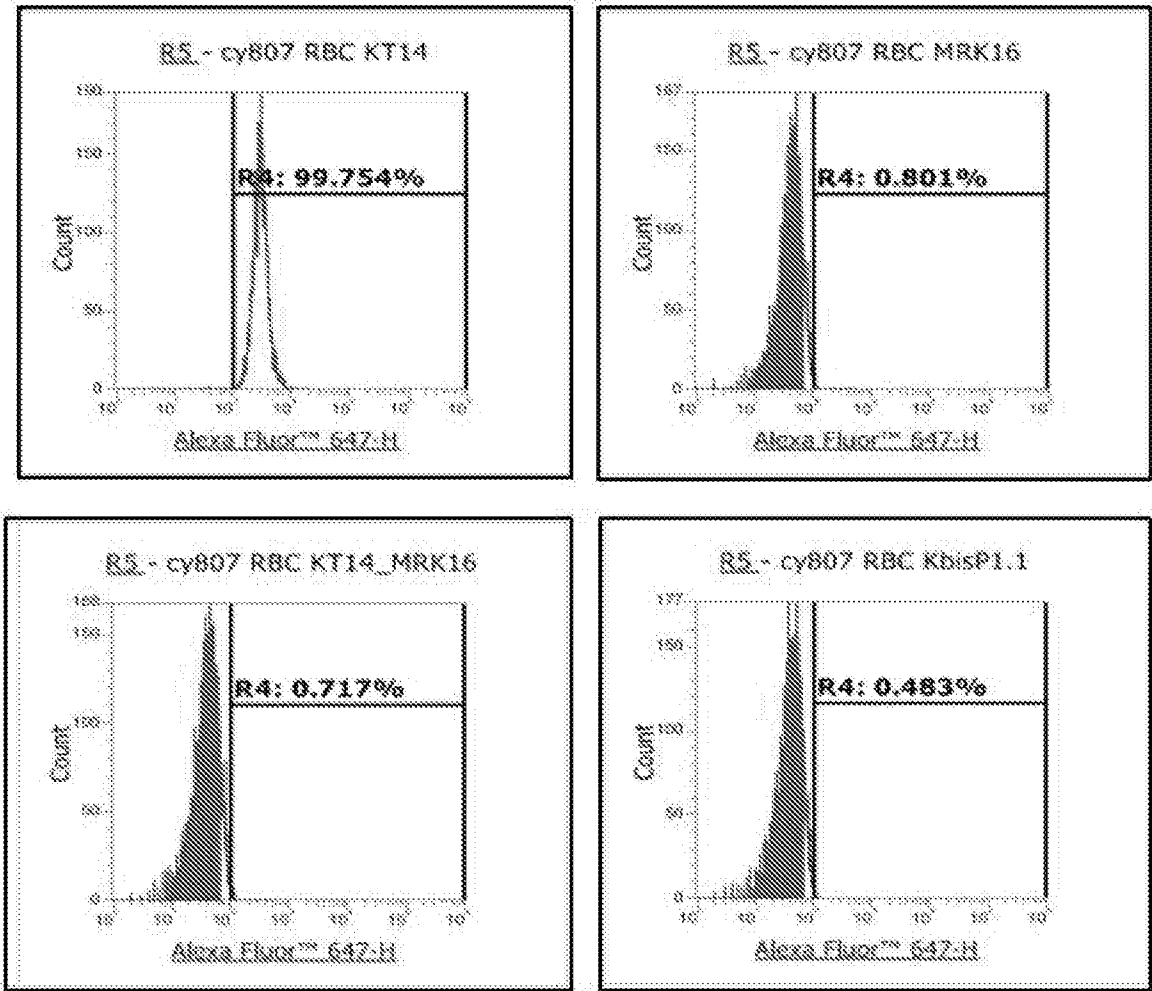


FIG. 22B

```
m15D3      evkvvesggvlvrpggslklascaasgftfsrytmswvrqtpekrlewatissgggntyy 60
15D3H20     evqlvesggvvvqpggslrlascaasgftfsrytmswvrqapgkglewatissgggntyy 60
15D3H21     evqlvesggvvvqpggslrlascaasgftfsrytmswvrqapgkglewatissgggntyy 60
15D3H22     evqlvesggvvvqpggslrlascaasgftfsrytmswvrqapgkglewatissgggntyy 60
            **:*****;*****;*****;* * *****;***
m15D3      pdsvkgrftvsrdnamsslylqmsslrstedtalyycaryagadawfaywgqgtlvtvss 119
15D3H20     pdsvkgrftvsrdnsknslylqmnslrtdetalyycaryagadawfaywgqgtlvtvss 119
15D3H21     pdsvkgrftvsrdnsknslylqmnslrtdetalyycaryagadawfaywgqgtlvtvss 119
15D3H22     pdsvkgrftvsrdnsknslylqmnslrtdetalyycaryagadawfaywgqgtlvtvss 119
            *****; .*****;*****;*****;*****;*****;*****
```

FIG. 23

MRK16m	dvlmtqtpvslsvslgdqasiscrssqsihstgntylewylqkpgqspklliyklsnrf	60
MRK16Hm20	divmtqtpvslsvslgdqasiscrssqsihstgntylewylqkpgqspklliyklsnrf	60
MRK16Hm21	divmtqtpvslsvslgdqasiscrssqsihstgntylewylqkpgqspklliyklsnrf	60
MRK16Hm22	divmtqtpvslsvslgdqasiscrssqsihstgntylewylqkpgqspklliyklsnrf	60
	*;***** *;***** *;***** *;***** *;*****	
MRK16m	sgvpdrfsgsgagtdftlkisrveaedlgvyyycfqashfprtfgggtkleikr	113
MRK16Hm20	sgvpdrfsgsgagtdftlkisrveaedlgvyyycfqashfprtfgggtkleikr	113
MRK16Hm21	sgvpdrfsgsgagtdftlkisrveaedlgvyyycfqashfprtfgggtkleikr	113
MRK16Hm22	sgvpdrfsgsgagtdftlkisrveaedlgvyyycfqashfprtfgggtkleikr	113
	*****;*****;*****;*****;***** ***** *****	

FIG. 24

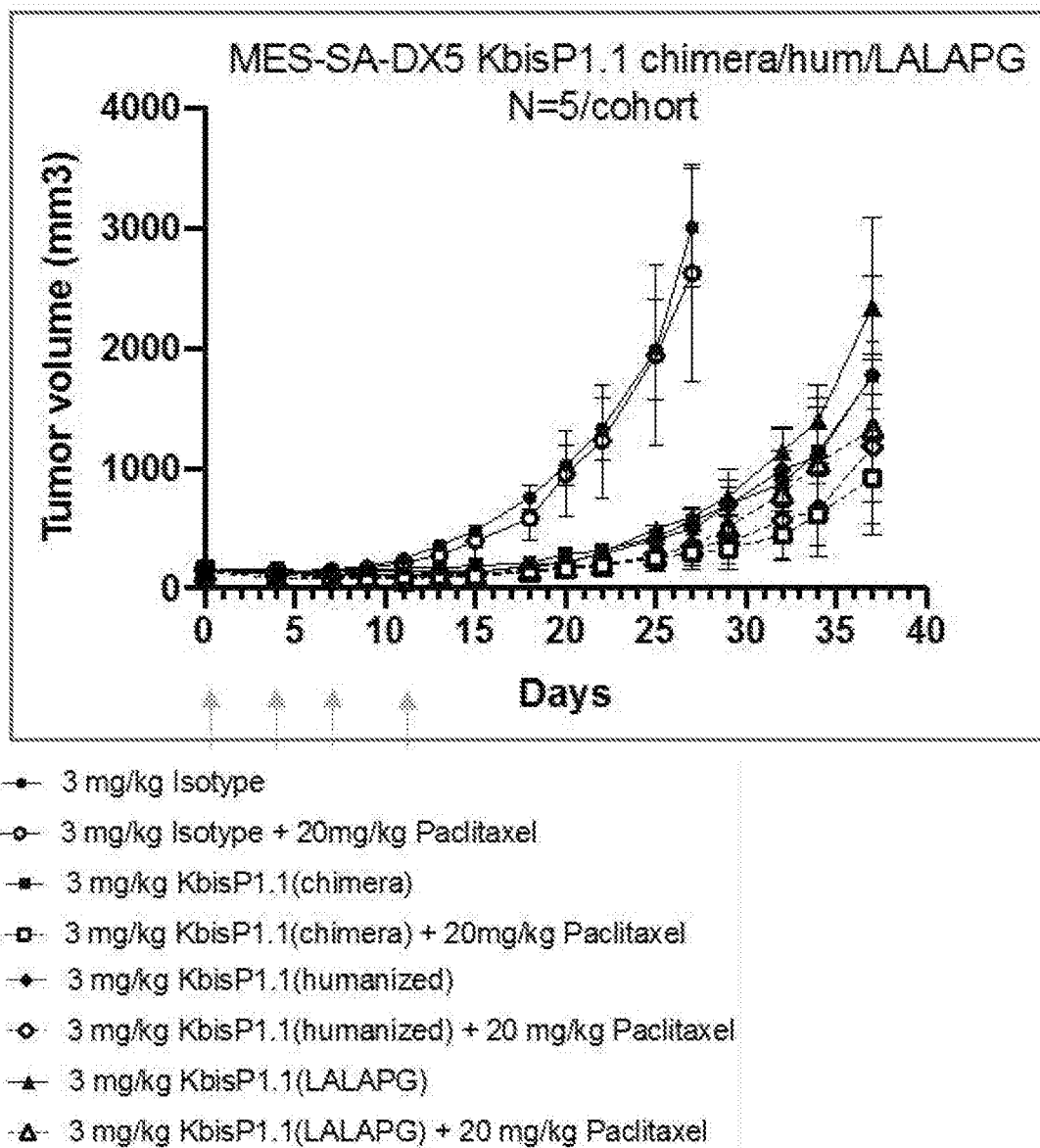


FIG. 25

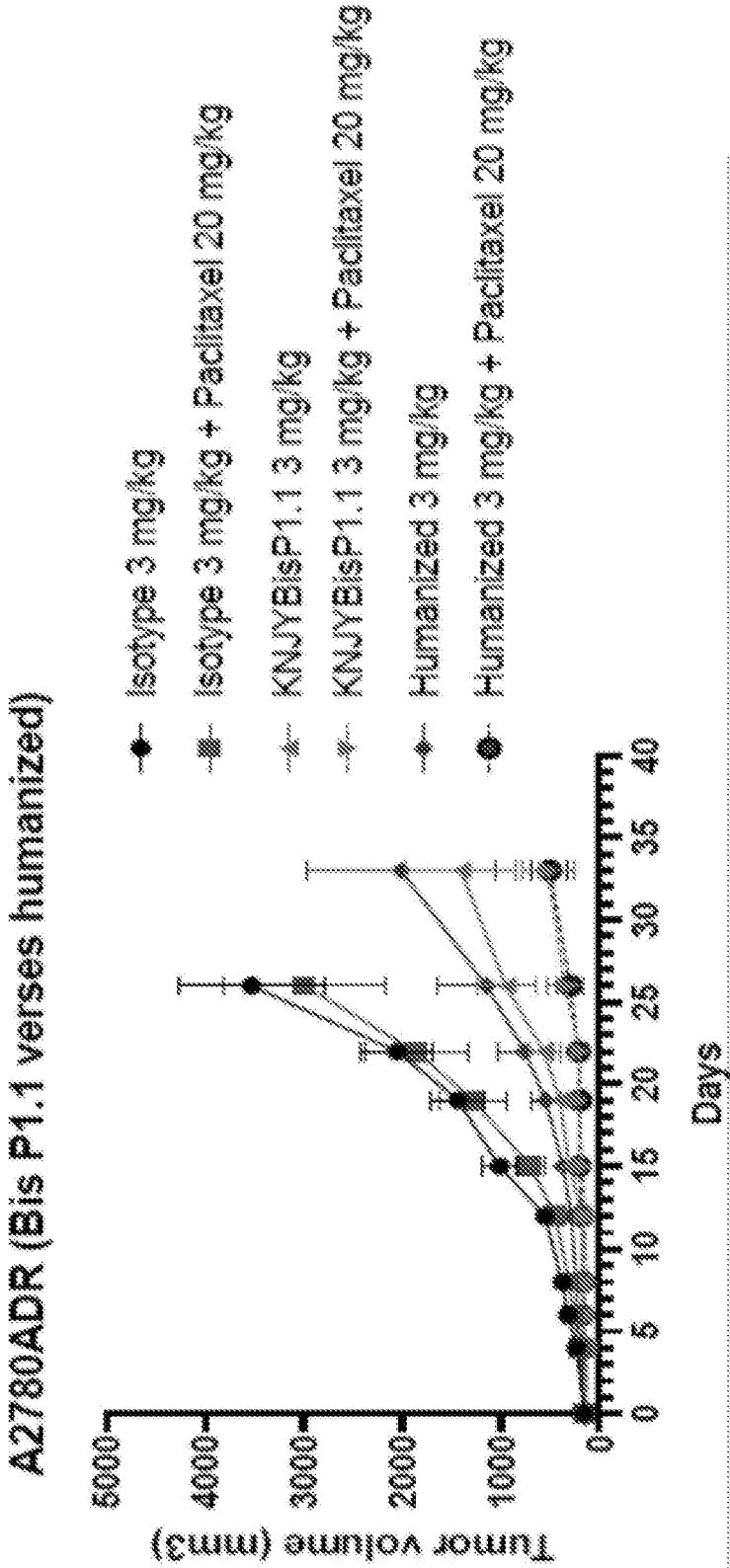


FIG. 26

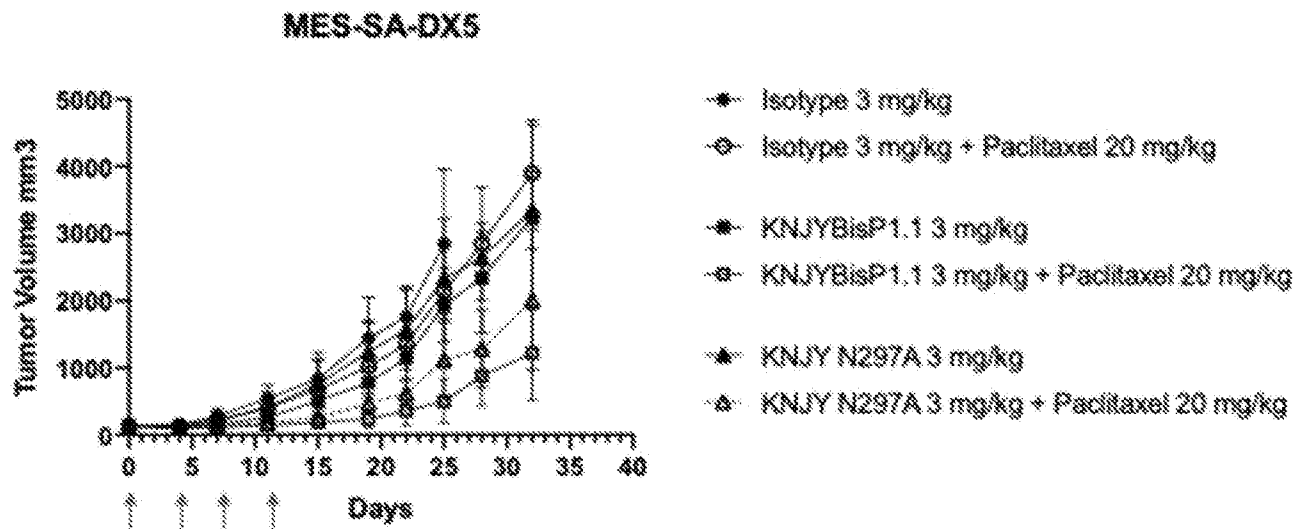


FIG. 27A

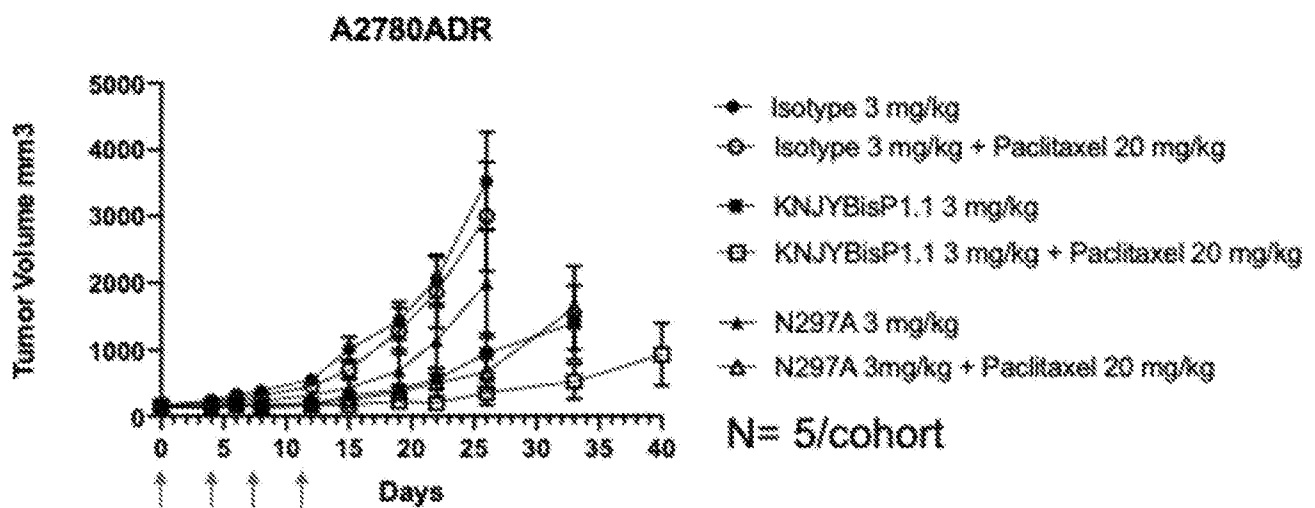


FIG. 27B

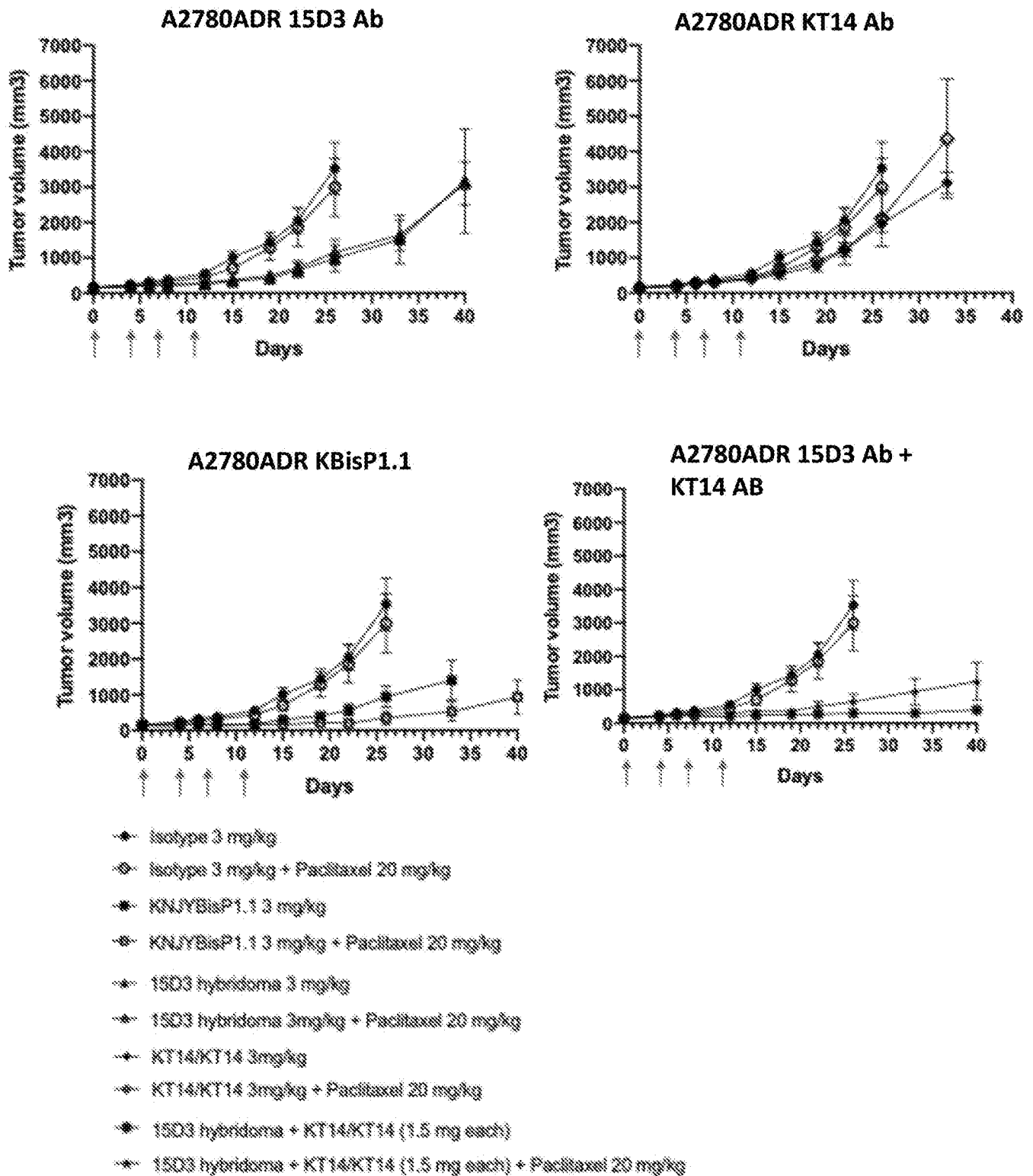


FIG. 28

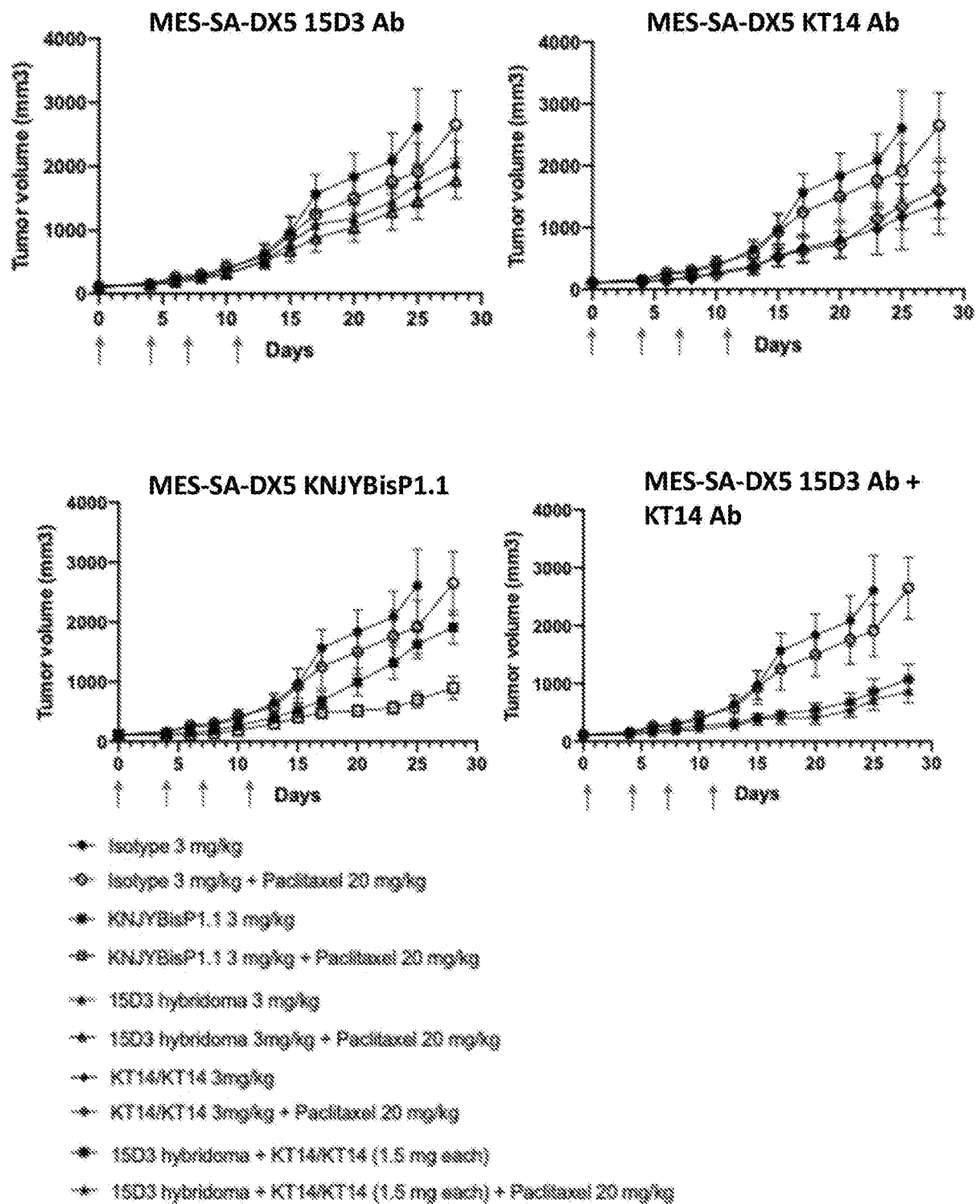


FIG. 29

**KBisP1.1 antibody chemosensitizes breast PDX-CTG-2616
to Paclitaxel**

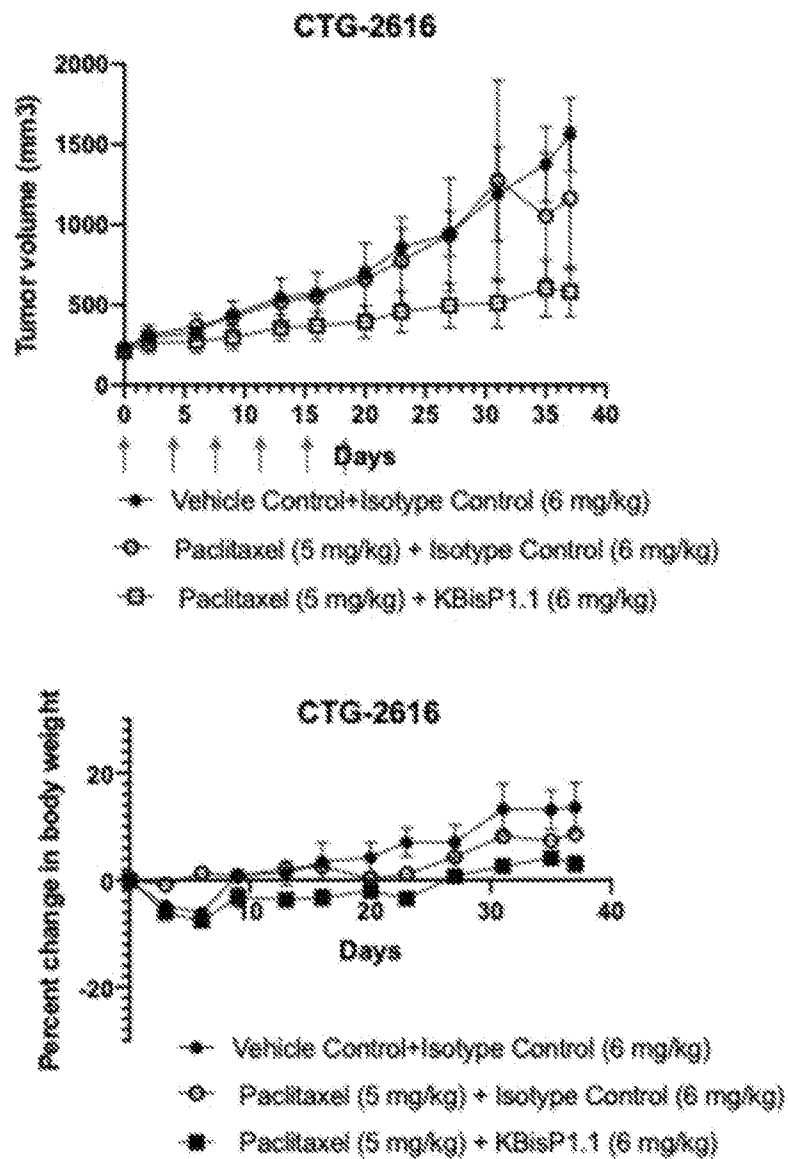


FIG. 30

HC1	HC2	LC	CD47 ELISA	MDR1 FACS	PDL1 ELISA	EGFR ELISA	EGFR FACS
15D3 hmz	5F9 (aCD47)	MRK16 hmz	+	+			
15D3	Cetuximab (aEGFR)	15D3		+		+	
15D3	Cetuximab (aEGFR)	MRK16		+		±	
MRK16	Cetuximab (aEGFR)	15D3		+		+	
MRK16	Cetuximab (aEGFR)	MRK16		+		±	
UIC2	Cetuximab (aEGFR)	MRK16		-		+	
UIC2	Cetuximab (aEGFR)	15D3		-		+	
15D3	Necitumum ab (aEGFR)	MRK16		+		-	+
15D3	Atezolizum ab (aPDL1)	MRK16		+	+		
15D3 hmz	Atezolizum ab (aPDL1)	MRK16 hmz		+	+		

FIG. 31

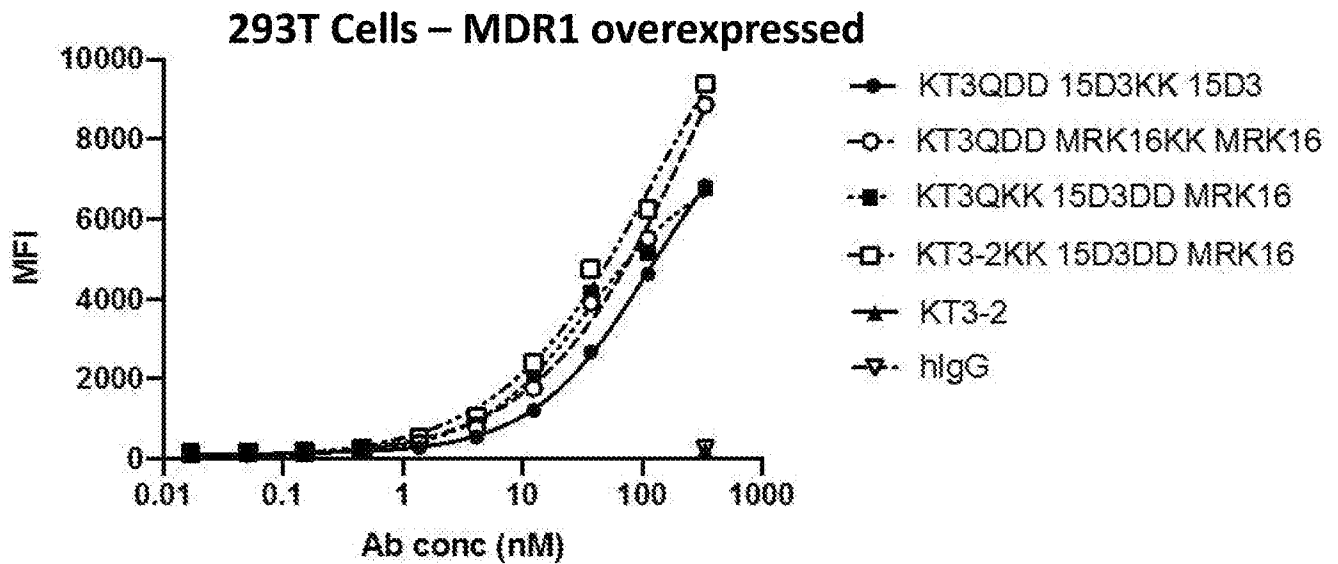


FIG. 32A

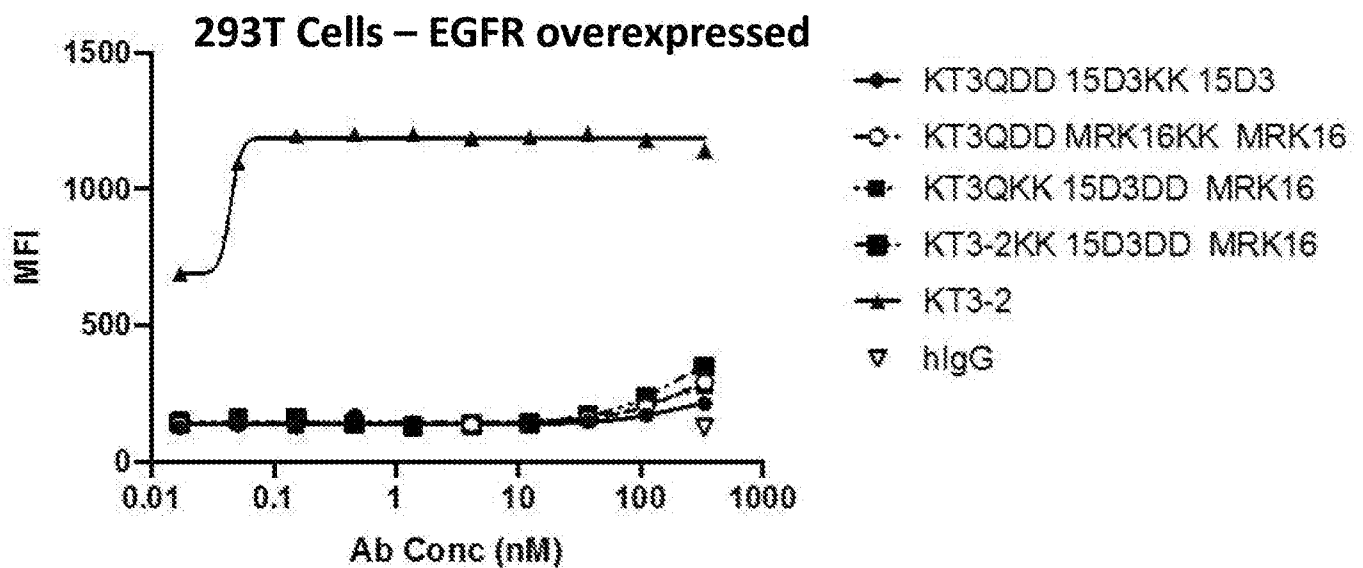


FIG. 32B

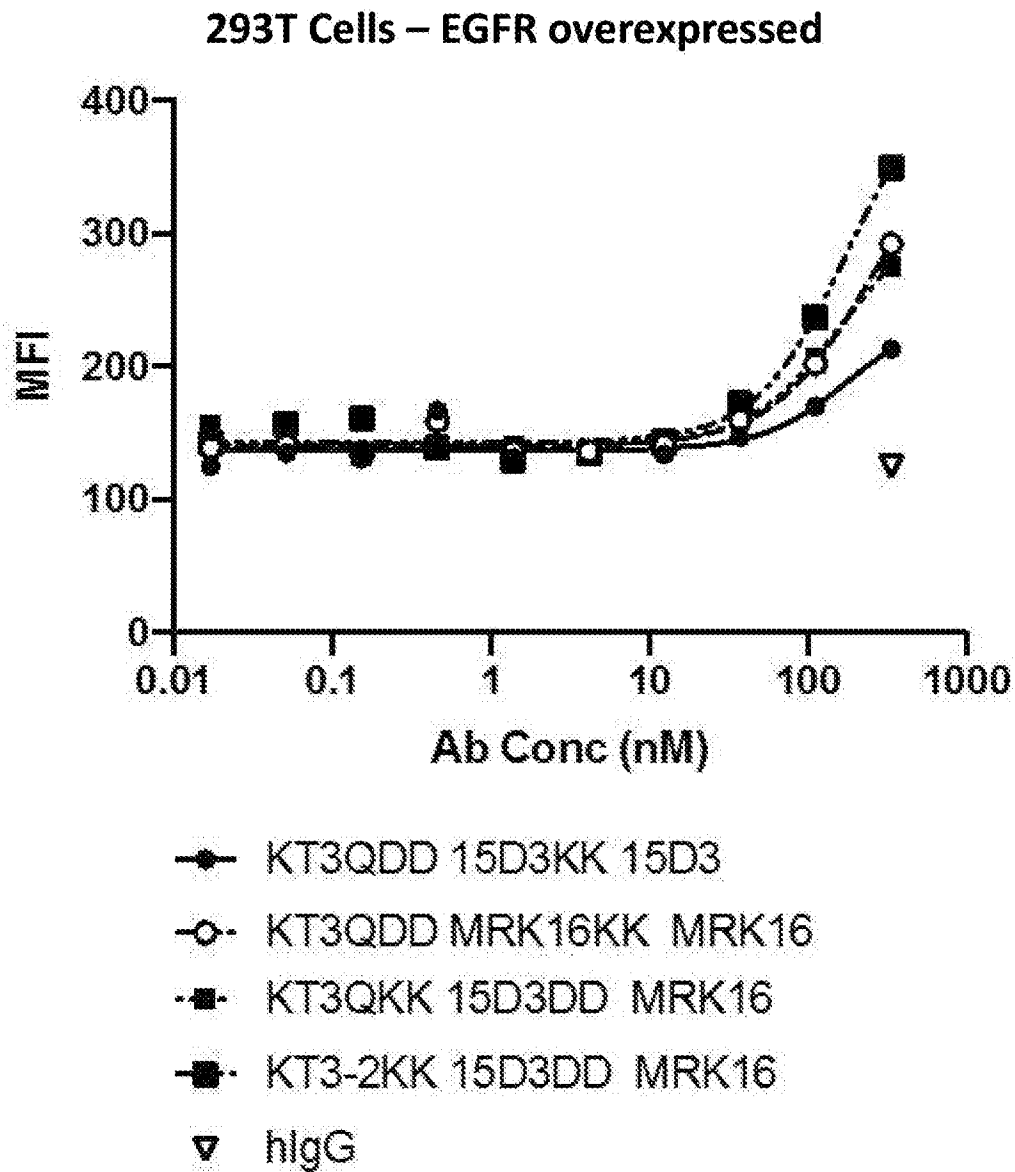


FIG. 32C

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/026261

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P35/00 C07K16/28 A61K39/395
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K A61P C07K

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

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

EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Y GAO ET AL: "Efficient inhibition of multidrug-resistant human tumors with a recombinant bispecific anti-P-glycoprotein x anti-CD3 diabody", BLOOD CANCER JOURNAL, vol. 18, no. 3, 1 March 2004 (2004-03-01), pages 513-520, XP055713772, London ISSN: 0887-6924, DOI: 10.1038/sj.leu.2403267 the whole document in particular, pages 513-515 -----	1-72, 97-138
Y	WO 2006/108070 A2 (CEDARS SINAI MEDICAL CENTER [US]; SZABO GABOR [HU]; GODA KATALIN [HU]) 12 October 2006 (2006-10-12) the whole document in particular, pages 12 and ----- -/-	1-72, 97-138



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

20 July 2020

Date of mailing of the international search report

15/09/2020

Name and mailing address of the ISA/

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Authorized officer

Pérez-Mato, Isabel

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2020/026261

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 3 243 840 A1 (SUZHOU ALPHAMAB CO LTD [CN]) 15 November 2017 (2017-11-15) the whole document in particular, pages 5, 10 and 11 -----	1-72, 97-138
Y	Maurizio Cianfriglia: "The biology of MDR1-P-glycoprotein (MDR1-Pgp) in designing functional antibody drug conjugates (ADCs): the experience of gemtuzumab ozogamicin", Annali dell'Istituto superiore di sanita, 1 January 2013 (2013-01-01), pages 150-168, XP055715361, DOI: 10.4415/ANN_13_02_07 Retrieved from the Internet: URL: http://old.iss.it/publ/anna/2013/2/492150.pdf the whole document in particular, pages 151 and 152 -----	1-72, 97-138
Y	J. JACKMAN ET AL: "Development of a Two-part Strategy to Identify a Therapeutic Human Bispecific Antibody That Inhibits IgE Receptor Signaling", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 285, no. 27, 2 July 2010 (2010-07-02) , pages 20850-20859, XP055002016, ISSN: 0021-9258, DOI: 10.1074/jbc.M110.113910 the whole document in particular, page 20853 -----	1-72
Y	HIROTAKE SHIRAIWA ET AL: "Engineering a bispecific antibody with a common light chain: Identification and optimization of an anti-CD3 epsilon and anti-GPC3 bispecific antibody, ERY974", METHODS, vol. 154, 1 February 2019 (2019-02-01), pages 10-20, XP055715064, NL ISSN: 1046-2023, DOI: 10.1016/j.ymeth.2018.10.005 the whole document in particular, pages 10-12 ----- -/--	1-72, 97-138

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/026261

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SIMON KRAH ET AL: "Generation of human bispecific common light chain antibodies by combining animal immunization and yeast display", PROTEIN ENGINEERING, DESIGN AND SELECTION, 5 January 2017 (2017-01-05), XP055690141, GB ISSN: 1741-0126, DOI: 10.1093/protein/gzw077 the whole document in particular, page 293-295 -----	1-72, 97-138
Y	THOMAS VAN BLARCOM ET AL: "Productive common light chain libraries yield diverse panels of high affinity bispecific antibodies", MABS, vol. 10, no. 2, 14 December 2017 (2017-12-14), pages 256-268, XP055531386, US ISSN: 1942-0862, DOI: 10.1080/19420862.2017.1406570 the whole document in particular, pages 261-263 -----	1-72, 97-138
A	CHRISTIAN KLEIN ET AL: "Progress in overcoming the chain association issue in bispecific heterodimeric IgG antibodies", MABS, vol. 4, no. 6, 1 November 2012 (2012-11-01), pages 653-663, XP055106060, ISSN: 1942-0862, DOI: 10.4161/mabs.21379 the whole document see for instance, pages 656-658 -----	1-72, 97-138

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2020/026261

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.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-72(completely); 97-138(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-72(completely); 97-138(partially)

directed to a bispecific antibody that binds MDR1 and a tumor associated antigen.

2. claims: 73-92(completely); 97-138(partially)

directed to an antibody that binds MDR1.

3. claims: 93-138(partially)

directed to antibodies that bind CD47.

4. claims: 93-138(partially)

directed to antibodies that bind PD-L1.

5. claims: 93-138(partially)

directed to antibodies that bind EGFR.

6. claims: 139-142

directed to a method of generating a multi-specific antibody binding MDR1 and a TAA.

7. claims: 143-146

directed to a genetically modified human cell line expressing a tumor associated antigen and comprising an exogenous nucleic acid sequence comprising a sequence encoding MDR1 for overexpression thereof and method to obtain said cell.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2020/026261

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006108070 A2	12-10-2006	EP 1865987 A2	19-12-2007
		WO 2006108070 A2	12-10-2006

EP 3243840 A1	15-11-2017	CN 105820251 A	03-08-2016
		CN 107108747 A	29-08-2017
		CN 110655582 A	07-01-2020
		CN 110658340 A	07-01-2020
		DK 3243840 T3	24-08-2020
		EP 3243840 A1	15-11-2017
		JP 2018504113 A	15-02-2018
		US 2019031782 A1	31-01-2019
		WO 2016110267 A1	14-07-2016
