TRIVALENT MULTISPECIFIC BINDING MOLECULES AND METHODS OF USE THEREOF

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. provisional application no. 63/567,615, filed March 20, 2024, the contents of which are incorporated herein in their entirety by reference thereto.

2. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML Sequence Listing, created on March 19, 2025, is named RGN-032WO_SL.xml and is 158,673 bytes in size.

3. BACKGROUND

[0003] Numerous biological therapeutics have been developed for the prevention and/or treatment of diseases including but not limited to proliferative diseases (e.g., cancers), infectious diseases (e.g., viral, bacterial, fungal, or protozoal infections), and inflammatory diseases (e.g., Crohn's disease). Multispecific antigen-binding molecules that bind to both a target antigen (e.g. a cancerous antigen or an infectious antigen) and an immune cell antigen (e.g., a T cell antigen) are promising biological therapeutics, useful for triggering an immune response against the ailment associated with the target antigen, often with the goal of selective destruction of the cells expressing the target antigen. For example, multispecific antibody therapeutics have been actively pursued as means to induce an immune response specifically against tumor cells expressing a particular antigen.

[0004] A common use for multispecific antibodies is the activation of immune cells (e.g., T cells) by an antibody containing one arm targeting a cancer cell (e.g., tumor-associated antigens, TAAs) and another arm targeting a T cell (e.g., CD3). However, the task of generating multispecific molecules suitable for treatment provides several technical challenges related to toxicity, as TAAs are typically expressed on normal cells as well as tumor cells. Antigens specifically expressed by tumor cells are optimal for these therapeutics, however such tumor-specific antigens are often present at a low density on the

cancer cells, for example by virtue of being expressed as HLA-bound peptides, posing difficulties for effective targeting.

[0005] Thus, there is a need for multispecific antigen-binding molecules that will trigger an immune response with high specificity and efficacy even for low copy number target antigens such as HLA-bound peptide antigens.

4. SUMMARY

[0006] The present disclosure relates to multispecific binding molecules (MBMs) comprising two T cell antigen (TCA) targeting moieties flanking an antigen targeting moiety (e.g., tumor antigen, low-density antigen, and/or HLA-bound peptide antigen targeting moiety), often referred to herein for convenience as "primary MBMs". As disclosed herein, it was surprisingly found that this MBM configuration significantly enhanced T cell activation and decreased immunogenicity relative to other formats, notably including where the antigen targeted by the antigen targeting moiety was a low-density antigen such as an HLA-bound peptide antigen. Primary MBMs described herein are useful in various therapeutic and prophylactic methods where stimulation of the immune system of the host is beneficial, including, for example, in treatment of proliferative disorders such as cancer.

[0007] In some cases, primary MBMs may be administered for therapeutic applications in combination (together or separately) with one or more MBMs comprising a targeting moiety that binds to a tumor antigen and a targeting moiety that binds to a costimulatory molecule such as CD28 (referred to herein for convenience as "secondary MBMs"). Exemplary secondary MBMs that can be included in combination with a primary MBM are described in Section 6.3. Administration may further include additional therapeutics, for example an immunotherapeutic such as a checkpoint inhibitor.

[0008] Exemplary TCA targeting moieties that can be used in the primary MBMs of the disclosure are described in Section 6.5.

[0009] Exemplary antigen targeting moieties at can be used in the primary MBMs of the disclosure are described in Section 6.6, including tumor antigen targeting moieties (Section 6.6.1), low-density antigen targeting moieties (Section 6.6.2), and HLA-bound peptide antigen targeting moieties (Section 6.6.3).

[0010] In some cases, primary MBMs disclosed herein comprise a multimerization moiety such as an Fc region. Exemplary Fc regions that can be used in the primary MBMs of the disclosure are described in Section 6.8, including Fc regions comprising Fc domains with altered effector function (Section 6.8.1.1) and Fc domains that confer heterodimerization capability to the MBM (Section 6.8.1.2).

[0011] Linkers that can be used to connect different components of the primary MBMs of the disclosure are described in Section 6.7.

[0012] Various exemplary configurations of the primary MBMs of the disclosure are described in specific embodiments 1 to 87, *infra*.

[0013] The disclosure further provides nucleic acids encoding the primary MBMs of the disclosure. The nucleic acids encoding the primary MBMs can be a single nucleic acid (*e.g.*, a vector encoding all polypeptide chains of a primary MBMs) or a plurality of nucleic acids (*e.g.*, two or more vectors encoding the different polypeptide chains of a primary MBMs). The disclosure further provides host cells and cell lines engineered to express the nucleic acids and primary MBMs of the disclosure. The disclosure further provides methods of producing a primary MBM of the disclosure. Exemplary nucleic acids, host cells, and cell lines, and methods of producing a primary MBM are described in Section 6.9 and specific embodiments 88 to 91, *infra*.

[0014] The disclosure further provides pharmaceutical compositions comprising the primary MBMs of the disclosure, optionally in addition to one or more secondary MBMs (*e.g.*, as described in Section 6.3). Exemplary pharmaceutical compositions are described in Section 6.10 and specific embodiments 95 to 102, *infra*.

[0015] Further provided herein are methods of using the primary MBMs and the pharmaceutical compositions of the disclosure, *e.g.,* for treating or preventing cancer. Exemplary methods are described in 6.11 and include therapeutic methods and prophylactic methods. The primary MBMs of the disclosure are useful in combination therapy, for example in combination with a secondary MBM (*e.g.*, as described in Section 6.3). Exemplary combination therapy methods are disclosed in Section 6.11.1. Specific embodiments of the methods of treatment of the disclosure are described in specific embodiments 103 to 114, *infra*.

5. BRIEF DESCRIPTION OF THE FIGURES

[0016] **FIGS. 1A-1D** show exemplary structures of multispecific binding molecules (MBMs) of the disclosure. **FIG. 1A** shows the structural components of MBMs - TCA TM: T cell antigen targeting moiety, Antigen TM: antigen targeting moiety. **FIG. 1B** shows the structure of an MBM comprising two half antibodies. The first half antibody comprises, from N- to C-terminus, a T cell antigen (TCA) targeting moiety in Fab format – optional linker – antigen targeting moiety in scFv format – optional linker – Fc domain, and the second half antibody comprises from N- to C-terminus, a TCA targeting moiety Fab – optional linker – Fc domain that interacts with the Fc domain of the first half antibody to form an Fc region. **FIG. 1C** shows the structure of an MBM comprising a first half antibody, which comprises, from N- to C-terminus, a TCA targeting moiety scFv – optional linker – antigen targeting moiety Fab – optional linker – Fc domain, and a second half antibody comprising, from N- to C-terminus, a TCA targeting moiety scFv – optional linker – Fc domain that interacts with the Fc domain of the first half antibody to form an Fc region. **FIG. 1D** shows the structure of an MBM comprising a first half antibody, which comprises, from N- to C-terminus, a TCA targeting moiety Fab – optional linker – antigen targeting moiety Fab – optional linker – Fc domain, and a second half antibody comprising from N- to C-terminus, a TCA targeting moiety Fab – optional linker – Fc domain that interacts with the Fc domain of the first half antibody to form an Fc region. Although illustrated as having a particular N- to C-terminal orientation in FIGs. 1B, 1C, and 1D, an scFv can be any scFv, e.g., as described in Section 6.4.1 (e.g., may have orientation VL-linker-VH or VH-linker-VL). Although not depicted, asymmetrical constructs may include one or more Fc heterodimerization variants (e.g., as described in Section 6.8.1.2), such as knob and hole mutations and/or “star” mutations.

[0017] **FIGS. 2A-2K** depict structures of exemplary multispecific binding molecules evaluated in the examples of the present disclosure. **FIGS. 2A-2E** depict structures of MBMs comprising an antigen targeting moiety that specifically binds to HLA-A2-PRAME(425-433). **FIGS. 2F-2H** depict structures of MBMs comprising an antigen targeting moiety that specifically binds to HLA-A2-MAGE-A4(230-239). **FIGS. 2I-2K** depict structures of MBMs comprising a targeting moiety that specifically binds to HLA-A2-PRAME(425-433).

[0018] **FIGS. 3A-3B** show differences in cytotoxicity exerted by exemplary MBMs against target cells. **FIG. 3A** displays the exemplary MBM structures and descriptions associated

with the symbols used in FIG. 3B. **FIG. 3B** is a graph showing the percent viability of wild-type OVCAR3 cells treated with an MBM over a range of concentrations in the presence of anti-PD1 and EGFRxCD28 antibodies.

[0019] FIGS. 4A-4M show differences in potency of exemplary MBMs against wild-type OVCAR3, wild-type SK-MEL-24, and wild-type COLO205 cell lines. **FIG. 4A** displays the exemplary MBM structures and descriptions associated with the symbols used in FIGS. 4B-4M. **FIG. 4B** is a graph showing the percent viability of wild-type OVCAR3 cells treated with different concentrations of an MBM only. **FIG. 4C** is a graph showing the percent viability of wild-type SK-MEL-24 cells treated with different concentrations of an MBM only. **FIG. 4D** is a graph showing the percent viability of wild-type COLO205 cells treated with different concentrations of an MBM only. **FIG. 4E** is a graph showing the percent viability of wild-type OVCAR3 cells treated with different concentrations of an MBM in the presence of anti-PD1 and EGFRxCD28 antibodies. **FIG. 4F** is a graph showing the percent viability of wild-type SK-MEL-24 cells treated with different concentrations of an MBM in the presence of anti-PD1 and EGFRxCD28 antibodies. **FIG. 4G** is a graph showing the percent viability of wild-type COLO205 cells treated with different concentrations of an MBM in the presence of anti-PD1 and EGFRxCD28 antibodies. **FIG. 4H** is a graph showing T cell activation following treatment with different concentrations of an MBM only in the presence of wild-type OVCAR3 cells. **FIG. 4I** is a graph showing T cell activation following treatment with different concentrations of an MBM only in the presence of wild-type SK-MEL-24cells. **FIG. 4J** is a graph showing T cell activation following treatment with different concentrations of an MBM only in the presence of wild-type COLO205 cells. **FIG. 4K** is a graph showing T cell activation following treatment with different concentrations of an MBM, together with an anti-PD1 antibody and an EGFRxCD28 antibody, in the presence of wild-type OVCAR3 cells. **FIG. 4L** is a graph showing T cell activation following treatment with different concentrations of an MBM, together with an anti-PD1 antibody and an EGFRxCD28 antibody, in the presence of wild-type SK-MEL-24 cells. **FIG. 4M** is a graph showing T cell activation following treatment with different concentrations of an MBM, together with an anti-PD1 antibody and an EGFRxCD28 antibody, in the presence of wild-type COLO205 cells.

[0020] FIG. 5 shows the reactivity to pre-existing ADA associated with two MBMs, a positive control antibody construct (REGN9930), and a negative control antibody construct (REGN8503).

[0021] FIGS. 6A-6E show differences in potency of exemplary MBMs against wild-type A375 cells. **FIG. 6A** displays the exemplary MBM structures and descriptions associated with the symbols used in FIGS. 6B-6E. **FIG. 6B** is a graph showing the percent viability of wild-type A375 cells treated with different concentrations of an MBM only. **FIG. 6C** is a graph showing the percent viability of wild-type A375 cells treated with different concentrations of an MBM in the presence of anti-PD1 (REGN2810) and EGFRxCD28 (REGN7075) antibodies. **FIG. 6D** is a graph showing T cell activation following treatment with different concentrations of an MBM only in the presence of wild-type A375 cells. **FIG. 6E** is a graph showing T cell activation following treatment with different concentrations of an MBM, together with an anti-PD1 antibody (REGN2810) and an EGFRxCD28 antibody (REGN7075), in the presence of wild-type A375 cells.

[0022] FIGS. 7A-7D show potency of exemplary MBMs against HLA-A2 PRAME (425-433) overexpressing cells and HLA-A2 PRAME (425-433) knockout cells. **FIG. 7A** displays the exemplary MBM structures and descriptions associated with the symbols used in FIGS. 7B-7D. **FIG. 7B** is a graph showing the percent viability of HLA-A2 PRAME (425-433) overexpressing A375 cells treated with a range of MBM concentrations. **FIG. 7C** is a graph showing the percent viability of HLA-A2 PRAME (425-433) knockout A375 cells treated with a range of MBM concentrations in the presence of anti-PD1 (REGN2810) and EGFRxCD28 (REGN7075) antibodies. **FIG. 7D** is a graph showing T cell activation following treatment with a range of MBM concentrations, together with anti-PD1 (REGN2810) and EGFRxCD28 (REGN7075) antibodies, in the presence of HLA-A2 PRAME (425-433) overexpressing A375 cells.

[0023] FIGS. 8A-8C show binding activity of exemplary MBMs against A375 cells overexpressing HLA-A2 PRAME (425-433) and Jurkat E6 cells. **FIG. 8A** displays the exemplary MBM structures and descriptions associated with the symbols used in FIGS. 8B-8C. **FIG. 8B** is a graph showing the binding profiles of MBMs and control constructs against A375 cells overexpressing HLA-A2 PRAME (425-433). **FIG. 8C** is a graph showing the binding profiles of MBMs and control constructs against Jurkat E6 cells.

6. DETAILED DESCRIPTION

6.1. Definitions

[0024] **About, Approximately:** The terms "about", "approximately" and the like are used throughout the specification in front of a number to show that the number is not necessarily exact (*e.g.*, to account for fractions, variations in measurement accuracy and/or precision, timing, etc.). It should be understood that a disclosure of "about X" or "approximately X" where X is a number is also a disclosure of "X." Thus, for example, a disclosure of an embodiment in which one sequence has "about X% sequence identity" to another sequence is also a disclosure of an embodiment in which the sequence has "X% sequence identity" to the other sequence.

[0025] **And, or:** Unless indicated otherwise, an "or" conjunction is intended to be used in its correct sense as a Boolean logical operator, encompassing both the selection of features in the alternative (A or B, where the selection of A is mutually exclusive from B) and the selection of features in conjunction (A or B, where both A and B are selected). In some places in the text, the term "and/or" is used for the same purpose, which shall not be construed to imply that "or" is used with reference to mutually exclusive alternatives.

[0026] **Antibody:** The term "antibody" as used herein refers to a polypeptide (or set of polypeptides) of the immunoglobulin family that is capable of binding an antigen non-covalently, reversibly and specifically. For example, a naturally occurring "antibody" of the IgG type is a tetramer comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain (abbreviated herein as CL). The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The

constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system. The term "antibody" includes, but is not limited to, monoclonal antibodies, human antibodies, humanized antibodies, camelized antibodies, chimeric antibodies, bispecific or multispecific antibodies and anti-idiotypic (anti-id) antibodies. The antibodies can be of any isotype/class (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY) or subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2). Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding domain or amino-terminus of the antibody. The N-terminus is a variable region and at the C-terminus is a constant region; the CH3 and CL domains represent the carboxy-terminus of the heavy and light chain, respectively, of natural antibodies. For convenience, and unless the context dictates otherwise, the reference to an antibody also refers to antibody fragments as well as engineered antibodies that include non-naturally occurring antigen-binding domains and/or antigen-binding domains having non-native configurations.

[0027] **Antigen-binding Domain:** The term "antigen-binding domain" or "ABD" as used herein refers to the portion of a targeting moiety that is capable of specific, non-covalent, and reversible binding to a target molecule.

[0028] **Associated:** The term "associated" in the context of a multispecific binding molecule refers to a functional relationship between two or more polypeptide chains or portions of a polypeptide chain. In particular, the term "associated" means that two or more polypeptides are associated with one another, *e.g.,* non-covalently through molecular interactions or covalently through one or more disulfide bridges or chemical cross-linkages, so as to produce a functional multispecific binding molecule. Examples of associations that might be present in a multispecific binding molecule of the disclosure include (but are not limited to)

associations between homodimeric or heterodimeric Fc domains in an Fc region, associations between VH and VL regions in a Fab or scFv, associations between CH1 and CL in a Fab, and associations between CH3 and CH3 in a domain substituted Fab.

[0029] Bivalent: The term "bivalent" as used herein in the context of a multispecific binding molecule (MBM) refers to an MBM that has two antigen-binding domains. The domains can be the same or different. Accordingly, a bivalent antigen-binding molecule can be monospecific or bispecific.

[0030] Cancer: The term "cancer" refers to a disease characterized by the uncontrolled (and often rapid) growth of aberrant cells. Cancer cells can spread locally or through the bloodstream and lymphatic system to other parts of the body. Examples of various cancers are described herein and include but are not limited to, breast cancer, prostate cancer, ovarian cancer, cervical cancer, skin cancer, pancreatic cancer, colorectal cancer, renal cancer, liver cancer, brain cancer, adrenal gland cancer, autonomic ganglial cancer, biliary tract cancer, bone cancer, endometrial cancer, eye cancer, fallopian tube cancer, genital tract cancers, large intestinal cancer, cancer of the meninges, oesophageal cancer, peritoneal cancer, pituitary cancer, penile cancer, placental cancer, pleura cancer, salivary gland cancer, small intestinal cancer, stomach cancer, testicular cancer, thymus cancer, thyroid cancer, upper aerodigestive cancers, urinary tract cancer, vaginal cancer, vulva cancer, lymphoma, leukemia, lung cancer and the like.

[0031] Complementarity Determining Region or CDR: The terms "complementarity determining region" or "CDR," as used herein, refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. In general, there are three CDRs in each heavy chain variable region (CDR-H1, CDR-H2, CDR-H3) and three CDRs in each light chain variable region (CDR1-L1, CDR-L2, CDR-L3). Exemplary conventions that can be used to identify the boundaries of CDRs include, *e.g.,* the Kabat definition, the Chothia definition, the ABD definition and the IMGT definition. See, *e.g.,* Kabat, 1991, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (Kabat numbering scheme); Al-Lazikani *et al.*, 1997, J. Mol. Biol. 273:927-948 (Chothia numbering scheme); Martin *et al.*, 1989, Proc. Natl. Acad. Sci. USA 86:9268-9272 (ABD numbering scheme); and Lefranc *et al.*, 2003, Dev. Comp. Immunol. 27:55-77 (IMGT numbering scheme). For example, for classic formats, under Kabat, the

CDR amino acid residues in the heavy chain variable domain (VH) are numbered 31-35 (CDR-H1), 50-65 (CDR- H2), and 95-102 (CDR-H3); and the CDR amino acid residues in the light chain variable domain (VL) are numbered 24-34 (CDR-L1), 50-56 (CDR-L2), and 89-97 (CDR-L3). Under Chothia, the CDR amino acids in the VH are numbered 26-32 (CDR-H1), 52-56 (CDR-H2), and 95-102 (CDR-H3); and the amino acid residues in VL are numbered 26-32 (CDR-L1), 50-52 (CDR-L2), and 91-96 (CDR-L3). By combining the CDR definitions of both Kabat and Chothia, the CDRs consist of amino acid residues 26-35 (CDR-H1), 50-65 (CDR-H2), and 95-102 (CDR-H3) in human VH and amino acid residues 24-34 (CDR-L1), 50-56 (CDR-L2), and 89-97 (CDR-L3) in human VL. Under IMGT the CDR amino acid residues in the VH are numbered approximately 26-35 (CDR-H1), 51-57 (CDR-H2) and 93-102 (CDR-H3), and the CDR amino acid residues in the VL are numbered approximately 27-32 (CDR-L1), 50-52 (CDR-L2), and 89-97 (CDR-L3) (numbering according to "Kabat"). Under IMGT, the CDR regions of an antibody can be determined using the program IMGT/DomainGap Align. Public databases are available for identifying CDR sequences within an antibody.

[0032] **Costimulatory Molecule:** The term "costimulatory molecule" refers to a molecule present on the surface of a T cell, other than the T cell receptor, that can contribute to the activation of the T cell, generally via what is known as a "costimulatory signal" or "signal 2". Example costimulatory molecules include: CD28, ICOS, CD27, HVEM, LIGHT, CD40, 4-1BB, OX40, GITR, CD30, TIM1, SLAM, CD2, and CD226. Costimulatory molecules and costimulatory signaling are described in detail in Chen L, Flies DB. 2013 Nat Rev Immunol. 13(4):227-242, incorporated herein by reference.

[0033] **EC50:** The term "EC50" refers to the half maximal effective concentration of a molecule, such as a multispecific binding molecule, which induces a response halfway between the baseline and maximum after a specified exposure time. The EC50 essentially represents the concentration of a molecule where 50% of its maximal effect is observed. Thus, reduced or weaker binding is observed with an increased EC50, or half maximal effective concentration value.

[0034] **Epitope:** An epitope (or "antigenic determinant") is a portion of an antigen (e.g., polypeptide antigen) recognized by an antibody or other antigen-binding moiety as described herein. An epitope can be linear or conformational.

[0035] **Fab:** The term "Fab" in the context of a multispecific binding molecule of the disclosure of the disclosure refers to a pair of polypeptide chains, the first comprising a variable heavy (VH) domain of an antibody N-terminal to a first constant domain (referred to herein as C1), and the second comprising variable light (VL) domain of an antibody N-terminal to a second constant domain (referred to herein as C2) capable of pairing with the first constant domain. In a native antibody, the VH is N-terminal to the first constant domain (CH1) of the heavy chain and the VL is N-terminal to the constant domain of the light chain (CL). The Fabs of the disclosure can be arranged according to the native orientation or include domain substitutions or swaps that facilitate correct VH and VL pairings. For example, it is possible to replace the CH1 and CL domain pair in a Fab with a CH3-domain pair to facilitate correct modified Fab-chain pairing in heterodimeric molecules. It is also possible to reverse CH1 and CL, so that the CH1 is attached to VL and CL is attached to the VH, a configuration generally known as Crossmab (a type of "domain exchanged" arrangement). Alternatively, or in addition to, the use of substituted or swapped constant domains, correct chain pairing can be achieved by the use of universal light chains that can pair with both variable regions of a heterodimeric multispecific binding molecule of the disclosure. The term "Fab" encompasses single chain Fabs.

[0036] **Fc Domain and Fc Region:** The term "Fc domain" refers to a portion of the heavy chain that pairs with the corresponding portion of another heavy chain. The term "Fc region" refers to the region of antibody-based binding molecules formed by association of two heavy chain Fc domains. The two Fc domains within the Fc region may be the same or different from one another. In a native antibody the Fc domains are typically identical, but one or both Fc domains might advantageously be modified to allow for heterodimerization, *e.g.,* via a knob-in-hole interaction and/or for purification, *e.g.*, via star mutations.

[0037] **Fv:** The term "Fv" refers to the minimum antibody fragment derivable from an immunoglobulin that contains a complete target recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in a tight, noncovalent association (VH-VL dimer). It is in this configuration that the three CDRs of each variable domain interact to define a target binding site on the surface of the VH-VL dimer. Often, the six CDRs confer target binding specificity to the antibody. However, in some instances even a single variable domain (or half of an Fv comprising only three CDRs specific for a target)

can have the ability to recognize and bind target. The reference to a VH-VL dimer herein is not intended to convey any particular configuration. When present on a single polypeptide chain (*e.g.*, a scFv), the VH and be N- terminal or C-terminal to the VL.

[0038] **Half Antibody:** The term "half antibody" refers to a molecule that comprises at least one Fc domain and can associate with another molecule comprising an Fc through, *e.g.*, a disulfide bridge or molecular interactions. A half antibody can be composed of one polypeptide chain or more than one polypeptide chains (*e.g.*, the two polypeptide chains of a Fab). An example of a half antibody is a molecule comprising a heavy and light chain of an antibody (e.g., an IgG antibody). Another example of a half antibody is a molecule comprising a first polypeptide comprising a VL domain and a CL domain, and a second polypeptide comprising a VH domain, a CH1 domain, a hinge domain, a CH2 domain, and a CH3 domain, wherein said VL and VH domains form an ABD. Yet another example of a half antibody is a polypeptide comprising an scFv domain, a CH2 domain and a CH3 domain.

[0039] A half antibody suitable for incorporating into a primary MBM or secondary MBM of the disclosure might include one or more than one targeting moiety. Half antibodies with one targeting moiety are described herein as "Type 1" half antibodies for convenience. Half antibodies with more than one targeting moiety are described herein as "Type 2" half antibodies for convenience.

[0040] In some embodiments, a half antibody comprises a) a first polypeptide chain comprising (in N- to C-terminal order) a VH domain, a CH1 domain, a CH2 domain, and a CH3 domain, and b) a second polypeptide chain comprising (in N- to C-terminal order) a VL domain and a CL domain (as depicted in, e.g., FIG. 1B or 1D, right half antibody). This half antibody is referred herein to as a "Type 1A" half antibody for convenience.

[0041] In some embodiments, a half antibody comprises a polypeptide chain comprising (in N- to C-terminal order) an scFv (e.g., VL-linker-VH or VH-linker-VL), a CH2 domain, and a CH3 domain (as depicted in, e.g., FIG. 1C, right half antibody). This half antibody is referred herein to as a "Type 1B" half antibody for convenience.

[0042] In some embodiments, a half antibody comprises a) a first polypeptide chain comprising (in N- to C-terminal order) a VH domain, a CH1 domain, an scFv (e.g., VL-linker-VH or VH-linker-VL), a CH2 domain, and a CH3 domain, and b) a second polypeptide chain

comprising (in N- to C-terminal order) a VL domain and a CL domain (as depicted in, e.g., FIG. 1B, left half antibody). This half antibody is referred herein to as a "Type 2A" half antibody for convenience.

[0043] In some embodiments, a half antibody comprises a) a first polypeptide comprising (in N- to C-terminal order) an scFv (e.g., VL-linker-VH or VH-linker-VL), a VH domain, a CH1 domain, a CH2 domain, and a CH3 domain, and b) a second polypeptide comprising (in N- to C-terminal order) a VL domain and a CL domain (as depicted in, e.g., FIG. 1C, left half antibody). This half antibody is referred herein to as a "Type 2B" half antibody for convenience.

[0044] In some embodiments, a half antibody comprises a) a first polypeptide comprising (in N- to C-terminal order) a first VH domain, a first CH1 domain, a second VH domain, a second CH1 domain, a CH2 domain, and a CH3 domain, b) a second polypeptide comprising (in N- to C-terminal order) a first VL domain and a first CL domain, and c) a third polypeptide comprising (in N- to C-terminal order) a second VL domain and a second CL domain (as depicted in, e.g., FIG. 1D, left half antibody). This half antibody is referred herein to as a "Type 2C" half antibody for convenience.

[0045] The term "half antibody" is intended for descriptive purposes only and does not connote a particular configuration or method of production. Descriptions of a half antibody as a "first" half antibody, a "second" half antibody, a "left" half antibody, a "right" half antibody or the like are merely for convenience and descriptive purposes.

[0046] **Host Cell or Recombinant Host Cell**: The terms "host cell" and "recombinant host cell" as used herein refer to a cell that has been genetically engineered, *e.g.*, through introduction of a heterologous nucleic acid. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications can occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. A host cell can carry the heterologous nucleic acid transiently, *e.g.,* on an extrachromosomal heterologous expression vector, or stably, *e.g.,* through integration of the heterologous nucleic acid into the host cell genome. For purposes of expressing a multispecific binding molecule, a host cell can be a cell line of mammalian origin or mammalian-like characteristics, such as

monkey kidney cells (COS, *e.g.,* COS-1 , COS- 7), HEK293, baby hamster kidney (BHK, *e.g.*, BHK21), Chinese hamster ovary (CHO), NSO, PerC6, BSC-1 , human hepatocellular carcinoma cells (*e.g.*, Hep G2), SP2/0, HeLa, Madin-Darby bovine kidney (MDBK), myeloma and lymphoma cells, or derivatives and/or engineered variants thereof. The engineered variants include, *e.g.,* glycan profile modified and/or site-specific integration site derivatives.

[0047] **Immune Response:** The term "immune response" refers to an integrated bodily response to an antigen and preferably refers to a cellular immune response or a cellular as well as a humoral immune response. The immune response may be protective (also "preventive" or "prophylactic") and/or therapeutic. The terms "inducing an immune response," "eliciting an immune response," and the like, as used herein, can indicate that there was no immune response against a particular antigen before administration of a particular composition (*e.g.*, an MBM of the disclosure), but it may also indicate that there was a certain level of immune response against a particular antigen before such administration, and that after induction the immune response is enhanced. Thus, "inducing an immune response" also includes "enhancing an immune response". Preferably, after inducing an immune response in a subject, said subject is protected from developing a disease (*e.g.*, cancer) or the disease condition is ameliorated (*e.g.*, tumor reduction, reduction in cancer cell number, etc.) by inducing an immune response. For example, an immune response against a tumor antigen may be induced in a patient having cancer or in a subject at risk of developing a cancer. Inducing an immune response in this case may mean that the disease condition of the subject is ameliorated, that the subject does not develop metastases, or that the subject at risk of developing cancer does not develop cancer.

[0048] **Low-density Antigen**: The term "low-density antigen" as used herein refers to a polypeptide antigen present on the surface of a target cell or population of target cells (either as a full-length polypeptide or presented as an HLA-bound peptide) at an average of no more than 5000 copies per cell. In some embodiments, a low-density antigen is present at an average of no more than 4000, no more than 3000, no more than 2000, no more than 1000, no more than 900, no more than 800, no more than 700, no more than 600, no more than 500, no more than 400, no more than 300, no more than 200, no more than 100, or no more than 50 copies per cell. Low-density antigens include, for example, full length proteins having at least a portion present on a cell surface as well as HLA-bound peptide antigens.

[0049] **Major Histocompatibility Complex and MHC:** These terms refer to naturally occurring MHC molecules, individual chains of MHC molecules (*e.g.,* MHC class I α (heavy) chain, β2 microglobulin, MHC class II α chain, and MHC class II β chain), individual subunits of such chains of MHC molecules (*e.g.,* α1, α2, and/or α3 subunits of MHC class I α chain, α1-α2 subunits of MHC class II α chain, β1-β2 subunits of MHC class II β chain) as well as portions (*e.g.,* the peptide-binding portions, *e.g.,* the peptide-binding grooves), mutants and various derivatives thereof (including fusions proteins), wherein such portion, mutants and derivatives retain the ability to display an antigenic peptide for recognition by a T cell receptor (TCR), *e.g.,* an antigen-specific TCR. An MHC class I molecule comprises a peptide binding groove formed by the α1 and α2 domains of the heavy chain that can stow a peptide of around 8-10 amino acids. Despite the fact that both classes of MHC bind a core of about 9 amino acids (*e.g.,* 5 to 17 amino acids) within peptides, the open-ended nature of MHC class II peptide binding groove (the α1 domain of a class II MHC a polypeptide in association with the β1 domain of a class II MHC β polypeptide) allows for a wider range of peptide lengths. Peptides binding MHC class II usually vary between 13 and 17 amino acids in length, though shorter or longer lengths are not uncommon. As a result, peptides may shift within the MHC class II peptide binding groove, changing which 9-mer sits directly within the groove at any given time. Conventional identifications of particular MHC variants are used herein. The terms encompass "human leukocyte antigen" or "HLA". An MHC complex containing a bound antigenic peptide (either as a single polypeptide chain or multiple polypeptide chains) may be referred to herein as an "HLA-bound peptide," "peptide MHC complex," or "pMHC".

[0050] **Multispecific Binding Molecule:** The term "multispecific binding molecule" (also "MBM" or "multispecific antigen-binding molecule") as used herein refers to a molecule (*e.g.*, assembly of multiple polypeptide chains) comprising two half antibodies and which specifically bind to at least two different epitopes (and in some instances three, four, or more different epitopes). A multispecific binding molecule of the disclosure may be bivalent, trivalent, or otherwise multivalent, and may be monospecific, bispecific, or otherwise multispecific. A multispecific binding molecule of the disclosure may specifically bind to epitopes on one, two, or more different antigens.

[0051] **Multivalent:** The term "multivalent" as used herein refers to a multispecific binding molecule comprising two or more ABDs, on one, two or more polypeptide chains.

[0052] **Operably Linked:** The term "operably linked" as used herein refers to a functional relationship between two or more regions of a polypeptide chain in which the two or more regions are linked so as to produce a functional polypeptide, or two or more nucleic acid sequences, *e.g.,* to produce an in-frame fusion of two polypeptide components or to link a regulatory sequence to a coding sequence. In the context of a fusion protein or other polypeptide, the term "operably linked" means that two or more amino acid segments are linked so as to produce a functional polypeptide. In the context of a nucleic acid encoding a fusion protein, such as a multispecific binding molecule of the disclosure, "operably linked" means that the two nucleic acids are joined such that the amino acid sequences encoded by the two nucleic acids remain in-frame. In the context of transcriptional regulation, the term refers to the functional relationship of a transcriptional regulatory sequence to a transcribed sequence. For example, a promoter or enhancer sequence is operably linked to a coding sequence if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system.

[0053] **Polypeptide, Peptide and Protein:** The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues.

[0054] **Single Chain Fab or scFab:** The term "single chain Fab" or "scFab" as used herein refers an ABD comprising a VH domain, a CH1 domain, a VL domain, a CL domain and a linker. In some embodiments, the foregoing domains and linker are arranged in one of the following orders in a N-terminal to C-terminal orientation: (a) VH-CH1-linker-VL-CL, (b) VL-CL-linker-VH-CH1, (c) VH-CL-linker-VL-CH1 or (d) VL-CH1-linker-VH-CL. Linkers are suitably noncleavable linkers of at least 30 amino acids, preferably between 32 and 50 amino acids. Single chain Fab fragments are typically stabilized via the natural disulfide bond between the CL domain and the CH1 domain. In addition, these single chain Fab molecules might be further stabilized by generation of interchain disulfide bonds via insertion of cysteine residues (*e.g.*, at position 44 in the VH domain and position 100 in the VL domain according to Kabat numbering).

[0055] **Single Chain Fv or scFv:** The term "single chain Fv" or "scFv" as used herein refers to a polypeptide chain comprising the VH and VL domains of antibody, where these

domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen-binding. For a review of scFv see Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. (1994), Springer-Verlag, New York, pp. 269-315.

[0056] **Specifically (or Selectively) Binds:** The term "specifically (or selectively) binds" to an antigen or an epitope refers to a binding reaction that is determinative of the presence of a cognate antigen or an epitope in a heterogeneous population of proteins and other molecules. The binding reaction can be but need not be mediated by an antibody or antibody fragment. The term "specifically binds" does not exclude cross-species reactivity. For example, an antigen-binding domain (e.g., an antigen-binding fragment of an antibody) that "specifically binds" to an antigen from one species may also "specifically bind" to that antigen in one or more other species. Thus, such cross-species reactivity does not itself alter the classification of an antigen-binding domain as a "specific" binder. In certain embodiments, an antigen-binding domain of the disclosure that specifically binds to a human antigen has cross-species reactivity with one or more non-human mammalian species, e.g., a primate species (including but not limited to one or more of Macaca fascicularis, Macaca mulatta, and Macaca nemestrina) or a rodent species, e.g., Mus musculus.

[0057] **Subject:** The term "subject" includes human and non-human animals. Non-human animals include all vertebrates, *e.g.,* mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, and reptiles. In certain embodiments, the subject is human. Except when noted, the terms "patient" or "subject" are used herein interchangeably.

[0058] **T Cell Antigen**: The term "T cell antigen" as used herein refers to any biological molecule (*e.g.,* protein, carbohydrate, lipid or combination thereof) that is present on and/or expressed by a T cell. In some embodiments, at least a portion of a T cell antigen is extracellular (*e.g.*, is a cell surface protein or transmembrane protein having at least one extracellular domain). Particular T cell antigens contemplated herein include, but are not limited to, CD3 and CD28. In some embodiments, the T cell antigen is CD3.

[0059] **Targeting Moiety**: The term "targeting moiety" as used herein refers to any molecule or binding portion thereof that can specifically bind to an antigen. Exemplary targeting moieties include, but are not limited to, antibodies and antigen binding portions thereof (e.g., Fab, scFv, etc.). A targeting moiety may be described with reference to the antigen to which it specifically binds. Thus, for example, a "T cell antigen targeting moiety" (or "TCA targeting moiety") refers to a molecule or binding portion thereof that can specifically bind to a T cell antigen. The TCA targeting moiety can also have a functional activity in addition to binding a T cell antigen. For example, a TCA targeting moiety that is a CD3 targeting moiety (*e.g.*, an anti-CD3 antibody or an antigen binding portion thereof) may facilitate clustering and activation of CD3 on a surface of a T cell, while a TCA targeting moiety that is a CD28 targeting moiety (*e.g.*, an anti-CD28 antibody or an antigen binding portion thereof) may activate CD28 signaling in a T cell. Similarly a "TCR targeting moiety" refers to a molecule or binding portion thereof that can specifically bind to a T cell receptor.

[0060] **Trivalent**: The term "trivalent" as used herein refers to a multispecific binding molecule that has three antigen-binding sites. In certain embodiments, all three of the antigen-binding sites bind to the same epitope. In some embodiments, two of the antigen-binding sites bind to the same epitope and the other antigen-binding site binds to a different epitope, whether of the same target molecule or different target molecules. In other embodiments, all three of the antigen-binding sites bind to different epitopes, whether on the same target molecule or on any combination of two or more different target molecules. Accordingly, a trivalent multispecific binding molecule may be monospecific, bispecific, or trispecific.

[0061] **Tumor:** The term "tumor" is used interchangeably with the term "cancer" herein, *e.g.*, both terms encompass solid and liquid, *e.g.,* diffuse or circulating, tumors. As used herein, the term "cancer" or "tumor" includes premalignant, as well as malignant cancers and tumors.

[0062] **Tumor-Associated Antigen:** The term "tumor-associated antigen" (also "tumor associated antigen") or "TAA" refers to a molecule (typically a protein, carbohydrate, lipid or some combination thereof) that is expressed on the surface of a cancer cell, either entirely or as a fragment (*e.g.,* as a peptide presented by an MHC molecule), and which is useful for the preferential targeting of a pharmacological agent to the cancer cell. In some

embodiments, a TAA is a marker expressed by both normal cells and cancer cells, *e.g.*, a lineage marker, *e.g.*, CD19 on B cells. In some embodiments, a TAA is a cell surface molecule that is overexpressed in a cancer cell in comparison to a normal cell, for instance, 1-fold over expression, 2-fold overexpression, 3-fold overexpression or more in comparison to a normal cell. In some embodiments, a TAA is a cell surface molecule that is inappropriately synthesized in the cancer cell, for instance, a molecule that contains deletions, additions or mutations in comparison to the molecule expressed on a normal cell. In some embodiments, a TAA will be expressed exclusively on the cell surface of a cancer cell, entirely or as a fragment (*e.g.,* as a peptide presented by an MHC molecule), and not synthesized or expressed on the surface of a normal cell. Accordingly, the term "TAA" encompasses antigens that are specific to cancer cells, sometimes known in the art as tumor-specific antigens ("TSAs").

[0063] **Universal Light Chain, ULC**: The term "universal light chain" or "ULC" as used herein in the context of an antigen-biding domain refers to a light chain polypeptide capable of pairing with the heavy chain region of the antigen-biding domain and also capable of pairing with other heavy chain regions. Universal light chains are also known as "common light chains."

[0064] **VH:** The term "VH" refers to the variable region of an immunoglobulin heavy chain of an antibody, including the heavy chain of an Fv, scFv, dsFv or Fab.

[0065] **VL:** The term "VL" refers to the variable region of an immunoglobulin light chain, including the light chain of an Fv, scFv, dsFv or Fab.

6.2. Primary Multispecific Binding Molecules

[0066] The present disclosure provides multispecific binding molecules (MBMs) comprising two T cell antigen (TCA) targeting moieties flanking an antigen targeting moiety, generally referred to herein for convenience as "primary" MBMs. The primary MBMs typically comprise a multimerization moiety such as an Fc region. The use of the term "primary MBMs" is intended for convenience only and does not connote any particular activity or functional property. Such primary MBMs may, in some cases, be administered for therapeutic applications in combination with one or more MBMs comprising a targeting moiety that binds to a tumor antigen and a targeting moiety that binds to a costimulatory molecule such as

CD28, generally referred to herein for convenience as secondary MBMs, described in more detail in Section 6.3, below.

[0067] Primary MBMs of the disclosure typically comprise a type 1 half antibody (e.g., a Type 1A or a Type 1B half antibody) comprising a TCA targeting moiety and a Type 2 half antibody (e.g., a Type 2A, Type 2B or Type 2C half antibody) comprising both a TCA targeting moiety and an antigen targeting moiety.

[0068] Accordingly, primary MBMs of the disclosure generally comprise:

(a) a Type 2 half antibody comprising:

(i) a first TCA targeting moiety;

(ii) an antigen targeting moiety; and

(iii) a first Fc domain; and

(b) a Type 1 half antibody comprising:

(i) a second TCA targeting moiety; and

(ii) a second Fc domain associated with the first Fc domain to form an Fc region.

[0069] Exemplary TCA targeting moieties suitable for use in the primary MBMs of the disclosure are described in Section 6.5. In some embodiments, the TCA targeting moieties of a primary MBM all specifically bind to the same T cell antigen (e.g., both of the two TCA targeting moieties specifically bind to CD3). In such cases, the TCA targeting moieties may bind to the same epitope (e.g., may have identical ABDs) or may bind to different epitopes (e.g., may have different ABDs). In further embodiments, the TCA targeting moieties bind to different T cell antigens (e.g., one TCA targeting moiety binds to one component of the T cell receptor TCR, while a second binds to a separate component of the TCR).

[0070] Exemplary antigen targeting moieties suitable for use in the primary MBMs of the disclosure are described in Section 6.6. In general, as used herein, the term "antigen targeting moiety" refers to a targeting moiety that binds to an antigen that is not a T cell antigen; that is, "antigen targeting moiety" refers to a targeting moiety that is not a TCA targeting moiety. In some embodiments, the antigen targeting moiety is a tumor antigen targeting moiety, e.g., as described in Section 6.6.1. In some embodiments, the antigen

targeting moiety is a low-density antigen targeting moiety, e.g., as described in Section 6.6.2. In some embodiments, the antigen targeting moiety is an HLA-bound peptide antigen targeting moiety, e.g., as described in Section 6.6.3.

[0071] In the primary MBMs of the disclosure, where the targeting moieties comprise an antigen binding domain ("ABD") of an antibody, each targeting moiety can be composed of two polypeptide chains, one polypeptide chain bearing the heavy chain variable region and the other polypeptide chain bearing the light chain variable region. Thus, the targeting moiety itself can comprise heavy and light chain variable domains on separate polypeptide chains. For example, a polypeptide comprising the targeting moiety can be composed of two polypeptide chains, where one chain contains the heavy chain variable domain of a targeting moiety – optional linker – multimerization moiety (e.g., Fc domain), and the other chain comprises the light chain variable domain of the targeting moiety. Alternatively, an scFv can be used in which the heavy and light chain variable regions are fused to one another in a single polypeptide. Various targeting moiety formats are described in more detail in Section 6.4.

[0072] The primary MBM generally contains a multimerization moiety to facilitate multimerization of two half antibodies. Exemplary multimerization moieties include Fc regions, e.g., as described in Section 6.8.

[0073] The primary MBM can include one or more linker sequences connecting the various components of the molecule, for example connecting a TCA targeting moiety to a multimerization moiety, connecting a TCA targeting moiety to an antigen targeting moiety, or connecting an antigen targeting moiety to a multimerization moiety. Exemplary linkers are described in Section 6.7.

[0074] Below are some illustrative, non-limiting configurations of the two half antibodies present in primary MBMs of the disclosure:

[0075] Configuration 1 refers to a primary MBM of the disclosure comprising a Type 2A half antibody and a Type 1A half antibody (e.g., as depicted in FIG. 1B). Accordingly, a primary MBM of the disclosure can comprise:

(a) a first half antibody comprising: i) a first polypeptide comprising (in N- to C-terminal orientation) a VH domain, a CH1 domain, an optional linker, an

scFv (e.g., VL-linker-VH or VH-linker-VL), an optional hinge region, a CH2 domain, and a CH3 domain, and ii) a second polypeptide comprising (in N- to C-terminal orientation) a VL domain and a CL domain associated with the VH and CH1 domains (see, e.g., FIG. 1B, left half antibody); and

(b) a second half antibody comprising: i) a first polypeptide comprising (in N- to C-terminal orientation) a VH domain, a CH1 domain, an optional hinge region, a CH2 domain, and a CH3 domain; and ii) a second polypeptide comprising (in N- to C-terminal orientation) a VL domain and a CL domain associated with the VH and CH1 domains (see, e.g., FIG. 1B, right half antibody)

[0076] Configuration 2 refers to a primary MBM of the disclosure comprising a Type 2B half antibody and a Type 1B half antibody (e.g., as depicted in FIG. 1C). Accordingly, a primary MBM can comprise:

(a) a first half antibody comprising: i) a first polypeptide comprising (in N- to C-terminal orientation) an scFv (e.g., VL-linker-VH or VH-linker-VL), an optional linker, a VH domain, a CH1 domain, an optional hinge region, a CH2 domain, and a CH3 domain, and ii) a second polypeptide comprising (in N- to C-terminal orientation) a VL domain and a CL domain associated with the VH and CH1 domains (see, e.g., FIG. 1C, left half antibody); and

(b) a second half antibody comprising: a polypeptide comprising (in N- to C-terminal orientation) an scFv (e.g., VL-linker-VH or VH-linker-VL), an optional hinge region, a CH2 domain, and a CH3 domain (see, e.g., FIG. 1C, right half antibody)

[0077] Configuration 3 refers to a primary MBM of the disclosure comprising a Type 2C half antibody and a Type 1A half antibody (e.g., as depicted in FIG. 1D). Accordingly, a primary MBM can comprise:

(a) a first half antibody comprising: i) a first polypeptide comprising (in N- to C-terminal orientation) a first VH domain, a first CH1 domain, an optional linker, a second VH domain, a second CH1 domain, an optional hinge region, a CH2 domain, and a CH3 domain, ii) a second polypeptide

comprising (in N- to C-terminal orientation) a first VL domain and a first CL domain associated with the first VH and CH1 domains, and iii) a third polypeptide comprising (in N- to C-terminal orientation) a second VL domain and a second CL domain associated with the second VH and CH1 domains (see, e.g., FIG. 1D, left half antibody); and

(b) a second half antibody comprising: i) a first polypeptide comprising (in N- to C-terminal orientation) a VH domain, a CH1 domain, an optional hinge region, a CH2 domain, and a CH3 domain; and ii) a second polypeptide comprising (in N- to C-terminal orientation) a VL domain and a CL domain associated with the VH and VL (see, e.g., FIG. 1D, right half antibody)

[0078] In addition to the above illustrative configurations, further configurations are contemplated herein comprising one or more of the above-described half antibodies. For example, additional configurations include:

Configuration 5: (a) a Type 2A half antibody (e.g., as depicted in FIG. 1B, left half antibody), and (b) a Type 1B half antibody (e.g., as depicted in FIG. 1C, right half antibody).

Configuration 6: (a) a Type 2B half antibody (e.g., as depicted in FIG. 1C, left half antibody), and (b) a Type 1A half antibody (e.g., as depicted in FIG. 1B, right half antibody).

Configuration 7: (a) a Type 2C half antibody (e.g., as depicted in FIG. 1D, left half antibody), and (b) a Type 1B half antibody (e.g., as depicted in FIG. 1C, right half antibody).

6.3. Secondary Multispecific Binding Molecules

[0079] Certain aspects of the present disclosure are directed to compositions comprising a primary MBM and a secondary MBM, as well as to methods comprising administration (together or separately) of primary MBM and a secondary MBM. As used herein, a "secondary multispecific binding molecule" or "secondary MBM" refers to a multispecific binding molecule comprising a targeting moiety that binds to a tumor antigen (e.g., a tumor-associated antigen) and a targeting moiety that binds to a costimulatory molecule (e.g., CD28). In certain aspects, the tumor antigen to which a secondary MBM specifically binds is an antigen that is different from, but expressed on the same cell as, that bound by the antigen targeting moiety of the primary MBM. In particular, the tumor antigen to which a secondary MBM specifically binds is generally neither a low-density antigen nor an HLA-

bound peptide antigen, whereas the antigen targeting moiety of the primary MBM may, in some embodiments, be a low-density antigen and/or HLA-bound peptide antigen targeting moiety. For example, a primary MBM may comprise a tumor antigen targeting moiety that specifically binds to low-density, HLA-bound peptide antigen (e.g., HLA-A2-PRAME(425-433), MAGE-A4 HLA-A2 (230-239), or other HLA-bound peptide set forth in Table P-1) while the secondary MBM comprises a targeting moiety that specifically binds to a non-low density, non-HLA bound peptide antigen (e.g., a TAA set forth in Table A below).

[0080] Accordingly, disclosed herein, in some embodiments, are cancer treatment methods comprising administration (together or separately) of (a) a primary MBM comprising a tumor antigen targeting moiety (which may also be a low-density and/or HLA-bound peptide antigen targeting moiety) and two CD3 targeting moieties, and (b) a secondary MBM comprising a targeting moiety that binds to a TAA (e.g., EGFR) and a targeting moiety that binds to a costimulatory molecule (e.g., CD28). Without being bound by theory, such a combination is understood to provide both the primary signal and costimulatory signal necessary for robust activation of T cells, while also bringing the T cells into proximity with cancer cells expressing the TAA.

[0081] A secondary MBM of the disclosure may be bivalent, containing only two targeting moieties. Bivalent secondary MBMs disclosed herein typically comprise two Type 1 half antibodies (e.g., two Type 1A half antibodies, two Type 1B half antibodies, or one Type 1A half antibody and one Type 1B half antibody). In some embodiments, a bivalent secondary MBM comprises: (a) a first Type 1A half antibody comprising a targeting moiety that specifically binds to a tumor antigen, and (b) a second Type 1A half antibody comprising a targeting moiety that specifically binds to a costimulatory molecule (e.g., CD28). In some embodiments, a bivalent secondary MBM comprises: (a) a first Type 1B half antibody comprising a targeting moiety that specifically binds to a tumor antigen, and (b) a second Type 1B half antibody comprising a targeting moiety that specifically binds to a costimulatory molecule (e.g., CD28). In some embodiments, a bivalent secondary MBM comprises: (a) a first Type 1A half antibody comprising a targeting moiety that specifically binds to a tumor antigen, and (b) a second Type 1B half antibody comprising a targeting moiety that specifically binds to a costimulatory molecule (e.g., CD28). In some embodiments, a bivalent secondary MBM comprises: (a) a first Type 1B half antibody comprising a targeting moiety

that specifically binds to a tumor antigen, and (b) a second Type 1A half antibody comprising a targeting moiety that specifically binds to a costimulatory molecule (e.g., CD28).

[0082] A secondary MBM of the disclosure may be trivalent, containing three targeting moieties. In some aspects, trivalent secondary MBMs disclosed herein comprise one Type 2 half antibody (e.g., Type 2A, 2B, or 2C half antibody) and one Type 1 half antibody (e.g., Type 1A or 1B half antibody). In some embodiments, a trivalent secondary MBM comprises (a) two targeting moieties that specifically bind to a tumor antigen (e.g., in the context of a Type 2A, 2B or 2C half antibody) and (b) one targeting moiety that specifically binds to a costimulatory molecule (e.g., in the context of a Type 1A or Type 1B half antibody). In further embodiments, a trivalent secondary MBM comprises (a) one targeting moiety that specifically binds to a tumor antigen (e.g., in the context of a Type 1A or Type 1B half antibody) and (b) two targeting moieties that specifically bind to a costimulatory molecule (e.g., in the context of a Type 2A, 2B or 2C half antibody).

[0083] A secondary MBM of the disclosure may be tetravalent, containing four targeting moieties. In some aspects, tetravalent secondary MBMs disclosed herein comprise two Type 2 half antibodies (e.g., Type 2A, 2B, or 2C half antibody). In some embodiments, a tetravalent secondary MBM comprises (a) two targeting moieties that specifically bind to a tumor antigen (e.g., in the context of a Type 2A, 2B or 2C half antibody) and (b) two targeting moieties that specifically bind to a costimulatory molecule (e.g., in the context of a Type 2A, 2B or 2C half antibody).

[0084] A secondary MBM of the disclosure comprises a targeting moiety that binds to a tumor antigen; such a targeting moiety may be a tumor antigen targeting moiety as described in Section 6.6.1. In some embodiments, a secondary MBM comprises a targeting moiety that specifically binds to a tumor antigen of Table A. In some embodiments, the TAA is EGFR. In some embodiments, the TAA is PSMA. In some embodiments, the TAA is BCMA. In some embodiments, the TAA is CD19. In some embodiments, the TAA is CD20. In some embodiments, the TAA is CD22.

[0085] A secondary MBM of the disclosure further comprises a targeting moiety that binds to a costimulatory molecule. Various costimulatory molecules are recognized in the art and

are described in, e.g., Chen L, Flies DB. 2013 Nat Rev Immunol. 13(4):227-242. In some embodiments, the costimulatory molecule is CD28, ICOS, CD27, HVEM, LIGHT, CD40, 4-1BB, OX40, GITR, CD30, TIM1, SLAM, CD2, or CD226.

[0086] In some embodiments, the costimulatory molecule is CD28. Accordingly, in some embodiments, the targeting moiety that binds to a costimulatory molecule is a CD28 targeting moiety. The CD28 targeting moiety may be or comprise an antigen-binding domain from an anti-CD28 antibody. Exemplary anti-CD28 antibodies or antibody sequences are set forth in Table K-1, upon which a CD28 targeting moiety in a secondary MBM can be based.

TABLE K-1
Exemplary Anti-CD28 Antibodies

Target	Antibody Name and/or Binding Sequences
CD28	Theralizumab (TGN1412)
CD28	FK734
CD28	CD28A-49
CD28	E1P2 (described in
CD28	Anti-CD28 antibody sequences in WO 2020/127618 A1
CD28	Anti-CD28 antibodies and CD28 portion of anti-PSMA/anti-CD28 antibodies described in WO 2019/246514 A1
CD28	The CD28 portion of anti-CD3/anti-CD28 antibodies described in WO 2021/260064 A1
CD28	The CD28 portion of anti-CD3/anti-CD28 antibodies described in WO 2009/062001 A1
CD28	The CD28 portion of anti-EGFR/anti-CD28 antibody described in WO 2020/198009 A1
CD28	The CD28 portion of anti-CD3/anti-CD28/anti-CD137 antibodies described in WO 2021/173307 A1
CD28	The CD28 portion of anti-CD38/anti-CD28/anti-CD3 antibodies described in WO 2019/074973 A2
CD28	The CD28 portion of anti-CEA/anti-CD3/anti-CD28 antibodies described in WO 2005/095456 A1
CD28	The CD28 portion of anti-MUC16/anti-CD28 antibodies described in WO 2020/132024 A1
CD28	The CD28 portion of anti-CD28/anti-CD22 antibodies described in WO 2020/132066 A1
CD28	Anti-CD28 antibody sequences in WO 2020/127628 A1
CD28	Anti-CD28 antibody sequences in WO 2022/061098 A1
CD28	Anti-CD28 antibody sequences in WO 2003/042231 A2

TABLE K-1 Exemplary Anti-CD28 Antibodies	
Target	**Antibody Name and/or Binding Sequences**
CD28	Anti-CD28 antibody sequences in WO 2014/209868 A1
CD28	Anti-CD28 antibody sequences in WO 2022/096536 A1

[0087] In some embodiments, the costimulatory molecule is ICOS. In some embodiments, the costimulatory molecule is CD27. In some embodiments, the costimulatory molecule is HVEM. In some embodiments, the costimulatory molecule is LIGHT. In some embodiments, the costimulatory molecule is CD40. In some embodiments, the costimulatory molecule is 4-1BB. In some embodiments, the costimulatory molecule is OX40. In some embodiments, the costimulatory molecule is GITR. In some embodiments, the costimulatory molecule is CD30. In some embodiments, the costimulatory molecule is TIM1. In some embodiments, the costimulatory molecule is SLAM. In some embodiments, the costimulatory molecule is CD2. In some embodiments, the costimulatory molecule is CD226.

[0088] Certain example secondary MBMs are provided in Table K-2. In some embodiments, a secondary MBM of the disclosure is a bispecific antigen binding molecule of Table K-2. In some embodiments, a secondary MBM comprises one or more CDR, VH, and/or VL sequences from a bispecific antigen binding molecule of Table K-2.

TABLE K-2 Exemplary Secondary Multispecific Binding Molecules	
Targets	**Antibody Name and/or Binding Sequences**
CD28 x CD38	Bispecific antibodies bsAb6031 and bsAb7945 described in US 2022/0089766 A1
CD28 x EGFR	Bispecific antibodies bsAb7075, bsAb6321, bsAb6322, and bsAb6323 described in US 2020/0299388 A1
CD28 x MUC16	Bispecific antibodies bs24963D and bs32897D described in US 11453721 B2
CD28 x PSMA	Bispecific antibodies bs16429D, bs16430D, and bs16431D described in US 2019/0389951 A1
CD28 x CD22	Bispecific antibodies REGN5837 and REGN5838 described in WO 2020/132066 A1
CD28 x PSMA	Bispecific antibodies described in WO 2023/164510 A1, (e.g., having a first and second antigen binding domain as laid out in claim 3).
CD28 x TROP2	Bispecific antibodies described in WO 2023/164640 (e.g., having a first and second antigen binding domain as laid out in FIGs. 17-20 (CD28) and FIGS 23-28 (TROP2)).

TABLE K-2 Exemplary Secondary Multispecific Binding Molecules	
Targets	**Antibody Name and/or Binding Sequences**
CD28 x MSLN	Bispecific antibodies described in WO 2023/164627 (e.g., having a first and second antigen binding domain as laid out in FIGs. 27-30 and 33 (CD28) and FIGS 15-19 and 23 (MSLN)).

[0089] The patent publications set forth in Table K-2, which disclose exemplary secondary MBMs, are incorporated herein by reference in their entirety.

6.4. Targeting Moiety Formats

[0090] In certain aspects, a targeting moiety that can be used in a primary MBM and/or secondary MBM can be any type of antibody or fragment thereof that retains specific binding to an antigenic determinant. In some embodiments, a targeting moiety is a full-length antibody. In some embodiments a targeting moiety is an immunoglobulin molecule, particularly an IgG class immunoglobulin molecule, more particularly an IgG1 or IgG4 immunoglobulin molecule. In some embodiments, a targeting moiety is an antibody fragment. Antibody fragments include, but are not limited to, VH (or VH) fragments, VL (or VL) fragments, Fab fragments, F(ab')2 fragments, scFv fragments, Fv fragments, minibodies, diabodies, triabodies, and tetrabodies.

6.4.1. scFv

[0091] Single chain Fv or "scFv" antibody fragments comprise the VH and VL domains of an antibody in a single polypeptide chain, are capable of being expressed as a single chain polypeptide, and retain the specificity of the intact antibodies from which they are derived. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domain that enables the scFv to form the desired structure for target binding. Examples of linkers suitable for connecting the VH and VL chains of an scFv are the linkers identified in Section 6.7.

[0092] Unless specified, as used herein an scFv may have the VL and VH variable regions in either order, e.g., with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

[0093] The scFv can comprise VH and VL sequences from any suitable species, such as murine, human or humanized VH and VL sequences.

[0094] To create an scFv-encoding nucleic acid, the VH and VL-encoding DNA fragments are operably linked to another fragment encoding a linker, e.g., encoding any of the linkers described in Section 6.7 (typically a repeat of a sequence containing the amino acids glycine and serine, such as the amino acid sequence $(Gly_4Ser)_3$ (SEQ ID NO:1)), such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see, e.g., Bird et al., 1988, Science 242:423-426; Huston et al., 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883; McCafferty et al., 1990, Nature 348:552-554).

6.4.2. Fab

[0095] Fab domains were traditionally produced by proteolytic cleavage of immunoglobulin molecules using enzymes such as papain. In the primary and secondary multispecific binding molecules of the disclosure, the Fab domains are typically recombinantly expressed as part of the multispecific binding molecule.

[0096] The Fab domains can comprise constant domain and variable region sequences from any suitable species, and thus can be murine, chimeric, human or humanized.

[0097] Fab domains typically comprise a CH1 domain attached to a VH domain which pairs with a CL domain attached to a VL domain. In a wild-type immunoglobulin, the VH domain is paired with the VL domain to constitute the Fv region, and the CH1 domain is paired with the CL domain to further stabilize the binding module. A disulfide bond between the two constant domains can further stabilize the Fab domain.

[0098] For the primary and secondary multispecific binding molecules of the disclosure, particularly when the light chain is not a common or universal light chain, it is advantageous to use Fab heterodimerization strategies to permit the correct association of Fab domains belonging to the same ABD and minimize aberrant pairing of Fab domains belonging to different ABDs. For example, the Fab heterodimerization strategies shown in Table H below can be used:

TABLE H
Fab Heterodimerization Strategies

STRATEGY	VH	CH1	VL	CL	REFERENCE
CrossMabCH1-CL	WT	CL domain	WT	CH1 domain	Schaefer *et al.*, 2011, Cancer Cell 2011;

TABLE H Fab Heterodimerization Strategies					
STRATEGY	**VH**	**CH1**	**VL**	**CL**	**REFERENCE**
					20:472-86; PMID:22014573.
orthogonal Fab VHVRD1CH 1CRD2 - VLVRD1CλC RD2	39K, 62E	H172A, F174G	1R, 38D, (36F)	L135Y, S176W	Lewis *et al.*, 2014, Nat Biotechnol 32:191-8
orthogonal Fab VHVRD2CH 1wt - VLVRD2Cλw t	39Y	WT	38R	WT	Lewis *et al.*, 2014, Nat Biotechnol 32:191-8
TCR CαCβ	39K	TCR Cα	38D	TCR Cβ	Wu *et al.*, 2015, MAbs 7:364-76
CR3	WT	T192E	WT	N137K, S114A	Golay *at al.*, 2016, J Immunol 196:3199-211.
MUT4	WT	L143Q, S188V	WT	V133T, S176V	Golay *at al.*, 2016, J Immunol 196:3199-211.
DuetMab	WT	F126C	WT	S121C	Mazor *et al.*, 2015, MAbs 7:377-89; Mazor *et al.*, 2015, MAbs 7:461-669.
Domain exchanged	WT	CH3 + knob or hole mutation	WT	CH3 + hole or knob mutation	Wozniak-Knopp *et al.*, 2018, PLoSONE13(4):e019 5442

[0099] Accordingly, in certain embodiments, correct association between the two polypeptides of a Fab is promoted by exchanging the VL and VH domains of the Fab for each other or exchanging the CH1 and CL domains for each other, *e.g.,* as described in WO 2009/080251.

[0100] Correct Fab pairing can also be promoted by introducing one or more amino acid modifications in the CH1 domain and one or more amino acid modifications in the CL

domain of the Fab and/or one or more amino acid modifications in the VH domain and one or more amino acid modifications in the VL domain. The amino acids that are modified are typically part of the VH:VL and CH1:CL interface such that the Fab components preferentially pair with each other rather than with components of other Fabs.

[0101] In one embodiment, the one or more amino acid modifications are limited to the conserved framework residues of the variable (VH, VL) and constant (CH1, CL) domains as indicated by the Kabat numbering of residues. Almagro, 2008, Frontiers In Bioscience 13:1619-1633 provides a definition of the framework residues on the basis of Kabat, Chothia, and IMGT numbering schemes.

[0102] In one embodiment, the modifications introduced in the VH and CH1 and/or VL and CL domains are complementary to each other. Complementarity at the heavy and light chain interface can be achieved on the basis of steric and hydrophobic contacts, electrostatic/charge interactions or a combination of the variety of interactions. The complementarity between protein surfaces is broadly described in the literature in terms of lock and key fit, knob into hole, protrusion and cavity, donor and acceptor etc., all implying the nature of structural and chemical match between the two interacting surfaces.

[0103] In one embodiment, the one or more introduced modifications introduce a new hydrogen bond across the interface of the Fab components. In one embodiment, the one or more introduced modifications introduce a new salt bridge across the interface of the Fab components. Exemplary substitutions are described in WO 2014/150973 and WO 2014/082179, the contents of which are hereby incorporated by reference.

[0104] In some embodiments, the Fab domain comprises a 192E substitution in the CH1 domain and 114A and 137K substitutions in the CL domain, which introduces a salt-bridge between the CH1 and CL domains (see, *e.g.,* Golay *et al.*, 2016, J Immunol 196:3199-211).

[0105] In some embodiments, the Fab domain comprises a 143Q and 188V substitutions in the CH1 domain and 113T and 176V substitutions in the CL domain, which serves to swap hydrophobic and polar regions of contact between the CH1 and CL domain (see, *e.g.,* Golay *et al.*, 2016, J Immunol 196:3199-211).

[0106] In some embodiments, the Fab domain can comprise modifications in some or all of the VH, CH1, VL, CL domains to introduce orthogonal Fab interfaces which promote correct

assembly of Fab domains (Lewis *et al.*, 2014 Nature Biotechnology 32:191-198). In an embodiment, 39K, 62E modifications are introduced in the VH domain, H172A, F174G modifications are introduced in the CH1 domain, 1 R, 38D, (36F) modifications are introduced in the VL domain, and L135Y, S176W modifications are introduced in the CL domain. In another embodiment, a 39Y modification is introduced in the VH domain and a 38R modification is introduced in the VL domain.

[0107] Fab domains can also be modified to replace the native CH1:CL disulfide bond with an engineered disulfide bond, thereby increasing the efficiency of Fab component pairing. For example, an engineered disulfide bond can be introduced by introducing a 126C in the CH1 domain and a 121 C in the CL domain (see, *e.g.,* Mazor *et al.*, 2015, MAbs 7:377-89).

[0108] Fab domains can also be modified by replacing the CH1 domain and CL domain with alternative domains that promote correct assembly. For example, Wu *et al.*, 2015, MAbs 7:364-76, describes substituting the CH1 domain with the constant domain of a T cell receptor and substituting the CL domain with the b domain of the T cell receptor, and pairing these domain replacements with an additional charge-charge interaction between the VL and VH domains by introducing a 38D modification in the VL domain and a 39K modification in the VH domain.

[0109] In lieu of, or in addition to, the use of Fab heterodimerization strategies to promote correct VH-VL pairings, the VL of common light chain (also referred to as a universal light chain) can be used for each Fab VL region of a multispecific binding molecule of the disclosure. In various embodiments, employing a common light chain as described herein reduces the number of inappropriate species of multispecific binding molecules as compared to employing original cognate VLs. In various embodiments, the VL domains of the multispecific binding molecules are identified from monospecific antibodies comprising a common light chain. In various embodiments, the VH regions of the multispecific binding molecules comprise human heavy chain variable gene segments that are rearranged *in vivo* within mouse B cells that have been previously engineered to express a limited human light chain repertoire, or a single human light chain, cognate with human heavy chains and, in response to exposure with an antigen of interest, generate an antibody repertoire containing a plurality of human VHs that are cognate with one or one of two possible human VLs, wherein the antibody repertoire specific for the antigen of interest. Common light chains are

those derived from a rearranged human Vκ1-39Jκ5 sequence or a rearranged human Vκ3-20Jκ1 sequence, and include somatically mutated (*e.g.,* affinity matured) versions. See, for example, U.S. Patent No. 10,412,940.

6.5. T Cell Antigen (TCA) Targeting Moieties

[0110] As disclosed herein, a primary MBM of the present disclosure comprises two T cell antigen (TCA) targeting moieties. A TCA targeting moiety generally binds to a specific T cell antigen. In some embodiments, both of the TCA targeting moieties specifically bind to the same T cell antigen (e.g., both TCA targeting moieties specifically bind to CD3). In such cases, each TCA targeting moiety may bind to the same epitope (e.g., the same epitope on CD3) or different epitopes (e.g., different epitopes of CD3). Where the TCA targeting moieties each bind to the same epitope, they may have identical antigen-binding domains (ABDs), or different ABDs. In some embodiments, the TCA targeting moieties each bind to a different T cell antigen (e.g., one TCA targeting moiety binds to one component of the TCR while the other TCA targeting moiety binds to a different component of the TCR). Exemplary target molecules recognized by the TCA targeting moieties of the disclosure, and sequences of ABDs of such targeting moieties, are described in Section 6.5.1.

[0111] The TCA targeting moieties of the disclosure include targeting moieties (*e.g.*, antibodies and antigen-binding fragments thereof) that bind the TCA (*e.g.*, CD3) with high affinity. The TCA targeting moieties of the disclosure also include targeting moieties (*e.g.*, antibodies and antigen-binding fragments thereof) that bind the TCA (*e.g.*, CD3) with moderate or low affinity, depending on the therapeutic context and particular targeting properties that are desired. In some embodiments, "low affinity" (also "weak affinity") describes TCA targeting moieties that bind the TCA with a K_D or EC_{50} (e.g., as measured in a surface plasmon resonance assay) of greater than 10^{-6} M, greater than 10^{-7} M, greater than 10^{-8} M, or greater than 10^{-9} M.

[0112] The TCA targeting moiety is generally an antigen binding moiety, for example an antibody or an antigen-binding portion of an antibody, *e.g.,* an scFv, as described in Section 6.4.1, or a Fab, as described in Section 6.4.2.

6.5.1. T Cell Antigens

[0113] In general, a target for a TCA targeting moiety is any molecule present on or expressed by a T cell. In certain embodiments, a TCA targeting moiety of the disclosure targets a cell surface molecule of a T cell. Example targets for a TCA targeting moiety of the disclosure include, but are not limited to, CD3, the T cell receptor (*e.g.*, TCRαβ or TCRγδ), CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, and B7-H3. In some embodiments, the target for the TCA targeting moiety is CD3. In some embodiments, the target for the TCA targeting moiety is the T cell receptor (*e.g.*, TCRαβ or TCRγδ). The epitope of the TCA targeting moiety can be an individual polypeptide (*e.g.*, CD3 epsilon) or a multimeric component of a protein complex (*e.g.*, the TCRαβ dimer or the TCRγδ dimer of the T cell receptor complex).

6.5.1.1. CD3 and TCR targeting moieties

[0114] In particular embodiments, a TCA targeting moiety of the present disclosure is a CD3 targeting moiety and/or a TCR targeting moiety. A CD3 targeting moiety may be or comprise an antigen-binding domain from an anti-CD3 antibody. A TCR targeting moiety may be or comprise an antigen-binding domain from an anti-TCR antibody.

[0115] Exemplary anti-CD3 and anti-TCR antibodies or antibody sequences are set forth in Table T below, upon which the TCA targeting moiety can be based.

TABLE T
Exemplary Anti-CD3 and Anti-TCR Antibodies

Target	Antibody Name and/or Binding Sequences
CD3	The CD3-binding portion of Catumaxomab
CD3	The CD3-binding portion of ertumaxomab
CD3	The CD3-binding portion of anti-PSMA/anti-CD3 antibodies described in WO2011121110A1
CD3	Anti-CD3 antibody sequences in US10266593B2
CD3	Anti-CD3 antibody sequences in US 8846042B2
CD3	Anti-CD3 antibody sequences US 2016/0355600
CD3	Anti-CD3 antibody sequences in WO 2014/110601
CD3	Anti-CD3 antibody sequences in WO 2014/145806
CD3	Anti-CD3 antibody sequences in US 10,066,015
CD3	Anti-CD3 antibody sequences in WO 2019/034580

TABLE T Exemplary Anti-CD3 and Anti-TCR Antibodies	
Target	**Antibody Name and/or Binding Sequences**
CD3	Anti-CD3 antibody sequences in WO 2014/056783
CD3	Anti-CD3 antibody sequences in WO 2013/055809 A1
CD3	Anti-CD3 antibody sequences in US 10,066,016
CD3	Anti-CD3 antibody sequences in US 2010/0150918
CD3	The CD3-binding portion of MT110
CD3	The CD3-binding portion of Acapatamab (AMG160)
CD3	The CD3-binding portion of AMG199
CD3	The CD3-binding portion of AMG330
CD3	The CD3-binding portion of AMG427 (Emirodatamab)
CD3	The CD3-binding portion of AMG562
CD3	The CD3-binding portion of AMG596
CD3	The CD3-binding portion of AMG673
CD3	The CD3-binding portion of AMG701 (Pavurutamab)
CD3	The CD3-binding portion of Tarlatamab (AMG757)
CD3	The CD3-binding portion of AMG910 (Gresonitamab)
CD3	The CD3-binding portion of BAY2010112 (Pasotuxizumab)
CD3	The CD3-binding portion of AMG420
CD3	The CD3-binding portion of AMG424
CD3	The CD3-binding portion of AMG509
CD3	The CD3-binding portion of AMV564
CD3	The CD3-binding portion of APVO436
CD3	The CD3-binding portion of Alnuctamab (CC-93269; BMS-986349)
CD3	The CD3-binding portion of ERY974
CD3	The CD3-binding portion of A-319
CD3	The CD3-binding portion of GEM333
CD3	The CD3-binding portion of GEM3PSCA
CD3	The CD3-binding portion of Cevostamab
CD3	The CD3-binding portion of Runimotamab
CD3	The CD3-binding portion of GEN1044
CD3	Epcoritamab (GEN3013)

TABLE T Exemplary Anti-CD3 and Anti-TCR Antibodies	
Target	**Antibody Name and/or Binding Sequences**
CD3	The CD3-binding portion of HPN424
CD3	The CD3-binding portion of ISB1302
CD3	The CD3-binding portion of ISB1342
CD3	The CD3-binding portion of IGM-2323
CD3	The CD3-binding portion of IMC-F106C
CD3	The CD3-binding portion of IMC-C103C
CD3	The CD3-binding portion of IMCnyeso
CD3	The CD3-binding portion of JNJ-63709178
CD3	The CD3-binding portion of JNJ-63898081 (JNJ-081)
CD3	The CD3-binding portion of Teclistamab
CD3	The CD3-binding portion of Talquetamab (JNJ-64407564)
CD3	The CD3-binding portion of JNJ-67571244
CD3	The CD3-binding portion of MGD007
CD3	The CD3-binding portion of Orlotamab (MGD009)
CD3	The CD3-binding portion of Duvortuxizumab
CD3	The CD3-binding portion of Flotetuzumab (MGD006)
CD3	The CD3-binding portion of MCLA-117
CD3	The CD3-binding portion of PF-06671008
CD3	The CD3-binding portion of Elranatamab
CD3	The CD3-binding portion of Odronextamab
CD3	The CD3-binding portion of REGN5458
CD3	The CD3-binding portion of REGN5459
CD3	The CD3-binding portion of REGN4018
CD3	The CD3-binding portion of Glofitamab (RO7082859)
CD3	The CD3-binding portion of RO6958688 (RG7802)
CD3	The CD3-binding portion of SAR440234
CD3	The CD3-binding portion of TNB-383B
CD3	The CD3-binding portion of M802
CD3	The CD3-binding portion of Xmab13676
CD3	The CD3-binding portion of Xmab18087

TABLE T Exemplary Anti-CD3 and Anti-TCR Antibodies	
Target	**Antibody Name and/or Binding Sequences**
CD3	The CD3-binding portion of Vibecotamab (XmAb14045)
CD3	The CD3-binding portion of Nivatrotamab (Hu3F8-BsAb)
CD3	Anti-CD3 antibody sequences in US20190211100
CD3	Anti-CD3 antibody sequences in EP1629011B
CD3	VH of SEQ ID NOS. 90 and 98 disclosed in US 2021/0206865 A1 CDR-H1 of SEQ ID NO: 92 and 100 disclosed in US 2021/0206865 A1 CDR-H2 of SEQ ID NO: 94 and 102 disclosed in US 2021/0206865 A1 CDR-H3 of SEQ ID NO: 96 and 104 disclosed in US 2021/0206865 A1 HC of SEQ ID NO: 127 or SEQ ID NO: 128 disclosed in US 2021/0206865 A1 LC of SEQ ID NO: 129 or SEQ ID NO: 132 disclosed in US 2021/0206865 A1
CD3	Anti-CD3 Heavy chain of SEQ ID NO: 2 disclosed in US 2021/0206870 A1 Anti-CD3 VH SEQ ID NO: 5 disclosed in US 2021/0206870 A1 Anti-CD3 VL of SEQ ID NO: 6 disclosed in US 2021/0206870 A1 Anti-CD3 CDR-H1 of SEQ ID NO: 10 disclosed in US 2021/0206870 A1 Anti-CD3 CDR-H2 of SEQ ID NO: 11 disclosed in US 2021/0206870 A1 Anti-CD3 CDR-H3 of SEQ ID NO: 12 disclosed in US 2021/0206870 A1
CD3	Anti-CD3 VH of SEQ ID NO: 92, 102, 112, 122, 132, 142, 156, 166, 176, 186, 196 or 206 disclosed in US 2022/0119525 A1 Anti-CD3 CDR-H1 of SEQ ID NO: 93, 103, 113, 123, 133, 143, 157, 167, 177, 187, 197 or 207 disclosed in US 2022/0119525 A1 Anti-CD3 CDR-H2 of SEQ ID NO: 94, 104, 114, 124, 134, 144, 158, 168, 178, 188, 198 or 208 disclosed in US 2022/0119525 A1 Anti-CD3 CDR-H3 of SEQ ID NO: 95, 105, 115, 125, 135, 145, 159, 169, 179, 189, 199 or 209 disclosed in US 2022/0119525 A1 Anti-CD3 VL of SEQ ID NO: 96, 106, 116, 126, 136, 146, 152, 162, 172, 182, 192 or 202 disclosed in US 2022/0119525 A1 Anti-CD3 CDR-L1 of SEQ ID NO: 97, 107, 117, 127, 137, 147, 153, 163, 173, 183, 193 or 203 disclosed in US 2022/0119525 A1 Anti-CD3 CDR-L2 of SEQ ID NO. 98, 108, 118, 128, 138, 148, 154, 164, 174, 184, 194 or 204 disclosed in US 2022/0119525 A1 Anti-CD3 CDR-L3 of SEQ ID NO. 99, 109, 119, 129, 139, 149, 155, 165, 175, 185, 195 or 205 disclosed in US 2022/0119525 A1
CD3	L2K
CD3	A2J
CD3	6G12
CD3	1A4
CD3	OKT3 (Ortho Kung T3; Muromonab-CD3)

TABLE T Exemplary Anti-CD3 and Anti-TCR Antibodies	
Target	**Antibody Name and/or Binding Sequences**
CD3	Teplizumab (PRV-031; MGA03)
CD3	Otelixizumab (TRX4)
CD3	Anti-CD3 VH of SEQ ID NO: 2, 18, 34, 50, 66, 82, 98, 114, 130, 146, 162, 178, 194, 210, 226, 242, 258, 274, 290, 306, 322, 338, 354, 370, 386, 402, 418, 434, 450, 466, 482, 498, 514, 530, 546, 562, 578, 594, 610, 626, 642, 658, 674, 690, 706, 722, 738, 754, 770, 786, 802, 818, 834, 850, 866, 882, 898, 914, 930, 946, 962, 978, 994, 1010, 1026, 1042, 1050, 1058, 1066, 1074, 1082, 1090, 1098, 1106, 1114, 1122, 1130, 1138, 1146, 1154, 1162, 1170, 1178, 1186, 1194, 1202, 1210, 1218, or 1226 disclosed in US 9657102 B2 Anti-CD3 CDR-H1 of SEQ ID NO: 4, 20, 36, 52, 68, 84, 100, 116, 132, 148, 164, 180, 196, 212, 228, 244, 260, 276, 292, 308, 324, 340, 356, 372, 388, 404, 420, 436, 452, 468, 484, 500, 516, 532, 548, 564, 580, 596, 612, 628, 644, 660, 676, 692, 708, 724, 740, 756, 772, 788, 804, 820, 836, 852, 868, 884, 900, 916, 932, 948, 964, 980, 996, 1012, 1028, 1044, 1052, 1060, 1068, 1076, 1084, 1092, 1100, 1108, 1116, 1124, 1132, 1140, 1148, 1156, 1164, 1172, 1180, 1188, 1196, 1204, 1212, or 1220, 1228 disclosed in US 9657102 B2 Anti-CD3 CDR-H2 of SEQ ID NO: 6, 22, 38, 54, 70, 86, 102, 118, 134, 150, 166, 182, 198, 214, 230, 246, 262, 278, 294, 310, 326, 342, 358, 374, 390, 406, 422, 438, 454, 470, 486, 502, 518, 534, 550, 566, 582, 598, 614, 630, 646, 662, 678, 694, 710, 726, 742, 758, 774, 790, 806, 822, 838, 854, 870, 886, 902, 918, 934, 950, 966, 982, 998, 1014, 1030, 1046, 1054, 1062, 1070, 1078, 1086, 1094, 1102, 1110, 1118, 1126, 1134, 1142, 1150, 1158, 1166, 1174, 1182, 1190, 1198, 1206, 1214, or 1222, 1230 disclosed in US 9657102 B2 Anti-CD3 CDR-H3 of SEQ ID NO: 8, 24, 40, 56, 72, 88, 104, 120, 136, 152, 168, 184, 200, 216, 232, 248, 264, 280, 296, 312, 328, 344, 360, 376, 392, 408, 424, 440, 456, 472, 488, 504, 520, 536, 552, 568, 584, 600, 616, 632, 648, 664, 680, 696, 712, 728, 744, 460, 776, 792, 808, 824, 840, 856, 872, 888, 904, 920, 936, 952, 968, 984, 1000, 1016, 1032, 1048, 1056, 1064, 1072, 1080, 1088, 1096, 1104, 1112, 1120, 1128, 1136, 1144, 1152, 1160, 1168, 1176, 1184, 1192, 1200, 1208, 1216, or 1224, 1232 disclosed in US 9657102 B2 Anti-CD3 VL of SEQ ID NO: 10, 26, 42, 58, 74, 90, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, 378, 394, 410, 426, 442, 458, 474, 490, 506, 522, 538, 554, 570, 586, 602, 618, 634, 650, 666, 682, 698, 714, 730, 746, 762, 778, 794, 810, 826, 842, 858, 874, 890, 906, 922, 938, 954, 970, 986, 1002, 1018, 1034, 1234, or 1234, 1234 disclosed in US 9657102 B2 Anti-CD3 CDR-L1 of SEQ ID NO: 12, 28, 44, 60, 76, 92, 108, 124, 140, 156, 172, 188, 204, 220, 236, 252, 268, 284, 300, 316, 332, 348, 364, 380, 396, 412, 428, 444, 460, 476, 492, 508, 524, 540, 556, 572, 588, 604, 620, 636, 652, 668, 684, 700, 716,

TABLE T Exemplary Anti-CD3 and Anti-TCR Antibodies	
Target	**Antibody Name and/or Binding Sequences**
	732, 748, 764, 780, 796, 812, 828, 844, 860, 876, 892, 908, 924, 940, 956, 972, 988, 1004, 1020, 1036, 1236, or 1236, 1236 disclosed in US 9657102 B2 Anti-CD3 CDR-L2 of SEQ ID NO. 14, 30, 46, 62, 78, 94, 110, 126, 142, 158, 174, 190, 206, 222, 238, 254, 270, 286, 302, 318, 334, 350, 366, 382, 398, 414, 430, 446, 462, 478, 494, 510, 526, 542, 558, 574, 590, 606, 622, 638, 654, 670, 686, 702, 718, 734, 750, 766, 782, 798, 814, 830, 846, 862, 878, 894, 910, 926, 942, 958, 974, 990, 1006, 1022, 1038, 1238, or 1238, 1238 disclosed in US 9657102 B2 Anti-CD3 CDR-L3 of SEQ ID NO. 16, 32, 48, 64, 80, 96, 112, 128, 144, 160, 176, 192, 208, 224, 240, 256, 272, 288, 304, 320, 336, 352, 368, 384, 400, 416, 432, 448, 464, 480, 496, 512, 528, 544, 560, 576, 592, 608, 624, 640, 656, 672, 688, 704, 720, 736, 752, 768, 784, 800, 816, 832, 848, 864, 880, 896, 912, 928, 944, 960, 976, 992, 1008, 1024, 1040, 1240, or 1240, 1240 disclosed in US 9657102 B2 (see also US 9657102 B2 at Table 1, incorporated herein by reference)
TCRαβ	BMA031 sequences disclosed in US 2012/0034221
TCRγδ	6TCS1 antibody disclosed in US 5,980,892

[0116] In some aspects, the TCA targeting moiety competes with an antibody set forth in Table T for binding to the target (*e.g.*, CD3 or a T cell receptor). In further aspects, the TCA targeting moiety comprises CDRs having CDR sequences of an antibody set forth in Table T. In some embodiments, the TCA targeting moiety comprises all 6 CDR sequences of an antibody set forth in Table T. In other embodiments, the TCA targeting moiety comprises at least the heavy chain CDR sequences (CDR-H1, CDR-H2, CDR-H3) or an antibody set forth in Table T and the light chain CDR sequences of a universal light chain. In further aspects, a TCA targeting moiety comprises a VH comprising the amino acid sequence of the VH of an antibody set forth in Table T. In some embodiments, the TCA targeting moiety further comprises a VL comprising the amino acid sequence of the VL of an antibody set forth in Table T. In other embodiments, the TCA targeting moiety further comprises a universal light chain VL sequence.

[0117] The CD3 targeting moieties of the disclosure include targeting moieties (*e.g.*, antibodies and antigen-binding fragments thereof) that bind CD3 (*e.g.*, human CD3) with high affinity. The CD3 targeting moieties of the disclosure also include targeting moieties (*e.g.*, antibodies and antigen-binding fragments thereof) that bind the CD3 (*e.g.*, human CD3) with moderate or low affinity, depending on the therapeutic context and particular targeting properties that are desired. Accordingly, in some embodiments, a CD3 targeting moiety of the present disclosure has a K_D as measured in a surface plasmon resonance assay (*e.g.*, at 25 °C) of greater than 10^{-6} M, greater than 10^{-7} M, greater than 10^{-8} M, or greater than 10^{-9} M, including any range or value derivable therein (*e.g.*, between 10^{-6} M and 10^{-7} M, between 10^{-6} M and 10^{-8} M, between 10^{-6} M and 10^{-9} M, between 10^{-7} M and 10^{-8} M, or between 10^{-7} M and 10^{-9} M, or between 10^{-8} M and 10^{-8} M).

6.6. Antigen Targeting Moieties

[0118] Primary MBMs of the present disclosure contain one antigen targeting moiety, flanked by two TCA targeting moieties. In general, as used herein, the term "antigen targeting moiety" refers to a targeting moiety that binds to an antigen that is not a T cell antigen; that is, "antigen targeting moiety" refers to a targeting moiety that is not a TCA targeting moiety. In some embodiments, the antigen targeting moiety is a tumor antigen targeting moiety, e.g., as described in Section 6.6.1. In some embodiments, the antigen targeting moiety is a low-density antigen targeting moiety, e.g., as described in Section 6.6.2. In some embodiments, the antigen targeting moiety is an HLA-bound peptide antigen targeting moiety, e.g., as described in Section 6.6.3.

[0119] As will be recognized by the skilled artisan, an antigen targeting moiety of a primary MBM of the disclosure may be considered as being of multiple types based on the antigen to which the antigen targeting moiety specifically binds. For example, an antigen targeting moiety may be both a tumor antigen targeting moiety and an HLA-bound peptide antigen targeting moiety where the HLA-bound peptide is a tumor-specific peptide. Similarly, an antigen targeting moiety may be both a low-density antigen targeting moiety and an HLA-bound peptide antigen targeting moiety, for example, where the HLA-bound peptide is present at no more than 5000 copies on the surface of a target cell.

[0120] Exemplary antigen targeting moieties are described in further detail below.

6.6.1. Tumor Antigen Targeting Moieties

[0121] In some embodiments, an MBM of the present disclosure comprises at least one targeting moiety that binds specifically to a target molecule expressed by a tumor cell or in the tumor cell environment, e.g., an extracellular matrix ("ECM") antigen, a tumor reactive lymphocyte antigen, a cell surface molecule of tumor or viral lymphocytes, a checkpoint inhibitor, or a tumor-associated antigen (TAA), collectively referred to herein as a "tumor antigen targeting moieties." The skilled artisan would recognize that the foregoing categories of target molecules are not mutually exclusive and thus a given target molecule may fall into more than one of the foregoing categories of target molecules. For example, some molecules may be considered both TAAs and ECM proteins. Preferably, the ECM antigen, tumor reactive lymphocyte antigen, cell surface molecule of tumor or viral lymphocytes, checkpoint inhibitor, or TAA is a human antigen. The antigen may or may not be present on normal cells. Certain aspects are directed to MBMs comprising at least one targeting moiety that binds specifically to a TAA.

[0122] It is anticipated that any type of tumor and any type of ECM antigen, tumor reactive lymphocyte antigen, cell surface molecule of tumor or viral lymphocytes, checkpoint inhibitor, or TAA may be targeted by an antigen targeting moiety of the disclosure. Exemplary types of cancers that may be targeted include acute lymphoblastic leukemia, acute myelogenous leukemia, biliary cancer, B-cell leukemia, B-cell lymphoma, biliary cancer, bone cancer, brain cancer, breast cancer, triple-negative breast cancer, cervical cancer, Burkitt lymphoma, chronic lymphocytic leukemia, chronic myelogenous leukemia, colorectal cancer, endometrial cancer, esophageal cancer, gall bladder cancer, gastric cancer, gastrointestinal tract cancer, glioma, hairy cell leukemia, head and neck cancer, Hodgkin's lymphoma, liver cancer, lung cancer, medullary thyroid cancer, melanoma, multiple myeloma, ovarian cancer, non-Hodgkin's lymphoma, pancreatic cancer, prostate cancer, pulmonary tract cancer, renal cancer, sarcoma, skin cancer, testicular cancer, urothelial cancer, and other urinary bladder cancers. However, the skilled artisan will realize that TAAs and other target molecules associated with the tumor microenvironment are known for virtually any type of cancer.

[0123] Non-limiting examples of ECM antigens include syndecan, heparanase, integrins, osteopontin, link, cadherins, laminin, laminin type EGF, lectin, fibronectin, notch, nectin (e.g., nectin-4), tenascin, collagen (e.g., collagen type X) and matrixin.

[0124] In particular embodiments, the target molecules are checkpoint inhibitors, for example CTLA-4, PD1, PDL1, PDL2, B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK1, CHK2. In particular embodiments, the target molecule is PD1. In other embodiments, the target molecule is LAG3. In some embodiments, where the target molecule is a checkpoint inhibitor, the tumor antigen targeting moiety is non-blocking or poorly-blocking of ligand-receptor binding. Examples of non-blocking or poorly-blocking anti-PD1 antibodies includes antibodies having VH/VL amino acid sequences of SEQ ID Nos: 2/10 of PCT Pub. No. WO2015/112800A1; SEQ ID Nos: 16/17 of US Patent No. 11,034,765 B2; SEQ ID Nos. 164/178, 165/179, 166/180, 167/181, 168/182, 169/183, 170/184, 171/185, 172/186, 173/187, 174/188, 175/189, 176/190 and 177/190 of US Patent No. 10,294,299 B2. Examples of non-blocking or poorly-blocking anti-LAG3 antibodies includes antibodies having VH/VL amino acid sequences of SEQ ID Nos 23/24, 3/4 and 11/12 of US Pub. US2022/0056126A1.

[0125] Exemplary tumor antigens are set forth in Table A below, together with references to exemplary antibodies or antibody sequences upon which the tumor antigen targeting moiety can be based.

TABLE A
Exemplary Target Molecules

Target (Tumor Antigen)	Antibody Name and/or Binding Sequences
1-92-LFA-3	Amevive™ (alefacept)
5T4	GEN1044
Activin Receptor Type II	Bimagrumab VH: SEQ ID Nos:107, 109 of US Patent No. 8,388,968 B2 VL: SEQ ID Nos:93, 95 of US Patent No. 8,388,968 B2
B7-H3	Obrindatamab (MGD009)
B7-H3 (CD276)	Enoblituzumab (MGA271)
B7-H3 (CD276)	MGC018
B7-H3 (CD276)	MGA012
B7-H3 (CD276)	8H9
B7-H3 (CD276)	VH: the VH sequence of the heavy chain of SEQ ID NO:21, 26 or 31 of US 2021/0171641 A1.

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	VL: the VL sequence of the light chain of SEQ ID NO:20, 22 or 30 of US 2021/0171641 A1.
B7-H3 (CD276)	VH: the VH sequence of the heavy chain of SEQ ID NO:21, 29 or 37 of US 2019/0002563 A1. VL: the VL sequence of the light chain of SEQ ID NO:17, 25 or 33 of US 2019/0002563 A1.
B7-H3 (CD276)	VH: the VH sequence of the heavy chain of SEQ ID NO:146, 147 or 148 of US Patent No. 10,640,563. VL: the VL sequence of the light chain of SEQ ID NO:143, 144 or 145 of US Patent No. 10,640,563.
BAFF/B Lymphocyte Stimulator	Benlysta™ (velimumab)
BAFF/B Lymphocyte Stimulator	VH: amino acids 1-123 of SEQ ID NO:327 of US Patent No. 7,138,501 VL: amino acids 139-249 of SEQ ID NO:327 of US Patent No. 7,138,501.
BAFF/B Lymphocyte Stimulator	VH: amino acids 1-126 of SEQ ID NO:1321 of US Patent No. 7,605,236; VL: amino acids 143-251 of SEQ ID NO:1049 of US Patent No. 7,605,236.
BAFF/B Lymphocyte Stimulator	Belimumab
BCMA	VH: the VH sequence of the heavy chain of SEQ ID NO. 126 of US 2021/0206865 A1 VL: the VL sequence of the light chain of SEQ ID NO. 129 or SEQ ID NO. 132 of US 2021/0206865 A1
CA125	Igobumab
CA125	OvaRex™ (oregobumab)
Cadherin	The antibodies described in US Pub. No. US 2006/0039915.
N-cadherin	An antibody that binds to the amino acid sequence of SEQ ID NO: 10, 17 or 18 of US Pub. No. US 2010/0278821.
CD11a	Raptiva™ (efalizumab) Sequence in Werther *et al.*, 1996, The Journal of Immunology 157(11): 4986-4995.
CD19	Blincyto™ (blinatumomab)
CD19	SGN-CD19A
CD20	Bexxar™ (tositumomab) VH: the VH sequence of the heavy chain of SEQ ID NO:124 of US Patent Pub. US 2017/0002060 A1

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	VL: the VL sequence of the light chain of SEQ ID NO:125 of US Patent Pub. US 2017/0002060 A1
CD20	Zevalin™ (ibritumomab tiuxetan) VH: SEQ ID NO:9 of US Patent No. 5,736,137 VL: SEQ ID NO:6 of US Patent No. 5,736,137
CD20	Rituxan™ (rituximab) VH: SEQ ID NO:9 of US Patent No. 5,736,137 VL: SEQ ID NO:6 of US Patent No. 5,736,137
CD20	Ocrevus™ (ocrelizumab)
CD20	Okaratuzumab
CD20	Arzerra™ (ofatumumab) VH: SEQ ID NO:2 of US Patent No. 8,529,902 VL: SEQ ID NO:4 of US Patent No. 8,529,902
CD20	Gazyva™ (obinutuzumab)
CD20	VH: SEQ ID NO: 4 of US 2021/0206870 A1 VL of SEQ ID NO:6 of US 2021/0206870 A1
CD20	Epcoritamab
CD22	Belimumab
CD22	Epratuzumab
CD22	Besponsa ™ (inotuzumab ozogamicin)
CD22	Lumoxiti ™ (moxetumumab pasudox)
CD22	pinatuzumab vedotin
CD25	Zenapax™ (daclizumab) VH: SEQ ID NO:9 of US Patent No. 7,060,269 VL: SEQ ID NO:10 of US Patent No. 7,060,269
CD30	Adcetris™ (brentuximab vedotin) VH: SEQ ID NO:2 of US Patent No. 7,090,843 VL: SEQ ID NO:10 of US Patent No. 7,090,843
CD33	Myelotarg™ (gemtuzumab) Sequence in Man Sung, *et al.*, 1993, Molecular immunology 30:1361-1367
CD33	Lintuzumab
CD38	Darzalex™ (daratumumab)
CD38	IB4, HB7 CS/2, clone 90 and NIM-R5 as disclosed in PCT Pub. WO2015/009726A2 and references cited therein.
CD40	Lukatumumab
CD40	Dacetuzumab
CD40L	Hu5c8 (ruplizumab)
CD44v6	vibatuzumab mertansine
CD52	Campath™ (alemtuzumab)

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	VH: SEQ ID NO:1 of US Patent Pub. US 2017/0002060 A1 VL: SEQ ID NO:2 of US Patent Pub. US 2017/0002060 A1
CD70	Blenrep ™ (borsetuzumab mafodotin)
CD123	Flotetuzumab
CD206	Anti-CD206 antibodies having a VH a of SEQ ID NO. 2 and a VL of SEQ ID NO:4 of WO2003/040169A2
CD221	Tepezza ™ (teprotumumab)
CEA	Hybri-Ceaker® (altumomab pentetate)
CEA	Scintimun™ (besilesomab)
CEA	CEA-CIDE™ (labetuzumab))
CEA	CEA-Scan ™ (arcitumomab)
CEA	hMN-15 CDR-H1, CDR-H2 and CDR-H3 sequences of SEQ ID Nos:4-6 of US Patent No. 8,771,690 B2 CDR-L1, CDR-L2 and CDR-L3 sequences of SEQ ID Nos:1-3 of US Patent No. 8,771,690 B2
CEA	CEA binding portion of RO6958688/RG7802 from clinical trial NCT02324257
CEA	Cibisatamab
CEA	CEA binding portion of MEDI-565/MT110/AMG211 from clinical trials NCT01284231 and NCT02291614 VH: SEQ ID NO:49 or 51 of PCT Publication No. WO 2013/012414 A1 VL: SEQ ID NO:48 of PCT Publication No. WO 2013/012414 A1.
CEA	Rabetuzumab
CEA	Atezolizumab
CEA	Cibisatamab
CEA	MEDI-565 (AMG211, MT111)
CEA	RO6958688
CEA	VH: SEQ ID No. 9 described in WO2022/048883A1 VL: SEQ ID No. 10 described in WO2022/048883A1
CLDN18.2	AMG910
Clec9a	Anti-Clec9a antibodies having VH and VL amino acids of SEQ ID Nos. 43 and 48 of PCT Pub. No. WO2009/026660A1
Clec9a	Anti-Clec9a antibodies having a VH a of SEQ ID NO. 38 and a VL of SEQ ID NO:37 or SEQ ID NO:43 of PCT Pub. No. WO2022/073062A1

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	Anti-Clec9a antibodies having a VH a of SEQ ID NO. 8 and a VL of SEQ ID NO:7 of PCT Pub. No. WO2022/073062A1
Collagen alpha-4 chain	TRC093 (MT293)
Collagen	The collagen binding antibody fragment described in Liang *et al.*, 2016, Sci. Rep. 5, 18205; doi: 10.1038/srep18205 (2016).
Collagen Type I	Cetuximab (Erbitux)
Collagen type X	The amino acid sequences of SEQ ID NO: 1 or 2 of PCT Pub No. WO 2019/020797.
Collagen type X	The amino acid sequences of SEQ ID NO: 1 of PCT Pub No. WO 2014/180992.
Collagen type X	Antibody X34 as described in I. Girkontaite et al., "Immunolocalization of type X collagen in normal fetal and adult osteoarthritic cartilage with monoclonal antibodies," Matrix Biol 15, 231-238 (1996).
Collagen type X	Antibodies X53 or 1H8 or ARC0659 or JF0961 collagen X polyclonal antibody sold under catalog number PA5-115039 or PA5-116871 or PA5-97603 or PA5-49198 from ThermoFisher Scientific.
Collagen type X	Antibody sold under catalog number RDI-COLL10abr from RDI.
Complement C5	Soliris™ (eculizumab) VH: amino acids 1-122 of SEQ ID NO: 10 of US Patent No. 6,355,245 VL: amino acids 3-110 of SEQ ID NO: 9 of US Patent No. 6,355,245
CTLA-4	Yervoy™ (ipilimumab) VH: SEQ ID NO: 17 of WO 2001/014424 A2 VL: SEQ ID NO: 7 of WO 2001/014424 A2
CTLA-4	(tremelimumab)
CTLA-4	Orencia™ (abatacept)
DEC-205	Anti-DEC-205 antibodies having a VH/VL pair of SEQ ID NOS. 4/10, 16/22, 28/34, 40/46, 52/58, and 76/82 of PCT Pub. WO2009/061996A2
DLL3	AMG757
EGFR	Erbitux™ (cetuximab) VH: SEQ ID NO: 11 of US Patent No. 6,217,866 VL: SEQ ID NO: 13 of US Patent No. 6,217,866
EGFR	Vectibix™ (panitumumab) VH: SEQ ID NO: 37 of US Patent No. 6,235,883 VL: SEQ ID NO: 38 of US Patent No. 6,235,883
EGFR	Zalutumumab

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	VH: SEQ ID NO: 64 of WO 2018/140831 A2 VL: SEQ ID NO: 69 of WO 2018/140831 A2
EGFR	Mapatumumab
EGFR	Matuzumab
EGFR	Nimotuzumab VH: SEQ ID NO: 51 of WO 2018/140831 A2 VL: SEQ ID NO: 56 of WO 2018/140831 A2
EGFR	ICR62
EGFR	mAb 528
EGFR	CH806
EGFRv3	AMG596
EGFRv3	AMG404
EGFR/CD64	MDX-447
EpCAM	Panorex ™ (edrecolomab) VH: SEQ ID NO:129 of WO 2018/140831 A2 VL: SEQ ID NO:134 of WO 2018/140831 A2
EpCAM	Adecatumumab VH: SEQ ID NO:142 of WO 2018/140831 A2 VL: SEQ ID NO:147 of WO 2018/140831 A2
EpCAM	tucotuzumab celmoleukin
EpCAM	citatuzumab bogatox
EpCAM	EP1629013 B1 VH: SEQ ID Nos: 80, 84, 88, 92 or 96 VL: SEQ ID Nos: 82, 86, 90, 94 or 98
EpCAM	G8.8 HC: SEQ ID NO: 4 of US Patent Pub. No. US 2020/0317806 A1 HL: SEQ ID NO: 3 of US Patent Pub. No. US 2020/0317806 A1
EpCAM	VH: SEQ ID Nos: 17-22 of WO 2021/211510 A2. VL: SEQ ID NO: 15-16 of WO 2021/211510 A2.
EpCAM	Removab ™(catumaxomab)
EpCAM	Vicineum ™ (oportuzumab monatox)
EpCAM	M701
F protein of RSV	Synagic™ (palivizumab)
GD2	3F8
Glycoprotein receptor IIb/IIIa	ReoPro™ (abiciximab)
gpA33	MGD007
GPC3	ERY974

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
GUCY2C	PF-07062119
Heparanase	An antibody selected from HP130, HP 239, HP 108.264, HP 115.140, HP 152.197, HP 110.662, HP 144.141, HP 108.371, HP 135.108, HP 151.316, HP 117.372, HP 37/33, HP3/17, HP 201 or HP 102 or an amino acid sequence of SEQ ID NO: 1-11 described in US Patent Pub. US 2004/0170631.
Her2	Herceptin™ (trastuzumab)
Her2	Aldesleukin (proleukine)
Her2	Sargramustim (Leucine)
Her2	M802
Her2	Runimotamab (BTRC4017A, R07227780)
Her2	ISB1302
Her2-neu	Perjeta™ (pertuzumab) VH: SEQ ID NO:16 of WO 2013/096812 A1. VL: SEQ ID NO:15 of WO 2013/096812 A1.
Her2-neu	Rexomun™ (ertumaxomab)
IgE	Xolair™ (omalizumab)
IGFIR	(figitumumab)
IL1β	Ilaris™ (canakinumab) VH: SEQ ID NO:1 of US Patent No. 7,446,175. VL: SEQ ID NO: 2 of US Patent No. 7,446,175
IL12/IFN3	Stelara™ (ustekinumab)
IL1Ra	Antril™, Kineret™ (ankinra)
IFNR	Simulect™ (basiliximab) VH: SEQ ID NO: 3 of US Patent No. 6,383,487 VL: SEQ ID NO: 6 of US Patent No. 6,383,487
IL6	Clazakizumab
IL6 receptor	Actemra™ (tocilizumab) VH: SEQ ID NO: 31 of US Patent No. 7,479,543 VL: SEQ ID NO: 29 of US Patent No. 7,479,543
IL12/IFN3 p40 subunit	Stelara™ (ustekinumab) VH: SEQ ID NO: 7 of US Patent No. 6,902,734 VL: SEQ ID NO: 8 of US Patent No. 6,902,734
Integrin α4	Tysabri™ (natalizumab) VH: SEQ ID Nos: 11-13 of US Patent No. 5,840,299 VL: SEQ ID Nos: 7-8 of US Patent No. 5,840,299
Integrin α4 β7	Entyvio ™ (vedolizumab) HC: SEQ ID NO: 2 of US Patent Pub. US 2012/0282249. LC: SEQ ID NO: 4 of US Patent Pub. US 2012/0282249.
Integrin α5 β1	VH: SEQ ID NO: 2 of European Patent No. 1 755 659. VL: SEQ ID NO: 4 of European Patent No. 1 755 659.

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
Integrin β1	VH: SEQ ID NO:2, 6, 8, 10, 12, 14, 29-43 or 91-100 of US Patent Pub. US 2022/0089744. VL: SEQ ID NO:4, 16, 18, 20, 22, 44-57 or 107-116 of US Patent Pub. US 2022/0089744.
KIR	Anti-KIR antibodies having a VH of SEQ ID NO: 5 and a VL of SEQ ID NO: 3 of PCT Pub. WO2014/066532A1
KIR	Anti-KIR antibodies having a VH of SEQ ID NO: 1 and a VL of SEQ ID NO: 2 of PCT Pub. WO2012/160448A2 Anti-KIR antibodies having a VH of SEQ ID NO: 3 and a VL of SEQ ID NO: 4 of PCT Pub. WO2012/160448A2
LAG3	Relatlimab (BMS-98016)
LAG3	Sym022
LAG3	HLX26
LAG3	TSR-033
LAG3	ABL501
LAG3	INCAGN02385
LAG3	Fianlimab (REGN3767)
LAG3	RO7247669
LAG3	EMB-02
LAG3	FS118
LAG3	GSK2831781
LAG3	IBI323
LAG3	IBI110
LAG3	LAG525
LAG3	XmAb®22841
LAG3	LBL-007
LAG3	VH: SEQ ID NO:1, 8, 10 or 12 of US Patent No. 9,902,772. VL: SEQ ID NO:2, 3, 4, 5, 6, 7, 9, 11, 13 or 14 of US Patent No. 9,902,772.
LAG3	VH: SEQ ID NO: 182 of US Patent Pub. US 2021/0095026. VL: SEQ ID NO: 88 of US Patent Pub. US 2021/0095026.
LAG3	Antibodies having VH/VL amino acid sequences of SEQ ID Nos 23/24, ¾ and 11/12 of US Pub. US2022/0056126A1.
Laminin	Lam-89 from Sigma Aldrich
Mesothelin	Amatuximab
Mesothelin	HPN536
MUC1	civatuzumab tetraxetane
MUC1	Pankomab ™ (gatipotuzumab)
MUC1	Femtumumab
MUC1	Cantuzumab ravtansine

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
MUC16 (CA125)	Anti-MUC16 antibodies having VH and VL sequences having the amino acid sequences of any one of the following SEQ ID NO: pairs from US 2018/0118848A1: 18/26; 82/858; 98/170
MUC17	AMG199
Nectin-4	Enfortumab (ASP7465, ASG-22CE, ASG-22ME) VH: SEQ ID NO:3 of PCT Pub. WO 2021/151984. VL: SEQ ID NO: 4 of PCT Pub. WO 2021/151984.
Nectin-4	SBT290
Nectin-4	VH: SEQ ID NO:1 of US Patent No. 11,274,160. VL: SEQ ID NO:2 of US Patent No. 11,274,160.
NGF	(tanezumab)
NKH1A	The monoclonal antibody deposited with ATCC and assigned accession no. HB8564, as described in US Patent No. 4,772,552A
NKP46	Anti-NKP46 antibodies having CDR-H1, CDR-H2 and CDR-H3 sequences of SEQ ID Nos:4, 6 and 8 and CDR-L1, CDR-L2 and CDR-L3 sequences of SEQ ID Nos:12, 14 and 16 of PCT Pub. WO2018/047154A1
Osteopontin	HC: SEQ ID NO: 22 of PCT Pub. WO 2021/030209. LC: SEQ ID NO: 24 of PCT Pub. WO 2021/030209.
PD1	MDX-1106 / BMS-936558 (nivolumab), a human IgG4 mAb with the structure described in WHO Drug Information, Vol. 27, No. 1, pages 68-69 (2013) and whose heavy and light chain sequences are disclosed in FIG. 7 of US Pub. No. US20190270812A1 HC: SEQ ID NO: 23 of US Pub. No. US20190270812A1 LC: SEQ ID NO: 24 of US Pub. No. US20190270812A1
PD1	MK-3475 (pembrolizumab), a humanized IgG4 mAb with the structure described in WHO Drug Information, Vol. 27, No. 2, pages 161-162 (2013) and whose heavy and light chain sequences are disclosed in FIG. 6 of US Pub. No. US20190270812A1 HC: SEQ ID NO: 21 of US Pub. No. US20190270812A1 LC: SEQ ID NO: 22 of US Pub. No. US20190270812A1
PD1	REGN2810 (disclosed as H4H7798N in US Pub No. 20150203579) HC: SEQ ID NO: 330 of US Pub. No. 20150203579 LC: SEQ ID NO: 331 of US Pub. No. 20150203579
PD1	H2M7791N disclosed in US Pub No. 20150203579 as having the following heavy and light chain variable domains: VH: SEQ ID NO: 98 of US Pub. No. 20150203579 VL: SEQ ID NO: 106 of US Pub. No. 20150203579

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
PD1	Anti-PD1 antibodies having CDR H1-H3 and CDR L1-L3 sequences corresponding to the following SEQ ID Nos. of US Patent No. 11,034,765 B2: a) SEQ ID Nos: 18, 19, 20, 21, 22, and 23, respectively; b) SEQ ID Nos: 24, 25, 26, 27, 28, and 29, respectively; c) SEQ ID Nos: 30, 31, 32, 33, 34, and 35, respectively; d) SEQ ID Nos: 36, 37, 38, 39, 40, and 41, respectively; e) SEQ ID Nos: 42, 43, 44, 45, 46, and 47, respectively; f) SEQ ID Nos: 48, 49, 50, 51, 52, and 53, respectively; g) SEQ ID Nos: 54, 55, 56, 57, 58, and 59, respectively; and h) SEQ ID Nos: 60, 61, 62, 63, 64, and 65, respectively.
PD1	Anti-PD1 antibodies disclosed in Tables 1-3 of PCT Pub. WO2015112800A1, including but not limited to anti-PD1 antibodies having VH/VL pairs having SEQ ID Nos: 2/10, 18/26, 34/42, 50/58, 66/74, 82/90, 98/106, 1 14/122, 130/138, 146/154, 162/170, 178/186, 194/202, 210/202, 218/202, 226/202, 234/202, 242/202, 250/202, 258/202, 266/202, 274/202, 282/202, 290/202, 298/186, 306/186 and 314/186 of PCT Pub. WO2015112800A1.
PD1	Anti-PD1 antibodies disclosed in US Patent No. 10,294,299 B2 as having the following SEQ ID NO. pairs for heavy and light chain variable domains: SEQ ID Nos. 164/178 SEQ ID Nos. 165/179 SEQ ID Nos. 166/180 SEQ ID Nos. 167/181 SEQ ID Nos. 168/182 SEQ ID Nos. 169/183 SEQ ID Nos. 170/184 SEQ ID Nos. 171/185 SEQ ID Nos. 172/186 SEQ ID Nos. 173/187 SEQ ID Nos. 174/188 SEQ ID Nos. 175/189 SEQ ID Nos. 176/190 SEQ ID Nos. 177/190
PD1	MEDI-0680 (AMP-514)
PD1	PDR001 (spartalizumab), a humanized IgG4 mAb whose heavy and light chain sequences are disclosed as BAP049-Clone-E in US Patent No: 9683048 B2. HC: SEQ ID NO: 91 of US Patent No: 9,683,048 LC: SEQ ID NO: 72 of US Patent No: 9,683,048
PD1	BGB-108
PD1	h409A11, described in WO2008/156712

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	HC: SEQ ID NO: 31 of PCT Pub. WO2008/156712 LC: SEQ ID NO: 36 of PCT Pub. WO2008/156712
PD1	h409A16, described in WO2008/156712 HC: SEQ ID NO: 31 of PCT Pub. WO2008/156712 LC: SEQ ID NO: 37 of PCT Pub. WO2008/156712
PD1	h409A17, described in WO2008/156712 HC: SEQ ID NO: 31 of PCT Pub. WO2008/156712 LC: SEQ ID NO: 38 of PCT Pub. WO2008/156712
PD1	Anti-PD1 antibodies described in US Patent No. 7,488,802 as having the following SEQ ID NO. pairs for heavy and light chain variable domains: SEQ ID Nos. 2/4 SEQ ID Nos. 6/8 SEQ ID Nos. 10/12 SEQ ID Nos. 14/16 SEQ ID Nos. 47/49
PD1	Anti-PD1 antibodies described in US Patent No. 7,521,051 as having the following SEQ ID NO. pairs for heavy and light chain variable domains: SEQ ID Nos. 2/4 SEQ ID Nos. 6/8 SEQ ID Nos. 10/12 SEQ ID Nos. 14/16 SEQ ID Nos. 47/49
PD1	Anti-PD1 antibodies described in US Patent No. 8,008,449 as having the following SEQ ID NO. pairs for heavy and light chain variable domains: SEQ ID Nos. 1/8 SEQ ID Nos. 2/9 SEQ ID Nos. 3/10 SEQ ID Nos. 4/11 SEQ ID Nos. 5/12 SEQ ID Nos. 6/13 SEQ ID Nos. 7/14
PD1	Anti-PD1 antibodies described in US Patent No. 8,354,509 as having the following SEQ ID NO. pairs for heavy and light chain full-length domains: SEQ ID Nos. 31/36 SEQ ID Nos. 31/37 SEQ ID Nos. 31/38
PD1	Anti-PD1 antibodies described in US Patent No. 8,168,757 as having the following SEQ ID NO. pairs for heavy and light chain variable domains:

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	SEQ ID Nos. 4/5 SEQ ID Nos. 12/13 SEQ ID Nos. 18/19 SEQ ID Nos.40/41 SEQ ID Nos.47/48 SEQ ID Nos.26/27 SEQ ID Nos.34/35 SEQ ID Nos.55/56 SEQ ID Nos.67/68
PD1	Anti-PD1 antibodies described in PCT Pub. No. WO2004/004771
PD1	Anti-PD1 antibodies described in PCT Pub. No. WO2004/056875 as having the following SEQ ID NO. pairs for heavy and light chain variable domains: SEQ ID Nos. 2/4 SEQ ID Nos. 6/8 SEQ ID Nos. 10/12 SEQ ID Nos. 14/16 SEQ ID Nos. 47/49
PD1	Anti-PD1 antibodies described in PCT Pub. No. WO2004/072286
PD1	VH: SEQ ID NO: 25, 26, 27, 28, or 29 of US Pub. No. US2011/0271358 VL: SEQ ID NO: 30, 31, 32, or 33 of US Pub. No. US2011/0271358
PD1	SHR-1210 (Camrelizumab) described in PCT Publication No: WO 2015/085847 as having the following heavy and light chain variable domains: HC: SEQ ID NO: 9 LC: SEQ ID NO: 10
PD1	4A11 described in U.S. Patent No. 8,008,449 as having the following heavy and light chain variable domains: VH: SEQ ID NO: 5 VL: SEQ ID NO: 12
PD1	h1H3 Var 6 described in U.S. Patent No. 9,205,148 as having the following heavy and light chain variable domains: VH: SEQ ID NO: 106 VL: SEQ ID NO: 98
PD1	PD1AB-6 described in U.S. Patent No. 10,428,145 as having the following heavy and light chain variable domains: VH: SEQ ID NO: 13 VL: SEQ ID NO: 8
PD1	A1.5 described in U.S. Patent No. 10,513,558 as having the following heavy and light chain variable domains:

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	VH: SEQ ID NO: 21 VL: SEQ ID NO: 47
PD1	Anti-PD1 antibodies 244C8-2 and 388D4-3, described in U.S. Patent No. 10,544,217 as having the following SEQ ID NO. pairs for heavy and light chain variable domains, respectively: SEQ ID NOs: 85/93 SEQ ID NOs: 88/96
PD1	Anti-PD1 antibodies 9B2C6C9 and 76G5B3, described in PCT Publication No. WO 2017/016497 as having the following SEQ ID NO. pairs for heavy and light chain variable domains, respectively: SEQ ID NOs: 1/5 SEQ ID NOs: 9/13
PD1	Anti-PD1 antibodies 1.153.7 hAb and 1.103.11 hAbv2 described in PCT Publication No. WO 2017/025051 as having the following SEQ ID NO. pairs for heavy and light chain variable domains, respectively: SEQ ID NOs: 61/63 SEQ ID NOs: 53/55
PD1	Clone 19 described in U.S. Patent No. 8,927,697 as having the following heavy and light chain variable domains: VH: SEQ ID NO: 14 VL: SEQ ID NO: 12
PD1	PD1-0103-312 described in PCT Publication No. WO 2017/055443 as having the following heavy and light chain variable domains: VH: SEQ ID NO: 57 VL: SEQ ID NO: 58
PD1	Anti-PD1 antibodies h4B10-3 and 1353-G08 described in PCT Publication No. WO 2016/077397 as having the following SEQ ID NO. pairs for heavy and light chain variable domains, respectively: SEQ ID NOs: 264/288 SEQ ID NOs: 251/275
PD1	PD-1-27 described in PCT Publication No. WO 2016/210129 as having the following heavy and light chain variable domains: VH: SEQ ID NO: 51 VL: SEQ ID NO: 49
PD1	Anti-PD1 antibodies RG1H10 and RG1H10-16C described in PCT Publication No. WO 2014/194302 as having the following

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	SEQ ID NO. pairs for heavy and light chain variable domains, respectively: SEQ ID NOs: 23/24 SEQ ID NOs: 40/24
PD1	HuEH12-VH4-VK3 described in U.S. Patent No. 9,102,727 as having the following heavy and light chain variable domains: VH: SEQ ID NO: 28 VL: SEQ ID NO: 32
PD1	Clone 39 described in U.S. Patent No. 10,066,013 as having the following heavy and light chain variable domains: VH: SEQ ID NO: 35 VL: SEQ ID NO: 34
PD1	tislelizumab
PD1	retifanlimab
PD1	balstilimab
PD1	dostarlimab
PD1	sasanlimab
PD1	sintilimab
PD1	cemiplimab
PDL1	Durvalumab (MEDI4736) HC: SEQ ID NO: 26 of PCT application No. WO2020225552 LC: SEQ ID NO: 27 of PCT application No. WO2020225552
PDL1	Atezolizumab (Tecentriq, MPDL3280A, RG7446) HC: SEQ ID NO: 20 of US Patent No. 8,217,149 LC: SEQ ID NO: 21 of US Patent No. 8,217,149
PDL1	MDX 1105 (BMS-936559)
PDL1	Anti-PDL1 antibodies described in U.S. Patent No. 7,943,743 as having the following SEQ ID NO. pairs for heavy and light chain variable domains: SEQ ID Nos: 1/11 SEQ ID Nos: 2/12 SEQ ID Nos: 3/13 SEQ ID Nos: 4/14 SEQ ID Nos: 5/15 SEQ ID Nos: 6/16 SEQ ID Nos: 7/17 SEQ ID Nos: 8/18 SEQ ID Nos: 9/19 SEQ ID Nos: 10/20
PDL1	Avelumab, described in US Patent No: 9,624,298 as having the following heavy and light chain variable domains:

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	HC: SEQ ID NO: 24 LC: SEQ ID NO: 25
PDL1	ZKAB001 (Socazolimab)
PDL1	TQB2450 (APL-502 or CBT-502)
PDL1	HLX20 CDR-H1, CDR-H2 and CDR-H3 sequences of SEQ ID Nos: 52, 56, and 77 of PCT Pub. No. 2018/080812 CDR-L1, CDR-L2 and CDR-L3 sequences of SEQ ID Nos: 65, 42, and 71 of PCT Pub. No. 2018/080812
PDL1	KN035 (Envafolimab) is a nanobody described as Hu56V2 in US Patent No. 11,225,522 as having the VHH SEQ ID NO: 34
PDL1	LY3434172
PDL1	LY3300054 (Iodapolimab) described in PCT Pub No: WO 2017/034916 as having the following heavy and light chain variable domains: HC: SEQ ID NO: 10 L: SEQ ID NO: 11
PDL1	LDP (Iesabelimab, ADG104) described in CN Patent No: 114225023 as having the following heavy and light chain variable domains: HC: SEQ ID NO: 10 LC: SEQ ID NO: 9
PDL1	EMB-09
PDL1	ABL501
PDL1	INBRX-105
PDL1	STI-3031 (IMC-001) described in US Patent No: 10,118,963 as having the following heavy and light chain variable domains: HC: SEQ ID NO: 1 LC: SEQ ID NO: 2
PDL1	BGB-A333 (garivulimab) described in US Patent No: 11,512,132 as having the following heavy and light chain variable domains: HC: SEQ ID NO: 22 LC: SEQ ID NO: 23
PDL1	HLX301
PDL1	Y101D
PDL1	ES101
PDL1	IBI322
PDL1	VH: SEQ ID NO:46, 48, 50 or 52 of US Patent No. 11,168,144. VL: SEQ ID NO:58, 137 or 12 of US Patent No. 11, 168,144.

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
PDL1	VH: SEQ ID NO:23, 124, 126, 127, 128, 130, 140 or 145 of US Patent No. 11,208,486. VL: SEQ ID NO:24 or 125 of US Patent No. 11,208,486.
Phosphatidylserine	(bavituximab)
PSCA	GEM3PSCA
PSMA	huJ591
PSMA	Anti-PSMA antibodies having VH and VL sequences having the amino acid sequences of any one of the following SEQ ID NO: pairs from WO 2017/023761A1: 2/1642; 10/1642; 18/1642; 26/1642; 34/1642; 42/1642; 50/1642; 58/1642; 66/1642; 74/1642; 82/1642; 90/1642; 98/1642; 106/1642; 1 14/1642; 122/130; and 138/146.
PSMA	An antibody such as: PSMA 3.7, PSMA 3.8, PSMA 3.9, PSMA 3.11, PSMA 5.4, PSMA 7.1, PSMA 7.3, PSMA 10.3, PSMA 1.8.3, PSMA A3.1.3, PSMA A3.3.1, Abgenix 4.248.2, Abgenix 4.360.3, Abgenix 4.7.1, Abgenix 4.4.1, Abgenix 4.177.3, Abgenix 4.16.1, Abgenix 4.22.3, Abgenix 4.28.3, Abgenix 4.40.2, Abgenix 4.48.3, Abgenix 4.49.1, Abgenix 4.209.3, Abgenix 4.219.3, Abgenix 4.288.1, Abgenix 4.333.1, Abgenix 4.54.1, Abgenix 4.153.1, Abgenix 4.232.3, Abgenix 4.292.3, Abgenix 4.304.1, Abgenix 4.78.1 and Abgenix 4.152.1 described in WO2003034903A2 A hybridoma cell line such as: PSMA 3.7 (PTA-3257), PSMA 3.8, PSMA 3.9 (PTA- 3258), PSMA 3.11 (PTA-3269), PSMA 5.4 (PTA-3268), PSMA 7.1 (PTA-3292), PSMA 7.3 (PTA-3293), PSMA 10.3 (PTA-3247) , PSMA 1.8.3 (PTA-3906), PSMA A3.1.3 (PTA- 3904), PSMA A3.3.1 (PTA-3905), Abgenix 4.248.2 (PTA-4427), Abgenix 4.360.3 (PTA- 4428), Abgenix 4.7.1 (PTA-4429), Abgenix 4.4.1 (PTA-4556), Abgenix 4.177.3 (PTA-4557), Abgenix 4.16.1 (PTA-4357), Abgenix 4.22.3 (PTA-4358), Abgenix 4.28.3 (PTA-4359), Abgenix 4.40.2 (PTA-4360), Abgenix 4.48.3 (PTA-4361), Abgenix 4.49.1 (PTA-4362), Abgenix 4.209.3 (PTA-4365), Abgenix 4.219.3 (PTA-4366), Abgenix 4.288.1 (PTA-4367), Abgenix 4.333.1 (PTA-4368), Abgenix 4.54.1 (PTA-4363), Abgenix 4.153.1 (PTA-4388), Abgenix 4.232.3 (PTA-4389), Abgenix 4.292.3 (PTA-4390), Abgenix 4.304.1 (PTA-4391), Abgenix 4.78.1 (PTA-4652), and Abgenix 4.152.1(PTA-4653) described in WO 2003/034903A2. VH of SEQ ID Nos: 2-7 described in WO 2003/034903A2 VL of SEQ ID Nos: 8-13 described in WO 2003/034903A2

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
PSMA	VH: SEQ ID Nos: 225, 239, 253, 267, 281, 295, 309, 323, 337, 351, 365, 379, 393, 407, 421, 435, 449, 463, 477, 491, 505, 519, 533, 547, 561, 575, 589, 603 or 617 described in WO 2011/121110A1. VL SEQ ID Nos: 230, 244, 258, 272, 286, 300, 314, 328, 342, 356, 370, 384, 398, 412, 426, 440, 454, 468, 482, 496, 510, 524, 538, 552, 566, 580, 594, 608 or 622 described in WO 2011/121110A1. VH and VL SEQ ID Nos: 235, 249, 263, 277, 291, 305, 319, 333, 347, 361, 375, 389, 403, 417, 431, 445, 459, 473, 487, 501, 515, 529, 543, 557, 571, 585, 599, 613 or 627 described in WO 2011/121110A1.
PSMA	An anti-PSMA antibody having a VL amino acid sequence of any one of SEQ ID Nos: 229-312 of US 2022/0119525 A1 and a VH of SEQ ID NO: 217 of US 2022/0119525 A1.
PSMA	ES414
PSMA	BAY2010112 (pasotuxizumab)
PSMA	CCW702
PSMA	JNJ-63898081
PSMA	CC-1
PSMA	Acapatamab
PSMA	HPN424
RAAG12	RAV12
RANKL	Prolia™ (denosumab) VH: SEQ ID NO:51 of US Patent Pub. 2017/0002060 VL: SEQ ID NO:52 of US Patent Pub. 2017/0002060
SLAMF7	Empliciti™ (elotuzumab)
SSTR2	XmAb®18087
STEAP1	VHCDR1 SEQ ID Nos: 14, 33, 182, 184 or 185 described in US20210179731A1. VHCDR2 SEQ ID Nos: 15, 21, 34, 182, 184 or 185 described in US20210179731A1. VHCDR3 SEQ ID Nos: 16 and 35 described in US20210179731A1. VH SEQ ID Nos: 182 or 184 described in US20210179731A1. VLCDR1 SEQ ID Nos: 11 or 30 described in US20210179731A1. VLCDR2 SEQ ID Nos: 12 or 31 described in US20210179731A1. VLCDR3 SEQ ID Nos: 13 or 32 described in US20210179731A1.

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
	VL SEQ ID Nos: 183 or 186 described in US20210179731A1.
STEAP1	AMG509
STEAP2	Anti-STEAP 2 antibodies having CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3 sequences selected from SEQ ID NOS: (1) 4-6-8-12-14-16; (2) 20-22-24-28-30-32; (3) 36-38-40-44-46-48; (4) 52-54-56-60-62-64; (5) 68-70-72-60-62-64; (6) 76-78-80-60-62-64; (7) 84-86-88-60-62-64; (8) 92-94-96-60-62-64; (9) 100-102-104-60-62-64; (10) 108-110-112-116-118-120; (11) 124-126-128-132-134-136; (12) 140-142-144-148-150-152; (13) 156-158-160-164-166-168; (14) 172-174-176-180-182-184; (15) 188-190-192-196-198-200; (16) 204-206-208-212-214-216; (17) 220-222-224-228-230-232; (18) 236-238-240-244-246-248; (19) 252-254-256-260-262-264; (20) 268-270-272-276-278-280; (21) 284-286-288-292-294-296; (22) 300-302-304-308-310-312; (23) 316-318-320-324-326-328; (24) 332-334-336-340-342-344; (25) 348-350-352-356-358-360; (26) 364-366-368-372-374-376; and (27) 380-382-384-388-390-392 of US Patent No. 10,772,972 B2. Anti-STEAP 2 antibodies having (a) a VH comprising the amino acid of any one of SEQ ID Nos: 2, 18, 34, 50, 66, 74, 82, 90, 98, 106, 122, 138, 154, 170, 186, 202, 218, 234, 250, 266, 282, 298, 314, 330, 346, 362, and 378 of US Patent No. 10,772,972 B2; and (b) a VL comprising the amino acid sequence of any one of SEQ ID Nos: 10; 26; 42; 58; 114; 130; 146; 162; 178; 194; 210; 226, 242; 258; 274; 290; 306; 322; 338; 354; 370; and 386 of US Patent No. 10,772,972 B2. Anti-STEAP 2 antibodies having a VH/VL pair comprising the amino acid sequences of any of the following pairs of SEQ ID Nos of US Patent No. 10,772,972 B2: 2/10; 18/26; 34/42; 50/58; 66/58; 74/58; 82/58; 90/58; 98/58; 106/114; 122/130; 138/146; 154/162; 170/178; 186/194; 202/210; 218/226; 234/242; 250/258; 266/274; 282/290; 298/306; 314/322; 330/338; 346/354; 362/370; and 378/386.
Syndecan-1 (CD 138)	The B-B4 antibody described in Wijdenes et al. (1996) Br. J. Haematol., 94: 318-323
Syndecan-4	The amino acid sequence of amino acids 93 and 121 of SEQ ID NO: 1 or the amino acid sequence of amino acids 92 and 122 of SEQ ID NO:2 described in European Patent Pub. EP 2 603 236.

TABLE A Exemplary Target Molecules	
Target (Tumor Antigen)	**Antibody Name and/or Binding Sequences**
TGFβ	GC1008
TNFR	Enbrel™ (etanercept)
TNFα	Remicade™ (infliximab) VH: SEQ ID NO:2 of Int. Patent Publication WO201/3087911 A1 VH: SEQ ID NO:3 of Int. Patent Publication WO2013/ A1087911
TNFα	Humira™ (adalimumab) VH: SEQ ID NO:4 of US Patent No. 6,258,562 VL: SEQ ID NO:3 of US Patent No. 6,258,562
TNFα	Cimzia ™ (certolizumab pegol) VH: SEQ ID NO:14 of US Patent No. 7,012,135 VL: SEQ ID NO:9 of US Patent No. 7,012,135
TNFα	Simponi ™ (golimumab) VH: SEQ ID NO:7 of US Patent No. 7,250,165 VL: SEQ ID NO:8 of US Patent No. 7,250,165
VEGF	Avastin™ (bevacizumab) VH: SEQ ID NO:9 of US Patent No. 7,060,269 VL: SEQ ID NO:10 of US Patent No. 7,060,269
VEGF	Lucentis™ (ranibizumab) VH: SEQ ID NO:4 of US Patent No. 9,914,770 VL: SEQ ID NO:2 of US Patent No. 9,914,770
XCR1	Anti-XCR1 antibodies disclosed in US Patent No. 9,371, 389 B2, including: - The antibodies designated 2H6, 5G7, 11H2, HK1L2 and HK5L5 - Antibodies having CDR-H1, CDR-H2 and CDR-H3 sequences of SEQ ID Nos:53-55 and CDR-L1, CDR-L2 and CDR-L3 sequences of SEQ ID Nos:56-58. - Antibodies having CDR-H1, CDR-H2 and CDR-H3 sequences of SEQ ID Nos:41-43 and CDR-L1, CDR-L2 and CDR-L3 sequences of SEQ ID Nos:44-46.

[0126] In some aspects, the tumor antigen targeting moiety competes with an antibody set forth in Table A for binding to the tumor antigen. In further aspects, the tumor antigen targeting moiety comprises CDRs having CDR sequences of an anti-tumor antigen antibody set forth in Table A. In some embodiments, the tumor antigen targeting moiety comprises all 6 CDR sequences of the anti-tumor antigen antibody set forth in Table A. In other embodiments, the tumor antigen targeting moiety comprises at least the heavy chain CDR sequences (CDR-H1, CDR-H2, CDR-H3 and the light chain CDR sequences of a universal

light chain. In further aspects, a tumor antigen targeting moiety comprises a VH comprising the amino acid sequence of the VH of an anti-tumor antigen antibody set forth in Table A. In some embodiments, the tumor antigen targeting moiety further comprises a VL comprising the amino acid sequence of the VL of the anti-tumor antigen antibody set forth in Table A. In other embodiments, the tumor antigen targeting moiety further comprises a universal light chain VL sequence.

[0127] Additional tumor antigens that can be targeted by a tumor antigen targeting moiety are disclosed in, *e.g.*, Hafeez *et al.*, 2020, Molecules 25:4764, doi:10.3390/molecules25204764, particularly in Table 1. Table 1 of Hafeez *et al.* is incorporated by reference in its entirety here.

[0128] Additional exemplary TAAs include Fibroblast Activation Protein (FAP), the A1 domain of Tenascin-C (TNC A1), the A2 domain of Tenascin-C (TNC A2), the Extra Domain B of Fibronectin (EDB), the Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP), MART-1/Melan-A, gp100, Dipeptidyl peptidase IV (DPPIV), adenosine deaminase-binding protein (ADAbp), cyclophilin b, colorectal associated antigen (CRC)-C017-1A/GA733, Carcinoembryonic Antigen (CEA) and its immunogenic epitopes CAP-1 and CAP-2, etv6, aml1, Prostate Specific Antigen (PSA) and its immunogenic epitopes PSA-1, PSA-2, and PSA-3, prostate-specific membrane antigen (PSMA), T cell receptor/CD3-zeta chain, MAGE-family of tumor antigens (*e.g.*, MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A5, MAGE-A6, MAGE-A7, MAGE-A8, MAGE-A9, MAGE-A10, MAGE-A11, MAGE-A12, MAGE-Xp2 (MAGE-B2), MAGE-Xp3 (MAGE-B3), MAGE-Xp4 (MAGE-B4), MAGE-C1, MAGE-C2, MAGE-C3, MAGE-C4, MAGE-C5), GAGE-family of tumor antigens (*e.g.*, GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7, GAGE-8, GAGE-9), BAGE, RAGE, LAGE-1, NAG, GnT-V, MUM-1, CDK4, tyrosinase, p53, MUC family, HER2/neu, p21ras, RCAS1, α-fetoprotein, E-cadherin, α-catenin, β-catenin and γ-catenin, p120ctn, gp100 Pmel117, PRAME, NY-ESO-1, cdc27, adenomatous polyposis coli protein (APC), fodrin, Connexin 37, Ig-idiotype, p15, gp75, GM2 and GD2 gangliosides, viral products such as human papilloma virus proteins, Smad family of tumor antigens, Imp-1, P1A, EBV-encoded nuclear antigen (EBNA)-1, brain glycogen phosphorylase, SSX-1, SSX-2 (HOM-MEL-40), SSX-1, SSX-4, SSX-5, SCP-1 and CT-7, c-erbB-2, Her2, EGFR, IGF-1R, CD2 (T cell surface antigen), CD3 (heteromultimer associated with the TCR), CD22 (B-cell

receptor), CD23 (low affinity IgE receptor), CD30 (cytokine receptor), CD33 (myeloid cell surface antigen), CD40 (tumor necrosis factor receptor), IL-6R-(IL6 receptor), CD20, MCSP, PDGFβR (β-platelet-derived growth factor receptor), ErbB2 epithelial cell adhesion molecule (EpCAM), EGFR variant III (EGFRvIII), CD19, disialoganglioside GD2, ductal-epithelial mucine, gp36, TAG-72, glioma-associated antigen, β-human chorionic gonadotropin, alphafetoprotein (AFP), lectin-reactive AFP, thyroglobulin, MN-CA IX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostase, prostate specific antigen (PSA), PAP, LAGA-1a, p53, prostein, PSMA, surviving and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), ELF2M, neutrophil elastase, ephrin B2, insulin growth factor (IGF1)-I, IGF-II, IGFI receptor, 5T4, ROR1, Nkp30, NKG2D, tumor stromal antigens, the extra domain A (EDA) and extra domain B (EDB) of fibronectin and the A1 domain of tenascin-C (TnC A1).

6.6.2. Low-density Antigen Targeting Moieties

[0129] Many cancers are characterized by low-density antigens, for example by virtue of the cancer-specific antigen being present only as an HLA-bound peptide. Targeting of such cancers is particularly challenging due to the low expression of the target antigen, making targeting difficult using traditional bispecific antibodies having a single T cell antigen (e.g., CD3) targeting moiety and a single target antigen targeting moiety. Accordingly, in some embodiments, a primary MBM of the present disclosure comprises at least one targeting moiety that binds specifically to a polypeptide antigen present on the surface of a cell or population of cells at an average of no more than 5000 copies per cell (a "low-density antigen"), referred to herein as "low-density antigen targeting moieties." In general, a low-density antigen targeting moiety of the disclosure specifically binds to a low-density antigen present at no more than 5000, 4000, 3000, 2000, 1000, 500, 400, 300, 200, 100, 50, 40, 30, 20, or 10 copies per cell, or less. In some embodiments, the low-density antigen is present at between 10 and 100, between 50 and 200, between 100 and 300, between 200 and 500, between 300 and 750, between 500 and 1000, between 750 and 1500, or between 1000 and 2000 copies per cell. In particular embodiments, the low-density antigen is present at no more than 200, no more than 100, or no more than 50 copies per cell.

[0130] As described herein, primary MBMs comprising a low-density antigen targeting moiety and two TCA (e.g., CD3) targeting moieties are capable of significantly enhancing T

cell activation relative to a traditional bispecific antibody targeting the same low-density antigen and CD3. Without being bound by theory, it is understood that the inclusion of two TCA targeting moieties and one low-density antigen targeting moiety, arranged as in the primary MBMs described herein (e.g., as depicted in FIGS. 1B, 1C, or 1D), confers a particular advantage in activating T cells relative to other formats.

[0131] In some embodiments, the low-density antigen targeting moiety is a tumor antigen targeting moiety, e.g., as described in Section 6.6.1. In some embodiments, the low-density antigen targeting moiety is an HLA-bound peptide antigen targeting moiety, e.g., as described in Section 6.6.3.

6.6.3. HLA-bound Peptide Antigen Targeting Moieties

[0132] Many cancers are characterized by HLA-bound peptide antigens which are uniquely presented by the cancer cells. These include so-called "neoantigens", which are generally either mutations relative to a wild type allele or result from the expression of a new open reading frame in cancer cells (as shown in Table 1 of Fritsch *et al.*, 2014, Cancer Immunol Res 2:522-529, incorporated by reference herein in its entirety), as well as peptides from viral-associated cancers, such as HPV. In addition, viral infections generally result in HLA presentation of peptides from intracellular processing of viral proteins, serving as a unique marker of infected cells relative to uninfected cells. However, such HLA-bound peptides are often expressed on the surface of the target cell (e.g., cancer cell, virally infected cell) at low copy number relative to other cell surface proteins, making targeting via traditional bispecific antibodies difficult. Accordingly, in some embodiments, a primary MBM of the present disclosure comprises at least one targeting moiety that binds specifically to an HLA-bound peptide antigen.

[0133] As described herein, primary MBMs comprising an HLA-bound peptide antigen targeting moiety and two TCA (e.g., CD3) targeting moieties are capable of significantly enhancing T cell activation relative to a traditional bispecific antibody targeting the same low-density antigen and CD3. Without being bound by theory, it is understood that the inclusion of two TCA targeting moieties and one low-density antigen targeting moiety, arranged as in the primary MBMs described herein (e.g., as depicted in FIGS. 1B, 1C, or 1D), confers a particular advantage in activating T cells relative to other formats.

[0134] In some embodiments, the HLA-bound peptide antigen targeting moiety is a tumor antigen targeting moiety, e.g., as described in Section 6.6.1. In some embodiments, the HLA-bound peptide antigen targeting moiety is a low-density antigen targeting moiety, e.g., as described in Section 6.6.2.

[0135] Exemplary HLA-bound peptide antigens which may be targeted by a targeting moiety of the disclosure are set forth in Table P-1, below.

Table P-1

Peptide	HLA allele	Peptide sequence	Type
KRAS (6-14) G12V	A 02:01	LVVVGAVGV (SEQ ID NO: 2)	Cancer antigen
KRAS (5-14) G12D	A 02:01	KLVVVGADGV (SEQ ID NO: 3)	Cancer antigen
KRAS (5-14) G12V	A 02:01	KLVVVGAVGV (SEQ ID NO: 4)	Cancer antigen
KRAS (10-18) G12D	C 08:02	GADGVGKSA (SEQ ID NO: 5)	Cancer antigen
KRAS (10-19) G12D	C 08:02	GADGVGKSAL (SEQ ID NO: 6)	Cancer antigen
KRAS (8-16) G12D	A 11:01	VVGADGVGK (SEQ ID NO: 7)	Cancer antigen
KRAS (8-16) G12V	A 11:01	VVGAVGVGK (SEQ ID NO: 8)	Cancer antigen
KRAS (7-16) G12D	A 11:01	VVVGADGVGK (SEQ ID NO: 9)	Cancer antigen
KRAS (7-16) G12V	A 11:01	VVVGAVGVGK (SEQ ID NO: 10)	Cancer antigen
MAGE-A3 (168-176)	A 01:01	EVDPIGHLY (SEQ ID NO: 11)	Cancer antigen
MAGE-A3 (271-279)	A 02:01	FLWGPRALV (SEQ ID NO: 12)	Cancer antigen
MAGE-A3 (108-116)	A 02:01	ALSRKVAEL (SEQ ID NO: 13)	Cancer antigen
MAGE-A10 (137-145)	A 02:01	KVTDLVQFL (SEQ ID NO: 14)	Cancer antigen
MAGE-A10 (254-262)	A 02:01	GLYDGMEHL (SEQ ID NO: 15)	Cancer antigen
MAGE-A10 (310-318)	A 02:01	SLLKFLAKV (SEQ ID NO: 16)	Cancer antigen
WT-1 (10-18)	A 02:01	ALLPAVPSL (SEQ ID NO: 17)	Cancer antigen
PRAME (312-320)	A 02:01	RLDQLLRHV (SEQ ID NO: 18)	Cancer antigen
PRAME (425-433)	A 02:01	SLLQHLIGL (SEQ ID NO: 19)	Cancer antigen
PSA (79-89)	A 02:01	SLFHPEDTGQV (SEQ ID NO: 39)	Cancer antigen
PSA (159-169)	A 11:01	SIEPEEFLTPK (SEQ ID NO: 40)	Cancer antigen
PSA (241-249)	A 11:01	SLYTKVVHY (SEQ ID NO: 41)	Cancer antigen
EBNA-1 (562-570)	A 02:01	FMVFLQTHI (SEQ ID NO: 42)	Cancer antigen
LMP2 (426-434)	A 02:01	CLGGLLTMV (SEQ ID NO: 43)	Cancer antigen
DNTT (475-483)	A 02:01	ALYDKTKRI (SEQ ID NO: 44)	Cancer antigen
DNTT (250-258)	A 02:01	KLFTSVFGV (SEQ ID NO: 45)	Cancer antigen
MPO (132-140)	A 02:01	SLWRRPFNV (SEQ ID NO: 46)	Cancer antigen
MPO (549-557)	A 02:01	GLMATPAKL (SEQ ID NO: 47)	Cancer antigen
MPO (571-579)	A 02:01	RLFEQVMRI (SEQ ID NO: 48)	Cancer antigen
AFP (58-68)	A 11:01	ATYKEVSKMVK (SEQ ID NO: 49)	Cancer antigen
AFP (173-181)	A 24:02	LYAPTILLW (SEQ ID NO: 50)	Cancer antigen
IGF2BP3 (552-560)	A 02:01	KIQEILTQV (SEQ ID NO: 51)	Cancer antigen
KLK4 (215-223)	A 24:02	GYLQGLVSF (SEQ ID NO: 52)	Cancer antigen
KLK4 (105-113)	B 07:02	HPEYNRPLL (SEQ ID NO: 53)	Cancer antigen

Table P-1			
Peptide	**HLA allele**	**Peptide sequence**	**Type**
ACP3 (PAP) (112-120)	A 02:01	TLMSAMTNL (SEQ ID NO: 54)	Cancer antigen
KLK3 (PSA) (82-90)	HLA-B	HPEDTGQVF (SEQ ID NO: 55)	Cancer antigen
BALF-4 (276-284)	A 02:01	FLDKGTYTL (SEQ ID NO: 56)	Cancer antigen
CEA (571-579)	A 02:01	YLSGANLNL (SEQ ID NO: 57)	Cancer antigen
Her2/neu (369-377;V2v9)	A 02:01	KIFGSLAFL (SEQ ID NO: 58)	Cancer antigen
LMP1 (125-133)	A 02:01	YLLEMLWRL (SEQ ID NO: 59)	Cancer antigen
MAGE-A3 (112-120)	A 02:01	KVAELVHFL (SEQ ID NO: 60)	Cancer antigen
MAGE-A4 (230-239)	A 02:01	GVYDGREHTV (SEQ ID NO: 61)	Cancer antigen
MAGE-A4 (286-294)	A 02:01	KVLEHVVRV (SEQ ID NO: 62)	Cancer antigen
NY-ESO-1 (157-165, C165V)	A 02:01	SLLMWITQV (SEQ ID NO: 63)	Cancer antigen
NY-ESO-1 (157-165, C165A)	A 02:01	SLLMWITQA (SEQ ID NO: 64)	Cancer antigen
p53 (264-272)	A 02:01	LLGRNSFEV (SEQ ID NO: 65)	Cancer antigen
PSMA (4-12)	A 02:01	LLHETDSAV (SEQ ID NO: 66)	Cancer antigen
Survivin (96-104)	A 02:01	LTLGEFLKL (SEQ ID NO: 67)	Cancer antigen
Tyrosinase (369-377, 371D)	A 02:01	YMDGTMSQV (SEQ ID NO: 68)	Cancer antigen
WT1 (126-134)	A 02:01	RMFPNAPYL (SEQ ID NO: 69)	Cancer antigen
HIV P17 (77-85)	A 02:01	SLYNTVATL (SEQ ID NO: 70)	Viral antigen
HIV RT (896-904)	A 02:01	ILKEPVHGV (SEQ ID NO: 71)	Viral antigen
HIV GAG (41-49)	HLA-E	AISPRTLNA (SEQ ID NO: 72)	Viral antigen
ENV (183-191)	A 02:01	FLLTRILTI (SEQ ID NO: 73)	Viral antigen
HBV Pol (616-626 variant 5)	A 11:01	GTLPQDHIVQK (SEQ ID NO: 74)	Viral antigen
HBV Core (18-27)	A 02:01	FLPSDFFPSV (SEQ ID NO: 75)	Viral antigen
HPV E7 (11-20)	A 02:01	YMLDLQPETT (SEQ ID NO: 76)	Cancer antigen; Viral antigen
HPV E7 (11-19)	A 02:01	YMLDLQPET (SEQ ID NO: 77)	Cancer antigen; Viral antigen
HPV E7 (82-90)	A 02:01	LLMGTLGIV (SEQ ID NO: 78)	Cancer antigen; Viral antigen

[0136] Example HLA-bound peptide antigen targeting moieties, including antibodies (e.g., multispecific antibodies) and T-cell receptors (TCRs), which may be incorporated into a primary MBM disclosed herein are set forth in Table P-2, below.

Table P-2 Example HLA-bound Peptide Antigen Targeting Moieties	
Target (HLA-bound peptide)	**Antibody Name and/or Binding Sequences**
MAGE-A4 286-294	Anti-MAGE-A4 antibody sequences described in PCT Pub. No. WO 2023/183758 A2 as having the following SEQ ID NO. pairs for heavy and light chain variable domains:

Table P-2 Example HLA-bound Peptide Antigen Targeting Moieties	
Target (HLA-bound peptide)	**Antibody Name and/or Binding Sequences**
	SEQ ID NOs. 172/189 SEQ ID NOs. 172/180 SEQ ID NOs. 191/199
NY-ESO-1	Anti-HLA-A2:NY-ESO-1 amino acid sequences described in PCT Pub. No. WO 2021/003357 A1 as having the following SEQ ID NO. pairs for heavy and light chain variable domains: SEQ ID NOs. 2/10 SEQ ID NOs. 22/30 SEQ ID NOs. 42/50 SEQ ID NOs. 62/70 SEQ ID NOs. 82/90 SEQ ID NOs. 102/110 SEQ ID NOs. 122/130 SEQ ID NOs. 142/150 SEQ ID NOs. 162/170 SEQ ID NOs. 180/186 SEQ ID NOs. 196/203 SEQ ID NOs. 211/219 SEQ ID NOs. 230/238 SEQ ID NOs. 250/258
PRAME (312-320)	TCR-alpha and TCR-beta CDR amino acid sequences described in US. Pub. No. 2023/0060095 A1 that were identified with PRAME (312-320) TCR-alpha CDR1 of SEQ ID NOs. 1, 7, 13, 19, 25, 31, 37, 43, 49, 55, 61, 67, 73, 79, 85, 91, 97, 103 TCR-alpha CDR2 of SEQ ID NOs. 2, 8, 14, 20, 26, 32, 38, 44, 50, 56, 62, 68, 74, 80, 86, 92, 98, 104 TCR-alpha CDR3 of SEQ ID NOs. 3, 9, 15, 21, 27, 33, 39, 45, 51, 57, 63, 69, 75, 81, 87, 93, 99, 105 TCR-beta CDR1 of SEQ ID NOs. 4, 10, 16, 22, 28, 34, 40, 46, 52, 58, 64, 70, 76, 82, 88, 94, 100, 106 TCR-beta CDR2 of SEQ ID NOs. 5, 11, 17, 23, 29, 35, 41, 47, 53, 59, 65, 71, 77, 83, 89, 95, 101, 107 TCR-beta CDR3 of SEQ ID NOs. 6, 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 96, 102, 108
PRAME (425-433)	TCR-alpha and TCR-beta CDR amino acid sequences described in US. Pub. No. 2023/0060095 A1 that were identified with PRAME (425-433) TCR-alpha CDR1 of SEQ ID NOs. 289, 295, 301, 307, 313, 319, 325, 331, 337, 343, 349, 355, 361, 367, 373, 379, 385, 391, 397, 403, 409, 415, 421, 427, 433, 439, 445, 451, 457, 463, 469, 475, 481, 487, 493, 499, 505, 511, 517, 523 TCR-alpha CDR2 of SEQ ID NOs. 290, 296, 302, 308, 314, 320, 326, 332, 338, 344, 350, 356, 362, 368, 374, 380, 386, 392, 398, 404, 410,

Table P-2 Example HLA-bound Peptide Antigen Targeting Moieties	
Target (HLA-bound peptide)	**Antibody Name and/or Binding Sequences**
	416, 422, 428, 434, 440, 446, 452, 458, 464, 470, 476, 482, 488, 494, 500, 506, 512, 518, 524 TCR-alpha CDR3 of SEQ ID NOs. 291, 297, 303, 309, 315, 321, 327, 333, 339, 345, 351, 357, 363, 369, 375, 381, 387, 393, 399, 405, 411, 417, 423, 429, 435, 441, 447, 453, 459, 465, 471, 477, 483, 489, 495, 501, 507, 513, 519, 525 TCR-beta CDR1 of SEQ ID NOs. 292, 298, 304, 310, 316, 322, 328, 334, 340, 346, 352, 358, 364, 370, 376, 382, 388, 394, 400, 406, 412, 418, 424, 430, 436, 442, 448, 454, 460, 466, 472, 478, 484, 490, 496, 502, 508, 514, 520, 526 TCR-beta CDR2 of SEQ ID NOs. 293, 299, 305, 311, 317, 323, 329, 335, 341, 347, 353, 359, 365, 371, 377, 383, 389, 395, 401, 407, 413, 419, 425, 431, 437, 443, 449, 455, 461, 467, 473, 479, 485, 491, 497, 503, 509, 515, 521, 527 TCR-beta CDR3 of SEQ ID NOs. 294, 300, 306, 312, 318, 324, 330, 336, 342, 348, 354, 360, 366, 372, 378, 384, 390, 396, 402, 408, 414, 420, 426, 432, 438, 444, 450, 456, 462, 468, 474, 480, 486, 492, 498, 504, 510, 516, 522, 528
MAGE-A4 (286-294)	Anti-MAGE-A4(286-294) VH and VL amino acid sequences described in US Pub. No. 2022/0031748 A1 VH: SEQ ID NO:21 VL: SEQ ID NO:17
NY-ESO (156-165)	Anti-NY-ESO (156-165) VH and VL amino acid sequences described in US Pub. No. 2022/0031748 A1 VH: SEQ ID NO:22 VL: SEQ ID NO:18
Tyrosinase D11 (369-377)	Anti-Tyr (369-377) VH and VL amino acid sequences described in US Pub. No. 2022/0031748 A1 VH: SEQ ID NO:23 VL: SEQ ID NO:19
MAGE-A4 (230-239)	Anti-MAGE-A4 (230-239) VH and VL amino acid sequences described in US Pub. No. 2022/0031748 A1 as having the following SEQ ID NO. pairs for heavy and light chain variable domains: SEQ ID NOs: 32/31 SEQ ID NOs: 38/37
MAGE-A4 (230-239)	Anti-MAGE-A4 (230-239) VH and VL amino acid sequences described in US Pat. No. 11,497,768 VH: SEQ ID NO:36 VL: SEQ ID NO:38

[0137] In some aspects, the HLA-bound peptide antigen targeting moiety competes with an antibody or TCR set forth in Table P-2 for binding to the HLA-bound peptide antigen. In

further aspects, the HLA-bound peptide antigen targeting moiety comprises CDRs having CDR sequences of an antibody or TCR set forth in Table P-2. In some embodiments, the HLA-bound peptide antigen targeting moiety comprises all 6 CDR sequences of an antibody or TCR set forth in Table P-2. In other embodiments, the HLA-bound peptide antigen targeting moiety comprises at least the heavy chain CDR sequences (CDR-H1, CDR-H2, CDR-H3 and the light chain CDR sequences of a universal light chain. In further aspects, the HLA-bound peptide antigen targeting moiety comprises a VH comprising the amino acid sequence of the VH of an antibody or TCR set forth in Table P-2. In some embodiments, the HLA-bound peptide antigen targeting moiety further comprises a VL comprising the amino acid sequence of the VL of an antibody or TCR set forth in Table P-2. In other embodiments, the tumor antigen targeting moiety further comprises a universal light chain VL sequence.

6.7. Linkers

[0138] In certain aspects, the present disclosure provides recombinant polypeptides in which two or more components of the recombinant polypeptide are connected to one another by a linker (also "peptide linker"). By way of example and not limitation, linkers can be used to connect (a) a targeting moiety and an Fc domain; (b) a first targeting moiety and a second targeting moiety (e.g. a Fab and an scFv, or a first scFv and a second scFv); or (c) different domains within a targeting moiety (e.g., the VH and VL domains in an scFv).

[0139] A peptide linker can range from 2 amino acids to 60 or more amino acids, and in certain aspects a peptide linker ranges from 3 amino acids to 50 amino acids, from 4 to 30 amino acids, from 5 to 25 amino acids, from 10 to 25 amino acids, 10 amino acids to 60 amino acids, from 12 amino acids to 20 amino acids, from 20 amino acids to 50 amino acids, or from 25 amino acids to 35 amino acids in length.

[0140] In particular aspects, a peptide linker is at least 5 amino acids, at least 6 amino acids or at least 7 amino acids in length and optionally is up to 30 amino acids, up to 40 amino acids, up to 50 amino acids or up to 60 amino acids in length.

[0141] In some embodiments of the foregoing, the linker ranges from 5 amino acids to 50 amino acids in length, e.g., ranges from 5 to 50, from 5 to 45, from 5 to 40, from 5 to 35, from 5 to 30, from 5 to 25, or from 5 to 20 amino acids in length. In other embodiments of the foregoing, the linker ranges from 6 amino acids to 50 amino acids in length, e.g., ranges

from 6 to 50, from 6 to 45, from 6 to 40, from 6 to 35, from 6 to 30, from 6 to 25, or from 6 to 20 amino acids in length. In yet other embodiments of the foregoing, the linker ranges from 7 amino acids to 50 amino acids in length, e.g., ranges from 7 to 50, from 7 to 45, from 7 to 40, from 7 to 35, from 7 to 30, from 7 to 25, or from 7 to 20 amino acids in length.

[0142] Charged (e.g., charged hydrophilic linkers) and/or flexible linkers are particularly preferred.

[0143] Examples of flexible linkers that can be used in the recombinant polypeptides of the disclosure include those disclosed by Chen *et al.*, 2013, Adv Drug Deliv Rev. 65(10): 1357-1369 and Klein *et al.*, 2014, Protein Engineering, Design & Selection 27(10): 325-330. Particularly useful flexible linkers are or comprise repeats of glycines and serines, e.g., a monomer or multimer of GnS (SEQ ID NO: 79) or SGn (SEQ ID NO: 80), where n is an integer from 1 to 10, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10. In one embodiment, the linker is or comprises a monomer or multimer of repeat of G_4S (SEQ ID NO: 81) e.g., $(GGGGS)_n$, where n is an integer from 1 to 10, e.g., 1 2, 3, 4, 5, 6, 7, 8, 9 or 10 (SEQ ID NO: 82). For example, in some embodiments, the linker is GGGGS (SEQ ID NO: 81), GGGGSGGGGS (SEQ ID NO: 83), GGGGSGGGGSGGGGS (SEQ ID NO: 1), GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 84), or GGGGSGGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 85).

[0144] Polyglycine linkers can suitably be used in the recombinant polypeptides of the disclosure. In some embodiments, a peptide linker comprises two consecutive glycines (2Gly), three consecutive glycines (3Gly), four consecutive glycines (4Gly), five consecutive glycines (5Gly), six consecutive glycines (6Gly), seven consecutive glycines (7Gly), eight consecutive glycines (8Gly) or nine consecutive glycines (9Gly).

[0145] Exemplary linker sequences are set forth in Table L below. An MBM of the disclosure may comprise one or more linkers of Table L.

TABLE L
Linker Sequences

Linker Sequence	Designation	SEQ ID NO:
$(GGGGS)_n$	L1	81
$(GGGS)_n$	L2	92
$(GGS)_n$	L3	
$(GS)_n$	L4	
$(GSGGS)_n$	L5	93

TABLE L Linker Sequences		
Linker Sequence	**Designation**	**SEQ ID NO:**
AAAGG	L6	94
ADAAP	L7	95
ADAAPTVSIFP	L8	96
ADAAPTVSIFPP	L9	97
AKTTAP	L10	98
AKTTAPSVYPLAP	L11	99
AKTTPKLEEGEFSEARV	L12	100
AKTTPKLGG	L13	101
AKTTPP	L14	102
AKTTPPSVTPLAP	L15	103
ASTKGP	L16	104
ASTKGPSVFPLAPASTKGPSVFPLAP	L17	105
EGKSSGSGSESKST	L18	106
GEGESGEGESGEGES	L19	107
GEGESGEGESGEGESGEGES	L20	108
GEGGSGEGGSGEGGS	L21	109
GENKVEYAPALMALS	L22	110
GGEGSGGEGSGGEGS	L23	111
GGGESGGEGSGEGGS	L24	112
GGGESGGGESGGGES	L25	113
GGGGSGGGGS	L26	83
GGGGSGGGGSGGGGS	L27	1
GGGGSGGGGSGGGGSGGGGS	L28	84
GGGKSGGGKSGGGKS	L29	114
GGGKSGGKGSGKGGS	L30	115
GGGS	L31	92
GGGSG	L32	116
GGKGSGGKGSGGKGS	L33	117
GGS	L34	
GGSG	L35	118
GGSGG	L36	119
GGSGG	L37	120
GGSGGGGSG	L38	121
GGSGGGGSGGGGS	L39	122
GHEAAAVMQVQYPAS	L40	123
GKGGSGKGGSGKGGS	L41	124
GKGKSGKGKSGKGKS	L42	125
GKGKSGKGKSGKGKSGKGKS	L43	126
GKPGSGKPGSGKPGS	L44	127
GKPGSGKPGSGKPGSGKPSGS	L45	128

TABLE L Linker Sequences		
Linker Sequence	**Designation**	**SEQ ID NO:**
GPAKELTPLKEAKVS	L46	129
GSAGSAAGSGEF	L47	130
GSGGG	L48	131
GSGSG	L49	132
GSS	L50	
GSSG	L51	133
GSSGGSGGSG	L52	134
GSSGGSGGSGG	L53	135
GSSGGSGGSGGS	L54	136
GSSGGSGGSGGSG	L55	137
GSSGGSGGSGGSGGGS	L56	138
GSSGGSGGSGS	L57	139
GSSGT	L58	140
GSSSG	L59	141
GSTSGSGKPGSGEGSTKG	L60	142
GTAAAGAGAAGGAAAGAAG	L61	143
GTSGSSGSGSGGSGSGGGG	L62	144
IRPRAIGGSKPRVA	L63	145
KESGSVSSEQLAQFRSLD	L64	146
KTTPKLEEGEFSEAR	L65	147
PRGASKSGSASQTGSAPGS	L66	148
QPKAAP	L67	149
QPKAAPSVTLFPP	L68	150
RADAAAA$(G4S)_4$	L69	151
RADAAAAGGPGS	L70	152
RADAAP	L71	153
RADAAPTVS	L72	154
SAKTTP	L73	155
SAKTTPKLEEGEFSEARV	L74	156
SAKTTPKLGG	L75	157
STAGDTHLGGEDFD	L76	158
TVAAP	L77	159
TVAAPSVFIFPP	L78	160
TVAAPSVFIFPPTVAAPSVFIFPP	L79	161

6.8. Fc Regions

[0146] In certain aspects, the primary and/or secondary multispecific binding molecules of the disclosure comprise a pair of Fc domains that associate to form an Fc region. In native antibodies, Fc regions comprise hinge regions at their N-termini to form a constant domain.

Throughout this disclosure, the reference to an Fc domain encompasses an Fc domain with a hinge domain at its N-terminus unless specified otherwise.

[0147] The Fc domains can be derived from any suitable species operably linked to an antigen-binding domain or component thereof. In one embodiment the Fc domain is derived from a human Fc domain. In preferred embodiments, an antigen-binding domain of a multispecific molecule of the disclosure is fused to an IgG Fc molecule. An antigen-binding domain may be fused to the N-terminus or the C-terminus of the IgG Fc domain or both.

[0148] The Fc domains can be derived from any suitable class of antibody, including IgA (including subclasses IgA1 and IgA2), IgD, IgE, IgG (including subclasses IgG1, IgG2, IgG3 and IgG4), and IgM. In one embodiment, the Fc domain is derived from IgG1, IgG2, IgG3 or IgG4. In one embodiment the Fc domain is derived from IgG1. In one embodiment the Fc domain is derived from IgG4.

[0149] The two Fc domains within the Fc region can be the same or different from one another. In a native antibody the Fc domains are typically identical, but for the purpose of producing multispecific binding molecules, *e.g.,* multispecific binding molecules described herein, the Fc domains might advantageously be different to allow for heterodimerization, as described in Section 6.8.1.2 below. In other embodiments, the two Fc domains of multispecific binding molecules disclosed herein are the same.

[0150] In native antibodies, the heavy chain Fc domain of IgA, IgD and IgG is composed of two heavy chain constant domains (CH2 and CH3) and that of IgE and IgM is composed of three heavy chain constant domains (CH2, CH3 and CH4). These dimerize to create an Fc region.

[0151] In multispecific binding molecules of the present disclosure, the Fc region, and / or the Fc domains within it, can comprise heavy chain constant domains from one or more different classes of antibody, for example one, two or three different classes.

[0152] In one embodiment the Fc region comprises CH2 and CH3 domains derived from IgG1.

[0153] In one embodiment the Fc region comprises CH2 and CH3 domains derived from IgG2.

[0154] In one embodiment the Fc region comprises CH2 and CH3 domains derived from IgG3.

[0155] In one embodiment the Fc region comprises CH2 and CH3 domains derived from IgG4.

[0156] In one embodiment the Fc region comprises a CH4 domain from IgM. The IgM CH4 domain is typically located at the C-terminus of the CH3 domain.

[0157] In one embodiment the Fc region comprises CH2 and CH3 domains derived from IgG and a CH4 domain derived from IgM.

[0158] It will be appreciated that the heavy chain constant domains for use in producing an Fc region for multispecific binding molecules of the present disclosure may include variants of the naturally occurring constant domains described above. Such variants may comprise one or more amino acid variations compared to wild type constant domains. In one example the Fc region of the present disclosure comprises at least one constant domain that varies in sequence from the wild type constant domain. It will be appreciated that the variant constant domains may be longer or shorter than the wild-type constant domain. Preferably the variant constant domains are at least 60% identical or similar to a wild-type constant domain. In another example the variant constant domains are at least 70% identical or similar. In another example the variant constant domains are at least 80% identical or similar. In another example the variant constant domains are at least 90% identical or similar. In another example the variant constant domains are at least 95% identical or similar.

[0159] IgM and IgA occur naturally in humans as covalent multimers of the common H2L2 antibody unit. IgM occurs as a pentamer when it has incorporated a J-chain, or as a hexamer when it lacks a J-chain. IgA occurs as monomer and dimer forms. The heavy chains of IgM and IgA possess an 18 amino acid extension to the C-terminal constant domain, known as a tailpiece. The tailpiece includes a cysteine residue that forms a disulfide bond between heavy chains in the polymer, and is believed to have an important role in polymerization. The tailpiece also contains a glycosylation site. In certain embodiments, the multispecific binding molecules of the present disclosure do not comprise a tailpiece.

[0160] The Fc domains that are incorporated into the primary and/or secondary multispecific binding molecules of the present disclosure may comprise one or more modifications that

alter the functional properties of the proteins, for example, binding to Fc-receptors such as FcRn or leukocyte receptors, binding to complement, modified disulfide bond architecture, or altered glycosylation patterns. Exemplary Fc modifications that alter effector function are described in Section 6.8.1.1.

[0161] The Fc domains can also be altered to include modifications that improve manufacturability of asymmetric multispecific binding molecules, for example by allowing heterodimerization, which is the preferential pairing of non-identical Fc domains over identical Fc domains. Heterodimerization permits the production of multispecific binding molecules in which different polypeptide components are connected to one another by an Fc region containing Fc domains that differ in sequence. Examples of heterodimerization strategies are exemplified in Section 6.8.1.2.

[0162] It will be appreciated that any of the modifications mentioned above can be combined in any suitable manner to achieve the desired functional properties and/or combined with other modifications to alter the properties of the multispecific binding molecules.

TABLE F-1
Fc Sequences

Fc	Sequence	SEQ ID NO
hIgG1 Fc (amino acids 99-330 of UniprotKB P01857-1)	EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	20
hIgG2 Fc (amino acids 99-326 of UniprotKB P01859-1)	ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEY KCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDISVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK	21
hIgG3 Fc (amino acids 99-377 of UniprotKB P01860-1)	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPK SCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVQFKWYVDGVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKLTVDKSRWQ QGNIFSCSVMHEALHNRFTQKSLSLSPGK	22

TABLE F-1
Fc Sequences

Fc	Sequence	SEQ ID NO
hIgG4 Fc (amino acids 99-327 of UniprotKB P01861-1)	ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLSLGK	23
hIgG4s Fc	ESKYGPPCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQE DPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQE GNVFSCSVMHEALHNHYTQKSLSLSLGK	24
hIgG1 PVA Fc	EPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	25
hIgG1 PVA star	EPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLT CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNRFTQKSLSLSPGK	26
hIgG1s	DKKVEPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSRDELTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	27
hIgG1 N180G, also referred to as N297G	EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVD VSHEDPEVKFNWYVDGVEVHNAKTKPREEQYGSTYRVVSVLTVLHQDWLN GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	28
hIgG2 variant	ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTVVHQDWLNGKEY KCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK	29
hIgG4 S108P	ESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQ EDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCL	30

TABLE F-1 Fc Sequences		
Fc	**Sequence**	**SEQ ID NO**
	VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQ EGNVFSCSVMHEALHNHYTQKSLSLSLGK	
IgG1PVA_hinge-Fc knob	EPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	31
IgG1PVA_hinge-Fc knob_star	EPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNRFTQKSLSLSPGK	32
IgG1PVA_hinge-Fc knob_Cys	EPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	33
IgG1PVA_hinge-Fc knob_Cys_star	EPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLW CLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNRFTQKSLSLSPGK	34
IgG1PVA_hinge-Fc Hole	EPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	35
IgG1PVA_hinge-Fc Hole_star	EPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSR WQQGNVFSCSVMHEALHNRFTQKSLSLSPGK	36
IgG1PVA_hinge-Fc Hole_Cys	EPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	37

TABLE F-1 Fc Sequences		
Fc	**Sequence**	**SEQ ID NO**
IgG1PVA_hinge-Fc Hole_Cys_star	EPKSCDKTHTCPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVVDV SHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVCTLPPSRDELTKNQVSLS CAVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSR WQQGNVFSCSVMHEALHNRFTQKSLSLSPGK	38

[0163] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to any one of the sequences disclosed in Table F-1.

[0164] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:20. In cases where an Fc domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:20 (*e.g.,* between 90% and 99% sequence identity to SEQ ID NO:20), an Fc domain may also comprise one or more amino acid substitutions described herein, for example one or more substitutions that reduce effector function (*e.g.*, as described in Section 6.8.1.1) and/or one or more substitutions that promote Fc heterodimerization (*e.g.*, as described in Section 6.8.1.2).

[0165] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:21. In cases where an Fc domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:21 (*e.g.,* between 90% and 99% sequence identity to SEQ ID NO:21), an Fc domain may also comprise one or more amino acid substitutions described herein, for example one or more substitutions that reduce effector function (*e.g.*, as described in Section 6.8.1.1)

and/or one or more substitutions that promote Fc heterodimerization (*e.g.*, as described in Section 6.8.1.2).

[0166] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:22. In cases where an Fc domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:22 (*e.g.,* between 90% and 99% sequence identity to SEQ ID NO:22), an Fc domain may also comprise one or more amino acid substitutions described herein, for example one or more substitutions that reduce effector function (*e.g.*, as described in Section 6.8.1.1) and/or one or more substitutions that promote Fc heterodimerization (*e.g.*, as described in Section 6.8.1.2).

[0167] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:23. In cases where an Fc domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:23 (*e.g.,* between 90% and 99% sequence identity to SEQ ID NO:23), an Fc domain may also comprise one or more amino acid substitutions described herein, for example one or more substitutions that reduce effector function (*e.g.*, as described in Section 6.8.1.1) and/or one or more substitutions that promote Fc heterodimerization (*e.g.*, as described in Section 6.8.1.2).

[0168] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:24.

[0169] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:25.

[0170] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:26.

[0171] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:27.

[0172] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:28.

[0173] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:29.

[0174] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:30.

[0175] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:31.

[0176] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:32.

[0177] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%,

at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:33.

[0178] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:34.

[0179] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:35.

[0180] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:36.

[0181] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:37.

[0182] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or 100% sequence identity to SEQ ID NO:38.

6.8.1.1. Fc Domains with Altered Effector Function

[0183] In some embodiments, the Fc domain comprises one or more amino acid substitutions that reduces binding to an Fc receptor and/or effector function.

[0184] In a particular embodiment the Fc receptor is an Fcγ receptor. In one embodiment the Fc receptor is a human Fc receptor. In one embodiment the Fc receptor is an activating Fc receptor. In a specific embodiment the Fc receptor is an activating human Fcγ receptor, more specifically human FcγRIIIa, FcγRI or FcγRIIa, most specifically human FcγRIIIa. In one embodiment the effector function is one or more selected from the group of complement

dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and cytokine secretion. In a particular embodiment, the effector function is ADCC.

[0185] In one embodiment, the Fc domain (*e.g.*, an Fc domain of a multispecific binding molecule) or the Fc region (*e.g.*, one or both Fc domains of a multispecific binding molecule that can associate to form an Fc region) comprises an amino acid substitution at a position selected from the group of E233, L234, L235, N297, P331 and P329 (numberings according to Kabat EU index). In a more specific embodiment, the Fc domain or the Fc region comprises an amino acid substitution at a position selected from the group of L234, L235 and P329 (numberings according to Kabat EU index). In some embodiments, the Fc domain or the Fc region comprises the amino acid substitutions L234A and L235A (numberings according to Kabat EU index). In one such embodiment, the Fc domain or region is an IgG Fc domain or region, particularly a human IgG Fc domain or region. In one embodiment, the Fc domain or the Fc region comprises an amino acid substitution at position P329. In a more specific embodiment, the amino acid substitution is P329A or P329G, particularly P329G (numberings according to Kabat EU index). In one embodiment, the Fc domain or the Fc region comprises an amino acid substitution at position P329 and a further amino acid substitution at a position selected from E233, L234, L235, N297 and P331 (numberings according to Kabat EU index). In a more specific embodiment, the further amino acid substitution is E233P, L234A, L235A, L235E, N297A, N297D or P331S. In particular embodiments, the Fc domain or the Fc region comprises amino acid substitutions at positions P329, L234 and L235 (numberings according to Kabat EU index). In more particular embodiments, the Fc domain comprises the amino acid mutations L234A, L235A and P329G ("P329G LALA", "PGLALA" or "LALAPG").

[0186] Typically, the same one or more amino acid substitution is present in each of the two Fc domains of an Fc region. Thus, in a particular embodiment, each Fc domain of the Fc region comprises the amino acid substitutions L234A, L235A and P329G (Kabat EU index numbering), *i.e.* in each of the first and the second Fc domains in the Fc region the leucine residue at position 234 is replaced with an alanine residue (L234A), the leucine residue at position 235 is replaced with an alanine residue (L235A) and the proline residue at position 329 is replaced by a glycine residue (P329G) (numbering according to Kabat EU index).

[0187] In one embodiment, the Fc domain is an IgG1 Fc domain, particularly a human IgG1 Fc domain. In some embodiments, the IgG1 Fc domain is a variant IgG1 comprising D265A, N297A mutations (EU numbering) to reduce effector function.

[0188] In another embodiment, the Fc domain is an IgG4 Fc domain with reduced binding to Fc receptors. Exemplary IgG4 Fc domains with reduced binding to Fc receptors may comprise an amino acid sequence selected from Table F-2 below: In some embodiments, the Fc domain includes only the bolded portion of the sequences shown below:

TABLE F-2		
Fc Domain	**Sequence**	**SEQ ID NO**
SEQ ID NO:1 of WO2014/121087	DKRVESKYGP PCPPCPAPPV AGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSQEDPEVQF NWYVDGVEVH NAKTKPREEQ FNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KGLPSSIEKT ISKAKGQPRE PQVYTLPPSQ EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSRLTVDKS RWQEGNVFSC SVMHEALHNH YTQKSLSLSL GK	165
SEQ ID NO:2 of WO2014/121087	DKKVEPKSCD KTHTCPPCPA PPVAGPSVFL FPPKPKDTLM ISRTPEVTCV VVDVSQEDPE VQFNWYVDGV EVHNAKTKPR EEQFNSTYRV VSVLTVLHQD WLNGKEYKCK VSNKGLPSSI EKTISKAKGQ PREPQVYTLP PSRDELTKNQ VSLTCLVKGF YPSDIAVEWE SNGQPENNYK TTPPVLDSDG SFFLYSKLTV DKSRWQQGNV FSCSVMHEAL HNHYTQKSLS LSPGK	27
SEQ ID NO:30 of WO2014/121087	ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKV**EP KSCDKTHTCP PCPAPPVAGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK**	166
SEQ ID NO:31 of WO2014/121087	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRV**ES KYGPPCPPCP APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKGLPSS IEKTISKAKG QPREPQVYTL PPSQEEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSRLT VDKSRWQEGN VFSCSVMHEA LHNHYTQKSL SLSLGK**	167

TABLE F-2		
Fc Domain	**Sequence**	**SEQ ID NO**
SEQ ID NO:37 of WO2014/121087	ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKV**EP KSCDKTHTCP PCPAPPVAGP SVFLFPPKPK DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSRDEL TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNRFTQ KSLSLSPGK**	168
SEQ ID NO:38 of WO2014/121087	ASTKGPSVFP LAPCSRSTSE STAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTKT YTCNVDHKPS NTKVDKRV**ES KYGPPCPPCP APPVAGPSVF LFPPKPKDTL MISRTPEVTC VVVDVSQEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTYR VVSVLTVLHQ DWLNGKEYKC KVSNKGLPSS IEKTISKAKG QPREPQVYTL PPSQEEMTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSRLT VDKSRWQEGN VFSCSVMHEA LHNRFTQKSL SLSLGK**	169

[0189] In a particular embodiment, the IgG4 with reduced effector function comprises the bolded portion of the amino acid sequence of SEQ ID NO:31 of WO2014/121087, sometimes referred to herein as IgG4s or hIgG4s.

[0190] For heterodimeric Fc regions, it is possible to incorporate a combination of the variant IgG4 Fc sequences set forth above, for example an Fc region comprising an Fc domain comprising the amino acid sequence of SEQ ID NO:30 of WO2014/121087 (or the bolded portion thereof) and an Fc domain comprising the amino acid sequence of SEQ ID NO:37 of WO2014/121087 (or the bolded portion thereof) or an Fc region comprising an Fc domain comprising the amino acid sequence of SEQ ID NO:31 of WO2014/121087 (or the bolded portion thereof) and an Fc domain comprising the amino acid sequence of SEQ ID NO:38 of WO2014/121087 (or the bolded portion thereof).

6.8.1.2. Fc Heterodimerization Variants

[0191] Certain multispecific binding molecules entail dimerization between two Fc domains that, unlike a native immunoglobulin, are operably linked to non-identical N-terminal or C-terminal regions. Inadequate heterodimerization of two Fc domains to form an Fc region can be an obstacle for increasing the yield of desired heterodimeric molecules and represents

challenges for purification. A variety of approaches available in the art can be used in for enhancing dimerization of Fc domains that might be present in the multispecific binding molecules of the disclosure, for example as disclosed in EP 1870459A1; U.S. Patent No. 5,582,996; U.S. Patent No. 5,731,168; U.S. Patent No. 5,910,573; U.S. Patent No. 5,932,448; U.S. Patent No. 6,833,441; U.S. Patent No. 7,183,076; U.S. Patent Application Publication No. 2006204493A1; and PCT Publication No. WO 2009/089004A1.

[0192] In some embodiments, the present disclosure provides multispecific binding molecules comprising Fc heterodimers, *i.e.*, Fc regions comprising heterologous, non-identical Fc domains. Typically, each Fc domain in the Fc heterodimer comprises a CH3 domain of an antibody. The CH3 domains are derived from the constant region of an antibody of any isotype, class or subclass, and preferably of IgG (IgG1, IgG2, IgG3 and IgG4) class, as described in the preceding section.

[0193] In a specific embodiment said modification promoting the formation of Fc heterodimers is a so-called "knob-into-hole" or "knob-in-hole" modification, comprising a "knob" modification in one of the Fc domains and a "hole" modification in the other Fc domain. The knob-into-hole technology is described *e.g.*, in U.S. Patent No. 5,731,168; US 7,695,936; Ridgway *et al.*, 1996, Prot Eng 9:617-621, and Carter, 2001, Immunol Meth 248:7-15. Generally, the method involves introducing a protuberance ("knob") at the interface of a first polypeptide and a corresponding cavity ("hole") in the interface of a second polypeptide, such that the protuberance can be positioned in the cavity so as to promote heterodimer formation and hinder homodimer formation. Protuberances are constructed by replacing small amino acid side chains from the interface of the first polypeptide with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory cavities of identical or similar size to the protuberances are created in the interface of the second polypeptide by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine).

[0194] Accordingly, in some embodiments, an amino acid residue in the CH3 domain of the first subunit of the Fc domain is replaced with an amino acid residue having a larger side chain volume, thereby generating a protuberance within the CH3 domain of the first subunit which is positionable in a cavity within the CH3 domain of the second subunit, and an amino acid residue in the CH3 domain of the second subunit of the Fc domain is replaced with an

amino acid residue having a smaller side chain volume, thereby generating a cavity within the CH3 domain of the second subunit within which the protuberance within the CH3 domain of the first subunit is positionable. Preferably said amino acid residue having a larger side chain volume is selected from the group consisting of arginine I, phenylalanine (F), tyrosine (Y), and tryptophan (W). Preferably said amino acid residue having a smaller side chain volume is selected from the group consisting of alanine (A), serine (S), threonine (T), and valine (V). The protuberance and cavity can be made by altering the nucleic acid encoding the polypeptides, *e.g.*, by site-specific mutagenesis, or by peptide synthesis. An exemplary substitution is Y470T.

[0195] In a specific such embodiment, in the first Fc domain the threonine residue at position 366 is replaced with a tryptophan residue (T366W), and in the Fc domain the tyrosine residue at position 407 is replaced with a valine residue (Y407V) and optionally the threonine residue at position 366 is replaced with a serine residue (T366S) and the leucine residue at position 368 is replaced with an alanine residue (L368A) (numbering according to Kabat EU index). In a further embodiment, in the first Fc domain additionally the serine residue at position 354 is replaced with a cysteine residue (S354C) or the glutamic acid residue at position 356 is replaced with a cysteine residue (E356C) (particularly the serine residue at position 354 is replaced with a cysteine residue), and in the second Fc domain additionally the tyrosine residue at position 349 is replaced by a cysteine residue (Y349C) (numbering according to Kabat EU index). In a particular embodiment, the first Fc domain comprises the amino acid substitutions S354C and T366W, and the second Fc domain comprises the amino acid substitutions Y349C, T366S, L368A and Y407V (numbering according to Kabat EU index).

[0196] In some embodiments, electrostatic steering (*e.g.*, as described in Gunasekaran *et al.*, 2010, J Biol Chem 285(25): 19637-46) can be used to promote the association of the first and the second Fc domains of the Fc region.

[0197] As an alternative, or in addition, to the use of Fc domains that are modified to promote heterodimerization, an Fc domain can be modified to allow a purification strategy that enables selections of Fc heterodimers. In one such embodiment, one polypeptide comprises a modified Fc domain that abrogates its binding to Protein A, thus enabling a purification method that yields a heterodimeric protein. See, for example, U.S. Patent No.

8,586,713. As such, the multispecific binding molecules comprise a first CH3 domain and a second Ig CH3 domain, wherein the first and second Ig CH3 domains differ from one another by at least one amino acid, and wherein at least one amino acid difference reduces binding of the multispecific binding molecules to Protein A as compared to a corresponding multispecific binding molecule lacking the amino acid difference. In one embodiment, the first CH3 domain binds Protein A and the second CH3 domain contains a mutation/modification that reduces or abolishes Protein A binding such as an H95R modification (by IMGT exon numbering; H435R by EU numbering). The second CH3 may further comprise a Y96F modification (by IMGT; Y436F by EU). This class of modifications is referred to herein as "star" mutations.

[0198] In some embodiments, the Fc can contain one or more mutations (*e.g.*, knob and hole mutations) to facilitate heterodimerization as well as star mutations to facilitate purification.

6.8.1.3. Hinge Domains

[0199] The multispecific binding molecules of the disclosure can comprise an Fc domain comprising a hinge domain at its N-terminus. The hinge region can be a native or a modified hinge region. Hinge regions are typically found at the N-termini of Fc regions. The term "hinge domain", unless the context dictates otherwise, refers to a naturally or non-naturally occurring hinge sequence that in the context of a single or monomeric polypeptide chain is a monomeric hinge domain and in the context of a dimeric polypeptide (*e.g.*, a homodimeric or heterodimeric multispecific binding molecule formed by the association of two Fc domains) can comprise two associated hinge sequences on separate polypeptide chains. Sometimes, the two associated hinge sequences are referred to as a "hinge region". In certain embodiments of multispecific binding molecules of the disclosure, additional iterations of hinge regions may be incorporated into the polypeptide sequence.

[0200] A native hinge region is the hinge region that would normally be found between Fab and Fc domains in a naturally occurring antibody. A modified hinge region is any hinge that differs in length and/or composition from the native hinge region. Such hinges can include hinge regions from other species, such as human, mouse, rat, rabbit, shark, pig, hamster, camel, llama or goat hinge regions. Other modified hinge regions may comprise a complete hinge region derived from an antibody of a different class or subclass from that of the heavy

chain Fc domain or Fc region. Alternatively, the modified hinge region may comprise part of a natural hinge or a repeating unit in which each unit in the repeat is derived from a natural hinge region. In a further alternative, the natural hinge region may be altered by converting one or more cysteine or other residues into neutral residues, such as serine or alanine, or by converting suitably placed residues into cysteine residues. By such means the number of cysteine residues in the hinge region may be increased or decreased. Other modified hinge regions may be entirely synthetic and may be designed to possess desired properties such as length, cysteine composition and flexibility.

[0201] A number of modified hinge regions have already been described for example, in U.S. Patent No. 5,677,425, WO 99/15549, WO 2005/003170, WO 2005/003169, WO 2005/003170, WO 98/25971 and WO 2005/003171 and these are incorporated herein by reference.

[0202] In one embodiment, a multispecific binding molecule of the disclosure comprises an Fc region in which one or both Fc domains possesses an intact hinge domain at its N-terminus.

[0203] In various embodiments, positions 233-236 within a hinge region may be G, G, G and unoccupied; G, G, unoccupied, and unoccupied; G, unoccupied, unoccupied, and unoccupied; or all unoccupied, with positions numbered by EU numbering.

[0204] In some embodiments, the multispecific binding molecules of the disclosure comprise a modified hinge region that reduces binding affinity for an Fcγ receptor relative to a wild-type hinge region of the same isotype (*e.g.*, human IgG1 or human IgG4).

[0205] In one embodiment, the multispecific binding molecules of the disclosure comprise an Fc region in which each Fc domain possesses an intact hinge domain at its N-terminus, where each Fc domain and hinge domain is derived from IgG4 and each hinge domain comprises the modified sequence CPPC (SEQ ID NO: 170). The core hinge region of human IgG4 contains the sequence CPSC (SEQ ID NO: 171) compared to IgG1 that contains the sequence CPPC (SEQ ID NO: 170). The serine residue present in the IgG4 sequence leads to increased flexibility in this region, and therefore a proportion of molecules form disulfide bonds within the same protein chain (an intrachain disulfide) rather than bridging to the other heavy chain in the IgG molecule to form the interchain disulfide. (Angel

et al., 1993, Mol Immunol 30(1):105-108). Changing the serine residue to a proline to give the same core sequence as IgG1 allows complete formation of inter-chain disulfides in the IgG4 hinge region, thus reducing heterogeneity in the purified product. This altered isotype is termed IgG4P.

6.8.1.3.1. Chimeric Hinge Sequences

[0206] The hinge domain can be a chimeric hinge domain (also "chimeric hinge region").

[0207] For example, a chimeric hinge domain may comprise an "upper hinge" sequence, derived from a human IgG1, a human IgG2 or a human IgG4 hinge region, combined with a "lower hinge" sequence, derived from a human IgG1, a human IgG2 or a human IgG4 hinge region.

[0208] In particular embodiments, a chimeric hinge region comprises the amino acid sequence EPKSCDKTHTCPPCPAPPVA (SEQ ID NO: 172) (previously disclosed as SEQ ID NO:8 of WO2014/121087, which is incorporated by reference in its entirety herein) or ESKYGPPCPPCPAPPVA (SEQ ID NO: 173) (previously disclosed as SEQ ID NO:9 of WO2014/121087). Such chimeric hinge sequences can be suitably linked to an IgG4 CH2 region (for example by incorporation into an IgG4 Fc domain, for example a human or murine Fc domain, which can be further modified in the CH2 and/or CH3 domain to reduce effector function, for example as described in Section 6.8.1.1).

6.8.1.3.2. Hinge Sequences with Reduced Effector Function

[0209] In further embodiments, the hinge region can be modified to reduce effector function, for example as described in WO2016161010A2, which is incorporated by reference in its entirety herein. In various embodiments, the positions 233-236 of the modified hinge region are G, G, G and unoccupied; G, G, unoccupied, and unoccupied; G, unoccupied, unoccupied, and unoccupied; or all unoccupied, with positions numbered by EU numbering (as shown in FIG. 1 of WO2016161010A2). These segments can be represented as GGG-, GG--, G--- or ---- with "-" representing an unoccupied position.

[0210] Position 236 is unoccupied in canonical human IgG2 but is occupied by in other canonical human IgG isotypes. Positions 233-235 are occupied by residues other than G in all four human isotypes (as shown in FIG. 1 of WO2016161010A2).

[0211] The hinge modification within positions 233-236 can be combined with position 228 being occupied by P. Position 228 is naturally occupied by P in human IgG1 and IgG2 but is occupied by S in human IgG4 and R in human IgG3. An S228P mutation in an IgG4 antibody is advantageous in stabilizing an IgG4 antibody and reducing exchange of heavy chain light chain pairs between exogenous and endogenous antibodies. Preferably positions 226-229 are occupied by C, P, P and C respectively.

[0212] Exemplary hinge regions have residues 226-236, sometimes referred to as middle (or core) and lower hinge, occupied by the modified hinge sequences designated GGG-(233-236), GG—(233-236), G---(233-236) and no G(233-236). Optionally, the hinge domain amino acid sequence comprises CPPCPAPGGG-GPSVF (SEQ ID NO: 174) (previously disclosed as SEQ ID NO:1 of WO2016161010A2), CPPCPAPGG—GPSVF (SEQ ID NO: 162) (previously disclosed as SEQ ID NO:2 of WO2016161010A2), CPPCPAPG---GPSVF (SEQ ID NO: 163) (previously disclosed as SEQ ID NO:3 of WO2016161010A2), or CPPCPAP----GPSVF (SEQ ID NO: 164) (previously disclosed as SEQ ID NO:4 of WO2016161010A2).

[0213] The modified hinge regions described above can be incorporated into a heavy chain constant region, which typically includes CH2 and CH3 domains, and which may have an additional hinge segment (*e.g.*, an upper hinge) flanking the designated region. Such additional constant region segments present are typically of the same isotype, preferably a human isotype, although can be hybrids of different isotypes. The isotype of such additional human constant regions segments is preferably human IgG4 but can also be human IgG1, IgG2, or IgG3 or hybrids thereof in which domains are of different isotypes. Exemplary sequences of human IgG1, IgG2 and IgG4 are shown in FIGS. 2-4 of WO2016161010A2.

[0214] In specific embodiments, the modified hinge sequences can be linked to an IgG4 CH2 region (for example by incorporation into an IgG4 Fc domain, for example a human or murine Fc domain, which can be further modified in the CH2 and/or CH3 domain to reduce effector function, for example as described in Section 6.8.1.1).

6.9. Nucleic Acids and Host Cells

[0215] In another aspect, the disclosure provides nucleic acids encoding the multispecific binding molecules (e.g., primary MBMs) of the disclosure. In some embodiments, the

multispecific binding molecules are encoded by a single nucleic acid. In other embodiments, for example in the case of a heterodimeric molecule or a molecule comprising a component composed of more than one polypeptide chain, the multispecific binding molecules can be encoded by a plurality (*e.g.,* two, three, four or more) nucleic acids.

[0216] A single nucleic acid can encode a multispecific binding molecule that comprises a single polypeptide chain, a multispecific binding molecule that comprises two or more polypeptide chains, or a portion of a multispecific binding molecule that comprises more than two polypeptide chains (for example, a single nucleic acid can encode two polypeptide chains of a multispecific binding molecule comprising three, four or more polypeptide chains, or three polypeptide chains of a multispecific binding molecule comprising four or more polypeptide chains). For separate control of expression, the open reading frames encoding two or more polypeptide chains can be under the control of separate transcriptional regulatory elements (*e.g.,* promoters and/or enhancers). The open reading frames encoding two or more polypeptides can also be controlled by the same transcriptional regulatory elements, and separated by internal ribosome entry site (IRES) sequences allowing for translation into separate polypeptides.

[0217] In some embodiments, a multispecific binding molecule comprising two or more polypeptide chains is encoded by two or more nucleic acids. The number of nucleic acids encoding a multispecific binding molecule can be equal to or less than the number of polypeptide chains in the multispecific binding molecule (for example, when more than one polypeptide chains are encoded by a single nucleic acid).

[0218] The nucleic acids of the disclosure can be DNA (*e.g.*, plasmid) or RNA (*e.g.,* mRNA).

[0219] In another aspect, the disclosure provides host cells and vectors containing the nucleic acids of the disclosure. The nucleic acids may be present in a single vector or separate vectors present in the same host cell or separate host cell, as described in more detail herein below.

6.10. Pharmaceutical Compositions

[0220] The multispecific binding molecules, e.g., a primary MBM and optionally a secondary MBM of the disclosure may be in the form of a composition comprising the primary MBM, optionally a secondary MBM, and one or more carriers, excipients and/or diluents. The

compositions may be formulated for specific uses, such as for veterinary uses or pharmaceutical uses in humans. The form of the composition (e.g., dry powder, liquid formulation, etc.) and the excipients, diluents and/or carriers used will depend upon the intended uses of the multispecific binding molecule and, for therapeutic uses, the mode of administration.

[0221] For therapeutic uses, the compositions may be supplied as part of a sterile, pharmaceutical composition that includes a pharmaceutically acceptable carrier. This composition can be in any suitable form (depending upon the desired method of administering it to a patient). The pharmaceutical composition can be administered to a patient by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intratumorally, intrathecally, topically, or locally. The most suitable route for administration in any given case will depend on the particular antibody, the subject, and the nature and severity of the disease and the physical condition of the subject. Typically, the pharmaceutical composition will be administered intravenously or subcutaneously.

[0222] Pharmaceutical compositions can be conveniently presented in unit dosage forms containing a predetermined amount of a multispecific binding molecule of the disclosure per dose. The quantity of primary MBM (and, if present, secondary MBM) included in a unit dose will depend on the disease being treated, as well as other factors as are well known in the art. Such unit dosages may be in the form of a lyophilized dry powder containing an amount of multispecific binding molecule suitable for a single administration, or in the form of a liquid. Dry powder unit dosage forms may be packaged in a kit with a syringe, a suitable quantity of diluent and/or other components useful for administration. Unit dosages in liquid form may be conveniently supplied in the form of a syringe pre-filled with a quantity of multispecific binding molecule suitable for a single administration.

[0223] The pharmaceutical compositions may also be supplied in bulk from containing quantities of primary MBMs (and optionally secondary MBMs) suitable for multiple administrations.

[0224] Pharmaceutical compositions may be prepared for storage as lyophilized formulations or aqueous solutions by mixing a multispecific binding molecule having the desired degree of purity with optional pharmaceutically-acceptable carriers, excipients or

stabilizers typically employed in the art (all of which are referred to herein as "carriers"), i.e., buffering agents, stabilizing agents, preservatives, isotonifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. See, Remington's Pharmaceutical Sciences, 16th edition (Osol, ed. 1980). Such additives should be nontoxic to the recipients at the dosages and concentrations employed.

[0225] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They may be present at a wide variety of concentrations, but will typically be present in concentrations ranging from about 2 mM to about 50 mM. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), succinate buffers (e.g., succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartrate buffers (e.g., tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g., gluconic acid-sodium gluconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium gluconate mixture, etc.), oxalate buffer (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc.), lactate buffers (e.g., lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers (e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

[0226] Preservatives may be added to retard microbial growth, and can be added in amounts ranging from about 0.2%-1 % (w/v). Suitable preservatives for use with the present disclosure include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalkonium halides (e.g., chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonicifiers sometimes known as "stabilizers" can be added to ensure isotonicity of liquid compositions of the present

disclosure and include polyhydric sugar alcohols, for example trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, etc., organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, a-monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (e.g., peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trehalose; and trisaccacharides such as raffinose; and polysaccharides such as dextran. Stabilizers may be present in amounts ranging from 0.5 to 10 wt % per wt of multispecific binding molecule.

[0227] Non-ionic surfactants or detergents (also known as "wetting agents") may be added to help solubilize the glycoprotein as well as to protect the glycoprotein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, etc.), polyoxamers (184, 188 etc.), and pluronic polyols. Non-ionic surfactants may be present in a range of about 0.05 mg/mL to about 1.0 mg/mL, for example about 0.07 mg/mL to about 0.2 mg/mL.

[0228] Additional miscellaneous excipients include bulking agents (*e.g.,* starch), chelating agents (*e.g.,* EDTA), antioxidants (*e.g.,* ascorbic acid, methionine, vitamin E), and cosolvents.

6.11. Methods of Use

[0229] The primary MBMs of the disclosure are useful in eliciting immune responses against cancer and thus treating cancer in a subject. The MBMs of the disclosure may be

administered per se or in any suitable pharmaceutical composition, including a composition as described in Section 6.10.

[0230] In one aspect, primary MBMs of the disclosure for use as a medicament are provided. In further aspects, primary MBMs of the disclosure for use in treating a disease are provided. In certain embodiments, primary MBMs of the disclosure for use in a method of treatment are provided. In one embodiment, the disclosure provides a primary MBM as described herein for use in the treatment of a disease in a subject in need thereof. In certain embodiments, the disclosure provides a primary MBM for use in a method of treating a subject having a disease comprising administering to the individual a therapeutically effective amount of the primary MBM. In certain embodiments the disease to be treated is a proliferative disorder. In a preferred embodiment the disease is cancer.

[0231] In further embodiments, the disclosure provides a primary MBM for use in stimulating the immune system, particularly against a tumor cell. In certain embodiments, the disclosure provides a primary MBM for use in a method of stimulating the immune system in a subject comprising administering to the individual an effective amount of the primary MBM to stimulate the immune system. An "individual" according to any of the above embodiments is a mammal, preferably a human. "Stimulation of the immune system" according to any of the above embodiments may include any one or more of a general increase in immune function, an increase in T cell function, and/or an increase in T cell proliferation.

[0232] In a further aspect, the disclosure provides for the use of a primary MBM of the disclosure in the manufacture or preparation of a medicament for the treatment or prevention of a disease in a subject in need thereof. In one embodiment, the medicament is for use in a method of treating a disease comprising administering to a subject having the disease a therapeutically effective amount of the medicament.

[0233] In a further embodiment, the medicament is for stimulating the immune system. In a further embodiment, the medicament is for use in a method of stimulating the immune system in a subject comprising administering to the individual an amount effective of the medicament to stimulate the immune system. An "individual" according to any of the above embodiments is a mammal, preferably a human. "Stimulation of the immune system" according to any of the above embodiments may include any one or more of a general

increase in immune function, an increase in T cell function, and/or an increase in T cell proliferation.

[0234] In a further aspect, the disclosure provides a method for treating a disease in a subject, comprising administering to said individual a therapeutically effective amount of a primary MBM of the disclosure. In one embodiment a composition is administered to said individual, comprising the primary MBM of the disclosure in a pharmaceutically acceptable form, e.g., as described in Section 6.10. In certain embodiments the disease to be treated is a proliferative disorder. In a preferred embodiment the disease is cancer. In a further aspect, the disclosure provides a method for stimulating the immune system in a subject, comprising administering to the individual an effective amount of a primary MBM (e.g., in the form of a pharmaceutical composition, e.g., as described in Section 6.10) to stimulate the immune system. An "individual" according to any of the above embodiments is a mammal, preferably a human.

[0235] In certain embodiments the disease to be treated is a proliferative disorder, preferably cancer. Non-limiting examples of cancers include bladder cancer, brain cancer, head and neck cancer, pancreatic cancer, lung cancer, breast cancer, ovarian cancer, uterine cancer, cervical cancer, endometrial cancer, esophageal cancer, colon cancer, colorectal cancer, rectal cancer, gastric cancer, prostate cancer, blood cancer, skin cancer, squamous cell carcinoma, bone cancer, and kidney cancer. Other cell proliferation disorders that can be treated using a primary MBM of the present disclosure include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic region, and urogenital system. Also included are pre-cancerous conditions or lesions and cancer metastases. In certain embodiments the cancer is chosen from the group consisting of renal cell cancer, skin cancer, lung cancer, colorectal cancer, breast cancer, brain cancer, head and neck cancer. A skilled artisan readily recognizes that in many cases the primary MBMs, when used in the context of therapeutic treatment, may not provide a cure but may only provide partial benefit. In some embodiments, a physiological change having some benefit is also considered therapeutically beneficial. Thus, in some embodiments, an amount of primary MBM that

provides a physiological change is considered an "effective amount" or a "therapeutically effective amount". The subject, patient, or individual in need of treatment is typically a mammal, more specifically a human.

[0236] The appropriate dosage of a primary MBM of the disclosure (when used alone or in combination with one or more other additional therapeutic agents, e.g., a secondary MBM as described below) will depend on the type of disease to be treated, the route of administration, the body weight of the patient, the particular primary MBM, the severity and course of the disease, previous or concurrent therapeutic interventions, the patient's clinical history and response to the primary MBM (and any adjunct therapies, such as a secondary MBM), and the discretion of the attending physician. The practitioner responsible for administration will, in any event, determine the concentration of active ingredient(s) in a composition(s) and appropriate dose(s) for the individual subject. Various dosing schedules including but not limited to single or multiple administrations over various time-points.

[0237] The primary MBMs of the disclosure will generally be used in an amount effective to achieve the intended purpose. For use to treat proliferative condition, the primary MBMs of the disclosure, or pharmaceutical compositions thereof, are administered or applied in a therapeutically effective amount. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein. For clarity, a therapeutically effective amount is an amount that can elicit a beneficial physiological change (whether in a patient population or in a specific patient), and not necessarily an amount that will lead to a cure or remission of a proliferative disorder.

6.11.1. Combination Therapy

[0238] The therapeutic methods described above encompass combination therapy methods, whereby the primary MBMs of the disclosure are administered in combination with one or more other agents in therapy.

[0239] For instance, a primary MBM of the disclosure may be co-administered with at least one additional therapeutic agent. The term "therapeutic agent" encompasses any agent administered to treat a symptom or disease in a subject in need of such treatment. Such additional therapeutic agent may comprise any active ingredients suitable for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. In certain embodiments, an additional therapeutic agent is an

immunomodulatory agent, a cytostatic agent, an inhibitor of cell adhesion, a cytotoxic agent, an activator of cell apoptosis, or an agent that increases the sensitivity of cells to apoptotic inducers. In a particular embodiment, the additional therapeutic agent is an anti-cancer agent, for example a microtubule disruptor, an antimetabolite, a topoisomerase inhibitor, a DNA intercalator, an alkylating agent, a hormonal therapy, a kinase inhibitor, a receptor antagonist, an activator of tumor cell apoptosis, or an antiangiogenic agent. In further embodiments, the additional therapeutic agent is an immunotherapeutic agent, for example a checkpoint inhibitor (e.g., anti-PD1 antibody, anti-PDL1 antibody, or the like).

[0240] Such other agents are suitably present in combination in amounts that are effective for the purpose intended. The effective amount of such other agents depends on the amount of primary MBM used, the type of disorder or treatment, and other factors discussed above.

[0241] In some embodiments, the primary MBMs of the disclosure stimulate T cells against tumor cells through stimulation of the T-cell receptor (TCR)–CD3 complex. The TCR-CD3 complex is composed of a diverse αβ TCR heterodimer noncovalently associated with the invariant CD3 dimers CD3εγ, CD3εδ, and CD3ζζ. The TCR mediates recognition of antigenic peptides bound to MHC molecules (pMHC; also described herein as "HLA-bound peptide"), whereas the CD3 molecules transduce activation signals to the T cell. See Mariuzza *et al.*, 2020, J Biol Chem. 295(4): 914–925. Thus, a primary MBM whose antigen targeting moiety binds to a pMHC present on the tumor cell and the TCA binds to a TCR-CD3 complex component such as CD3, the primary MBM can stimulate the T cell against the tumor cell.

[0242] This T cell stimulation elicited by a primary MBM of the disclosed can be boosted by use of a second therapeutic agent that promotes signaling via a costimulatory pathway such as CD28, ICOS or CTLA4. See, e.g., Rudd & Schneider, 2003, Nature Reviews Immunology 3:544–556. In some embodiments, the second therapeutic agent is an anti-CD28, anti-ICOS or anti-CTLA4 antibody. Advantageously, a second therapeutic agent that promotes signaling via a costimulatory pathway is a secondary MBM as described in Section 6.3 that comprises not only a costimulatory receptor targeting moiety but a second targeting moiety that binds to a tumor antigen, e.g., a tumor antigen bound present on the same tumor cell that is bound by the antigen targeting moiety in the primary MBM. The tumor

antigen bound by the secondary MBM may be different from the antigen bound by the targeting moiety in the primary MBM.

[0243] Thus, the present disclosure provides methods of stimulating a T cell, e.g., against a tumor cell, through both the TCR-CD3 complex and a costimulatory pathway, using a combination of a primary MBM and a secondary MBM of the disclosure.

[0244] Accordingly, the present disclosure provides a combination of a primary MBM and a secondary MBM for use in stimulating the immune system, particularly against a tumor cell. In certain embodiments, the disclosure provides a combination of a primary MBM and secondary MBM for use in a method of stimulating the immune system in a subject comprising administering to the individual an effective amount of the primary MBM and the secondary MBM to stimulate the immune system. An "individual" according to any of the above embodiments is a mammal, preferably a human. "Stimulation of the immune system" according to any of the above embodiments may include any one or more of a general increase in immune function, an increase in T cell function, and/or an increase in T cell proliferation.

[0245] In a further aspect, the disclosure provides a method for treating a proliferative disease in a subject, comprising administering to said individual a therapeutically effective amount of a combination of a primary MBM and a secondary MBM as described herein. In one embodiment a single composition comprising both the primary MBM and secondary MBM is administered to said individual. In another embodiment, separate compositions comprising the primary MBM and secondary MBM are administered. The primary MBM and/or secondary MBM can be administered in a pharmaceutically acceptable form, e.g., as described in Section 6.10. In a preferred embodiment, the proliferative disease is cancer, e.g., a cancer as described in any Section 6.11. An "individual" according to any of the above embodiments is a mammal, preferably a human.

[0246] The combination of primary MBM and secondary MBM will generally be used in an amount effective to treat a proliferative condition. Determination of a therapeutically effective amount is well within the capabilities of those skilled in the art, especially in light of the detailed disclosure provided herein. For clarity, a therapeutically effective amount of the combination is an amount of the combination that can elicit a beneficial physiological change (e.g., in a patient population or in a specific patient), and not necessarily an amount that will

lead to a cure or remission of a proliferative disorder. Further, the amounts of primary MBM and secondary MBM used in the combination may not be therapeutically effective amounts of the individual components, provided the combination as a whole is a therapeutically effective amount.

[0247] Such combination therapies noted above encompass combined administration (where two or more therapeutic agents are included in the same compositions or separate composition administered in the same treatment session), and separate administration, in which case, administration of the primary MBM of the disclosure can occur prior to, simultaneously, and/or following, administration of the additional therapeutic agent.

[0248] Primary MBMs and combination therapies of the disclosure can also be used in combination with radiation therapy and/or immunotherapy. In particular embodiments, combination therapies of the disclosure include combination of: (a) a primary MBM, (b) a secondary MBM, and (c) an immunotherapeutic agent such as a checkpoint inhibitor. In some embodiments, the immunotherapeutic agent is an anti-PD1 antibody (e.g., an anti-PD1 antibody set forth in Table A). In some embodiments, the immunotherapeutic agent is an anti-PDL1 antibody (e.g., an anti-PDL1 antibody set forth in Table A).

7. SPECIFIC EMBODIMENTS

[0249] While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the disclosure(s). The present disclosure is exemplified by the numbered embodiments set forth below.

[0250] In the numbered embodiments that follow, the TCA targeting moiety preferably binds to a mammalian (e.g., human) TCA, the antigen targeting moiety preferably binds to a mammalian (e.g., human) antigen, the Fc domains are preferably derived from a mammalian (e.g., human) antibody, and the subjects are preferably mammals (e.g., humans).

1. A multispecific binding molecule (MBM) comprising:
   (a) a first polypeptide comprising, in N- to C-terminal orientation:
      (i) a first TCA targeting moiety or a component thereof;
      (ii) an optional first linker;

(iii) an antigen targeting moiety or a component thereof;

(iv) an optional second linker; and

(v) a first Fc domain; and

(b) a second polypeptide comprising, in N- to C-terminal orientation:

(i) a second TCA targeting moiety or a component thereof;

(ii) an optional third linker; and

(iii) a second Fc domain associated with the first Fc domain to form an Fc region.

2. The MBM of embodiment 1, wherein the first TCA targeting moiety is a Fab comprising a VH associated with a VL domain on a separate polypeptide chain.

3. The MBM of embodiment 1, wherein the first TCA targeting moiety is a single chain Fab comprising a VH and a VL domain on a single polypeptide chain.

4. The MBM of embodiment 2 or 3, wherein the VL domain is a universal light chain VL domain.

5. The MBM of embodiment 1, wherein the first TCA targeting moiety is an scFv.

6. The MBM of embodiment 5, wherein the scFv has the configuration, in N- to C-terminal orientation, VH – linker – VL.

7. The MBM of embodiment 5, wherein the scFv has the configuration, in N- to C-terminal orientation, VL – linker – VH.

8. The MBM of any one of embodiments 1 to 7, wherein the second TCA targeting moiety is a Fab comprising a VH associated with a VL domain on a separate polypeptide chain.

9. The MBM of any one of embodiments 1 to 7, wherein the second TCA targeting moiety is a single chain Fab comprising a VH and a VL domain on a single polypeptide chain.

10. The MBM of embodiment 8 or 9, wherein the VL domain is a universal light chain VL domain.

11. The MBM of any one of embodiments 1 to 7, wherein the second TCA targeting moiety is an scFv.

12. The MBM of embodiment 11, wherein the scFv has the configuration, in N- to C-terminal orientation, VH – linker – VL.

13. The MBM of embodiment 11, wherein the scFv has the configuration, in N- to C-terminal orientation, VL – linker – VH.

14. The MBM of any one of embodiments 1 to 13, wherein the antigen targeting moiety is a Fab comprising a VH associated with a VL domain on a separate polypeptide chain.

15. The MBM of any one of embodiments 1 to 13, wherein the antigen targeting moiety is a single chain Fab comprising a VH and a VL domain on a single polypeptide chain.

16. The MBM of embodiment 14 or 15, wherein the VL domain is a universal light chain VL domain.

17. The MBM of any one of embodiments 1 to 16, wherein the antigen targeting moiety is an scFv.

18. The MBM of embodiment 17, wherein the scFv has the configuration, in N- to C-terminal orientation, VH – linker – VL.

19. The MBM of embodiment 17, wherein the scFv has the configuration, in N- to C-terminal orientation, VL – linker – VH.

20. The MBM of any one of embodiments 1 to 19, further comprising a first Fc domain and a second Fc domain associated to form an Fc region.

21. The MBM of embodiment 20, wherein the first Fc domain is an IgG1 Fc domain.

22. The MBM of embodiment 20, wherein the first Fc domain is an IgG4 Fc domain.

23. The MBM of embodiment 20, wherein the first Fc domain is an Fc domain set forth in Table F-1.

24. The MBM of one of embodiments 20 to 22, wherein the second Fc domain is an IgG1 Fc domain.

25. The MBM of one of embodiments 20 to 22, wherein the second Fc domain is an IgG4 Fc domain.

26. The MBM of any one of embodiments 20 to 22, wherein the second Fc domain is an Fc domain set forth in Table F-1.

27. The MBM of any one of embodiments 20 to 26, wherein the Fc region is an Fc heterodimer.

28. The MBM of any one of embodiments 1 to 27, wherein the first linker, if present, is a linker set forth in Table L.

29. The MBM of any one of embodiments 1 to 27, wherein the second linker, if present, is a linker set forth in Table L.

30. The MBM of any one of embodiments 1 to 27, wherein the third linker, if present, is a linker set forth in Table L.

31. An MBM, which is optionally an MBM of any one of embodiments 1 to 30, which has the configuration depicted in FIG. 1B.

32. An MBM, which is optionally an MBM of any one of embodiments 1 to 30, which has the configuration depicted in FIG. 1C.

33. An MBM, which is optionally an MBM of any one of embodiments 1 to 30, which has the configuration depicted in FIG. 1D.

34. An MBM, which is optionally an MBM according to any one of embodiments 1 to 30, comprising:

(a) a first polypeptide comprising:

(i) a first VH domain;

(ii) a first CH1 domain;

(iii) an scFv which is an antigen targeting moiety; and

(iv) a first Fc region;

(b) a second polypeptide comprising:

(i) a first VL domain associated with the first VH domain to form a first TCA targeting moiety; and

(ii) a first CL domain;

(c) a third polypeptide comprising:

(i) a second VH domain;

(ii) a second CH1 domain; and

(iii) a second Fc region associated with the first Fc region to form an Fc domain; and

(d) a fourth polypeptide comprising:

(i) a second VL domain associated with the second VH domain to form a second TCA targeting moiety; and

(ii) a second CL domain.

35. An MBM, which is optionally an MBM according to any one of embodiments 1 to 30, comprising:

(a) a first polypeptide comprising:

(i) a first scFv which is a first TCA targeting moiety;

(ii) a first VH domain;

(iii) a first CH1 domain; and

(iv) a first Fc region;

(b) a second polypeptide comprising:

(i) a first VL domain associated with the first VH domain to form an antigen targeting moiety; and

(ii) a first CL domain; and

(c) a third polypeptide comprising:

(i) a second scFv which is a second TCA targeting moiety; and

(ii) a second Fc region associated with the first Fc region to form an Fc domain.

36. An MBM, which is optionally an MBM according to any one of embodiments 1 to 30, comprising:

(a) a first polypeptide comprising:

(i) a first VH domain;

(ii) a first CH1 domain;

(iii) a second VH domain;

(iv) a second CH1 domain; and

(v) a first Fc region;

(b) a second polypeptide comprising:

(i) a first VL domain associated with the first VH domain to form a first TCA targeting moiety; and

(ii) a first CL domain;

(c) a third polypeptide comprising:

(i) a second VL domain associated with the second VH domain to form an antigen targeting moiety; and

(ii) a second CL domain;

(d) a fourth polypeptide comprising:

(i) a third VH domain;

(ii) a third CH1 domain; and

(iii) a second Fc region associated with the first Fc region to form an Fc domain; and

(e) a fifth polypeptide comprising:

(i) a third VL domain associated with the third VH domain to form a second TCA targeting moiety; and

(ii) a third CL domain.

37. The MBM of any one of embodiments 1 to 36, wherein the antigen targeting moiety is a tumor antigen targeting moiety.

38. The MBM of embodiment 37, wherein the tumor antigen targeting moiety is capable of binding to a tumor antigen set forth in Table A.

39. The MBM of embodiment 37, wherein the tumor antigen targeting moiety is capable of binding to a tumor antigen set forth in Table P-1.

40. The MBM of embodiment 37, wherein the tumor antigen targeting moiety (a) comprises the (i) CDR or (ii) VH and VL sequences of an antibody or TCR set forth in Table P-2 or (b) competes with an antibody or TCR set forth in Table P-2 for binding to its target.

41. The MBM of any one of embodiments 1 to 40, wherein the antigen targeting moiety is a low-density antigen targeting moiety.

42. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 5000 copies per cell.

43. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 4000 copies per cell.

44. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 3000 copies per cell.

45. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 2000 copies per cell.

46. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 1000 copies per cell.

47. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 500 copies per cell.

48. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 250 copies per cell.

49. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 100 copies per cell.

50. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 50 copies per cell.

51. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 40 copies per cell.

52. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 30 copies per cell.

53. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 20 copies per cell.

54. The MBM of embodiment 41, wherein the low-density antigen is an antigen expressed at no more than 10 copies per cell.

55. The MBM of any one of embodiments 1 to 54, wherein the antigen targeting moiety is an HLA-bound peptide antigen targeting moiety.

56. The MBM of embodiment 55, wherein the HLA-bound peptide is a viral peptide.

57. The MBM of embodiment 56, wherein the viral peptide is HPV16 E7 (11-20), HPV E716 (11-19), HPV16 E7 (82-90), CMV pp65 (495-503), HIV P17 (77-85), HIV RT (896-904), HIV GAG (41-49), or ENV (183-191).

58. The MBM of embodiment 56, wherein the viral peptide is a peptide set forth in Table P-1.

59. The MBM of embodiment 55, wherein the HLA-bound peptide is a tumor-associated peptide.

60. The MBM of embodiment 59, wherein the tumor-associated peptide is LCMV derived peptide gp33-41, ACP3 (PAP) (112-120), AFP (58-68), AFP (173-181), APF (126-134), BALF(276-284), CEA (571-579), CMV pp65 (495-503), DNTT (475-483), DNTT (250-258), EBNA-1 (562-570), FLU-M1 (58-66), gp100 (154-162), gp100 (209-217), HBV Core (18-27), Her2/neu (369-377;V2v9); HPV16 E7 (11-20), HPV E716 (11-19), HPV16 E7 (82-90), IGF2BP3 (552-560), KLK4 (11-19), KLK4 (215-223), KLK4 (105-113), KRAS (6-14; G12V), KRAS (4-12; K5L/G12V), KRAS (5-14; G12V), KRAS (5-14; G12D), KRAS (10-18; G12D), KRAS (10-19; G12D), KRAS (8-16; G12D), KRAS (8-16; G12D), KRAS (7-16; G12D), KRAS (7-16; G12D), LMP1 (125-133), LMP2 (426-434), MAGE-A3 (112-120), MAGE-A3 (168-176), MAGE-A3 (271-279), MAGE-A3 (108-116), MAGE-A4 (230-239), MAGE-A4 (286-294), MAGE-A10 (137-145), MAGE-A10 (254-262), MAGE-A10 (310-318), MPO (132-140), MPO (549-557), MPO (571-579), NYESO1 (157-165, C165A), NYESO1 (157-165, C165V), p54 WT (264-272), PAP-3 (136-143), PRAME (425-433), PRAME (312-320), PSA (159-169), PSA (241-249), PSA (79-89), PSA (170-178), PSA (82-90), PSMA (4-12), PSMA (135-145), Survivin (96-014), Tyrosinase (369-377, 371D), WT1 (10-18), WT1 (137-145), or WT1 (126-134).

61. The MBM of embodiment 59, wherein the HLA-bound peptide is PRAME (425-433).

62. The MBM of embodiment 59, wherein the HLA-bound peptide is MAGE-A4 (230-239).

63. The MBM of embodiment 59, wherein the tumor-associated peptide is a peptide set forth in Table P-1.

64. The MBM of embodiment 55, wherein the HLA-bound peptide antigen targeting moiety (a) comprises the (i) CDR or (ii) VH and VL sequences of an antibody or TCR set forth in Table P-2 or (b) competes with an antibody set forth in Table P-2 for binding to its target.

65. The MBM according to any one of embodiments 1 to 64, wherein the first TCA targeting moiety is a T cell engaging targeting moiety.

66. The MBM of any one of embodiments 1 to 65, wherein the first TCA targeting moiety is a T cell receptor (TCR) targeting moiety.

67. The MBM of embodiment 66, wherein the TCR targeting moiety is a CD3 targeting moiety.

68. The MBM of embodiment 67, wherein the CD3 targeting moiety specifically binds to human CD3.

69. The MBM of embodiment 66, wherein the TCR targeting moiety is a TCRαβ targeting moiety.

70. The MBM of embodiment 69, wherein the TCRαβ targeting moiety specifically binds to human TCRαβ.

71. The MBM of embodiment 66, wherein the TCR targeting moiety is a TCRγδ targeting moiety.

72. The MBM of embodiment 71, wherein the TCRγδ targeting moiety specifically binds to human TCRγδ.

73. The MBM according to any one of embodiments 1 to 72, wherein the first TCA targeting moiety (a) comprises the (i) CDR or (ii) VH and VL sequences of an antibody set forth in Table T or (b) competes with the antibody set forth in Table T for binding to its target.

74. The MBM according to any one of embodiments 1 to 73, wherein the second TCA targeting moiety is a T cell engaging targeting moiety.

75. The MBM according to any one of embodiments 1 to 74, wherein the second TCA targeting moiety is a T cell receptor (TCR) targeting moiety.

76. The MBM of embodiment 75, wherein the TCR targeting moiety is a CD3 targeting moiety.

77. The MBM of embodiment 76, wherein the CD3 targeting moiety specifically binds to human CD3.

78. The MBM of embodiment 75, wherein the TCR targeting moiety is a TCRαβ targeting moiety.

79. The MBM of embodiment 78, wherein the TCRαβ targeting moiety specifically binds to human TCRαβ.

80. The MBM of embodiment 75, wherein the TCR targeting moiety is a TCRγδ targeting moiety.

81. The MBM of embodiment 80, wherein the TCRγδ targeting moiety specifically binds to human TCRγδ.

82. The MBM according to any one of embodiments 1 to 81, wherein the second TCA targeting moiety (a) comprises the (i) CDR or (ii) VH and VL sequences of an antibody set forth in Table T or (b) competes with the antibody set forth in Table T for binding to its target.

83. The MBM according to any one of embodiments 1 to 82, wherein the MBM has reduced binding to anti-drug antibodies as measured by an ADA reactivity assay as presented in Section 8.1.3 relative to a control antigen-binding molecule.

84. A multispecific binding molecule (MBM), which is optionally an MBM according to any one of embodiments 1 to 83, comprising:

(a) a first polypeptide comprising, in N- to C-terminal orientation:
  (i) a first VH domain;
  (ii) a first CH1 domain;
  (iii) an scFv comprising:
    (A) means for binding a tumor antigen;
    (B) means for binding a low-density antigen; or
    (C) means for binding an HLA-bound peptide antigen; and
  (iv) a first Fc domain;

(b) a second polypeptide comprising:
  (i) a first VL domain; and
  (ii) a first CL domain,

wherein the first VL domain and the first CL domain are associated with the first VH domain and the first CH1 domain to form a first Fab comprising means for binding a T cell antigen;

(c) a third polypeptide comprising, in N- to C-terminal orientation:
  (i) a second VH domain;
  (ii) a second CH1 domain; and

(iii) a second Fc domain associated with the first Fc domain to form an Fc region; and

(b) a fourth polypeptide comprising:

(i) a second VL domain; and

(ii) a second CL domain,

wherein the second VL domain and the second CL domain are associated with the second VH domain and the second CH1 domain to form a second Fab comprising means for binding a T cell antigen.

85. A multispecific binding molecule (MBM), which is optionally an MBM according to any one of embodiments 1 to 83, comprising:

(a) a first polypeptide comprising:

(i) a first scFv comprising means for binding a T cell antigen;

(ii) a VH domain;

(iii) a CH1 domain; and

(iv) a first Fc domain;

(b) a second polypeptide comprising:

(i) a first VL domain; and

(ii) a first CL domain,

wherein the first VL domain and the first CL domain are associated with the first VH domain and the first CH1 domain to form a first Fab comprising:

(A) means for binding a tumor antigen;

(B) means for binding a low-density antigen; or

(C) means for binding an HLA-bound peptide antigen; and

(c) a third polypeptide comprising, in N- to C-terminal orientation:

(i) a second scFv comprising means for binding a T cell antigen; and

(iii) a second Fc domain associated with the first Fc domain to form an Fc region.

86. A multispecific binding molecule (MBM), which is optionally an MBM according to any one of embodiments 1 to 83, comprising:

(a) a first polypeptide comprising:

(i) a first VH domain;

(ii) a first CH1 domain;

(iii) a second VH domain;

(iv) a second CH1 domain; and

(v) a first Fc region;

(b) a second polypeptide comprising:

(i) a first VL domain; and

(ii) a first CL domain,

wherein the first VL domain and the first CL domain are associated with the first VH domain and the first CH1 domain to form a first Fab comprising means for binding a T cell antigen;

(c) a third polypeptide comprising:

(i) a second VL domain; and

(ii) a second CL domain,

wherein the second VL domain and the second CL domain are associated with the second VH domain and the second CH1 domain to form a second Fab comprising:

(A) means for binding a tumor antigen;

(B) means for binding a low-density antigen; or

(C) means for binding an HLA-bound peptide antigen;

(d) a fourth polypeptide comprising:

(i) a third VH domain;

(ii) a third CH1 domain; and

(iii) a second Fc region associated with the first Fc region to form an Fc domain; and

(e) a fifth polypeptide comprising:

(i) a third VL domain; and

(ii) a third CL domain,

wherein the third VL domain and the third CL domain are associated with the third VH domain and the third CH1 domain to form a third Fab comprising means for binding a T cell antigen.

87. The MBM according to any one of embodiments 84 to 86, wherein the means for binding a T cell antigen are means for binding human CD3.

88. A nucleic acid or plurality of nucleic acids encoding the MBM of any one of embodiments 1 to 87.

89. A host cell engineered to express the MBM of any one of embodiments 1 to 87.

90. A cell transfected with one or more expression vectors comprising one or more nucleic acid sequences encoding the MBM molecule of any one of embodiments 1 to 87 under the control of one or more promoters.

91. A method of producing the MBM of any one of embodiments 1 to 87, comprising culturing the host cell of embodiment 89 or 90 and recovering the MBM expressed thereby.

92. A method of killing a cancer cell comprising administering to the cell the MBM of any one of embodiments 1 to 87.

93. The method of embodiment 92, wherein the cell expresses less than 5000, 4000, 3000, 2000, 1000, 500, 250, 100, 50, 40, 30, 20, or 10 copies of the antigen targeted by the antigen targeting moiety.

94. A method of activating a T cell comprising administering to the T cell the MBM of any one of embodiments 1 to 87.

95. A pharmaceutical composition comprising the MBM of any one of embodiments 1 to 87 and an excipient.

96. The pharmaceutical composition of embodiment 95, further comprising a secondary multispecific antigen binding molecule (e.g., as described in Section 6.3) comprising a tumor antigen targeting moiety and a TCA targeting moiety.

97. The pharmaceutical composition of embodiment 96, wherein the tumor antigen targeting moiety of the multispecific antigen binding molecule is an EGFR targeting moiety.

98. The pharmaceutical composition of embodiment 97, wherein the EGFR targeting moiety specifically binds to human EGFR.

99. The pharmaceutical composition of any one of embodiments 96 to 98, wherein the TCA targeting moiety of the multispecific antigen binding molecule is a CD28 targeting moiety.

100. The pharmaceutical composition of embodiment 99, wherein the CD28 targeting moiety specifically binds to human CD28.

101. The pharmaceutical composition of any one of embodiments 95 to 100, further comprising an immune checkpoint inhibitor.

102. The pharmaceutical composition of embodiment 101, wherein the immune checkpoint inhibitor is an anti-PD1 antibody.

103. A method of treating cancer, comprising administering to a subject in need thereof a multispecific binding molecule (MBM) comprising:

(a) a first polypeptide comprising, in N- to C-terminal orientation:
  (i) a first TCA targeting moiety or a component thereof;
  (ii) an optional first linker;
  (iii) an antigen targeting moiety or a component thereof;
  (iv) an optional second linker; and
  (v) a first Fc domain; and

(b) a second polypeptide comprising, in N- to C-terminal orientation:

(i) a second TCA targeting moiety or a component thereof;

(ii) an optional third linker; and

(iii) a second Fc domain associated with the first Fc domain to form an Fc region.

104. A method of inhibiting growth of a tumor cell in a subject, comprising administering to a subject a multispecific binding molecule (MBM) comprising:

(a) a first polypeptide comprising, in N- to C-terminal orientation:

(i) a first TCA targeting moiety or a component thereof;

(ii) an optional first linker;

(iii) an antigen targeting moiety or a component thereof;

(iv) an optional second linker; and

(v) a first Fc domain; and

(b) a second polypeptide comprising, in N- to C-terminal orientation:

(i) a second TCA targeting moiety or a component thereof;

(ii) an optional third linker; and

(iii) a second Fc domain associated with the first Fc domain to form an Fc region.

105. A method of stimulating proliferation of cancer antigen specific T cells, comprising administering to a subject in need thereof a multispecific binding molecule (MBM) comprising:

(a) a first polypeptide comprising, in N- to C-terminal orientation:

(i) a first TCA targeting moiety or a component thereof;

(ii) an optional first linker;

(iii) an antigen targeting moiety or a component thereof;

(iv) an optional second linker; and

(v) a first Fc domain; and

(b) a second polypeptide comprising, in N- to C-terminal orientation:

(i) a second TCA targeting moiety or a component thereof;

(ii) an optional third linker; and

(iii) a second Fc domain associated with the first Fc domain to form an Fc region.

106. The method of any one of embodiments 103 to 105, wherein the MBM is the MBM of any one of embodiments 1 to 87.

107. The method of any one of embodiments 103 to 105, wherein the MBM is administered by administration of the pharmaceutical composition of any one of embodiments 95 to 102.

108. The method of any one of embodiments 103 to 107, further comprising administering to the subject a secondary multispecific antigen binding molecule (e.g., as described in Section 6.3) comprising a tumor antigen targeting moiety and a TCA targeting moiety.

109. The method of embodiment 108, wherein the tumor antigen targeting moiety of the secondary multispecific antigen binding molecule is an EGFR targeting moiety.

110. The method of embodiment 109, wherein the EGFR targeting moiety specifically binds to human EGFR.

111. The method of any one of embodiments 108 to 110, wherein the TCA targeting moiety of the secondary multispecific antigen binding molecule is a CD28 targeting moiety.

112. The method of embodiment 111, wherein the CD28 targeting moiety specifically binds to human CD28.

113. The method of any one of embodiments 103 to 111, further comprising administering to the subject an immune checkpoint inhibitor.

114. The method of embodiment 113, wherein the immune checkpoint inhibitor is an anti-PD1 antibody.

8. EXAMPLES

8.1. Materials and Methods

8.1.1. Design and Production of Multispecific Binding Molecules (MBMs)

[0251] Multispecific binding molecules (MBMs) were designed to comprise two polypeptides connected to one another via their Fc domains, one or both of which further comprise one or more antigen targeting and/or TCA targeting moieties. FIGS. 1A-1D show exemplary MBM structures and structural components. FIGS. 2A-2K show the exemplary MBM structures that were produced and evaluated in the Examples below.

[0252] All constructs were expressed in Expi293F™ cells by transient transfection (Thermo Fisher Scientific). Proteins in Expi293F supernatant were purified using the ProteinMaker system (Protein BioSolutions, Gaithersburg, MD) with HiTrap MabSelect SuRe Protein A columns (GE Healthcare). After single step elution with IgG Elution Buffer (Thermo Fisher Scientific), the proteins were neutralized, dialyzed into a final buffer of phosphate buffered saline (PBS) with 5% glycerol, aliquoted and stored at -80°C.

[0253] An overview of the constructs produced is provided in Table E-1, below.

Table E-1 - MBMs

Name	Description
PRAME-1	(PRAME)Fab-Fc x (CD3)scFv-Fc
PRAME-2	(CD3)Fab-(PRAME)scFv-Fc x (CD3)Fab-Fc
PRAME-3	(PRAME)Fab-(CD3)scFv-Fc x (PRAME)Fab-Fc
PRAME-4	(CD3)scFv-(PRAME)Fab-Fc x (CD3)scFv-Fc
PRAME-5	(PRAME)scFv-(CD3)Fab-Fc x (PRAME)scFv-Fc
PRAME-6	(PRAME)scFv-Fc x (CD3)Fab-Fc
PRAME-7	(CD3)Fab-(PRAME)scFv-Fc x Fc
PRAME-8	(CD3)scFv-(PRAME)Fab-Fc x Fc
MAGEA4-1	(CD3)Fab-(MAGEA4)scFv-Fc x (CD3)Fab-Fc
MAGEA4-2	(CD3)scFv-(MAGEA4)Fab-Fc x (CD3)scFv-Fc
MAGEA4-3	(CD3)Fab-(MAGEA4)Fab-Fc x (CD3)Fab-Fc

8.1.2. Cytotoxicity Assay and T cell Activation Assessment

[0254] Target cell lines were resuspended in PBS and stained with CellTrace™ Violet (Thermo Fisher Scientific) based on manufacturer protocols. Cells were resuspended in RPMI media containing 10% FBS and penicillin-streptomycin-glutamine supplementation (R10 media) and plated in a 96-well plate to provide 10,000 cells per well. Effector cells (isolated T cells) were plated to provide 60,000 cells per well, so that the Effector:Target cell ratio was 6:1. MBMs were diluted with a ratio of 1:10 and added to the assay plates with a final starting concentration of 6.7×10^{-08} M in R10 media. An antibody mixture was added to some assay plates for a final concentration of 2.5 µg/ml of an anti-EGFRxCD28 and 3 µg/ml anti-PD1. The plates were incubated in a cell culture incubator for 72 hours or longer. The supernatant was then removed and spun down at 300 x g for 4 minutes to pellet all cells in suspension. The tumor cells were washed and harvested with trypsin, and combined with the suspension cell pellet. The final cell pellet was washed with PBS and stained with LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (Thermo Fisher Scientific). After 2 washes with PBS, cells were resuspended in FACS wash and analyzed on a cytometer such as the BD FACSCelesta™ instrument or BD FACSCanto™ II instrument. For the assessment of killing specificity, cells were gated on live Violet labeled populations. The percentage of the live population was recorded and used for the calculation of survival. T cell activation was assessed by incubating cells with directly conjugated antibodies against CD2 and CD25 and by reporting the percentage of CD25+ T cells out of CD2+ T cells. Results were analyzed by FlowJo™. All appropriate compensation samples and fluorescence minus one (FMO) controls were included.

8.1.3. ADA Reactivity Assay

[0255] In order to assess antigenicity, antibodies were tested in an ADA reactivity assay using a SMCxPro™ Immunogenicity method for measuring binding to pre-existing anti-drug antibodies. Briefly, antibodies were labeled with biotin and Alexa647 according to the Millipore SMC™ Immunogenicity Plate Based Assay Development Kit protocol (Catalog #03-0189-00). Biotinylated-antibody at 1µg/mL, Alexa647-antibody at 0.063ug/mL, and human or monkey serum diluted 1:10 were combined 1:1:1 and incubated overnight shaking at 4°C. After incubation, the bridged complex was transferred to a pre-blocked streptavidin-coated assay plate and incubated for 1 hour shaking at RT. The plate was washed 6 times in 1X Wash Buffer. Elution Buffer B was added to the assay plate and incubated for 10

minutes shaking at RT. After 10 minutes the reaction was quenched with Buffer C. The neutralized solution was transferred to an SMCxPro™ Aurora plate, sealed, and read on the SMCxPro™.

8.1.4. Flow Cytometry Binding Assay

[0256] Cells were resuspended in FACS wash (PBS with 1% FBS and 0.5mM EDTA) at 1×10^6 cells/mL. The staining was performed in 1×10^5 cells per well. The antibodies were diluted with a ratio of 1:5 from a starting concentration of 1.3×10^{-07} M. The diluted antibodies were then added into the wells containing cells. Cells were stained for 30 min at 2-8°C and washed twice with FACS wash buffer. APC-conjugated goat anti-human IgG (Jackson Immuno Research, 109-607-003, 1:400) was added to stain the cells for 30 min at 2–8°C along with LIVE/DEAD™ Fixable Violet Dead Cell Stain Kit (Thermo Fisher Scientific). Following washing, cells were fixed in 2% paraformaldehyde for 30 min at 2–8°C. After two washes, stained cells were analyzed using BD FACSCanto™ II instrument. Cells were gated using forward scatter (FSC) and side scatter (SCC), and MFI values for fluorescent dye APC were calculated using FlowJo™ software and the results were analyzed by FlowJo™. MFI values were plotted using GraphPad Prism™ software and EC_{50} values for the antibodies were determined from a four-parameter logistic equation over a dose-response curve.

8.2. Example 1: Cytotoxic Potency of PRAME-CD3 MBMs against OVCAR3 Cells

[0257] Multispecific antigen binding molecules (MBMs) were generated as described in Section 8.1.1; those evaluated in this example are shown in FIG. 3A. A cytotoxicity assay was performed by incubating PRAME-expressing wild-type OVCAR3 and PRAME-knockout OVCAR3 cells with the MBMs for 88 hours in the presence of anti-PD1 and anti-EGFRxCD28 antibodies as described in Section 8.1.2.

[0258] Both MBMs with anti-CD3 and anti-HLA-A2-PRAME(425-433) ABDs, PRAME-1 and PRAME-2, displayed dose-dependent cytotoxicity against wild-type OVCAR3 cells. PRAME-2 showed improved cell toxicity relative to PRAME-1 (FIG. 3B).

8.3. Example 2: Cytotoxic Potency of PRAME-CD3 MBMs Against Cells With Different Copy Numbers of PRAME

[0259] Multispecific antigen binding molecules (MBMs) were generated as described in Section 8.1.1; those evaluated in this example are shown in FIG. 4A. A cytotoxicity assay was performed by incubating PRAME-expressing wild-type OVCAR3 and SK-MEL-24 wild-type cells, as well as PRAME-negative wild type COLO205 cells with the MBMs for 96 hours in the absence or presence of anti-PD1 and anti-EGFRxCD28 antibodies as described in Section 8.1.2. T cell activation assessments were carried out as described in Section 8.1.2.

[0260] Wild-type OVCAR3 cells express a low copy number (~50 copies) of PRAME HLA-A2 (425-433), whereas SK-MEL-24 cells express PRAME HLA-A2 (425-433) at relatively high levels (~2400 copies). In contrast, COLO205 cells do not express PRAME HLA-A2 (425-433). In the absence of anti-PD1 and anti-EGFRxCD28 antibodies, PRAME-2 displayed dose-dependent cytotoxicity against OVCAR3 cells, while PRAME-5 and PRAME-3 exerted cytotoxicity at high concentrations (FIG. 4B). All constructs except the isotype control and PRAME-4 displayed cytotoxic potency against SK-MEL-24 cells (FIG. 4C). None of the constructs, except the positive control comprising a Pan-HLA-A2 ABD exerted cytotoxicity against COLO205 cells (FIG. 4D). The cytotoxic potency of PRAME-CD3 MBMs was enhanced against OVCAR3 and SK-MEL-24 cells in the presence of anti-PD1 and anti-EGFRxCD28 antibodies relative to the MBM alone (FIGS. 4E and 4F), while no PRAME-CD3 MBM displayed cytotoxicity against COLO205 cells, even with the addition of anti-PD1 and anti-EGFRxCD28 antibodies (FIG. 4G).

[0261] Similar results were obtained for T cell activation. In the absence of anti-PD1 and anti-EGFRxCD28 antibodies, PRAME-2, PRAME-5, and PRAME-3 were associated with T cell activation in OVCAR3 cells to varying degrees (FIG. 4H). All constructs except the isotype control and PRAME-4 displayed cytotoxic potency against SK-MEL-24 cells (FIG. 4I). As was the case with cytotoxicity, none of the constructs, except the positive control, were associated with any T cell activation (FIG. 4J). T cell activation potency of PRAME-CD3 MBMs was greatly enhanced in the presence of anti-PD1 and anti-EGFRxCD28 antibodies in plates with OVCAR3 and SK-MEL-24 cells, especially for PRAME-2 (FIGS. 4K and 4L). No T cell activity was observed in COLO205 cell plates, except for the positive control, even in the presence of anti-PD1 and anti-EGFRxCD28 antibodies (FIG. 4M).

[0262] Taken together, these results suggest that PRAME-2, PRAME-3, and PRAME-5 have potency not only against cells that expresses PRAME HLA-A2 (425-433) at high

copies but also has potency against cells with low copy numbers of PRAME HLA-A2 (425-433).

8.4. Example 3: PRAME-CD3 MBMs Display Low ADA Reactivity

[0263] ADA reactivity of PRAME-CD3 MBMs PRAME-2 and PRAME-6 were assessed as described in Section 8.1.3.

[0264] Both PRAME-CD3 MBMs displayed levels of ADA similar to the ADA reactivity displayed by the negative control (FIG. 5), suggesting that these PRAME-CD3 have relatively low ADA reactivity.

8.5. Example 4: Cytotoxic Potency of PRAME-CD3 MBMs Against A375 Cells

[0265] Wild-type A375 cells express a low copy number of PRAME HLA-A2 (425-433) (~50 copies) and a medium copy number of MAGE-A4 HLA-A2 (230-239) (~550 copies). Therefore, these cells were used to assess the cytotoxic and T cell activating effects of PRAME-CD3 and MAGE-A4-CD3 MBMs.

[0266] Multispecific antigen binding molecules (MBMs) comprising one or more CD3 targeting moieties and further comprising: a targeting moiety that specifically binds to HLA-A2-PRAME(425-433), a targeting moiety that specifically binds to HLA-A2-MAGE-A4 (230-239), a positive control targeting moiety (anti-Pan-HLA-A2), or a negative control targeting moiety (FIGS. 6A and 7A) were generated as described in Section 8.1.1. A cytotoxicity assay was performed by incubating A375 cells with the MBMs for 96 hours in the absence or presence of anti-PD1 and anti-EGFRxCD28 antibodies as described in Section 8.1.2. T cell activation assessments were carried out as described in Section 8.1.2.

[0267] In the absence of anti-PD1 and anti-EGFRxCD28, all three MAGE-A4-CD3 MBMs displayed potent and dose-dependent cytotoxicity (FIG. 6B). Among PRAME-CD3 MBMs, only PRAME-2 displayed a dose-dependent cytotoxicity (FIG. 6B). The cytotoxicity of these constructs was enhanced in the presence of anti-PD1 and anti-EGFRxCD28 antibodies, and PRAME-1 displayed cell killing activity as well (FIG. 6C).

[0268] Similarly, in the absence of anti-PD1 and anti-EGFRxCD28 antibodies, the strongest levels of T cell activation were associated with MAGE-A4-CD3 MBMs, followed by PRAME-2, and to some degree, PRAME-1 (FIG. 6D). The presence of anti-PD1 and anti-EGFRxCD28 antibodies enhanced these effects (FIG. 6E).

[0269] Next, cytotoxicity and T cell activation assessments using PRAME-CD3 MBMs were conducted with PRAME-overexpressing and PRAME-knockout A375 cells. All constructs displayed potent dose-dependent cytotoxicity against the PRAME-overexpressing cells (FIG. 7B) and none of the constructs except the positive control had cytotoxic or T cell activating effects in PRAME-knockout cells (FIGS. 7C and 7D, respectively).

[0270] Taken together, these results suggest that PRAME-2 and PRAME-1 show specific cytotoxicity and T cell activation.

8.6. Example 5: Target Binding Properties of PRAME-CD3 MBMs

[0271] PRAME-CD3 MBMs and control constructs (FIG. 8A) were evaluated for their ability to binding PRAME and CD3 targets as described in Section 8.1.4.

[0272] In PRAME-expressing A375 cells, all PRAME-CD3 MBM constructs displayed dose-dependent binding (FIG. 8B). Similarly, all PRAME-CD3 MBM constructs and controls with anti-CD3 ABDs displayed dose-dependent binding to Jurkat E6 cells (FIG. 8C). These results suggested that the PRAME-CD3 MBMs were able to bind to both CD3 and HLA-A2 PRAME(425-433) targets.

WHAT IS CLAIMED IS

1. A multispecific binding molecule (MBM) comprising:
   (a) a first polypeptide comprising, in N- to C-terminal orientation:
      (i) a first TCA targeting moiety or a component thereof;
      (ii) an optional first linker;
      (iii) an antigen targeting moiety or a component thereof;
      (iv) an optional second linker; and
      (v) a first Fc domain; and
   (b) a second polypeptide comprising, in N- to C-terminal orientation:
      (i) a second TCA targeting moiety or a component thereof;
      (ii) an optional third linker; and
      (iii) a second Fc domain associated with the first Fc domain to form an Fc region.

2. The MBM of claim 1, wherein the first TCA targeting moiety is a Fab comprising a VH associated with a VL domain on a separate polypeptide chain.

3. The MBM of claim 1, wherein the first TCA targeting moiety is a single chain Fab comprising a VH and a VL domain on a single polypeptide chain.

4. The MBM of claim 2 or 3, wherein the VL domain is a universal light chain VL domain.

5. The MBM of claim 1, wherein the first TCA targeting moiety is an scFv.

6. The MBM of any one of claims 1 to 5, wherein the second TCA targeting moiety is a Fab comprising a VH associated with a VL domain on a separate polypeptide chain.

7. The MBM of any one of claims 1 to 5, wherein the second TCA targeting moiety is a single chain Fab comprising a VH and a VL domain on a single polypeptide chain.

8. The MBM of claim 6 or 7, wherein the VL domain is a universal light chain VL domain.

9. The MBM of any one of claims 1 to 5, wherein the second TCA targeting moiety is an scFv.

10. The MBM of any one of claims 1 to 9, wherein the antigen targeting moiety is a Fab comprising a VH associated with a VL domain on a separate polypeptide chain.

11. The MBM of any one of claims 1 to 9, wherein the antigen targeting moiety is a single chain Fab comprising a VH and a VL domain on a single polypeptide chain.

12. The MBM of claim 10 or 11, wherein the VL domain is a universal light chain VL domain.

13. The MBM of any one of claims 1 to 12, wherein the antigen targeting moiety is an scFv.

14. The MBM of any one of claims 1 to 13, further comprising a first Fc domain and a second Fc domain associated to form an Fc region.

15. The MBM of claim 14, wherein the first Fc domain is (a) an IgG1 Fc domain; (b) an IgG4 Fc domain; or (c) an Fc domain set forth in Table F-1.

16. The MBM of claim 14 or claim 15, wherein the second Fc domain is (a) an IgG1 Fc domain; (b) an IgG4 Fc domain; or (c) an Fc domain set forth in Table F-1.

17. The MBM of any one of claims 14 to 16, wherein the Fc region is an Fc heterodimer.

18. An MBM, which is optionally an MBM of any one of claims 1 to 17, which has the configuration depicted in FIG. 1B.

19. An MBM, which is optionally an MBM of any one of claims 1 to 17, which has the configuration depicted in FIG. 1C.

20. An MBM, which is optionally an MBM of any one of claims 1 to 17, which has the configuration depicted in FIG. 1D.

21. An MBM, which is optionally an MBM according to any one of claims 1 to 17, comprising:

(a) a first polypeptide comprising:

(i) a first VH domain;

(ii) a first CH1 domain;

(iii) an scFv which is an antigen targeting moiety; and

(iv) a first Fc region;

(b) a second polypeptide comprising:

(i) a first VL domain associated with the first VH domain to form a first TCA targeting moiety; and

(ii) a first CL domain;

(c) a third polypeptide comprising:

(i) a second VH domain;

(ii) a second CH1 domain; and

(iii) a second Fc region associated with the first Fc region to form an Fc domain; and

(d) a fourth polypeptide comprising:

(i) a second VL domain associated with the second VH domain to form a second TCA targeting moiety; and

(ii) a second CL domain.

22. An MBM, which is optionally an MBM according to any one of claims 1 to 17, comprising:

(a) a first polypeptide comprising:

(i) a first scFv which is a first TCA targeting moiety;

(ii) a first VH domain;

(iii) a first CH1 domain; and

(iv) a first Fc region;

(b) a second polypeptide comprising:

(i) a first VL domain associated with the first VH domain to form an antigen targeting moiety; and

(ii) a first CL domain;

(c) a third polypeptide comprising:

(i) a second scFv which is a second TCA targeting moiety; and

(ii) a second Fc region associated with the first Fc region to form an Fc domain.

23. An MBM, which is optionally an MBM according to any one of claims 1 to 17, comprising:

(a) a first polypeptide comprising:

(i) a first VH domain;

(ii) a first CH1 domain;

(iii) a second VH domain;

(iv) a second CH1 domain; and

(v) a first Fc region;

(b) a second polypeptide comprising:

(i) a first VL domain associated with the first VH domain to form a first TCA targeting moiety; and

(ii) a first CL domain;

(c) a third polypeptide comprising:

(i) a second VL domain associated with the second VH domain to form an antigen targeting moiety; and

(ii) a second CL domain.

(d) a fourth polypeptide comprising:

(i) a third VH domain;

(ii) a third CH1 domain; and

(iii) a second Fc region associated with the first Fc region to form an Fc domain; and

(e) a fifth polypeptide comprising:

(i) a third VL domain associated with the third VH domain to form a second TCA targeting moiety; and

(ii) a third CL domain.

24. The MBM of any one of claims 1 to 23, wherein the antigen targeting moiety is a tumor antigen targeting moiety.

25. The MBM of claim 24, wherein the tumor antigen targeting moiety: (a) is capable of binding to a tumor antigen set forth in Table A; (b) is capable of binding to a tumor antigen set forth in Table P-1; (c) comprises the (A) CDR or (B) VH and VL sequences of an antibody or TCR set forth in Table P-2; or (d) competes with an antibody or TCR set forth in Table P-2 for binding to its target.

26. The MBM of any one of claims 1 to 25, wherein the antigen targeting moiety is a low-density antigen targeting moiety.

27. The MBM of claim 26, wherein the low-density antigen is an antigen expressed at no more than 3000 copies per cell.

28. The MBM of claim 26, wherein the low-density antigen is an antigen expressed at no more than 1000 copies per cell.

29. The MBM of claim 26, wherein the low-density antigen is an antigen expressed at no more than 100 copies per cell.

30. The MBM of any one of claims 1 to 29, wherein the antigen targeting moiety is an HLA-bound peptide antigen targeting moiety.

31. The MBM of claim 30, wherein the HLA-bound peptide is a viral peptide.

32. The MBM of claim 30, wherein the HLA-bound peptide is a tumor-associated peptide.

33. The MBM of claim 32, wherein the HLA-bound peptide is PRAME (425-433).

34. The MBM of claim 32, wherein the HLA-bound peptide is MAGE-A4 (230-239).

35. The MBM of claim 30, wherein the HLA-bound peptide antigen targeting moiety (a) comprises the (i) CDR or (ii) VH and VL sequences of an antibody or TCR set forth in Table P-2 or (b) competes with an antibody set forth in Table P-2 for binding to its target.

36. The MBM according to any one of claims 1 to 35, wherein the first TCA targeting moiety is a CD3 targeting moiety.

37. The MBM according to any one of claims 1 to 36, wherein the first TCA targeting moiety (a) comprises the (i) CDR or (ii) VH and VL sequences of an antibody set forth in Table T or (b) competes with the antibody set forth in Table T for binding to its target.

38. The MBM according to any one of claims 1 to 37, wherein the second TCA targeting moiety is a CD3 targeting moiety.

39. The MBM according to any one of claims 1 to 38, wherein the second TCA targeting moiety (a) comprises the (i) CDR or (ii) VH and VL sequences of an antibody set forth in Table T or (b) competes with the antibody set forth in Table T for binding to its target.

40. The MBM according to any one of claims 1 to 39, wherein the MBM has reduced binding to anti-drug antibodies as measured by an ADA reactivity assay as presented in Section 8.1.3 relative to a control antigen-binding molecule.

41. A nucleic acid or plurality of nucleic acids encoding the MBM of any one of claims 1 to 40.

42. A host cell engineered to express the MBM of any one of claims 1 to 40.

43. A method of producing the MBM of any one of claims 1 to 40, comprising culturing the host cell of claim 42 and recovering the MBM expressed thereby.

44. A method of killing or inhibiting growth of a cancer cell comprising administering to the cell the MBM of any one of claims 1 to 40.

45. The method of claim 44, wherein the cell expresses less than 5000, 4000, 3000, 2000, 1000, 500, 250, 100, 50, 40, 30, 20, or 10 copies of the antigen targeted by the antigen targeting moiety.

46. A method of activating a T cell, optionally a cancer antigen specific T cell, the method comprising administering to the T cell the MBM of any one of claims 1 to 40.

47. A pharmaceutical composition comprising the MBM of any one of claims 1 to 40 and an excipient.

48. The pharmaceutical composition of claim 47, further comprising a secondary multispecific antigen binding molecule (e.g., as described in Section 6.3) comprising a tumor antigen targeting moiety and a TCA targeting moiety.

49. The pharmaceutical composition of claim 48, wherein the tumor antigen targeting moiety is an EGFR targeting moiety and the TCA targeting moiety is a CD28 targeting moiety.

50. The pharmaceutical composition of any one of claims 47 to 49, further comprising an immune checkpoint inhibitor.