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Antibody ID	Domain 1	Humanized	Domain 2	Humanized	IgG Fc	Domain 3	Humanized
	LH-scFv	Variant	LH-scFv	Variant		HL-scFv	Variant
SI-27833	R11	-	4420(F1TC)	-	n2	I2C	-
SI-27839	323H7	L1H4	PL230C6	H3L2	n2	I2C	-
SI-27845	338H4	L4H3	PL230C6	H3L2	n2	PL221	H2L1
SI-27862	R11	-	PL230C6	H3L2	n2	4420(F1TC)	-
SI-27863	323H7	L1H4	PL230C6	H3L2	n2	284A10	H1L1
SI-27869	338H4	L4H3	PL230C6	H3L2	n2	299F8	H2L1
SI-27873	338H4	L4H3	PL230C6	H3L2	n2	480C8	H2L1
SI-27875	4420(F1TC)	-	PL230C6	H3L2	n2	I2C	-
SI-278109	PL230C6	L2H3	4420(F1TC)	-	n2	480C8	H2L1
SI-37X1	PL230C6	L2H3	806	-	n2	480C8	H2L1
SI-37X2	806	-	PL230C6	H3L2	n2	480C8	H2L1
SI-37X3	4420(F1TC)	-	806	-	n2	480C8	H2L1
SI-37X4	PL230C6	L2H3	806	-	n2	4420(F1TC)	-
SI-37X5	806	-	4420(F1TC)	-	n2	480C8	H2L1
SI-37X6	806	-	PL230C6	H3L2	n2	4420(F1TC)	H2L1
SI-37X7	-	-	-	-	n2	480C8	H2L1
SI-34X2	2104	-	PL230C6	H3L2	n2	480C8	H2L1
SI-34X7	4420(F1TC)	-	PL230C6	H3L2	n2	480C8	H2L1
SI-34X8	2104	-	4420(F1TC)	-	n2	480C8	H2L1
SI-34X9	2104	-	PL230C6	H3L2	n2	4420(F1TC)	-

FIGURE 1

(57) Abstract: The application provides tri-specific antibody monomers having a N-terminal and a C-terminal, comprising in tandem from the N-terminal to the C-terminal, a first scFv domain at the N-terminal, a Fab domain, a Fc domain, and a second scFv domain at the C-terminal. In one embodiment, the first scFv domain, the Fab domain, and the second scFv domain each has a binding specificity against a different antigen.



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MULTI-SPECIFIC ANTIBODIES AND METHODS OF MAKING AND USING THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application No. 62524554, filed June 25, 2017, which application is expressly incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present disclosure generally relates to the technical field of biologic therapeutics, and more particularly relates to making and using multi-specific antibodies.

BACKGROUND

Cancer cells develop various strategies to evade destruction and elimination by the immune system of the host. One main mechanism is immune-editing where the immune response against the original cancer provides immunological pressure to select for variants of the original cancer cell that are not recognized by the immune system through the down-regulation of the Major Histocompatibility Complex (MHC) presentation of tumour specific antigens. Another mechanism resides within the tumour microenvironment where cells such as T regulatory cells (Treg) or Myeloid-derived suppressor cells (MDSC) actively inhibit the cytolytic activity of cytotoxic T cells or natural killer cells through the production of immune-suppressive cytokines such as Transforming Growth Factor Beta (TGF β) or Interleukin-10 (IL-10). Also, within the tumour microenvironment cancer cells often express one or more "Immune Checkpoint" receptors or molecules such as Programmed Death Ligand 1 (PD-L1) that bind to inhibitory receptors on T cells such as Programmed cell death protein 1 (PD-1) that plays an important role in down-regulating the immune response and promoting self-tolerance. [Vinay et al., 2015, *Semin. Cancer Biol.*,(35): S185-S198, Dunnet al., 2004, *Immunity*, 21(2): 137-48; Adachi & Tamada, 2015, *Cancer Sci.*, 106(8): 945-50].

As the mechanisms by which tumours evade recognition by the immune system continue to be better understood new treatment modalities that target these mechanisms have recently emerged. On March 25, 2011, the U. S. Food and Drug Administration (FDA) approved ipilimumab injection (Yervoy, Bristol-Myers Squibb) for the treatment of unresectable or metastatic melanoma. Yervoy binds to cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) expressed on activated T cells and blocks the interaction of CTLA-4 with CD80/86 on antigen-presenting cells thereby blocking the negative or inhibitory signal delivered into the T cell through CTLA-4 resulting in re-activation of the antigen-specific T cell leading to, in many patients, eradication of the tumour. A few years later in 2014 the FDA approved Keytruda (Pembrolizumab, Merck) and Opdivo (Nivolumab, Bristol-Myers Squibb) for treatment of advanced melanoma. These monoclonal antibodies bind to PD-1 which is expressed on activated and/or exhausted T cells and block the interaction of PD-1 with PD-L1 expressed on tumours thereby eliminating the inhibitory signal through PD-1 into the T cell resulting in re-activation of the antigen-specific T cell leading to again, in many patients, eradication of the tumour. Since then additional clinical trials have been performed comparing the single monoclonal antibody Yervoy to the combination of the monoclonal antibodies Yervoy and Opdivo in the treatment of advanced melanoma which showed

improvement in overall survival and progression-free survival in the patients treated with the combination of antibodies. (Hodi et al., 2016, Lancet Oncol. 17(11):1558-1568, Hellman et al., 2018, Cancer Cell 33(5):853-861). However, as many clinical trials have shown a great benefit of treating cancer patients with monoclonal antibodies that are specific for one or more immune checkpoint molecules data has emerged that only those patients with a high mutational burden that generates a novel T cell epitope(s) which is recognized by antigen-specific T cells show a clinical response (Snyder et al., 2014, NEJM 371:2189-2199). Those patients that have a low tumour mutational load mostly do not show an objective clinical response (Snyder et al., 2014, NEJM 371:2189-2199, Hellman et al., 2018, Cancer Cell 33(5):853-861).

In recent years other groups have developed an alternate approach that does not require the presence of neoepitope presentation by antigen-presenting cells to activate T cells. One example is the development of a bi-specific antibody where the binding domain of an antibody which is specific for a tumour associated antigen, e.g., CD19, is linked to an antibody binding domain specific for CD3 on T cells thus creating a bi-specific T cell engager or BiTe molecule. In 2014, the FDA approved a bi-specific antibody called Blinotumumab for the treatment of Precursor B-Cell Acute Lymphoblastic Leukemia. Blinotumumab links the scFv specific for CD19 expressed on leukemic cells with the scFv specific for CD3 expressed on T cells (Bejnjamin and Stein 2016, Ther Adv Hematol 7(3):142-146). However, despite an initial response rate of >50% in patients with relapsed or refractory ALL many patients are resistant to blinotumumab therapy or relapse after successful treatment with blinotumumab. Evidence is emerging that the resistance to blinotumumab or the relapse after blinotumumab treatment is attributable to the expression of immune checkpoint inhibitory molecules expressed on tumour cells such as PD-L1 that drives an inhibitory signal through PD-1 expressed on activated T cells (Feucht et al., 2016, Oncotarget 7(47):76902-76919). In a case study of a patient who was resistant to therapy with blinotumumab a second round of blinotumumab therapy was performed but with the addition of a monoclonal antibody, pembrolizumab (Keytruda, Merck), which is specific for PD-1 and blocks the interaction of T cell-expressed PD-1 with tumour cell expressed PD-L1 resulted in a dramatic response and reduction of tumour cells in the bone marrow from 45% to less than 5% in this one patient (Feucht et al., 2016, Oncotarget 7(47):76902-76919). These results show that combining a bi-specific BiTe molecule with one or more monoclonal antibodies can significantly increase clinical activity compared to either agent alone.

SUMMARY

In one aspect, the application provides tri-specific antibody monomers, the antigen-binding fragments thereof, and multi-specific antibodies.

In one embodiment, the tri-specific antibody monomer having a N-terminal and a C-terminal, comprising in tandem from the N-terminal to the C-terminal, a first scFv domain at the N-terminal, a Fab domain, a Fc domain, and a second scFv domain at the C-terminal. The first scFv domain, the Fab domain, and the second scFv domain each has a binding specificity against a different antigen.

The antigen may be a tumour antigen, an immune signaling antigen, or a combination thereof. In one embodiment, the first scFv domain, the Fab domain, and the second scFv domain each has a binding specificity against a tumour antigen. In one embodiment, the first scFv domain, the Fab domain, and the second scFv domain each has a binding specificity against an immune signaling antigen.

In one embodiment, the first scFv domain has a binding specificity against a tumour antigen. In one embodiment, the first scFv domain has a binding specificity against an immune signaling antigen. In one embodiment, the Fab domain has a binding specificity against a tumour antigen. In one embodiment, the Fab domain has a binding specificity against an immune signaling antigen. In one embodiment, the second scFv domain has a binding specificity against a tumour antigen. In one embodiment, the second scFv domain has a binding specificity against an immune signaling antigen.

In one embodiment, the first scFv domain, the Fab domain, and the second scFv domain each independently has a binding specificity against an antigen selected from ROR1, PD-L1, CD3, CD28, 41BB, CEA, HER2, EGFRvIII, EGFR, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypican-3, gpA33, GD2, TROP2, NKG2D, BCMA, CD19, CD20, CD33, CD123, CD22, CD30, PDL1, PD1, OX40, 4-1BB, GITR, TIGIT, TIM-3, LAG-3, CTLA4, CD40, VISTA, ICOS, BTLA, LIGHT, HVEM, CSF1R, CD73, and CD39.

In one embodiment, the first scFv domain, the Fab domain, and the second scFv domain each independently has a binding specificity against an antigen selected from ROR1, PD-L1, and CD3. In one embodiment, the first scFv domain has a binding specificity against ROR1. In one embodiment, the Fab domain has a binding specificity against PD-L1. In one embodiment, the second scFv domain has a binding specificity against CD3. In one embodiment, the first scFv domain has a binding specificity against ROR1, the Fab domain has a binding specificity against PD-L1, and the second scFv has a binding specificity against CD3.

The antibody monomers and the antibodies disclosed herein may be humanized. In one embodiment, the Fc domain is a human IgG1 Fc.

The application provides scFv domains for the tri-specific antibody monomer and antibodies disclosed herein. In one embodiment, the scFv domain includes an amino acid sequence having a percentage homology to SEQ ID NO. 13-34, 39-46. The percentage homology is not less than 70%, 80%, 90%, 95%, 98%, or 99%.

The application provides Fab domains for the tri-specific antibody monomers and antibodies disclosed herein. In one embodiment, the Fab domain includes an amino acid sequence having a percentage homology to SEQ ID NO. 13-16. The percentage homology is not less than 70%, 80%, 90%, 95%, 98%, or 99%.

The application provides Fc domains for the tri-specific antibody monomers and antibodies disclosed herein. In one embodiment, the Fab domain includes an amino acid sequence having a percentage homology to SEQ ID NO.47 and 48. The percentage homology is not less than 70%, 80%, 90%, 95%, 98%, or 99%.

The first scFv domain, the second scFv domain, the Fab domain and the Fc domain in the tri-specific antibody monomers and antibodies may be any combination of sequences disclosed herein.

In one embodiment, the application provides a multi-specific antibody, comprising a tri-specific antibody monomer. In one embodiment, the multi-specific antibody includes a first tri-specific monomer and a second tri-specific monomer. In one embodiment, the first and the second tri-specific monomers are the same and the multi-specific antibody is a symmetric tri-specific antibody. In one embodiment, the first and the second tri-specific monomers are different and the multi-specific antibody is an asymmetric antibody. In one embodiment, the multi-specific antibody is a tri-specific antibody, a tetra-specific antibody, a penta-specific antibody, or a hexa-specific antibody.

In one embodiment, the multi-specific antibody includes a first tri-specific antibody monomer and a second tri-specific antibody monomer. The first tri-specific antibody monomer has a N-terminal and a C-terminal and includes, in tandem from the N-terminal to the C-terminal, a first scFv domain at the N-terminal, a first Fab domain, a first Fc domain, and a second scFv domain at the C-terminal. The second multi-specific antibody monomer has a N-terminal and a C-terminal and includes, in tandem from the N-terminal to the C-terminal, a third scFv domain at the N-terminal, a second Fab domain, a second Fc domain, and a fourth scFv domain at the C-terminal. In one embodiment, the third scFv domain, a second Fab domain and a fourth scFv domain each has a binding specificity against a different antigen. In one embodiment, the second scFv domain and the fourth scFv domain each has a binding specificity against a different antigen. In one embodiment, the first scFv domain and the third scFv domain each has a binding specificity against a different antigen. In one embodiment, the first Fab domain and the second Fab domain each has a binding specificity against a different antigen.

In one embodiment, the multi-specific antibody is a tri-specific antibody. In one embodiment, the application provides an engineered antibody with 3 different binding domains or a "tri-specific" antibody. One binding domain is specific for CD3 on T cells, a second binding domain is specific for a tumour associated antigen including but not limited to ROR1, CEA, HER2, EGFR, EGFRvIII, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypican-3, gpA33, GD2, TROP2, BCMA, CD19, CD20, CD33, CD123, CD22, CD30, and a third binding domain which is specific for an immune checkpoint modulator such as PDL1, PD1, OX40, 4-1BB, GITR, TIGIT, TIM-3, LAG-3, CTLA4, CD40, VISTA, ICOS, BTLA, Light, HVEM, CD73, CD39, etc. In one embodiment, the tri-specific antibody includes an amino acid sequence having a percentage homology to SEQ ID NO as disclosed in this application. The percentage homology is not less than 70%, 80%, 90%, 95%, 98%, or 99%.

In one embodiment, the antibody is purified, isolated, or non-natural existing.

The application further provides the isolated nucleic acid sequence encoding the tri-specific monomers, the antigen-binding fragments thereof, or the multi-specific antibodies disclosed herein. In one embodiment, the isolated nucleic acid sequence encodes the tri-specific antibody monomer disclosed herein.

The application further provides expression vector or host cells including the isolated nucleic acid sequence disclosed herein. In one embodiment, the host cell includes the expression vector. In one embodiment, the host cell is a prokaryotic cell or a eukaryotic cell.

The application further provides immuno-conjugates including a cytotoxic agent or an imaging agent linked to the tri-specific antibody monomers, the antigen-binding fragments, or the multi-specific antibodies through a linker.

The linker may be cleavable or non-cleavable. In one embodiment, the linker may be a covalent bond such as an ester bond, an ether bond, an amid bond, a disulphide bond, an imide bond, a sulfone bond, a phosphate bond, a phosphorus ester bond, a peptide bond, or a combination thereof. In one embodiment, the linker may include a hydrophobic poly(ethylene glycol) linker.

The cytotoxic agent may include a chemotherapeutic agent, a growth inhibitory agent, a cytotoxic agent from class of calicheamicin, an antimetabolic agent, a toxin, a radioactive isotope, a therapeutic agent, or a combination thereof. In one embodiment, the cytotoxic agent may include a calicheamicin, ozogamicin, monomethyl auristatin E, emtansine, a derivative or a combination thereof.

The imaging agent may be any compound useful for imaging purpose. In one embodiment, the imaging agent may be radionuclide, a fluorescent agent, a quantum dots, or a combination thereof.

In another aspect, the application provides methods of making the antibody monomers, their antigen-binding fragments, or antibodies disclosed herein. In one embodiment, the method for producing a multi-specific antibody includes the steps of culturing a host cell containing the nucleic acid sequences encoding the antibodies so that the DNA sequence encoding the antibody is expressed and purifying said antibody.

In a further aspect, the application provides pharmaceutical compositions. In one embodiment, the pharmaceutical composition includes a pharmaceutically acceptable carrier and the tri-specific antibody monomers, the multi-specific antibodies, the immuno-conjugate, or a combination thereof.

In one embodiment, the pharmaceutical composition further includes a therapeutic agent. The therapeutic agent may include a radioisotope, radionuclide, a toxin, a chemotherapeutic agent, an antibody, an enzyme, or a combination thereof. In one embodiment, the therapeutic agent comprises an anti-estrogen agent, a receptor tyrosine kinase inhibitor, a kinase inhibitor, a cell cycle inhibitor, a DNA, RNA or protein synthesis inhibitor, a RAS inhibitor, an immune check point inhibitor, or a combination thereof.

In a further aspect, the application provides methods for treating or preventing a cancer using the tri-specific antibody monomers, the multi-specific antibodies, the immuno-conjugate, or a combination thereof. In one embodiment, the method includes administering an effective amount of the tri-specific antibody monomers, the multi-specific antibodies, the immuno-conjugate, or a combination thereof to a subject in need of such treatment. In one embodiment, the method includes administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising the tri-

specific antibody monomers, the multi-specific antibodies, the immuno-conjugate, or a combination thereof. In one embodiment, the method of treating a subject with a cancer includes administering to the subject an effective amount of the tri-specific antibody disclosed herein.

In one embodiment, the method includes directly injecting into the tumour site an effective amount of multi-specific monomers, multi-specific antibodies, the immuno-conjugates, the antigen-binding fragment thereof.

In some embodiments, the advantages that the disclosed methods of treatment have over currently existing therapies include, without limitation: 1) Inclusion of an IgG Fc domain will confer the characteristic of a longer half-life in serum compared to a bi-specific BiTe molecule, 2) Inclusion of a binding domain which is specific for an immune checkpoint modulator e.g., PD-L1, may inhibit the suppressive effect of tumour expressed PD-L1 binding to PD-1 expressed on an activated T cell, and 3) Cross-link CD3 on T cells with tumour associated antigens thus "re-directing" T cells to kill the tumour without the need to remove T cells from the patient and genetically modify them to be specific for the tumour cell before re-introducing them back into the patient as done for chimeric antigen receptor T cells (CAR-T).

Varieties of cancer may be treated with the tri-specific antibody monomers, the multi-specific antibodies, the immuno-conjugate, or a combination thereof. Example cancers include without limitation breast cancer, colorectal cancer, anal cancer, pancreatic cancer, gallbladder cancer, bile duct cancer, head and neck cancer, nasopharyngeal cancer, skin cancer, melanoma, ovarian cancer, prostate cancer, urethral cancer, lung cancer, non-small lung cell cancer, small cell lung cancer, brain tumour, glioma, neuroblastoma, esophageal cancer, , , gastric cancer, liver cancer, kidney cancer, bladder cancer, cervical cancer, endometrial cancer, thyroid cancer, eye cancer, sarcoma, bone cancer, leukemia, myeloma or lymphoma. In one embodiment, the cancer comprises cells expressing ROR1, CEA, HER2, EGFR, EGFRvIII, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypican-3, gpA33, GD2, TROP2, NKG2D, BCMA, CD19, CD20, CD33, CD123, CD22, CD30.

The method of treating a subject may include co-administering an effective amount of a therapeutic agent. In one embodiment, the therapeutic agent comprises an antibody, a chemotherapy agent, an enzyme, or a combination thereof. In one embodiment, the therapeutic agent comprises an anti-estrogen agent, a receptor tyrosine kinase inhibitor, a kinase inhibitor, a cell cycle inhibitor, a DNA, RNA or protein synthesis inhibitor, a RAS inhibitor, or a combination thereof.

In one embodiment, the therapeutic agent comprises capecitabine, cisplatin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Mustine, vincristine, procarbazine, prednisolone, bleomycin, vinblastine, dacarbazine, etoposide, Epirubicin, pemetrexed, folinic acid, gemcitabine, oxaliplatin, irinotecan, topotecan, camptothecin, docetaxel, paclitaxel, , fulvestrant, tamoxifen, letrozole, exemestane, anastrozole, aminoglutethimide, testolactone, vorozole, formestane, fadrozole, erlotinib, lapatinib, dasatinib, gefitinib, osimertinib, vandertanib, afatinib, imatinib, pazopininib, lapatinib, sunitinib, nilotinib, sorafenib, nab-palitaxel, Everolimus, temsirolimus, Dabrafenib,

vemurafenib, trametinib, vintafolide, apatinib, crizotinib, periforsine, olaparib, Bortezomib, tofacitinib, trastuzumab, a derivative or a combination thereof.

In one embodiment, the therapeutic agent comprises a check point inhibitor. In one embodiment, the therapeutic agent comprises an inhibitor of PD1, PDL1, CTLA4, 4-1BB, OX40, GITR, ICOS, LIGHT, TIM3, LAG3, TIGIT, CD40, CD27, HVEM, BTLA, VISTA, B7H4, CSF1R, NKG2D, CD73, a derivative or a combination thereof.

The subject to be treated may be a human. In one embodiment, the application provides a solution comprising an effective concentration of the tri-specific antibody monomers, the antigen-binding fragments thereof, the multi-specific antibodies, of a combination thereof, and the solution is blood plasma in a subject.

Still other embodiments will become readily apparent to those skilled in the art from the following detailed description, wherein are described embodiments by way of illustrating the best mode contemplated. As will be realized, other and different embodiments are possible and the embodiments' several details are capable of modifications in various obvious respects, all without departing from their spirit and the scope. Accordingly, the drawings and detailed description are to be regarded as illustrative in nature and not as restrictive.

BRIEF DESCRIPTION OF THE DRAWINGS AND TABLES

The foregoing and other features of this disclosure will become more fully apparent from the following description and appended claims, taken in conjunction with the accompanying drawings. Understanding that these drawings depict only several embodiments arranged in accordance with the disclosure and are, therefore, not to be considered limiting of its scope, the disclosure will be described with additional specificity and detail through use of the accompanying drawings, in which:

FIGURE 1 shows the example tri-specific antibodies used in binding and RTCC assays.

FIGURE 2 shows the specificity of the antibody binding domains used in the example tri-specific antibodies.

FIGURE 3 is a diagram showing a symmetric tri-specific antibody according to at least one embodiment.

FIGURE 4 is a snapshot of experiment results showing FACS analysis of tri-specific antibodies binding to ROR1 expressing CHO cells according to some embodiments.

FIGURE 5 is a snapshot of experiment results showing FACS analysis of tri-specific antibody binding to PD-L1 expressing CHO cells according to some embodiments.

FIGURE 6 is a snapshot of experiment results showing re-directed T cell cytotoxicity assay with CD3+ T cells as effectors and ROR1 positive B-Acute Lymphoblastic Leukemia cell line Kasumi-2 as targets according to some embodiments.

FIGURE 7 is a snapshot of experiment results showing re-directed T cell cytotoxicity assay with CD4+ T regulatory cells as effectors and ROR1 positive B-Acute Lymphoblastic Leukemia cell line Kasumi-2 as targets according to some embodiments.

FIGURE 8 is a snapshot of experiment results showing re-directed T cell cytotoxicity assay with "exhausted" CD8+ T cells as effectors and ROR1 positive B-Acute Lymphoblastic Leukemia cell line Kasumi-2 as targets according to some embodiments.

FIGURE 9 is a snapshot of experiment results showing re-directed T cell cytotoxicity assay with peripheral blood mononuclear cells as effectors and the glioblastoma cell line U87-EGFRviii as targets according to some embodiments.

FIGURE 10 is a snapshot of experiment results showing re-directed T cell cytotoxicity assay with peripheral blood mononuclear cells as effectors and the glioblastoma cell line U87-EGFRviii as targets according to some embodiments.

FIGURE 11 is a snapshot of experiment results showing re-directed T cell cytotoxicity assay with PBMC cells as effectors and CD19 positive B-Acute Lymphoblastic Leukemia cell line Kasumi-2 as targets according to some embodiments.

DETAILED DESCRIPTION

In the following detailed description, reference is made to the accompanying drawings, which form a part hereof. In the drawings, similar symbols typically identify similar components, unless context dictates otherwise. The illustrative embodiments described in the detailed description, drawings, and claims are not meant to be limiting. Other embodiments may be utilized, and other changes may be made, without departing from the spirit or scope of the subject matter presented herein. It will be readily understood that the aspects of the present disclosure, as generally described herein, and illustrated in the Figures, can be arranged, substituted, combined, separated, and designed in a wide variety of different configurations, all of which are explicitly contemplated herein.

The disclosure provides, among others, isolated antibodies, methods of making such antibodies, bispecific or multi-specific molecules, antibody-drug conjugates and/or immuno-conjugates composed from such antibodies or antigen binding fragments, pharmaceutical compositions containing the antibodies, bispecific or multi-specific molecules, antibody-drug conjugates and/or immuno-conjugates, the methods for making the molecules and compositions, and the methods for treating cancer using the molecules and compositions disclosed herein.

The term "antibody" is used in the broadest sense and specifically covers single monoclonal antibodies (including agonist and antagonist antibodies), antibody compositions with polypeptopic specificity, as well as antibody fragments (e.g., Fab, F(ab')₂, and Fv), so long as they exhibit the desired biological activity. In some embodiments, the antibody may be monoclonal, polyclonal, chimeric, single chain, bispecific or bi-effective, simianized, human and humanized antibodies as well as active fragments thereof. Examples of active fragments of molecules that bind to known antigens include Fab, F(ab')₂,

scFv and Fv fragments, including the products of an Fab immunoglobulin expression library and epitope-binding fragments of any of the antibodies and fragments mentioned above. In some embodiments, antibody may include immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e. molecules that contain a binding site that immunospecifically bind an antigen. The immunoglobulin can be of any type (IgG, IgM, IgD, IgE, IgA and IgY) or class (IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclasses of immunoglobulin molecule. In one embodiment, the antibody may be whole antibodies and any antigen-binding fragment derived from the whole antibodies. A typical antibody refers to heterotetrameric protein comprising typically of two heavy (H) chains and two light (L) chains. Each heavy chain is comprised of a heavy chain variable domain (abbreviated as VH) and a heavy chain constant domain. Each light chain is comprised of a light chain variable domain (abbreviated as VL) and a light chain constant domain. The VH and VL regions can be further subdivided into domains of hypervariable complementarity determining regions (CDR), and more conserved regions called framework regions (FR). Each variable domain (either VH or VL) is typically composed of three CDRs and four FRs, arranged in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 from amino-terminus to carboxy-terminus. Within the variable regions of the light and heavy chains there are binding regions that interacts with the antigen.

The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present disclosure may be made by the hybridoma method first described by Kohler & Milstein, *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Pat. No. 4,816,567).

The monoclonal antibodies may include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al., *Proc. Natl. Acad. Sci. USA*, 81:6851-6855 [1984]).

Monoclonal antibodies can be produced using various methods including mouse hybridoma or phage display (see Siegel. *Transfus. Clin. Biol.* 9:15-22 (2002) for a review) or from molecular cloning of antibodies directly from primary B cells (see Tiller. *New Biotechnol.* 28:453-7 (2011)). In the present

disclosure antibodies were created by the immunization of rabbits with both human PD-L1 protein and cells transiently expressing human PD-L1 on the cell surface. Rabbits are known to create antibodies of high affinity, diversity and specificity (Weber et al. *Exp. Mol. Med.* 49:e305). B cells from immunized animals were cultured in vitro and screened for the production of anti-PD-L1 antibodies. The antibody variable genes were isolated using recombinant DNA techniques and the resulting antibodies were expressed recombinantly and further screened for desired features such as ability to inhibit the binding of PD-L1 to PD-1, the ability to bind to non-human primate PD-L1 and the ability to enhance human T-cell activation. This general method of antibody discovery is similar to that described in Seeber et al. *PLOS One.* 9:e86184 (2014).

The term "antigen- or epitope-binding portion or fragment" refers to fragments of an antibody that are capable of binding to an antigen (PD-L1 in this case). These fragments may be capable of the antigen-binding function and additional functions of the intact antibody. Examples of binding fragments include, but are not limited to a single-chain Fv fragment (scFv) consisting of the VL and VH domains of a single arm of an antibody connected in a single polypeptide chain by a synthetic linker or a Fab fragment which is a monovalent fragment consisting of the VL, constant light (CL), VH and constant heavy 1 (CH1) domains. Antibody fragments can be even smaller sub-fragments and can consist of domains as small as a single CDR domain, in particular the CDR3 regions from either the VL and/or VH domains (for example see Beiboer et al., *J. Mol. Biol.* 296:833-49 (2000)). Antibody fragments are produced using conventional methods known to those skilled in the art. The antibody fragments are can be screened for utility using the same techniques employed with intact antibodies.

The "antigen-or epitope-binding fragments" can be derived from an antibody of the present disclosure by a number of art-known techniques. For example, purified monoclonal antibodies can be cleaved with an enzyme, such as pepsin, and subjected to HPLC gel filtration. The appropriate fraction containing Fab fragments can then be collected and concentrated by membrane filtration and the like. For further description of general techniques for the isolation of active fragments of antibodies, see for example, Khaw, B. A. et al. *J. Nucl. Med.* 23:1011-1019 (1982); Rousseaux et al. *Methods Enzymology*, 121:663-69, Academic Press, 1986.

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, whose name reflects its 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.

The Fab fragment may contain the constant domain of the light chain and the first constant domain (CH1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH1 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.

“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 VH-VL 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, although at a lower affinity than the entire binding site.

The “light chains” of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda (λ), 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., IgG-1, IgG-2, IgG-3, and IgG-4; IgA-1 and IgA-2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α , delta, epsilon, γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

A “humanized antibody” refers to a type of engineered antibody having its CDRs derived from a non-human donor immunoglobulin, the remaining immunoglobulin-derived parts of the molecule being derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity. Methods to obtain “humanized antibodies” are well known to those skilled in the art. (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)).

The terms “polypeptide”, “peptide”, and “protein”, as used herein, are interchangeable and are defined to mean a biomolecule composed of amino acids linked by a peptide bond.

The terms “a”, “an” and “the” as used herein are defined to mean “one or more” and include the plural unless the context is inappropriate.

By “isolated” is meant a biological molecule free from at least some of the components with which it naturally occurs. “Isolated,” when used to describe the various polypeptides disclosed herein, means a polypeptide that has been identified and separated and/or recovered from a cell or cell culture from which it was expressed. Ordinarily, an isolated polypeptide will be prepared by at least one purification step. An “isolated antibody,” refers to an antibody which is substantially free of other antibodies having different antigenic a binding specificity.

“Recombinant” means the antibodies are generated using recombinant nucleic acid techniques in exogenous host cells.

The term "antigen" refers to an entity or fragment thereof which can induce an immune response in an organism, particularly an animal, more particularly a mammal including a human. The term includes immunogens and regions thereof responsible for antigenicity or antigenic determinants.

Also as used herein, the term "immunogenic" refers to substances which elicit or enhance the production of antibodies, T-cells or other reactive immune cells directed against an immunogenic agent and contribute to an immune response in humans or animals. An immune response occurs when an individual produces sufficient antibodies, T-cells and other reactive immune cells against administered immunogenic compositions of the present disclosure to moderate or alleviate the disorder to be treated.

"Specific binding" or "specifically binds to" or is "specific for" a particular antigen or an epitope means binding that is measurably different from a non-specific interaction. Specific binding can be measured, for example, by determining binding of a molecule compared to binding of a control molecule, which generally is a molecule of similar structure that does not have binding activity. For example, specific binding can be determined by competition with a control molecule that is similar to the target.

Specific binding for a particular antigen or an epitope can be exhibited, for example, by an antibody having a KD for an antigen or epitope of at least about 10^{-4} M, at least about 10^{-5} M, at least about 10^{-6} M, at least about 10^{-7} M, at least about 10^{-8} M, at least about 10^{-9} M, alternatively at least about 10^{-10} M, at least about 10^{-11} M, at least about 10^{-12} M, or greater, where KD refers to a dissociation rate of a particular antibody-antigen interaction. Typically, an antibody that specifically binds an antigen will have a KD that is 20-, 50-, 100-, 500-, 1000-, 5,000-, 10,000- or more times greater for a control molecule relative to the antigen or epitope.

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

"Homology" between two sequences is determined by sequence identity. If two sequences which are to be compared with each other differ in length, sequence identity preferably relates to the percentage of the nucleotide residues of the shorter sequence which are identical with the nucleotide residues of the longer sequence. Sequence identity can be determined conventionally with the use of computer programs. The deviations appearing in the comparison between a given sequence and the above-described sequences of the disclosure may be caused for instance by addition, deletion, substitution, insertion or recombination.

In one aspect, the application provides tri-specific antibody monomers, antigen-binding fragments thereof, and the multi-specific antibodies. In some embodiments, the application describes the generation of symmetric tri-specific antibodies and antigen-binding fragments thereof. In one embodiment, the tri-specific antibody is a dimer of a monomer having 3 different antibody binding domains.

The monomer has an N-terminal single-chain fragment variable (scFv) with one binding specificity, followed by the fragment antibody (Fab) domain with a second binding specificity, followed by a human IgG1 CH1, hinge, CH2, and CH3 domains, followed by an scFv with a third binding specificity at the C-terminal.

The corresponding kappa or lambda light chain is expressed as a separate VL-CL chain as with a typical antibody. The monomer has one domain binding to a tumour associated antigen, one domain binding to an immune checkpoint domain with either agonist or antagonist characteristics, and one domain binding to the CD3 complex on T cells, as shown in FIGURE 3.

In one embodiment, N-terminal scFv are oriented VL-VH, operably linked by a 20 amino acid G4S linker and C-terminal scFv are oriented VH-VL, linked by either a 15 or 20 amino acid G4S linker. Each of these linkers genetically fuses the C-terminus of one domain to the N-terminus of its partner domain. N-terminal scFv are fused to the Fab domain with a shorter, 10 amino acid G4S linker. Likewise, the C-terminal scFv are fused to the end of the human IgG1 Fc domain with a 10 amino acid G4S linker.

In one embodiment, the symmetric tri-specific antibodies were designed to bind a tumour associated antigen (TAA) such as sROR1 (Karvonen et al, 2017, *Biochem Soc Trans*, 45(2): 457-464.; Aghebati-Maleki et al, 2017, *Biomed Pharmacother*. 88:814-822; Shabani et al, 2015, *Expert Opin Ther Targets*. 19(7):941-55.) or CD19 (Johnsen et al., 2014 *Leuk Lymphoma*, 55(6):1251-60) or the viii mutant of the epidermal growth factor receptor (Gan et al., 2013, *FEBS J*. 280(21):5350-70).

In one embodiment, the tri-specific antibodies were designed to bind to a second antigen involved in the immune response against cancer cells such as PD-L1 which is expressed on many tumours in the tumour microenvironment (Dunn & Rao, 2017, *Mol Immunol*. 13;87:227-239; Balar & Weber. 2017, *Cancer Immunol Immunother*. 66(5):551-564.).

In one embodiment, the tri-specific antibodies were also designed to bind to a third antigen, CD3, which is T-cell specific. Antibodies of this class can bind to the tumour through the TAA and/or PD-L1 while engaging any CD3+ T-cells in the vicinity of the tumour through CD3, a process referred to as re-directed T-cell cytotoxicity or RTCC (Baeuerle and Reinhardt, 2009, *Cancer res*. 69(12):4941-4).

Cytotoxicity is generally achieved through the actions of CD8+ T-cells although a role for CD4+ T-cells has been demonstrated (Haas et al., 2009 *Immunobiology*, 214(6):441-53). In addition, the PD-L1 binding domain used in these tri-specific antibodies has been selected to block the interaction of PD-1 on T cells with PD-L1 expressed on the tumour cells thus reducing or eliminating this inhibitory pathway to T cell activation (Feucht et al., 2016, *Oncotarget* 7(47):76902-76919).

To eliminate cross-linking of tri-specific antibodies on the surface of cells expressing Fc-receptors, amino acid mutations (alanine) near the IgG1 hinge region were introduced at positions L234, L235, and G237 (Strohl WR, 2009, *Curr Opin Biotechnol*. 20(6):685-91) resulting in an Fc domain without antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC) effector function referred to as an "effector null" phenotype.

In one embodiment, the tri-specific molecules target either human ROR1 (SEQIDs 5-8), human CD19 (SEQIDs 43-46) or EGFR vIII (SEQIDs 39-42) as tumour associated antigens. Each of these targeted tri-specific proteins also carries an anti-human PD-L1 (SEQIDs 13-16) and anti-human CD3 binding domain (SEQIDs 21-24). These binding domains were converted to scFv, VLVH, for placement at the N-terminal Domain 1 (D1) or scFv, VHVL, for placement at the C-terminal Domain 3 (D3) of the peptide.

In one embodiment, the scFv molecules described herein contain a 20 amino acid flexible (G4S) X4 linker that operably links the VH and VL, regardless of the V-region orientation (LH or HL). The remaining position in the tri-specific protein, Domain 2 (D2), consists of an IgG1 heavy chain, VH-CH1-Hinge-CH2-CH3, and its corresponding light chain, VL-CL, which can be either a kappa or lambda chain. D1 and D2 are genetically linked through a 10 amino acid (G4S) x 2 linker, as are D2 and D3 resulting in a contiguous ~125 kDa heavy chain peptide.

When co-transfected with the appropriate light chain, the example symmetric tri-specific peptide can be subsequently purified through the IgG1 Fc (Protein A/Protein G) and assayed to assess functional activity. Heavy and light chain gene "cassettes" were previously constructed such that V-regions could be cloned using either restriction enzyme sites (HindIII/NheI for the heavy chain and HindIII/BsiWI for the light chain) or "restriction-free cloning" such as Gibson Assembly (SGI-DNA, La Jolla, CA), Infusion (Takara Bio USA), or NEBuilder (NEB, Ipswich, MA), the latter of which was used here.

The application provides methods for making the tri-specific antibody monomers, antigen-binding fragments thereof, and the multi-specific antibodies. In one embodiment, the antibody is a tri-specific antibody. The tri-specific antibody proteins are produced through a process that involves design of the intact molecule, synthesis and cloning of the nucleotide sequences for each domain, expression in mammalian cells and purification of the final product.

Nucleotide sequences were assembled using the Geneious 10.2.3 software package (Biomatters, Auckland, NZ) and broken up into their component domains for gene synthesis (Genewiz, South Plainsfield, NJ). In this example, SI-27X63 (SEQID 52) was split into its component domains where the anti-human ROR1 Ig domain-specific clone 338H4 VLVH scFv occupies D1, anti-human PD-L1 clone PL230C6 occupies D2 (Fab position) and anti-human CD3 clone 284A10 VHVL scFv occupies the C-terminal D3.

Using NEBuilder web-based tools, 5' and 3' nucleotides were appended to each of the domains depending on their position in the larger protein so that each domain overlaps its flanking domains by 20-30 nucleotides which direct site-specific recombination, thus genetically fusing each domain in a single gene assembly step.

A small aliquot was transformed into *E.coli* DH10b (Invitrogen, Carlsbad, CA) and plated on TB + carbenicillin 100ug/ml plates (Teknova, Hollister, CA) and incubated at 37C overnight. Resultant colonies were selected and 2ml overnight cultures inoculated in TB + carbenicillin. DNA was prepared (Thermo-Fisher, Carlsbad, CA) from overnight cultures and subsequently sequenced (Genewiz, South Plainsfield, NJ) using sequencing primers (Sigma, St. Louis, MO) flanking each domain. All DNA sequences were assembled and analysed in Geneious.

In another aspect, the application provides pharmaceutical compositions including the tri-specific antibody monomers, the multi-specific antibodies, the antigen-binding fragments, and the immuno-conjugates thereof. Formulation of the pharmaceutical composition can be accomplished according to standard methodology known to those of ordinary skill in the art.

In one embodiment, the antibodies and monomers according to the disclosure can be prepared in a physiologically acceptable formulation and may comprise a pharmaceutically acceptable carrier, diluent and/or excipient using known techniques. For example, the antibody as described herein may include any functionally equivalent antibody or functional parts thereof, in particular, the monoclonal antibody including any functionally equivalent antibody or functional parts thereof is combined with a pharmaceutically acceptable carrier, diluent and/or excipient to form a therapeutic composition.

With respect to the formulation of suitable compositions for administration to a subject such as a human patient in need of treatment, the antibodies disclosed herein may be mixed or combined with pharmaceutically acceptable carriers known in the art dependent upon the chosen route of administration. There are no particular limitations to the modes of application of the antibodies disclosed herein, and the choice of suitable administration routes and suitable compositions are known in the art without undue experimentation.

Suitable pharmaceutical carriers, diluents and/or excipients are well known in the art and include, for example, phosphate buffered saline solutions, water, emulsions such as oil/water emulsions.

“Pharmaceutically acceptable” refers to those compounds, materials, compositions, and dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings or animals without excessive toxicity, irritation, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

In one embodiment, the pharmaceutical composition may include proteinaceous carriers such as, for example, serum albumin or immunoglobulin, particularly of human origin. Further biologically active agents may be present in the pharmaceutical composition of the disclosure dependent on the intended use. In one embodiment, the proteinaceous pharmaceutically active matter may be present in amounts between 1 ng and 10 mg per dose. Generally, the regime of administration should be in the range of between 0.1 µg and 10 mg of the antibody according to the disclosure, particularly in a range 1.0 µg to 1.0 mg, and more particularly in a range of between 1.0 µg and 100 µg, with all individual numbers falling within these ranges also being part of the disclosure. If the administration occurs through continuous infusion a more proper dosage may be in the range of between 0.01 µg and 10 mg units per kilogram of body weight per hour with all individual numbers falling within these ranges also being part of the disclosure.

The compositions may be administered to a subject in the form of a solid, liquid or aerosol at a suitable, pharmaceutically effective dose. Examples of solid compositions include pills, creams, and implantable dosage units. Pills may be administered orally. Therapeutic creams may be administered topically. Implantable dosage units may be administered locally, for example, at a tumour site, or may be implanted for systematic release of the therapeutic composition, for example, subcutaneously.

Examples of liquid compositions include formulations adapted for injection intramuscularly, subcutaneously, intravenously, intra-arterially, and formulations for topical and intraocular administration. Examples of aerosol formulations include inhaler formulations for administration to the lungs.

It is well known to those of ordinary skill in the art that the dosage of the composition will depend on various factors such as, for example, the condition of being treated, the particular composition used, and other clinical factors such as weight, size, sex and general health condition of the patient, body surface area, the particular compound or composition to be administered, other drugs being administered concurrently, and the route of administration.

The term "therapeutically effective amount" or "effective amount" refers to the amount of antibody which, when administered to a human or animal, elicits a response which is sufficient to result in a therapeutic effect in said human or animal, e.g., to ameliorate disease in a subject. The effective amount is readily determined by one of ordinary skill in the art following routine procedures. Where the disease is a cancer, the effective amount of the drug may inhibit (for example, slow to some extent, inhibit or stop) one or more of the following example characteristics including, without limitation, cancer cell growth, cancer cell proliferation, cancer cell motility, cancer cell infiltration into peripheral organs, tumor metastasis, and tumor growth. Wherein the disease is a cancer, the effective amount of the drug may alternatively do one or more of the following when administered to a subject: slow or stop tumor growth, reduce tumor size (for example, volume or mass), relieve to some extent one or more of the symptoms associated with the cancer, extend progression free survival, result in an objective response (including, for example, a partial response or a complete response), and increase overall survival time. To the extent the drug may prevent growth and/or kill existing cancer cells, it is cytostatic and/or cytotoxic.

A person skilled in the art have the ability to determine the effective amount or concentration of the antibodies disclosed therein to effectively treat a condition such as a cancer. Other parameters such as the proportions of the various components in the pharmaceutical composition, administration dose and frequency may be obtained by a person skilled in the art without undue experimentation. For example, a suitable solution for injection may contain, without limitation, from about 1 to about 20, from about 1 to about 10 mg antibodies per ml. The example dose may be, without limitation, from about 0.1 to about 20, from about 1 to about 5mg/Kg body weight. The example administration frequency could be, without limitation, once per day or three times per week.

The compositions may be administered by standard routes of administration. In general, the composition may be administered by topical, oral, rectal, nasal, intradermal, intraperitoneal, or parenteral (for example, intravenous, subcutaneous, or intramuscular) routes. In addition, the composition may be incorporated into sustained release matrices such as biodegradable polymers, the polymers being implanted in the vicinity of where delivery is desired, for example, at the site of a tumour. The method includes administration of a single dose, administration of repeated doses at predetermined time intervals, and sustained administration for a predetermined period of time.

Although many forms of administration are possible, an example administration form would be a solution for injection, in particular for intravenous or intra-arterial injection. Usually, a suitable pharmaceutical composition for injection may include pharmaceutically suitable carriers or excipients such as, without limitation, a buffer, a surfactant, or a stabilizer agent. Example buffers may include, without limitation, acetate, phosphate or citrate buffer. Example surfactants may include, without limitation, polysorbate. Example stabilizer may include, without limitation, human albumin.

In one embodiment, the administration may be parenterally, e.g. intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions and emulsions. Non-aqueous solvents include without being limited to it, propylene glycol, polyethylene glycol, vegetable oil such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous solvents may be chosen from the group consisting of water, alcohol/aqueous solutions, emulsions or suspensions including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose) and others. Preservatives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, inert gases, etc.

The antibody monomers, antibodies, antigen-binding fragments and immuno-conjugates thereof may be used in combination with a therapeutic agent or a composition comprising a therapeutic agent for treatment purpose. In some embodiments, the multi-specific antibody molecule is used in combination with one or more additional therapeutic agents at an effective amount. The additional therapeutic agent includes an antibody, a chemotherapy agent, an enzyme, or a combination thereof. In some embodiment, the additional therapeutic agent can be an anti-estrogen agent, a receptor tyrosine kinase inhibitor, a kinase inhibitor, a cell cycle inhibitor, a DNA, RNA or protein synthesis inhibitor, a RAS inhibitor, or a combination thereof. In some embodiments, the additional therapeutic agent can be a check point inhibitor. In some embodiments, therapeutic agent comprises inhibitors of PD1, PDL1, CTLA4, 4-1BB, OX40, GITR, ICOS, LIGHT, TIM3, LAG3, TIGIT, CD40, CD27, HVEM, BTLA, VISTA, B7H4, CSF1R, NKG2D, CD73, a derivative or a combination thereof.

In one embodiment, the therapeutic agent may capecitabine, cisplatin, Cyclophosphamide, methotrexate, 5-fluorouracil, Doxorubicin, cyclophosphamide, Mustine, vincristine, procarbazine, prednisolone, bleomycin, vinblastine, dacarbazine, etoposide, Epirubicin, pemetrexed, folinic acid, gemcitabine, oxaliplatin, irinotecan, topotecan, camptothecin, docetaxel, paclitaxel, , fulvestrant, tamoxifen, letrozole, exemestane, anastrozole, aminoglutethimide, testolactone, vorozole, formestane, fadrozole, erlotinib, lapatinib, dasatinib, gefitinib, osimertinib, vandertanib, afatinib, imatinib, pazopinib, lapatinib, sunitinib, nilotinib, sorafenib, nab-palitaxel, Everolimus, temsirolimus, Dabrafenib, vemurafenib, trametinib, vintafolide, apatinib, crizotinib, periforsine, olaparib, Bortezomib, tofacitinib, trastuzumab, a derivative or a combination thereof.

Cancers, including breast cancer, colorectal cancer, pancreatic cancer, head and neck cancer, melanoma, ovarian cancer, prostate cancer, non-small lung cell cancer, glioma, esophageal cancer, nasopharyngeal cancer, anal cancer, rectal cancer, gastric cancer, bladder cancer, cervical cancer, or brain cancer, may

express cancer-associated genes. Inhibition of cancer-associated activity with specific monoclonal antibodies or antigen-binding fragment may have therapeutic effect on cancers. Furthermore, administering a therapeutically effective amount of composition comprising monoclonal antibodies or antigen-binding fragment specific for cancer-associated protein may cure, prevent, ameliorate, and delay the development or metastasis of cancers, through the effect of the cytotoxic agent.

The present disclosure may be understood more readily by reference to the following detailed description of specific embodiments and examples included herein. Although the present disclosure has been described with reference to specific details of certain embodiments thereof, it is not intended that such details should be regarded as limitations upon the scope of the disclosure.

EXAMPLES

Example 1: FACS analysis of tri-specific antibody binding to ROR1 expressing CHO cells.

The tri-specific antibodies listed in tables 1 and 2 were tested for binding to Chinese hamster ovary cells (CHO) cells stably expressing full length human ROR1. Antibodies were prepared at 2X final concentration and titrated 1:3 across 8 wells of a 96 well plate in 50 ul of PBS/2% FBS and then 5,000 ROR1-CHO cells in 50ul PBS/2%FBS were added. This mixture was incubated for 30 minutes on ice, washed once with 200 ul PBS/2%FBS, and then the secondary antibody PE Goat anti-Human IgG Fc at 1:1000 dilution of stock was added, and this mixture was incubated for 30 minutes on ice. Cells were washed 2 x 200 ul PBS/2%FBS, resuspended in 50 ul PBS/2%FBS and analyzed on a BD LSRFORTESSA and the binding profile is shown in FIGURE 4. All of the tri-specific antibodies that contained a ROR1 binding domain bound at varying levels to the CHO-ROR1 cells.

Example 2: FACS analysis of tri-specific antibody binding to PD-L1 expressing CHO cells

The tri-specific antibodies listed in tables 1 and 2 were tested for binding to Chinese hamster ovary cells (CHO) cells stably expressing full length human PD-L1. Antibodies were prepared at 2X final concentration and titrated 1:3 across 8 wells of a 96 well plate in 50 ul of PBS/2% FBS and then 5,000 PD-L1-CHO cells in 50ul PBS/2%FBS were added. This mixture was incubated for 30 minutes on ice, washed once with 200 ul PBS/2%FBS, and then the secondary antibody PE Goat anti-Human IgG Fc at 1:1000 dilution of stock was added, and this mixture was incubated for 30 minutes on ice. Cells were washed 2 x 200 ul PBS/2%FBS, resuspended in 50 ul PBS/2%FBS and analyzed on a BD LSRFORTESSA and the binding profile is shown in FIGURE 5. All of the tri-specific antibodies that contained a PD-L1 binding domain bound at similar levels to the CHO-PD-L1 cells.

Example 3: Re-directed T cell cytotoxicity (RTCC) assay with CD3+ T cells as effectors and B-Acute Lymphoblastic Leukemia (B-ALL) cell line Kasumi-2 as targets.

The tri-specific antibodies listed in tables 1 and 2 were tested for RTCC activity against the B-ALL cell line Kasumi 2 using human CD3+ T cells as effectors. The Kasumi 2 target cells, 5 x 10e6, were labeled with CFSE (Invitrogen, #C34554) at 0.5 uM in 10 ml of culture media for 20 minutes at 37C. The cells were washed 3 times with 50 ml of culture media before resuspending in 10 ml then counted again.

Antibodies were prepared at 4X final concentration and titrated 1:10 across 10 wells of a 96 well plate in 200 ul of RPMI + 10%FBS. Human CD3+ T cells were enriched from peripheral blood mononuclear cells from a normal donor using the EasySep™ Human T Cell Enrichment Kit (Stemcell Technologies, #19051) as per the manufacturers protocol. The final cell population was determined to be 98% CD3+ T cells by FACS analysis (data not shown). In the final destination 96 well plate the target cells, T cells, and serially titrated antibodies were combined by adding 100 ul of target cells (5,000), 50 ul of CD3+ T cells (50,000), and 50 ul of each antibody dilution to each well of the assay. The assay plate was incubated at 37C for approximately 72 hours and then the contents of each assay well were harvested and analyzed for the number of CFSE-labeled target cells remaining. As shown on FIGURE 6, all of the tri-specific antibodies that contained a ROR1 binding domain potently killed the target cells at less than 0.01 nM or 10 pM except for the control SI-27X62 which has a FITC binding domain in the C-terminal domain 3 position which does not bind to CD3+ T cells.

Example 4: Re-directed T cell cytotoxicity (RTCC) assay with CD4+ T regulatory cells as effectors and B-Acute Lymphoblastic Leukemia (B-ALL) cell line Kasumi-2 as targets.

The tri-specific antibodies listed in tables 1 and 2 were tested for RTCC activity against the B-ALL cell line Kasumi 2 using human CD4+ T regulatory cells as effectors. The Kasumi 2 target cells, 5 x 10e6, were labeled with CFSE (Invitrogen, #C34554) at 0.5 uM in 10 ml of culture media for 20 minutes at 37C. The cells were washed 3 times with 50 ml of culture media before resuspending in 10 ml then counted again. Antibodies were prepared at 4X final concentration and titrated 1:10 across 10 wells of a 96 well plate in 200 ul of RPMI + 10%FBS. Human CD4+ T regulatory cells were generated using the EasySep™ Human CD4+CD127lowCD25+ Regulatory T Cell Isolation Kit (Stemcell #18063) as per the manufacturers protocol. Enriched T regulatory cells were stimulated using Miltenyi CD3/CD28 activation beads (#-130-095-353) and cultured for 14 days in complete RPMI, supplemented with 5% human serum and 500U/ml recombinant human IL-2 (Peprotech #200-02). The final population of T regulatory cells were analyzed by FACS to be more than 90% CD127low CD25++ (Data not shown). In the final destination 96 well plate the target cells, T cells, and serially titrated antibodies were combined by adding 100 ul of target cells (5,000), 50 ul of CD4+ T regulatory cells (50,000), and 50 ul of each antibody dilution to each well of the assay. The assay plate was incubated at 37C for approximately 72 hours and then the contents of each assay well were harvested and analyzed for the number of CFSE-labeled target cells remaining. As shown on FIGURE 7, all of the tri-specific antibodies that contained a ROR1 binding domain potently killed the target cells at less than 0.1 nM or 100 pM except for the control SI-27X62 which has a FITC binding domain in the C-terminal domain 3 position which does not bind to CD3+ T cells.

Example 5: Re-directed T cell cytotoxicity (RTCC) assay with "Exhausted" CD8+ T cells as effectors and B-Acute Lymphoblastic Leukemia (B-ALL) cell line Kasumi-2 as targets.

The tri-specific antibodies listed in tables 1 and 2 were tested for RTCC activity against the B-ALL cell line Kasumi 2 using "exhausted" human CD8+ T cells as effectors. The Kasumi 2 target cells, 5 x 10e6, were labeled with CFSE (Invitrogen, #C34554) at 0.5 uM in 10 ml of culture media for 20 minutes at 37C. The cells were washed 3 times with 50 ml of culture media before resuspending in 10 ml then counted again. Antibodies were prepared at 4X final concentration and titrated 1:10 across 10 wells of a 96 well plate in

200 ul of RPMI + 10%FBS. Human CD8+ T cells were enriched using the EasySep™ Human CD8+ T Cell Enrichment Kit (Stemcell #19053) as per the manufacturers protocol. Enriched CD8+ T cells were stimulated using Miltenyi CD3/CD28 activation beads (#-130-095-353) and cultured for 7 days in complete RPMI, supplemented with 5% human serum and 500U/ml recombinant human IL-2 (Peprotech #200-02). The final population of exhausted CD8+ T cells were analyzed by FACS to be more than 95% CD8+ cells expressing several markers of exhaustion e.g., PD-1, TIGIT, TIM-3, and LAG-3 (Data not shown). In the final destination 96 well plate the target cells, T cells, and serially titrated antibodies were combined by adding 100 ul of target cells (5,000), 50 ul of exhausted CD8+ T cells (50,000), and 50 ul of each antibody dilution to each well of the assay. The assay plate was incubated at 37C for approximately 72 hours and then the contents of each assay well were harvested and analyzed for the number of CFSE-labeled target cells remaining. As shown on FIGURE 8, the tri-specific antibodies that contained the 338H4 ROR1 binding domain potently killed the target cells at less than 0.01 nM or 10 pM whereas those antibodies with the R11 or 323H7 ROR1 binding domain only weakly killed the target cells and the control SI-27X62 which has a FITC binding domain in the C-terminal domain 3 position which does not bind to CD3+ T cells showed negative killing activity.

Example 6: Re-directed T cell cytotoxicity (RTCC) assay with peripheral blood mononuclear cells as effectors and the U87 human glioblastoma cell line transfected with the epidermal growth factor viii (EGFRviii) mutant as targets.

The tri-specific antibodies listed in tables 1 and 2 were tested for RTCC activity against the U87-EGFRviii cell line using human PBMC as effectors. The U87-EGFR viii was transduced with the NuLight Red Lentivirus (IncuCyte®) to generate a cell line that stably expresses the fluorescent mKate2 protein (Shcherbo et al., 2009, J. Biochem, 418(3): 567–574). Antibodies were prepared at 4X final concentration and titrated 1:10 across 3 wells of a 96 well plate in 200 ul of RPMI + 10%FBS. Human PBMC were purified by standard ficoll density gradient from a “leukopak” which is an enriched leukapheresis product collected from normal human peripheral blood. In the final destination 96 well plate the target cells, PBMC, and serially titrated antibodies were combined by adding 100 ul of target cells (5,000), 50 ul of PBMC (200,000), and 50 ul of each antibody dilution to each well of the assay. The assay plate was incubated at 37C for approximately 72 hours and then the contents of each assay well were harvested and analyzed for the number of NuLight Red target cells remaining. As shown in FIGURE 9, the set of tri-specific molecules with the configuration of PL230 x 806 x 480C8 directed potent killing of target cells at 10 pM or more antibody. The control antibody, SI-37X3 FITC x 806 x 480C8 which does not contain the PD-L1 binding domain PL230C6 also potently killed target cells but with slightly lower potency than the tri-specific antibody with the PD-L1 binding domain, SI-37X1 PL230C6 x 806 x 480C8. The control tri-specific antibody SI-27X109 PL230C6 x FITC x 480C8 which does not contain the EGFRviii binding domain 806 showed much weaker killing of target cells and the control tri-specific antibody SI-37X4 PL230C6 x 806 x FITC that does not contain the CD3 binding domain 480C8 showed negligible killing of target cells. As shown in FIGURE 10, the set of tri-specific molecules with the configuration of 806 x PL230C6 x 480C8 directed potent killing of target cells at 10 pM or more antibody with the tri-specific antibody SI-37X2 806 x PL230C6 x 480C8 being more potent than the 3 control tri-specific antibodies with a FITC binding domain in each of the 3 positions of the tri-specific.

Example 7: Re-directed T cell cytotoxicity assay with PBMC cells as effectors and CD19 positive B-Acute Lymphoblastic Leukemia cell line Kasumi-2 as targets.

The tri-specific antibodies listed in tables 1 and 2 were tested for RTCC activity against the B-ALL cell line Kasumi 2 using PBMC as effectors. The Kasumi 2 target cells, 5×10^6 , were labeled with CFSE (Invitrogen, #C34554) at 0.5 μ M in 10 ml of culture media for 20 minutes at 37C. The cells were washed 3 times with 50 ml of culture media before resuspending in 10 ml then counted again. Antibodies were prepared at 4X final concentration and titrated 1:10 across 10 wells of a 96 well plate in 200 μ l of RPMI + 10%FBS. Human PBMC were purified by standard ficoll density gradient from a "leukopak" which is an enriched leukapheresis product collected from normal human peripheral blood. In the final destination 96 well plate the target cells, T cells, and serially titrated antibodies were combined by adding 100 μ l of target cells (5,000), 50 μ l of PBMC (50,000), and 50 μ l of each antibody dilution to each well of the assay. The assay plate was incubated at 37C for approximately 72 hours and then the contents of each assay well were harvested and analyzed for the number of CFSE-labeled target cells remaining. As shown on FIGURE 10, the tri-specific antibody SI34X2 21D4 x PL230C6 x 480C8 potently killed the target cells at less than 0.01 nM compared to the 3 control tri-specific antibodies with a FITC binding domain in each of the 3 positions of the tri-specific.

While the present disclosure has been described with reference to particular embodiments or examples, it may be understood that the embodiments are illustrative and that the disclosure scope is not so limited. Alternative embodiments of the present disclosure may become apparent to those having ordinary skill in the art to which the present disclosure pertains. Such alternate embodiments are considered to be encompassed within the scope of the present disclosure. Accordingly, the scope of the present disclosure is defined by the appended claims and is supported by the foregoing description. All references cited or referred to in this disclosure are hereby incorporated by reference in their entireties.

SEQUENCE LISTING

The antibody complementary Determining Regions (CDR) are defined using the KABAT numbering system and are underlined in the amino acid (aa) sequence listing:

Light Chain CDR1: residues 24-34

CDR2: residues 50-56

CDR3: residues 89-97

Heavy Chain CDR1: residues 31-35

CDR2: residues 50-65

CDR3: residues 95-102

> SEQ ID NO:1

anti-ROR1 R11 VH nt

CAGTCCGTGAAGGAGTCCGAGGGCGACCTGGTGACCCCCGCCGGCAACCTGACCTGACCTGCACCGCCTCCGGC
 TCCGACATCAACGACTACCCCATCTCCTGGGTGCGGCAGGCCCGCAAGGGCCTGGAGTGGATCGGCTTCATC
 AACTCCGGCGGCTCCACCTGGTACGCCTCCTGGGTGAAGGGCCGGTTCACCATCTCCCGGACCTCCACCACCGTGG
 ACCTGAAGATGACCTCCCTGACCACCGACGACACCGCCACCTACTTCTGCGCCCGGGGCTACTCCACCTACTACGG
 CGACTTCAACATCTGGGGCCCCGGCACCTGGTGACCATCTCCTCG

> SEQ ID NO:2

anti-ROR1 R11 VH aa

QSVKESEGLVTPAGNLTCTASGSDINDYPISWVRQAPGKGLEWIGFINSGGSTWYASWVKGRFTISRTSTTVDLKM
 TSLTDDTATYFCARGYSTYYGDFNIWGPGLVTISS

> SEQ ID NO:3

anti-ROR1 R11 VL nt

GAGCTGGTGATGACCCAGACCCCTCCTCCACCTCCGGCGCCGTGGGCGGCACCGTGACCATCAACTGCCAGGCC
 TCCCAGTCCATCGACTCCAACCTGGCCTGGTTCAGCAGAAGCCCGCCAGCCCCCACCCTGCTGATCTACCGGG
 CCTCAACCTGGCCTCCGGCGTGCCCTCCCGGTTCTCCGGCTCCCGGTCCGGCACCGAGTACACCTGACCATCTCC
 GCGTGCAGCGGGAGGACGCCGCCACCTACTACTGCCTGGGCGGCGTGGGCAACGTGTCCTACCGGACCTCCTTC
 GGCGGCGGCACCGAGGTGGTGGTGAAG

> SEQ ID NO:4

anti-ROR1 R11 VL aa

ELVMTQTPSSTSGAVGGTVTINCQASQSIDSNLAWFQQKPGQPPTLLIYRASNLASGVPSRFRSGRSRSGTEYTLTISGVQR
EDAATYYCLGGVGNVSYRTSFGGGTEVVVK

> SEQ ID NO:5

anti-ROR1 323H7 VHv4 nt

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCT
GGATTCACCATCAGTCGCTACCACATGACTTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGACAT
ATTTATGTTAATAATGATGACACAGACTACGCGAGCTCCGCGAAAGGCCGTTCCACCATCTCCAGAGACAATTCCA
AGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGCCACCTATTTCTGTGCGAGATTGGATG
TTGGTGGTGGTGGTCTTATATTGGGGACATCTGGGGCCAGGGAAGTCTGGTTACCGTCTCTTCA

> SEQ ID NO:6

anti-ROR1 323H7 VHv4 aa

EVQLLESGLLVQPGGSLRLSCAASGFTISRYHMTWVRQAPGKLEWIGHIYVNNDDTDYASSAKGRFTISRDNKNT
LYLQMNSLRAEDTATYFCARLDVGGGGAYIGDIWGGQTLVTSS

> SEQ ID NO:7

anti-ROR1 323H7 VLv1 nt

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCAGTCCA
GTCAGAGTGGTTATAACAACAACGACTTAGCCTGGTATCAGCAGAAACCAGGGAAAAGTTCCTAAGCTCCTGATCTA
TTATGCTTCCACTCTGGCATCTGGGGTCCCATCTCGGTTAGTGGCAGTGGATCTGGGACAGATTTCACTCTCACCA
TCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTGCAGGCGGTTATGATACGGATGGTCTTGATACGTT
TGCTTTCGGCGGAGGGACCAAGGTGGAGATCAA

> SEQ ID NO:8

anti-ROR1 323H7 VLv1 aa

DIQMTQSPSSLSASVGDRTITCQSSQSVYNNNDLAWYQQKPGKVPKLLIYASTLASGVPSRFRSGSGSGTDFLTISL
QPEDVATYYCAGGYDGLDFAFGGGTKVEIK

> SEQ ID NO:9

anti-ROR1 338H4 VHv3 nt

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTACTGCCTCT
GGATTCTCCCTCAGTAGCTATGCAATGAGCTGGGTCCGCCAGGCTCCAGGGAGGGGGCTGGAGTGGATCGGAAT
CATTATGCTAGTGGTAGCACATACTACGCGAGCTCGGCGAAAGGCAGATTCACCATCTCAAAGACAATACCAAG

AACACGGTGGATCTTCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAATTTATGAC
GGCATGGACCTCTGGGGCCAGGAACTCTGGTTACCGTCTCTTCA

> SEQ ID NO:10

anti-ROR1 338H4 VHv3 aa

EVQLVESGGGLVQPGGSLRLSCTASGFSLSSYAMSWVRQAPGRGLEWIGIIYASGSTYYASSAKGRFTISKDNTKNTVDL
QMNSLRAEDTAVYYCARIYDGMDLWGQGLTVTVSS

> SEQ ID NO:11

anti-ROR1 338H4 VLv4 nt

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGCCAGGCCA
GTCAGAACATTTACAGCTACTTATCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCGCCTGATCTATCTGGC
ATCTACTCTGGCATCTGGGGTCCCATCTCGGTTCAAGTGGCAGTGGATCTGGGACAGATTAACTCTCACCATCAGC
AGCCTGCAGCCTGAAGATGTTGCAACTTATTACTGTCAAAGCAATTATAACGGTAATTATGGTTTCGGCGGAGGGGA
CCAAGGTGGAGATCAAA

> SEQ ID NO:12

anti-ROR1 338H4 VLv4 aa

DIQMTQSPSSLSASVGRVTINCAASQNIYSYLSWYQQKPGKVPKRLIYLASTLASGVPSRFSGSGSGTDYTLTISSLQPE
DVATYYCQSNYNGNYGFGGGTKVEIK

>SEQ ID:13

anti-PD-L1 PL230C6 VHv3 nt

CAGTCGGTGGAGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCCTGTACAGCCTCTGG
AATCGACCTTAATACCTACGACATGATCTGGGTCCGCCAGGCTCCAGGCAAGGGGCTAGAGTGGGTTGGAATCAT
TACTTATAGTGGTAGTAGATACTACGCGAACTGGGCGAAAGGCCGATTACCATCTCAAAGACAATACCAAGAA
CACGGTGTATCTGCAAATGAACAGCCTGAGAGCTGAGGACACGGCTGTGTATTACTGTGCCAGAGATTATATGAG
TGTTCCCACTTGTGGGGCCAGGGAACCCTGGTCACCGTCTCTAGT

>SEQ ID:14

anti-PD-L1 PL230C6 VHv3 aa

QSVEESGGGLVQPGGSLRLSCTASGIDLNTYDMIWVRQAPGKGLEWVGIIYSGSRYYANWAKGRFTISKDNTKNTVY
LQMNSLRAEDTAVYYCARDYMSGSHLWGQGLTVTVSS

>SEQ ID:15

anti-PD-L1 PL230C6 VLv2 nt

GCCTATGATATGACCCAGTCTCCATCTTCCGTGTCTGCATCTGTAGGAGACAGAGTCACCATCAAGTGTCAGGCCA
 GTGAGGACATTTATAGCTTCTTGGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCCATTCTGC
 ATCCTCTCTGGCATCTGGGGTCCCATCAAGGTTACGCGGCAGTGGATCTGGGACAGATTTCACTCTACCATCAGC
 AGCCTGCAGCCTGAAGATTTGCAACTTACTATTGTCAACAGGGTTATGGTAAAAATAATGTTGATAATGCTTTCCG
 GCGGAGGGACCAAGGTGGAGATCAAA

>SEQ ID:16

anti-PD-L1 PL230C6 VLv2 aa

AYDMTQSPSSVSASVGDRTIKCQASEDIYSFLAWYQKPKGKAPKLLIHSASSLASGVPSRFSGSGSGTDFLTISLQPE
 DFATYYCQQGYGKNNVDNAFGGGTKVEIK

>SEQ ID:17

anti-CD3 I2C VH nt

CAGGTGCAATTGGTGGAAAGCGGAGGGGGACTGGTGCAGCCCCGGGGGAAGTCTGAAGCTGTCCTGTGCCGCCA
 GCGGCTTTACCTCAACAAGTACGCCATGAATTGGGTCCGACAGGCCCCAGGGAAAGGCCTGGAATGGGTGGCA
 CGGATTCGGTCCAAGTACAACAACCTACGCCACCTACTACGCTGACTCCGTGAAGGACAGATTCACCATCAGCCGGG
 ACGACTCTAAGAACCACCGCTATCTGCAGATGAACAACCTGAAAACCGAGGATACAGCTGTGTACTATTGTGTGCG
 GCACGGCAACTTCGGCAACTCCTACATCTCCTACTGGGCCTATTGGGGACAGGGAACACTGGTCACCGTGTCTAGC

>SEQ ID:18

anti-CD3 I2C VH aa

QVQLVESGGGLVQPGGSLKLSAASGFTFNKYAMN~~WVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDS~~
 KNTAYLQMNNLKTEDTAVYYCVRHGNFNGNSYISYWAYWVWGQTLVTVSS

>SEQ ID:19

anti-CD3 I2C VL nt

CAGACCGTGGTCACCCAGGAACCTTCCTGACCGTCTCCCCAGGCGGCACCGTGACCCTGACCTGTGGCTCCTCTA
 CCGGCGCTGTGACCTCCGGCAACTACCCTAACTGGGTGCAGCAGAAACCCGGACAGGCTCCTAGAGGCCTGATCG
 GCGGCACCAAGTTTCTGGCCCCTGGCACCCCTGCCAGATTCTCCGGCTCCCTGCTGGGAGGCAAGGCCGCTCTGAC
 CCTGTCTGGCGTGCAGCCTGAGGACGAGGCCGAGTACTACTGTGTGCTGTGGTACTCCAACAGATGGGTGTTCCG
 AGGCGGCACAAAGCTGACCGTGTCTCCTCG

>SEQ ID:20

anti-CD3 I2C VL aa

QTVVTQEPSLTVSPGGTVTLTCSSTGAVTSGNYPNWVQKPKGQAPRGLIGTKFLAPGTPARFSGSLLGGKAALTL
 GVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLSS

>SEQ ID:21

anti-CD3 284A10 VHv1 nt

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTC
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 TCATTACTGGTCGTGATATCACATACTACGCGAGCTGGGCGAAAGGCAGATTCACCATCTCCAGAGACAATTCCAA
 GAACACGCTGTATCTTCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGCGCGACGGTGG
 ATCATCTGCTATTACTAGTAACAACATTTGGGGCCAAGGAACTCTGGTCACCGTTTCTTCA

>SEQ ID:22

anti-CD3 284A10 VHv1 aa

EVQLVESGGGLVQPGGSLRLSCAASGFTISTNAMSWVROAPGKGLEWIGVITGRDITYYASWAKGRFTISRDNSKNTLY
 LQMNSLRAEDTAVYYCARDGGSSAITSNNIWGQGLTVTVSS

>SEQ ID:23

anti-CD3 284A10 VLv1 nt

GACGTCGTGATGACCCAGTCTCCTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAATTGCCAAGCCA
 GTGAGAGCATTAGCAGTTGGTTAGCCTGGTATCAGCAGAAACCAGGGAAGCCCTAAGCTCCTGATCTATGAAG
 CATCCAACTGGCATCTGGGGTCCCATCAAGGTTAGCGGCAGTGGATCTGGGACAGAGTTCACTCTCACCATCA
 GCAGCCTGCAGCCTGATGATTTGCAACTTATTACTGCCAAGGCTATTTTTATTTATTAGTCGTACTIONTATGTAAAT
 CTTTCGGCGGAGGGACCAAGGTGGAGATCAAA

>SEQ ID:24

anti-CD3 284A10 VLv1 aa

DVVMTQSPSTLSASVGDRTINCQASEISSWLAWYQKPKGKAPKLLIYEASKLASGVPSRFSGSGSGTEFTLTISLQPD
 DFATYYCQGYFYFISRTYVNSFGGGTKVEIK

>SEQ ID:25

anti-CD3 299F6 VHv2 nt

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTACGGCCTC
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 TCATTACTGGTCGTGATATCACATACTACGCGAGCTGGGCGAAAGGCAGATTCACCATCTCCAAAGACACCTCCAA
 GAACACGGTGGATCTTCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGCGCGACGGTG
 GATCATCTGCTATTACTAGTAACAACATTTGGGGCCAAGGAACTCTGGTCACCGTTTCTTCA

>SEQ ID:26

anti-CD3 299F6 VHv2 aa

EVQLVESGGGLVQPGGSLRLSCTASGFTISSNAMSWVRQAPGKGLEWIGVITGRDITYYASWAKGRFTISKDTSKNTVD
LQMNSLRAEDTAVYYCARDGGSSAITSNNIWGQGLTVTVSS

>SEQ ID:27

anti-CD3 299F6 VLv1 nt

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CATCCAACTGGCATCTGGGGTCCCATCAAGGTTGAGCGGCAGTGGATCTGGGACAGAGTTCCTCTCACCATCA
GCAGCCTGCAGCCTGATGATTTTGCAACTTATTACTGCCAAGGCTATTTTTATTTTATTAGTCGTAGTTATGTAATG
CTTTCGGCGGAGGGACCAAGGTGGAGATCAAA

>SEQ ID:28

anti-CD3 299F6 VLv1 aa

DIQMTQSPSTLSASVGDRTITCQASESISSWLAWYQKPKGKAPKLLIEASKLASGVPSRFSGSGSGTEFTLTISSLQPD
DFATYYCQGYFYFISRSYVNAFGGGTKVEIK

>SEQ ID:29

anti-CD3 480C8 VHv1 nt

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTC
TGGAATCGACCTCAGTAGCAATGCAATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGAG
TCATTACTGGTCGTGATATCACATACTACGCGAGCTGGGCGAAAGGCAGATTACCATCTCCAGAGACAATTCCAA
GAACACGCTGTATCTTCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGCGCGACGGTGG
ATCATCTGCTATTAATAGTAAGAACATTTGGGGCCAAGGAAGTCTGGTCCACCGTTTCTTCA

>SEQ ID:30

anti-CD3 480C8 VHv1 aa

EVQLVESGGGLVQPGGSLRLSCAASGIDLSSNAMSWVRQAPGKGLEWIGVITGRDITYYASWAKGRFTISRDNSKNTLY
LQMNSLRAEDTAVYYCARDGGSSAINSKNIWGQGLTVTVSS

>SEQ ID:31

anti-CD3 480C8 VHv2 nt

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGAGGGTCCCTGAGACTCTCCTGTACGGCCTC
TGGAATCGACCTCAGTAGCAATGCAATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGAG
TCATTACTGGTCGTGATATCACATACTACGCGAGCTGGGCGAAAGGCAGATTACCATCTCCAAAGACACCTCCAA
GAACACGGTGGATCTTCAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGCGCGACGGTGG
GATCATCTGCTATTAATAGTAAGAACATTTGGGGCCAAGGAAGTCTGGTCCACCGTTTCTTCA

>SEQ ID:32

anti-CD3 480C8 VHv2 aa

EVQLVESGGGLVQPGGSLRLSCTASGIDLSSNAMSWVRQAPGKGLEWIGVITGRDITYYASWAKGRFTISKDTSKNTV
DLQMNSLRAEDTAVYYCARDGGSSAINSKNIWGQGLTVTVSS

>SEQ ID:33

anti-CD3 480C8 VLv1 nt

GACATCCAGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCAAGCCA
GTGAGAGCATTAGCAGTTGGTTAGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTATGAAG
CATCCAAACTGGCATCTGGGGTCCCATCAAGGTTAGCGGCAGTGGATCTGGGACAGAGTTCACCTCACCATCA
GCAGCCTGCAGCCTGATGATTTGCAACTTATTACTGCCAAGGCTATTTTTATTTATTAGTCGTAATTATGTAAATG
CTTTCGGCGGAGGGACCAAGGTGGAGATCAAA

>SEQ ID:34

anti-CD3 480C8 VLv1 aa

DIQMTQSPSTLSASVGDRTITCQASESISWLAWYQKPKGKAPKLLIYEASKLASGVPSRFSGSGSGTEFTLTISLQPD
DFATYYCQGYFYFISRTYVNAFGGGTKVEIK

>SEQ ID:35

anti-FITC 4-4-2- (4420) VH nt

GAGGTGAAGCTGGATGAGACTGGAGGAGGCTTGGTGCAACCTGGGAGGCCATGAAACTCTCCTGTGTTGCCTCT
GGATTCACTTTTAGTGACTACTGGATGAACTGGGTCCGCCAGTCTCCAGAGAAAGGACTGGAGTGGGTAGCACAA
ATTAGAAACAAACCTTATAATTATGAAACATATTATTAGATTCTGTGAAAGGCAGATTCACCATCTCAAGAGATG
ATTCCAAAAGTAGTGTCTACCTGCAAATGAACAACCTAAGAGTTGAAGACATGGGTATCTATTACTGTACGGGTTT
TACTATGGTATGGACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA

>SEQ ID:36

anti-FITC 4-4-2- (4420) VH aa

EVKLDETGGGLVQPGRPMKLSVASGFTFSDYWMNWVRSPEKLEWVAQIRNKPYNYETYSDSVKGRFTISRDDS
KSSVYLQMNNLRVEDMGIYYCTGSYYGMDYWGQGTSTVTVSS

>SEQ ID:37

anti-FITC 4-4-2- (4420) VL nt

GATGTCGTGATGACCCAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAG
TCAGAGCCTTGTACACAGTAATGGAAACACCTATTTACGTTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAGGTC
CTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTAGTGGCAGTGGATCAGGGACAGATTTCA

CACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTCTGCTCTCAAAGTACACATGTTCCGTGGAC
GTTCCGGTGGAGGCACCAAGCTGGAAATCAA

>SEQ ID:38

anti-FITC 4-4-2- (4420) VL aa

DVVMQTPLSLPVS LGDQASISCRSSQSLVHSNGNTYLRWYLQKPGQSPKVLIIKVSNRFSGVPDRFSGSGSGDFTLKI
SRVEAEDLG VYFCSQSTHVPWTFGGGKLEIK

>SEQ ID:39

anti-EGFRvIII mAb 806 VH nt

GATGTGCAGCTTCAGGAGTCGGGACCTAGCCTGGTGAAACCTTCTCAGTCTCTGTCCCTCACCTGCACTGTCACTG
GCTACTCAATCACCAGTGATTTGCCTGGAAGTGGATTCGGCAGTTTCCAGGAAACAAGCTGGAGTGGATGGGCT
ACATAAGTTATAGTGGTAACACTAGGTACAACCCATCTCTCAAAGTGAATCTCTATCACTCGCGACACATCCAAG
AACCAATTCTTCTGCACTTGAAGTCTGTGACTATTGAGGACACAGCCACATATTACTGTGTAACGGCGGGACGCG
GGTTTCCTTATTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCA

>SEQ ID:40

anti-EGFRvIII mAb 806 VH aa

DVQLQESGPSLVKPSQSLTCTVTGYSITSDFAWNWIRQFPGNKLEWMGYISYSGNTRYNPSLKRISITRDTSKNQFF
LQLNSVTIEDTATYYCVTAGRGPYWGQGLTVTSA

>SEQ ID:41

anti-EGFRvIII mAb 806 VL nt

GACATCCTGATGACCCAATCTCCATCCTCCATGTCTGTATCTCTGGGAGACACAGTCAGCATCACTTGCCATTCAAG
TCAGGACATTAACAGTAATATAGGGTGGTTGCAGCAGAGACCAGGGAAATCATTTAAGGGCCTGATCTATCATGG
AACCAACTTGACGATGAAGTTCCATCAAGTTCAAGTGGCAGTGGATCTGGAGCCGATTATTCTCTCACCATCAGC
AGCCTGGAATCTGAAGATTTGCAGACTATTACTGTGTACAGTATGCTCAGTTTCCGTGGACGTTCCGGTGGAGGCA
CCAAGCTGGAAATCAA

>SEQ ID:42

anti-EGFRvIII mAb 806 VL aa

DILMTQSPSSMSVSLGDTVSITCHSSQDINSNIGWLQQRPGKSFKGLIYHGTNLDDEVPSRFSGSGSGADYSLTISSLESE
DFADYYCVQAQFPWTFGGGKLEIK

>SEQ ID:43

anti-CD19 21D4 VH nt

GAGGTGCAGCTGGTGCAGTCTGGAGCAGAGGTGAAGAAACCAGGAGAGTCTCTGAAGATCTCCTGTAAGGGTTC
 TGGATACAGCTTTAGCAGTTCATGGATCGGCTGGGTGCGCCAGGCACCTGGGAAAGGCCTGGAATGGATGGGGA
 TCATCTATCCTGATGACTCTGATAACCAGATACAGTCCATCCTTCCAAGGCCAGGTACCATCTCAGCCGACAAGTCC
 ATCAGGACTGCCTACCTGCAGTGGAGTAGCCTGAAGGCCTCGGACACCGCTATGTATTACTGTGCGAGACATGTT
 ACTATGATTTGGGGAGTTATTATTGACTTCTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

>SEQ ID:44

anti-CD19 21D4 VH aa

EVQLVQSGAEVKKPGESLKISCKGSGYSFSSWIGWVRQAPGKGLEWMGIYPDDSDTRYSPFQQVTVISADKSIRTA
 YLQWSSLKASDTAMYYCARHVTMIWGVIIDFWGGTLTVSS

>SEQ ID:45

anti-CD19 21D4 VL nt

GCCATCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCGGGCAA
 GTCAGGGCATTAGCAGTGCTTAGCCTGGTATCAGCAGAAACCAGGGAAAGCTCCTAAGCTCCTGATCTATGATG
 CCTCCAGTTTGGAAAGTGGGGTCCCATCAAGGTTCAGCGGCAGTGGATCTGGGACAGATTTCACTCTACCATCAG
 CAGCCTGCAGCCTGAAGATTTTGAACCTATTACTGTCAACAGTTAATAGTTACCCATTCACTTTCGGCCCTGGGA
 CCAAAGTGGATATCAAA

>SEQ ID:46

anti-CD19 21D4 VL aa

AIQLTQSPSSLSASVGDRTITCRASQGISSALAWYQQKPGKAPKLLIYDASSLESGVPSRFSGSGSGTDFTLTISSLQPED
 FATYYCQQFNSYPFTFGPGTKVDIK

>SEQ ID:47

human IgG1 null (G1m-fa with ADCC/CDC null mutations) nt

GCTAGACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTG
 GGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCCCTGACCAGCGGCGTG
 CACACCTTCCCGGTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTT
 GGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCA
 AATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAAGCCGCGGGGGCACCGTCAGTCTTCTCTT
 CCCCCAAAACCAAGGACACCCTCATGATCTCCCGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCA
 CGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGG
 AGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGG
 AGTACAAGTGC GCGGTCTCCAACAAAGCCCTCCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGGCAGC
 CCCGAGAACCACAGGTGTACACCCTGCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCC
 TGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACACTACAAG
 ACCACGCCTCCCGTGGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACCGTGGACAAGAGCAGGTGGC

AGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCT
GTCTCCGGGT

>SEQ ID:48

human IgG1 null (G1m-fa with ADCC/CDC null mutations) aa

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTY
ICNVNHNKPSNTKVDKRVKPKCDKHTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN
HYTQKSLSLSPG

>SEQ ID:49

human Ig Kappa nt

CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCC GCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGT
GTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAACGCCCTCCAATCGGGTAA
CTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCA
AAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGA
GCTTCAACAGGGGAGAGTGT

>SEQ ID:50

human Ig Kappa aa

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC

>SEQ ID:51

SI-27X63 (323H7-L1H4-scFv x PL230C6-H3-Fab x 284A10-H1L1-scFv) heavy chain nt

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACTTGCCAGTCCA
GTCAGAGTGTATAACAACAACGACTTAGCCTGGTATCAGCAGAAACCAGGGAAAGTTCCTAAGCTCCTGATCTA
TTATGCTTCCACTCTGGCATCTGGGGTCCCATCTCGGTTCAAGTGGCAGTGGATCTGGGACAGATTTCACTCTACCA
TCAGCAGCCTGCAGCCTGAAGATGTTGCAACTTACTGTGCAGGCGGTTATGATACGGATGGTCTTGATACGTT
TGCTTTCGGCGGAGGGACCAAGGTGGAGATCAAAGGCGGTGGCGGTAGTGGGGGAGGCGGTTCTGGCGGCGGA
GGGTCCGGCGGTGGAGGATCAGAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGATCACCATCAGTCGCTACCACATGACTTGGGTCCGCCAGGCTCCAGGGAA
GGGGCTGGAGTGGATCGGACATATTTATGTTAATAATGATGACACAGACTACGCGAGCTCCGCGAAAGGCCGGTT
CACCATCTCAGAGACAATCCAAGAACACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGCCAC
CTATTTCTGTGCGAGATTGGATGTTGGTGGTGGTGGTCTTATATTGGGGACATCTGGGGCCAGGGAACTCTGGT
TACCGTCTCTCAGGCGGTGGAGGGTCCGGCGGTGGTGGATCCCAGTCGGTGGAGGAGTCTGGGGGAGGCTTGG
TCCAGCCTGGGGGGTCCCTGAGACTCTCCTGTACAGCCTCTGGAATCGACCTAATACCTACGACATGATCTGGGT

CCGCCAGGCTCCAGGCAAGGGGCTAGAGTGGGTTGGAATCATTACTTATAGTGGTAGTAGATACTACGCGAACTG
GGCGAAAGGCCGATTACCCATCTCAAAGACAATACCAAGAACACGGTGTATCTGCAAATGAACAGCCTGAGAGC
TGAGGACACGGCTGTGTATTACTGTGCCAGAGATTATATGAGTGGTCCCCTTGTGGGGCCAGGGAACCCTGGT
CACCGTCTCTAGTGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCCTCTCCAAGAGCACCTCTGGGGGC
ACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAACCTCAGGCGCCCTG
ACCAGCGGCGTGACACCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTACCCTGTC
CCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGA
GAGTTGAGCCAAATCTTGTGACAAAACCTCACACATGCCACCGTGCCAGCACCTGAAGCCGCGGGGGCACCGT
CAGTCTTCTTCCCCCAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCCTGAGGTCAAGTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGA
CAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCCAGCGTCTCACCCTGCTGACCAGGACTGG
CTGAATGGCAAGGAGTACAAGTGC GCGGTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAA
GCCAAAGGGCAGCCCCGAGAACCACAGGTGTATACCCTGCCCCATCCCGGATGAGCTGACCAAGAACCAGGTC
AGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGA
GAACAATAAGACCACGCCTCCCGTGTGGACTCCGACGGCTCCTTCTTCTCTATAGCAAGCTCACCGTGGAC
AAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAG
AAGAGCCTCTCCCTGTCTCCGGTGGCGGTGGAGGGTCCGGCGGTGGTGGATCCGAGGTGCAGCTGGTGGAGTC
TGGGGGAGGCTTGGTCCAGCCTGGGGGGTCCCTGAGACTCTCTGTGCAGCCTCTGGATTACCATCAGTACCAA
TGCAATGAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGATCGGAGTCATTACTGGTCTGTATATCA
CATACTACGCGAGCTGGGCGAAAGGCAGATCACCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTTCAAAT
GAACAGCCTGAGAGCCGAGGACACGGCTGTGTATTACTGTGCGCGGACGGTGGATCATCTGCTATTACTAGTAA
CAACATTTGGGGCAAGGAACTCTGGTACCCTTTCTCAGGCGGTGGCGGTAGTGGGGGAGGCGGTTCTGGCG
GCGGAGGGTCCGGCGGTGGAGGATCAGACGTCGTGATGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAG
ACAGAGTCACCATCAATTGCCAAGCCAGTGAGAGCATTAGCAGTTGGTTAGCCTGGTATCAGCAGAAACCAGGGA
AAGCCCCTAAGCTCCTGATCTATGAAGCATCCAAACTGGCATCTGGGGTCCCATCAAGGTTACGCGGCAGTGGATC
TGGGACAGAGTTCATCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTATTACTGCCAAGGCTATTTT
ATTTTATTAGTCGTACTIONTGTAAATCTTTCGGCGGAGGGACCAAGGTGGAGATCAAA

>SEQ ID:52

SI-27X63 (323H7-L1H4-scFv x PL230C6-H3-Fab x 284A10-H1L1-scFv) heavy chain aa

DIQMTQSPSSLSASVGDRTITCQSSQSVYNNNDLAWYQQKPGKVPKLLIYYASTLASGVPSRFSGSGSGTDFLTISSL
QPEDVATYYCAGGYDGLDFAFGGGTKVEIK (anti-ROR1 323H7 VLv1)

GGGGSGGGSGGGSGGGGS (Gly₄Ser)x4 linker

EVQLLESGLVQPGGSLRLSCAASGFTISRYHMTWVRQAPGKLEWIGHIYVNNDDTDYASSAKGRFTISRDNKNT
LYLQMNSLRAEDTATYFCARLDVGGGGAYIGDIWGQGLVTVSS (anti-ROR1 323H7 VHv4)

GGGGSGGGGS (Gly₄Ser)x2 linker

QSVESGGGLVQPGGSLRLSCTASGIDLNTYDMIWVVRQAPGKGLEWVGIIITYSGSRYYANWAKGRFTISKDNTKNTVY
LQMNSLRAEDTAVYYCARDYMSGSHLWGQGTLTVSS (anti-PD-L1 PL230C6 VHv3)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTY
ICNVNHKPSNTKVDKRVKPKCDKHTHTCPPCPAPEAAGAPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCAVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRD
ELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCSCVMHEALHN
HYTQKSLSLSPG (human IgG1 null)

GGGGSGGGGS (Gly₄Ser)_{x2} linker

EVQLVESGGGLVQPGGSLRLSCAASGFTISTNAMSWVVRQAPGKGLEWIGVITGRDITYASWAKGRFTISRDNKNTLY
LQMNSLRAEDTAVYYCARDGGSSAITSNNIHWGQGTLTVSS (anti-CD3 284A10 VHv1)

GGGGSGGGSGGGSGGGGS (Gly₄Ser)_{x4} linker

DVVMTQSPSTLSASVGDVRTINCQASESISSWLAWYQKPKGKAPKLLIYEASKLASGVPSRFSGSGSGTEFTLTISLQPD
DFATYYCQGYFYFISRTYVNSFGGGTKVEIK (anti-CD3 284A10 VLv1)

>SEQ ID:53

SI-27X63 (323H7-L1H4-scFv x PL230C6-H3-Fab x 284A10-H1L1-scFv) light chain nt

GCCTATGATATGACCCAGTCTCCATCTTCCGTGTCTGCATCTGTAGGAGACAGAGTCACCATCAAGTGTCAGGCCA
GTGAGGACATTTATAGCTTCTTGGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCCATTCTGC
ATCCTCTCTGGCATCTGGGGTCCCATCAAGGTTACAGCGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGC
AGCCTGCAGCCTGAAGATTTGCAACTTACTATTGTCAACAGGGTTATGGTAAAAATAATGTTGATAATGCTTTCG
GCGGAGGGACCAAGGTGGAGATCAAACGTACGGTGGCTGCACCATCTGTCTTCATCTCCCGCCATCTGATGAGC
AGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAA
GGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACA
GCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATC
AGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGT

>SEQ ID:54

SI-27X63 (323H7-L1H4-scFv x PL230C6-H3-Fab x 284A10-H1L1-scFv) light chain aa

AYDMTQSPSSVSASVGDVRTIKQASEDIYSFLAWYQKPKGKAPKLLIHSASSLASGVPSRFSGSGSGTDFLTISLQPE
DFATYYCQGYGKNNVDNAFGGGTKVEIK (anti-PD-L1 PL230C6 VLv2)

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNFPYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYE
KHKVYACEVTHQGLSSPVTKSFNRGEC (human Ig Kappa)

CLAIMS**What is claimed is:**Antibody claims

1. A tri-specific antibody monomer having a N-terminal and a C-terminal, comprising in tandem from the N-terminal to the C-terminal,

a first scFv domain at the N-terminal,

a Fab domain,

a Fc domain, and

a second scFv domain at the C-terminal,

wherein the first scFv domain, the Fab domain, and the second scFv domain each has a binding specificity against a different antigen, and wherein the first scFv domain, the Fab domain, and the second scFv domain each has a specificity against a tumour antigen or an immune signaling antigen.

2. The tri-specific antibody monomer of Claim 1, wherein the first scFv domain, the Fab domain, and the second scFv domain each independently has a binding specificity against an antigen selected from ROR1, PD-L1, CD3, CD28, 41BB, CEA, HER2, EGFRvIII, EGFR, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypican-3, gpA33, GD2, TROP2, NKG2D, BCMA, CD19, CD20, CD33, CD123, CD22, CD30, PDL1, PD1, OX40, 4-1BB, GITR, TIGIT, TIM-3, LAG-3, CTLA4, CD40, VISTA, ICOS, BTLA, LIGHT, HVEM, CSF1R, CD73, and CD39.

3. The tri-specific antibody monomer of Claim 1, wherein the first scFv domain, the Fab domain, and the second scFv domain each independently has a binding specificity against an antigen selected from ROR1, PD-L1, and CD3.

4. The tri-specific antibody monomer of Claim 1, wherein the first scFv domain has a binding specificity against ROR1.

5. The tri-specific antibody monomer of Claim 1, wherein the Fab domain has a binding specificity against PD-L1.

6. The tri-specific antibody monomer of Claim 1, wherein the second scFv domain has a binding specificity against CD3.

7. The tri-specific antibody monomer of Claim 1, wherein the first scFv domain has a binding specificity against ROR1, wherein the Fab domain has a binding specificity against PD-L1, and wherein the second scFv has a binding specificity against CD3.

8. The tri-specific antibody monomer of Claim 1, wherein the Fc domain comprises a human IgG1 Fc.

9. The tri-specific antibody monomer of Claim 1, having a binding specificity to human PD-L1, human CD3, and one of human ROR1, human CD19 or EGFR VIII.
10. The tri-specific antibody monomer of Claim 1, wherein the first scFv domain comprises a (G4S)_n linker, wherein n is an integral from 2 to 4 and wherein the first scFv domain is linked to the Fab domain through the linker.
11. The tri-specific antibody monomer of Claim 1, having an amino acid sequence with a binding specificity to human ROR1, wherein the amino acid sequence has a percentage homology to SEQ ID 5, 6, 7 or 8, and wherein the percentage homology is not less than 90%.
12. The tri-specific antibody monomer of Claim 1, having an amino acid sequence with a binding specificity to human CD19, wherein the amino acid sequence has a percentage homology to SEQ ID 43, 44, 45 or 46, and wherein the percentage homology is not less than 90%.
13. The tri-specific antibody monomer of Claim 1, having an amino acid sequence with a binding specificity to EGFR VIII, wherein the amino acid sequence has a percentage homology to SEQ ID 39, 40, 41 or 42, and wherein the percentage homology is not less than 90%.
14. The tri-specific antibody monomer of Claim 1, having an amino acid sequence with a binding specificity to human PD-L1, wherein the amino acid sequence has a percentage homology to SEQ ID 13, 14, 15, or 16, and wherein the percentage homology is not less than 90%.
15. The tri-specific antibody monomer of Claim 1, having an amino acid sequence with a binding specificity to human CD3, wherein the amino acid sequence has a percentage homology to SEQ ID 21, 22, 23, or 24, and wherein the percentage homology is not less than 90%.
16. A scFv domain, comprising an amino acid sequence having a percentage homology to SEQ ID NO. 13-34, 39-46, wherein the percentage homology is not less than 90%.
17. A Fab domain, comprising an amino acid sequence having a percentage homology to SEQ ID NO. 13-16, wherein the percentage homology is not less than 90%.
18. A Fc domain, comprising an amino acid sequence having a percentage homology to SEQ ID NO. 47 and 48, wherein the percentage homology is not less than 90%.
19. A multi-specific antibody, comprising a tri-specific antibody monomer of Claim 1.
20. The multi-specific antibody of Claim 19, wherein the multi-specific antibody is a tri-specific antibody having a binding specific to human PD-L1, human CD3, or one of human ROR1, human CD19 or EGFR VIII.
21. The multi-specific antibody of Claim 20, having an amino acid sequence with a percentage homology to SEQ ID NO as disclosed in this application, wherein the percentage homology is not less than 90%.

22. The multi-specific antibody of Claim 20, wherein the antibody comprise a scFv domain linked to a Fab domain through a (G4S)₄ linker.
23. An isolated nucleic acid sequence encoding the tri-specific antibody monomer of Claim 1 or the multi-specific antibody of Claim 19.
24. An expression vector comprising the isolated nucleic acid sequence of Claim 23.
25. A host cell comprising the isolated nucleic acid sequence of Claim 22, wherein the host cell is a prokaryotic cell or a eukaryotic cell.
26. An immuno-conjugate, comprising a cytotoxic agent or an imaging agent linked to the multi-specific antibody of Claim 19 through a linker, wherein the linker comprises a covalent bond selected from an ester bond, an ether bond, an amid bond, a disulphide bond, an imide bond, a sulfone bond, a phosphate bond, a phosphorus ester bond, a peptide bond, or a combination thereof.
27. The immuno-conjugate of Claim 26, wherein the linker comprises a hydrophobic poly(ethylene glycol) linker.
28. The immunoconjugate of Claim 26, wherein the cytotoxic agent comprises a chemotherapeutic agent, a growth inhibitory agent, a cytotoxic agent from class of calicheamicin, an antimetabolic agent, a toxin, a radioactive isotope, a toxin, a therapeutic agent, or a combination thereof.
29. A pharmaceutical composition, comprising a pharmaceutically acceptable carrier and at least one of the multi-specific antibody of Claim 19 or the immuno-conjugate of Claim 26.
30. The pharmaceutical composition of Claim 29, further comprising a therapeutic agent selected from a radioisotope, radionuclide, a toxin, a chemotherapeutic agent or a combination thereof.
31. A method for treating or preventing a cancer in a human subject, said method comprising administering to the human subject an effective amount of a pharmaceutical composition comprising a purified multi-specific antibody of Claim 19, an immuno-conjugate of Claim 26, or a combination thereof.
32. The method of Claim 31, wherein the cancer comprises cells expressing ROR1, CEA, HER2, EGFR, EGFRvIII, LMP1, LMP2A, Mesothelin, PSMA, EpCAM, glypican-3, gpA33, GD2, TROP2, NKG2D, BCMA, CD19, CD20, CD33, CD123, CD22, or CD30.
33. The method of Claim 31, further comprising co-administering an effective amount of a therapeutic agent.
34. The method of Claim 35, wherein the therapeutic agent comprises an anti-estrogen agent, a receptor tyrosine kinase inhibitor, a kinase inhibitor, a cell cycle inhibitor, a DNA, RNA or protein synthesis inhibitor, a RAS inhibitor, a check point inhibitor, an inhibitor of PD1, PDL1, CTLA4, 4-1BB, OX40, GITR, ICOS, LIGHT, TIM3, LAG3, TIGIT, CD40, CD27, HVEM, BTLA, VISTA, B7H4, CSF1R, NKG2D, CD73, a derivative or a combination thereof.

35. A solution comprising an effective concentration of the multi-specific antibody of Claim 19, wherein the solution is blood plasma in a human subject.

Antibody ID	Domain 1	Humanized Variant	Domain 2	Humanized Variant	IgG Fc	Domain 3	Humanized Variant
	LH-scFv		LH-scFv			HL-scFv	
SI-27X33	R11	-	4428(FITC)	-	n2	I2C	-
SI-27X39	323H7	L1H4	PL230C6	H3L2	n2	I2C	-
SI-27X45	338H4	L4H3	PL230C6	H3L2	n2	PL221	H2L1
SI-27X62	R11	-	PL230C6	H3L2	n2	4420(FITC)	-
SI-27X63	323H7	L1H4	PL230C6	H3L2	n2	384A10	H2L1
SI-27X69	338H4	L4H3	PL230C6	H3L2	n2	299F6	H2L1
SI-27X73	338H4	L4H3	PL230C6	H3L2	n2	480C8	H2L1
SI-27X75	4420(FITC)	-	PL230C6	H3L2	n2	I2C	-
SI-27X109	PL230C6	L2H3	4420(FITC)	-	n2	480C8	H2L1
SI-37X1	PL230C6	L2H3	806	-	n2	480C8	H2L1
SI-37X2	806	-	PL230C6	H3L1	n2	480C8	H2L1
SI-37X3	4420(FITC)	-	806	-	n2	480C8	H2L1
SI-37X4	PL230C6	L2H3	806	-	n2	4420(FITC)	-
SI-37X5	806	-	4420(FITC)	-	n2	480C8	H2L1
SI-37X6	806	-	PL230C6	H3L2	n2	4420(FITC)	H2L1
SI-37X7	-	-	-	-	n2	480C8	H2L1
SI-34X2	21D4	-	PL230C6	H3L2	n2	480C8	H2L1
SI-34X7	4420(FITC)	-	PL230C6	H3L2	n2	480C8	H2L1
SI-34X8	21D4	-	4420(FITC)	-	n2	480C8	H2L1
SI-34X9	21D4	-	PL230C6	H3L2	n2	4420(FITC)	-

FIGURE 1

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Antibody Name	Specificity
21D4	CD19
806	EGFRviii
4420	FITC
R11	ROR1 Kringle Domain
323H7	ROR1 IgD Domain
338H4	ROR1 Frizzled Domain
PL230C6	PD-L1
I2C	CD3 complex Epsilon chain
284A10	CD3 complex Epsilon chain
299F6	CD3 complex Epsilon chain
480C8	CD3 complex Epsilon chain

FIGURE 2

Symmetric tri-specific Antibody

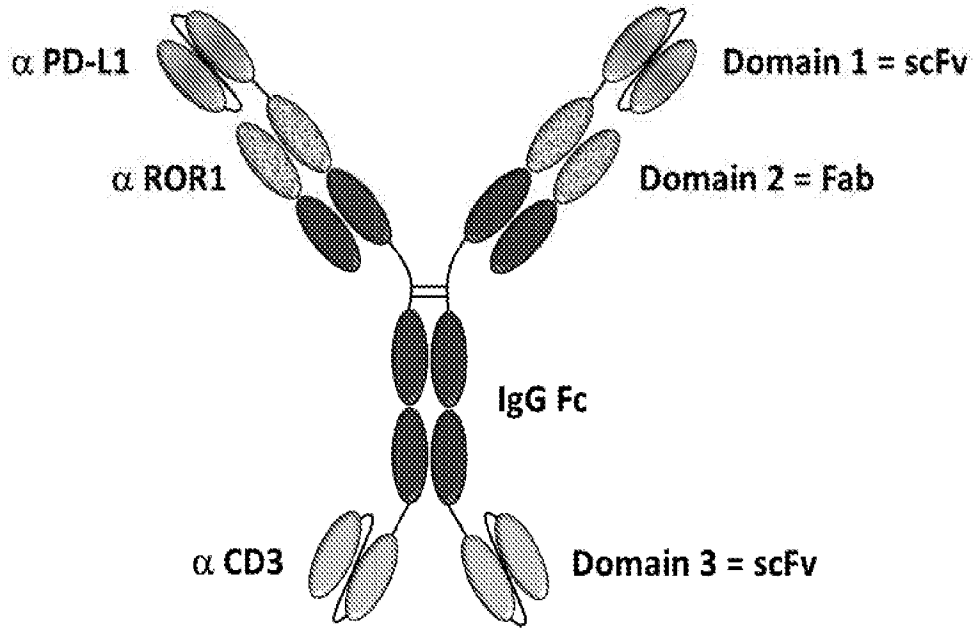


FIGURE 3

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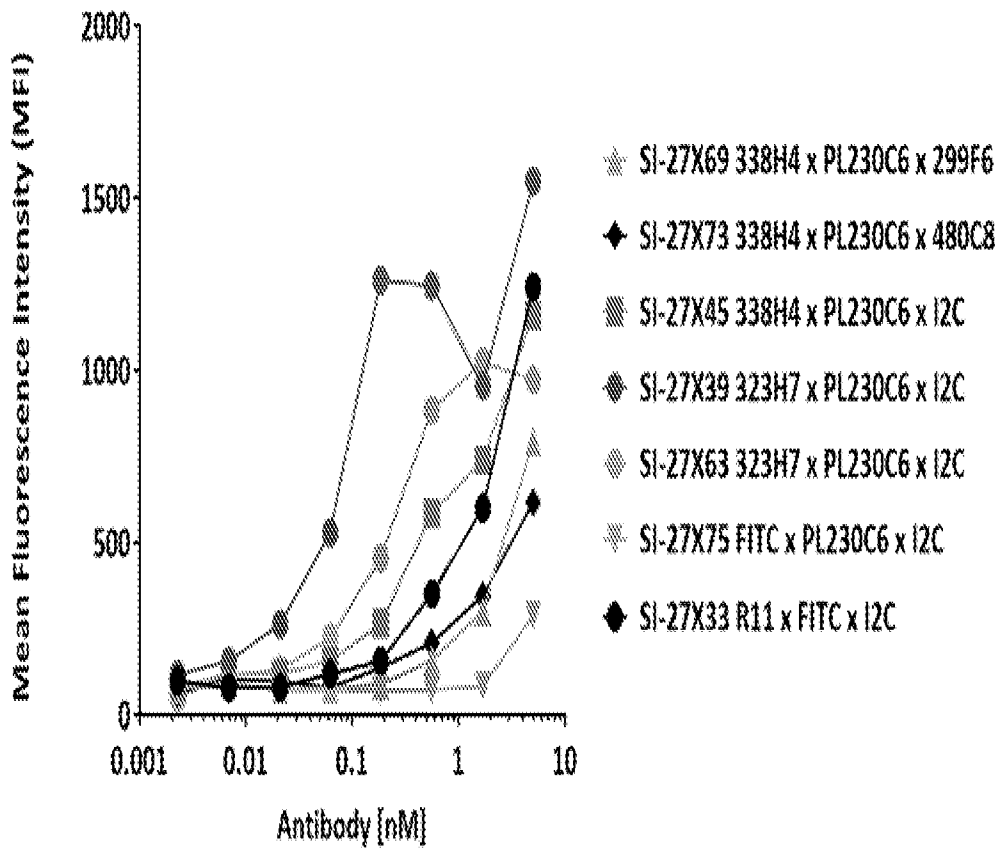


FIGURE 4

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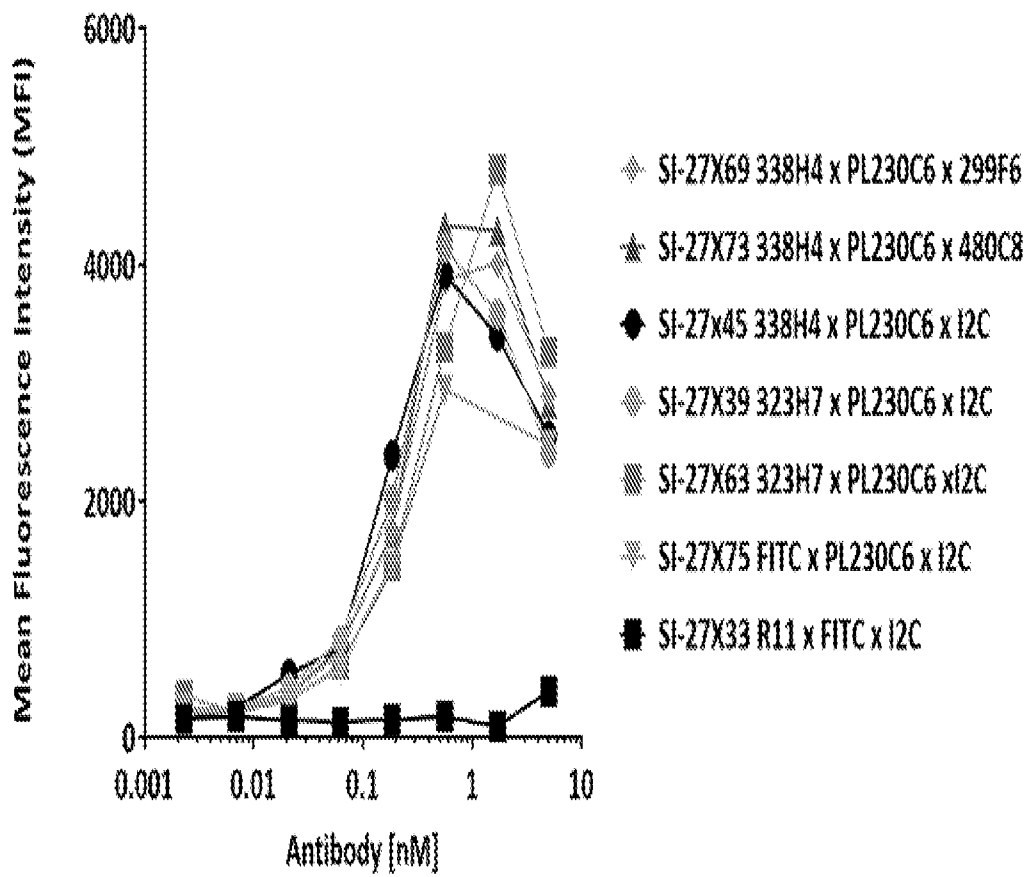


FIGURE 5

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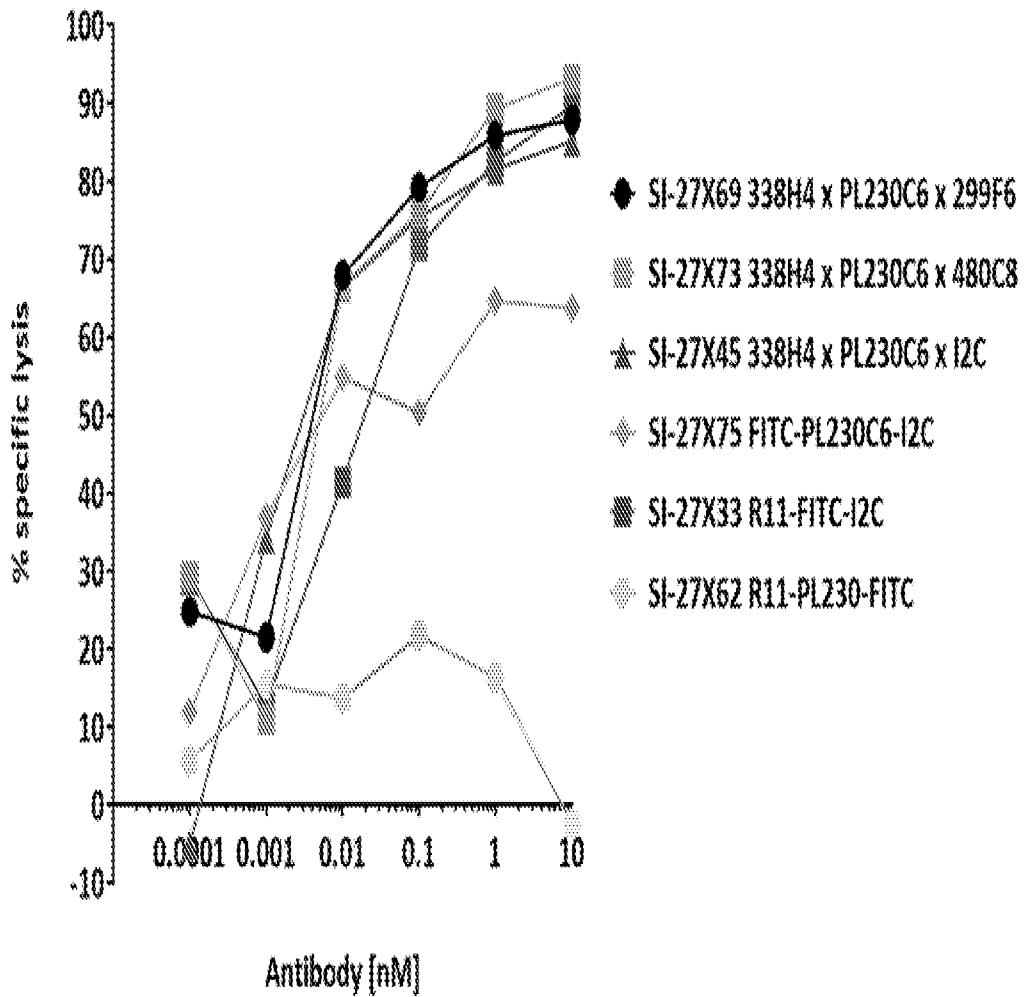


FIGURE 6

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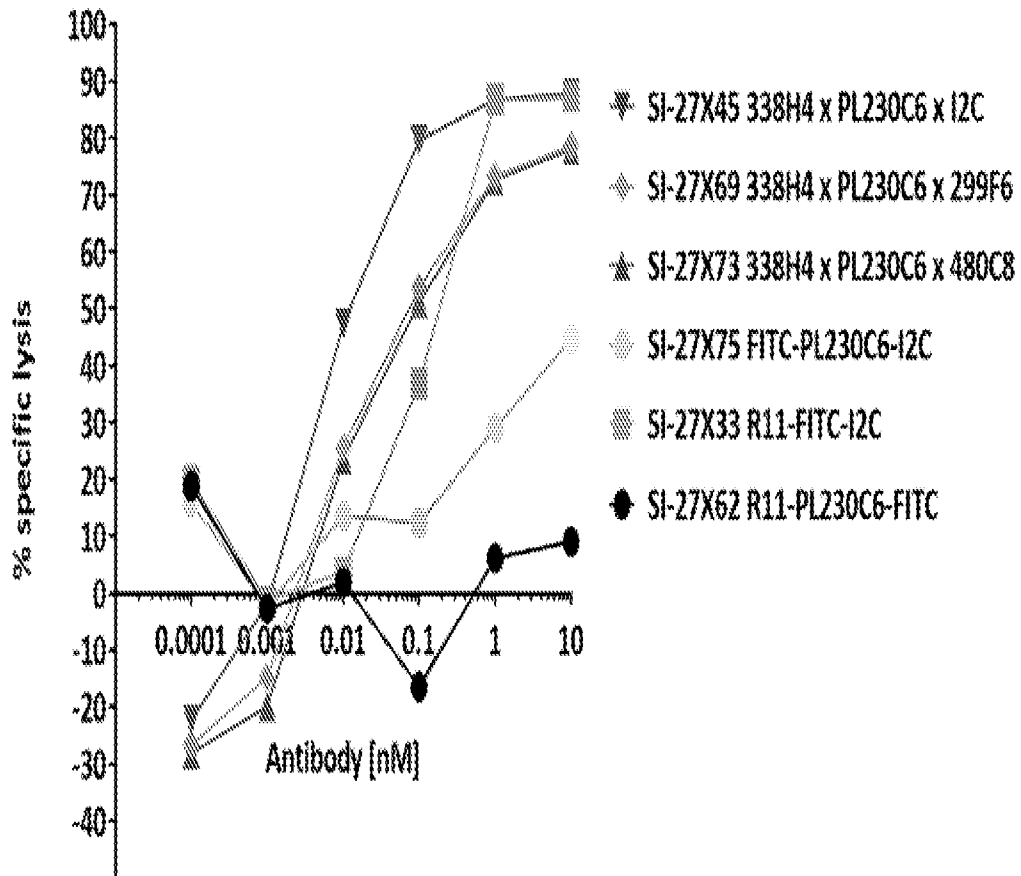


FIGURE 7

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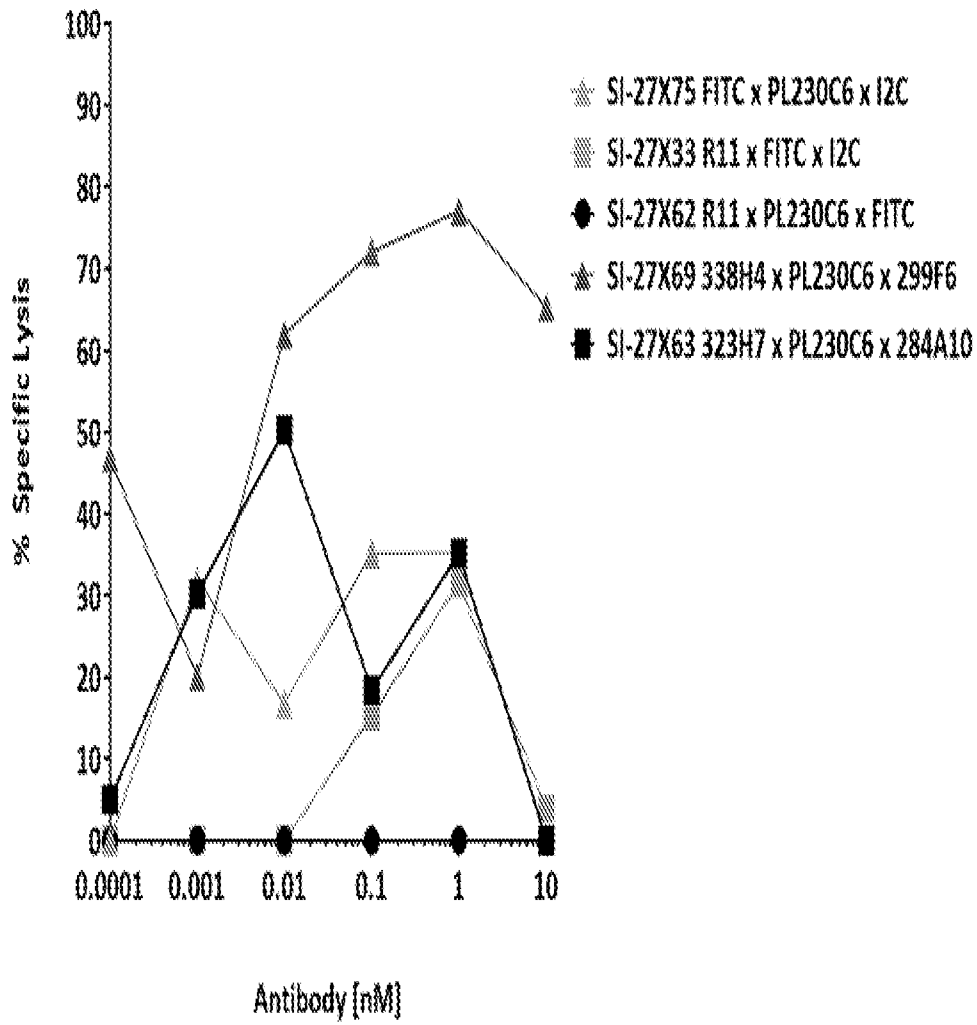


FIGURE 8

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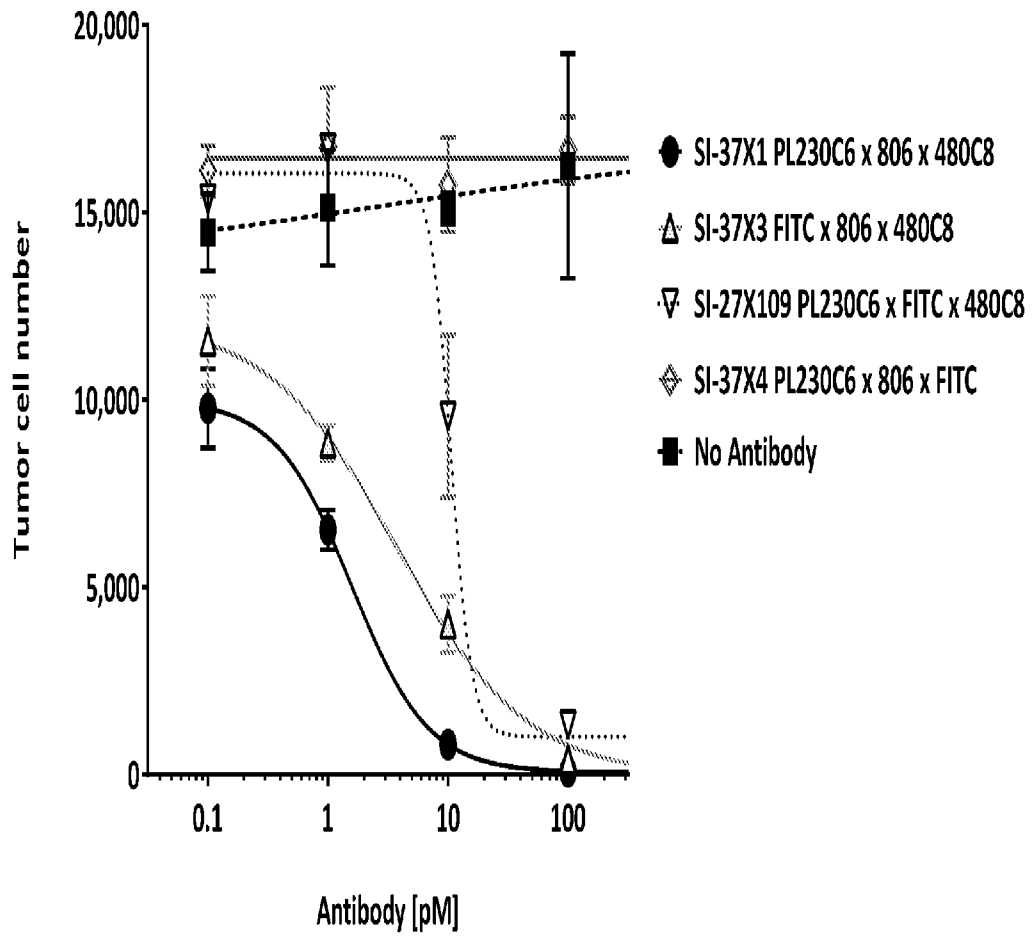


FIGURE 9

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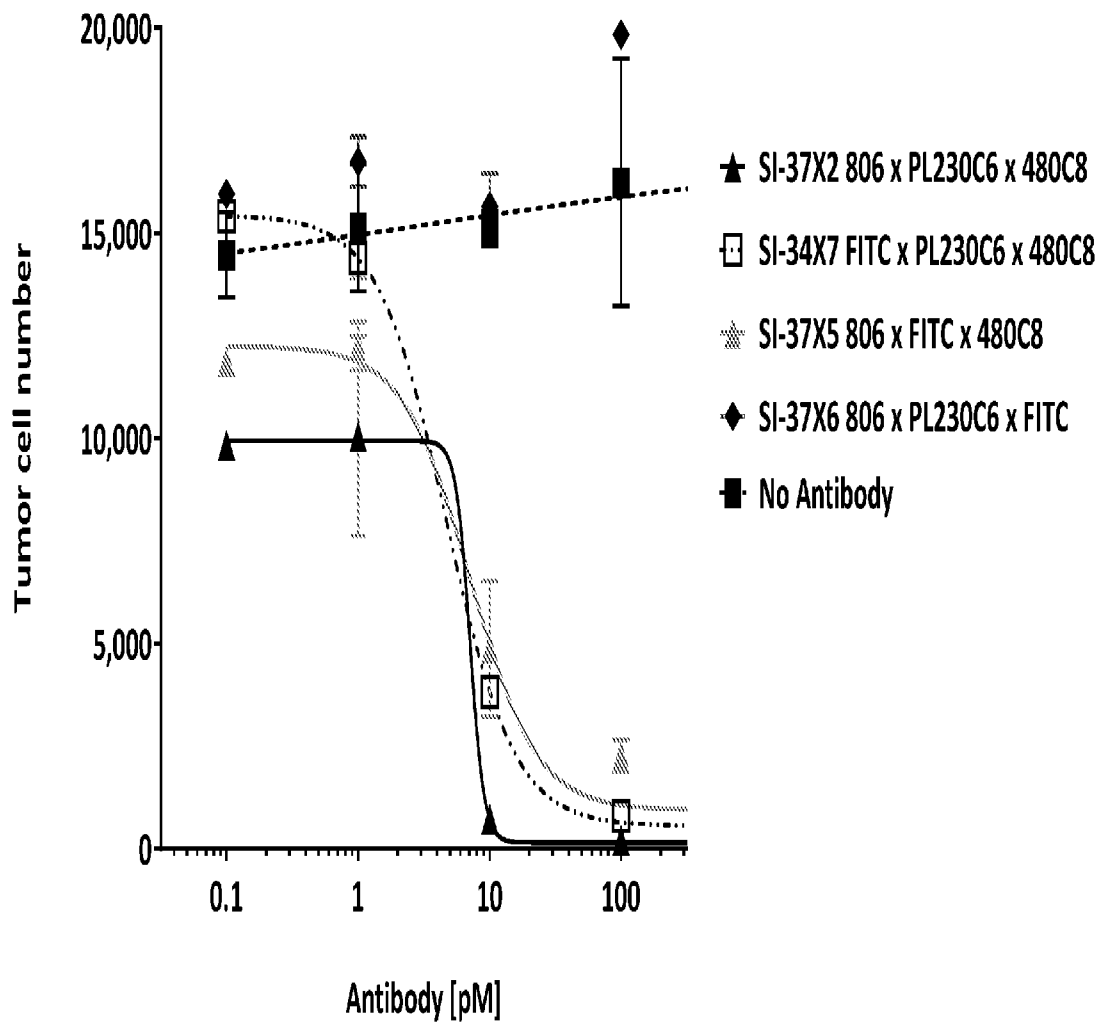


FIGURE 10

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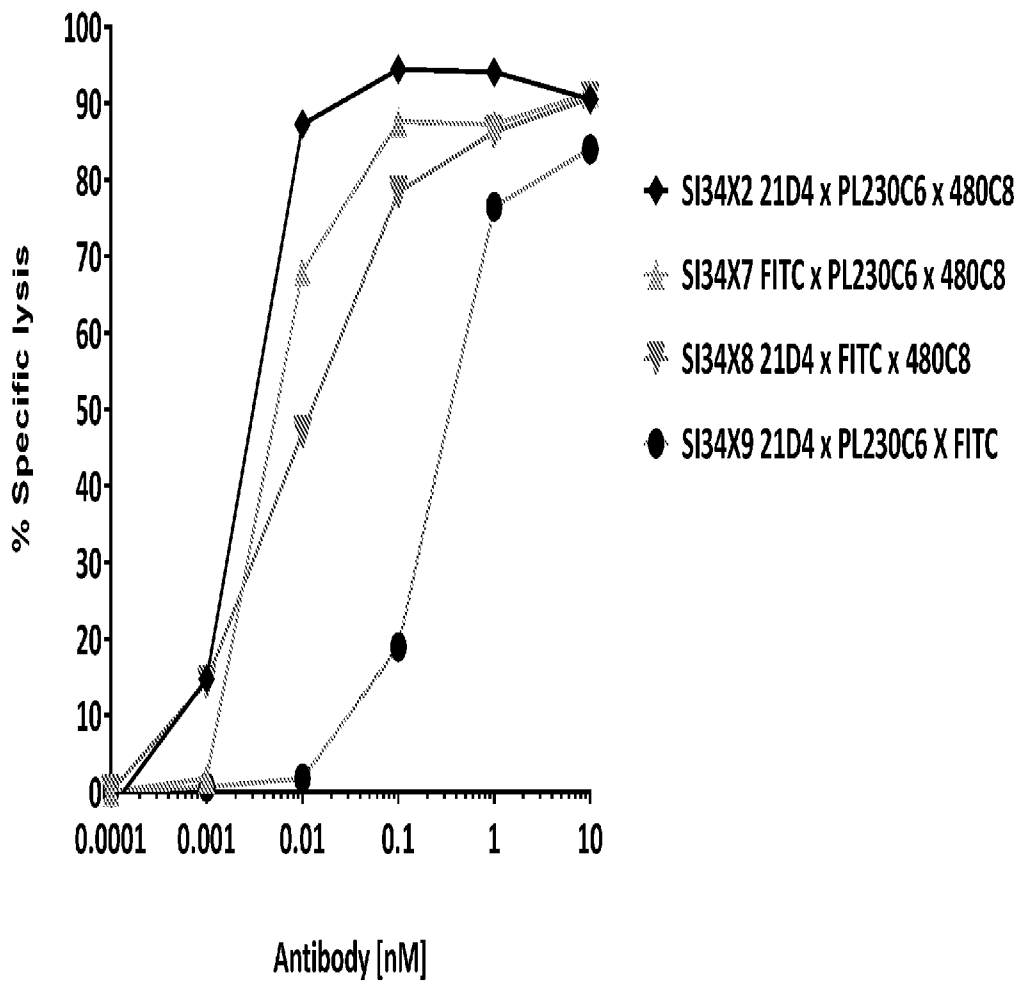


FIGURE 11