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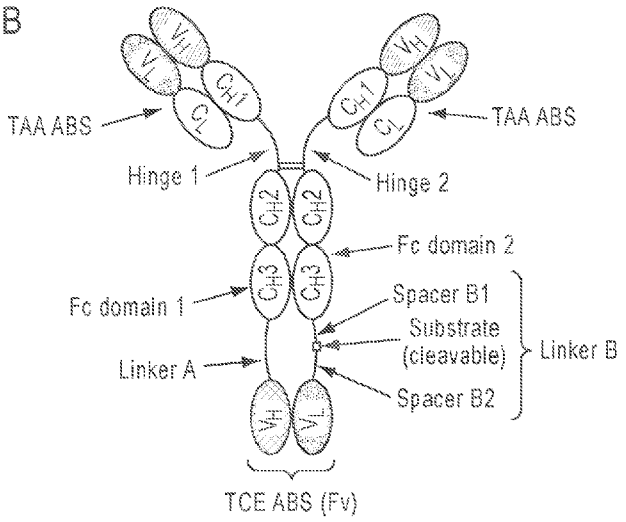
(54) Title: MULTISPECIFIC BINDING MOLECULE PROPROTEINS AND USES THEREOF

FIG. 3B



(57) Abstract: The present disclosure provides proproteins of antigen-binding molecules, including multispecific binding molecules (MBMs),. The antigen-binding molecule proproteins comprise an antigen-binding site, one or more components that inhibit the binding of the antigen-binding site to its target, and a protease-cleavable linker that can be cleaved by a protease and whose cleavage removes the inhibition of binding of the antigen-binding site to its target. The MBM proproteins and MBMs resulting from cleavage of a protease-cleavable linker, e.g., tandem Fab MBMs, comprise a T-cell engaging antigen-binding site and a tumor-associated antigen antigen-binding site. The MBM proproteins further comprise a component that inhibits the binding of the T-cell engaging antigen-binding site to its target and a protease-cleavable linker that can be cleaved by a protease in the tumor environment and whose cleavage removes the inhibition of binding of the T-cell engaging antigen-binding site to its target. The disclosure further provides pharmaceutical compositions comprising the antigen-binding molecule proproteins, and methods of use of the antigen-binding molecule proproteins in therapy, as well as nucleic acids encoding the antigen-binding molecule proproteins, recombinant cells that express the antigen-binding molecule proproteins, and methods of producing the antigen-binding molecule proproteins.

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[Continued on next page]

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— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

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DESCRIPTION

MULTISPECIFIC BINDING MOLECULE PROPROTEINS AND USES THEREOF

1. CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. provisional application No. 63/340,891, filed May 11, 2022, U.S. provisional application No. 63/481,291, filed January 24, 2023, and U.S. provisional application No. 63/489,812, filed March 13, 2023, the contents of each of which are incorporated herein in their entireties by reference thereto.

2. SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML file format and is hereby incorporated by reference in its entirety. Said XML copy, created on May 9, 2023, is named RGN-017WO_SL.xml and is 307,242 bytes in size.

3. BACKGROUND

[0003] The selective destruction of tumor cells, while leaving healthy cells and tissues intact and undamaged, is a goal of cancer immunotherapy. Bispecific antibody therapeutics have been developed to achieve this goal by inducing an immune response against the tumor. In this regard, bispecific antibodies are designed to both a tumor-associated antigen ("TAA") expressed preferentially on tumor cells and a component of the T cell receptor ("TCR") complex. The simultaneous binding of such an antibody to both of its targets results in activation of cytotoxic T cells and subsequent lysis of the TAA-expressing cell. Hence, the immune response is re-directed to the TAA-expressing cells.

[0004] Several bispecific antibody formats have been developed and their suitability for T cell mediated immunotherapy investigated. See You *et al.*, 2021, Vaccines 9:724, doi.org/10.3390/vaccines9070724.

[0005] The task of generating bispecific molecules suitable for treatment provides several technical challenges related to toxicity, as TAAs are typically expressed on normal cells as well as tumor cells. There is thus a need for efficacious T-cell activating bispecific molecules that unleash T-cell activation selectively in the tumor environment.

4. SUMMARY

[0006] The present invention generally relates to novel protease-activatable antigen-binding molecules ("ABMs"), referred to herein as antigen-binding molecule proproteins.

[0007] Generally, in their active state, the antigen-binding molecules comprise an antigen-binding site (ABS) that can bind to its target. However, in proprotein form, the ABS is masked by a masking moiety such that its ability to bind to its target is greatly diminished. The antigen-binding molecule proproteins are configured such that upon encountering a protease, e.g., a protease that is overexpressed in the tumor environment, the masking moiety is cleaved and a binding molecule is produced having enhanced target binding. This is achieved through the inclusion of one or more protease-cleavable linkers ("PCLs") that comprise one or more protease substrate sequences, *e.g.*, directly or indirectly between the ABS and the masking moiety. Proproteins can provide for reduced toxicity and adverse side effects that could otherwise result from binding of an ABM to normal tissues. Generally, antigen-binding molecule proproteins are described in Section 6.2 and Group A numbered embodiments 1 to 43, as well as Group B numbered embodiments 1 to 90.

[0008] In some embodiments, an antigen-binding molecule proprotein of the disclosure is a multispecific binding molecule (MBM) proprotein. Generally, in their active state, MBMs of the disclosure comprise a T-cell engaging antigen-binding site that binds to a component of the T-cell receptor complex (a "TCE ABS") and a tumor-associated antigen-binding site ("TAA ABS") that can simultaneously bind to their respective targets such that the T-cell bound by the TCE ABS is stimulated to attack the TAA-expressing tumor cell bound by the TAA ABS. However, when the MBM is in proprotein form, the TCE ABS is masked by a masking moiety such that its ability to bind its target is greatly diminished. The MBM proprotein is configured such that upon encountering a protease, *e.g.*, a protease that is overexpressed in the tumor environment, the masking moiety is cleaved and the inhibition of TCE ABS binding is reversed. This is achieved through the inclusion of protease-cleavable linkers ("PCLs") that comprise one or more protease substrate sequences in the MBM proprotein, *e.g.*, directly or indirectly between the masking moiety and the TCE ABS. Proproteins can provide for reduced toxicity and adverse side effects that could otherwise result from binding of an MBM to normal tissues. Generally, MBM proproteins are described in Section 6.3 and Group B numbered embodiments 1 to 5, 15, 54, and 69 to 72.

[0009] In certain aspects, the MBM proproteins (referred to as Type I MBMs) comprise an anti-idiotype of the TCE ABS which reversibly masks binding of the TCE ABS to its target, referred to herein as an "anti-TCE ABS". Thus, type I MBMs comprise anti-TCE ABS masking moieties. In some embodiments, the MBM proprotein is configured such that activation of the MBM proprotein releases an MBM (*e.g.*, a bispecific binding molecule) comprising a full Fc region. This type of MBM proprotein is referred to as Type IA MBM proprotein and exemplary embodiments of Type IA MBMs are depicted in FIGs. 1A-1C and described in Section 6.3.1.1 and Group B numbered embodiments 6 to 8 and 18 to 24. In

other embodiments, the MBM proprotein is configured such that activation of the MBM proprotein releases an MBM lacking an Fc region, such as a tandem Fab or a BiTe. This type of MBM proprotein is referred to as Type IB MBM proprotein and exemplary embodiments of Type IB MBMs are depicted in FIGs. 5A-5B and described in Section 6.3.1.2 and Group B numbered embodiments 16 and 34 to 40.

[0010] In other aspects, the MBM proproteins (referred to as Type II MBMs) the TCE ABS is sterically hindered from binding to its target by virtue of its proximity to an adjacent domain, *e.g.*, an Fc domain, that sterically hinders its binding to its targets. In some embodiments, the MBM proprotein is configured such that activation of the MBM proprotein releases an MBM (*e.g.*, a bispecific binding molecule) comprising a full Fc region. This type of MBM proprotein is referred to as Type IIA MBM proprotein and exemplary embodiments of Type IIA MBMs are depicted in FIGs. 3A-3F and described in Section 6.3.1.1 and Group B numbered embodiments 9 to 14 and 25 to 33. In other embodiments, the MBM proprotein is configured such that activation of the MBM proprotein releases an MBM lacking an Fc region, such as a tandem Fab or a BiTe. This type of MBM proprotein is referred to as Type IIB MBM proprotein and exemplary embodiments of Type IIB MBMs are depicted in FIG. 7 and described in Section 6.3.1.2 and Group B numbered embodiments 17 and 41 to 49.

[0011] Section 6.4, Group A numbered embodiments 29 to 31, and Group B numbered embodiments 50 to 53 describe exemplary protease-cleavable linkers that can be used in the antigen-binding molecule proproteins, including MBM proproteins, of the disclosure.

[0012] The present disclosure further provides tandem Fab MBMs comprising a TAA ABS and a TCE ABS. The tandem Fab may be produced by the activation of a Type IB or Type IIB MBM as a proprotein, or it can be generated through the expression of the tandem Fab protein (with relevant signal sequences but without a masking moiety). In such instances, the TAA ABS and TCE ABS preferably share a common light chain sequence. Section 6.11 and Group B numbered embodiments 75 to 83 describe exemplary tandem Fab MBMs.

[0013] Section 6.5 and Group B numbered embodiment 55 describe exemplary non-cleavable linkers that can be incorporated in the antigen-binding molecule proproteins, including MBM proproteins, and tandem Fab MBMs of the disclosure. Section 6.6, Group A numbered embodiment 23, and Group B numbered embodiments 56 to 64 describe exemplary TAA ABSs that can be incorporated into the antigen-binding molecule proproteins, including MBM proproteins, and tandem Fab MBMs of the disclosure. Section 6.7, Group A numbered embodiment 22, and Group B numbered embodiments 64 to 68 describe exemplary TCE ABSs that can be incorporated in the antigen-binding molecule proproteins, including MBM proproteins, and tandem Fab MBMs of the disclosure. Section

6.8 describes exemplary anti-TCE ABSs that can be incorporated in the antigen-binding molecule proproteins, including MBM proproteins, of the disclosure. Section 6.9 describes suitable formats for the ABSs that are incorporated in the antigen-binding molecule proproteins, including MBM proproteins, of the disclosure. Section 6.10 and Group B numbered embodiments 73 and 74 describe suitable Fc domains that can be incorporated in the antigen-binding molecule proproteins, including MBM proproteins, of the disclosure.

[0014] The disclosure further provides nucleic acids encoding the antigen-binding molecule proproteins, including MBM proproteins, and tandem Fab MBMs of the disclosure (either in a single nucleic acid or a plurality of nucleic acids) and recombinant host cells and cell lines engineered to express the nucleic acids and antigen-binding molecule proproteins, including MBM proproteins, of the disclosure. Exemplary nucleic acids, host cells, and cell lines are described in Section 6.12, Group A numbered embodiments 47 and 48, and Group B numbered embodiments 87 to 92.

[0015] Pharmaceutical compositions comprising the antigen-binding molecule proproteins, including MBM proproteins, and tandem Fab MBMs of the disclosure are also provided. Examples of pharmaceutical compositions are described in Section 6.13, Group A numbered embodiment 44 and Group B numbered embodiment 84.

[0016] Further provided herein are methods of using the antigen-binding molecule proproteins, including MBM proproteins, tandem Fab MBMs, and pharmaceutical compositions of the disclosure, for example for treating proliferative conditions (*e.g.*, cancers), on which the TAAs are expressed. Exemplary methods and indications are described in Section 6.14, Group A numbered embodiments 45 and 46, and Group b numbered embodiments 85 and 86.

5. BRIEF DESCRIPTION OF THE FIGURES

[0017] FIGS. 1A-1C are illustrations of embodiments of Type IA MBM proproteins, in which a TCE ABS is masked by an anti-TCE ABS. In the embodiments of FIG. 1A and FIG. 1B, the MBMs produced by the cleavage of Linker B are bivalent, with one TAA ABS and one TCE ABS. In the embodiment of FIG. 1C, the MBM produced by the cleavage of Linker B are trivalent, with two TAA ABSs and one TCE ABS. In some embodiments, the trivalent MBMs are bispecific. Although the ABSs in FIGS. 1A – 1C are illustrated as Fabs, the ABSs can be in other formats, *e.g.*, scFvs or other formats described in Section 6.9. In all embodiments depicted in this figure, an MBM comprising the Fc domains is released following cleavage of Linker B.

[0018] FIG. 2 illustrates the mechanism of activation of an exemplary MBM proprotein according to FIGs. 1A-1C.

[0019] FIGS. 3A-3G are illustrations of embodiments of Type IIA MBM proproteins, in which in which an Fc region acts as a masking moiety by sterically hindering the TCE ABS from binding to its target. In the embodiments of FIG. 3A and FIG. 3B, the MBMs produced by the cleavage of Linker B are trivalent, with two TAA ABSs and one TCE ABS. In some embodiments, the trivalent MBMs are bispecific. In the embodiments of FIG. 3C through 3F, the MBM produced by the cleavage of Linker B are bivalent, with one TAA ABS and one TCE ABS. Although the TAA ABSs in FIGS. 3A – 3F are illustrated as Fabs, the ABSs can be in other formats, *e.g.*, scFvs or other formats described in Section 6.9. The TCE ABSs can be in the forms of Fabs (*e.g.*, as illustrated in FIGS. 3A, 3C and 3E) or Fvs (*e.g.*, as illustrated in FIGS. 3B, 3D and 3G). In all embodiments depicted in this figure, an MBM comprising the Fc domains is released following cleavage of Linker B.

[0020] FIG. 4 illustrates the mechanism of activation of an exemplary MBM proprotein according to FIGs. 3A-3G.

[0021] FIGS. 5A-5B depict an embodiment of a Type IB MBM proprotein, in which a TCE ABS is masked by an anti-TCE ABS. The MBM produced by the cleavage of Linker B is bivalent, with one TAA ABS and one TCE ABS, and lacks the Fc domains. Although the ABSs in FIG. 5A are illustrated as Fabs, the ABSs can be in other formats, *e.g.*, scFvs or other formats described in Section 6.9.

[0022] FIG. 6 illustrates the mechanism of activation of an exemplary MBM proprotein according to FIGs. 5A-5B.

[0023] FIG. 7 is an illustration of an embodiment of a Type IIB MBM proprotein, in which an Fc region acts as a masking moiety by sterically hindering the TCE ABS from binding to its target. The MBM produced by the cleavage of Linker B is bivalent, with one TAA ABS and one TCE ABS, and lacks the Fc domains. Although the ABSs in FIG. 7 are illustrated as Fabs, the ABSs can be in other formats, *e.g.*, scFvs or other formats described in Section 6.9.

[0024] FIG. 8 illustrates the mechanism of activation of an exemplary MBM proprotein according to FIG. 7.

[0025] FIG. 9 shows the result of a study showing the correlation between linker length and the ability of an Fc region to sterically hinder an anti-CD3 TCE ABS from binding to its target in constructs having the TCE ABS C-terminal to the Fc domains configured as shown in FIGs. 3A-3F and 7. For the purpose of demonstrating the effect of linker length, the linkers

in this study did not include a substrate and were non-cleavable. FIG. 9 discloses SEQ ID NOs: 1 (1xG4S), 156 (3xG4S), and 153 (5xG4S).

[0026] FIG. 10 shows gel images displaying anti-TAAxCD3 antibodies run on non-reducing and reducing SDS-polyacrylamide gels after Protein A purification. Anti-TAAxCD3 bispecific antibodies tested had the configuration depicted in FIG. 7, where linkers A and B were either cleavable linkers as illustrated or replaced by noncleavable G4S (SEQ ID NO: 1) linkers.

[0027] FIG. 11 shows the results of a study demonstrating the inhibition of CD3 binding on JURKAT cells due to Fc steric hindrance in Type IIB MBM proprotein constructs configured as shown in FIG. 7. The curves show the inhibition of CD3 binding by Type IIB MBM proproteins relative to an anti-TAAxCD3 antibody in IgG format.

6. DETAILED DESCRIPTION

6.1. Definitions

[0028] As used herein, the following terms are intended to have the following meanings:

[0029] <u>ABM proprotein</u>: The term "ABM proprotein" (or "antigen-binding molecule proprotein") as used herein refers to an ABM having reduced or abrogated ability to bind to a target recognized by at least one of its ABSs, e.g., due to the presence of a masking moiety. The masking moiety hinders binding of an ABS to its target and is separated from the ABM by proteolytic cleavage, e.g., by proteolytic cleavage of a protease cleavable linker connecting the masking moiety to the ABM.

[0030] <u>ABS chain</u>: Individual ABSs can exist as one (*e.g.*, in the case of an scFv) polypeptide chain or form through the association of more than one polypeptide chains (*e.g.*, in the case of a Fab). As used herein, the term "ABS chain" refers to all or a portion of an ABS that exists on a single polypeptide chain. The use of the term "ABS chain" is intended for convenience and descriptive purposes only and does not connote a particular configuration or method of production. Further, the reference to an ABS when describing an ABM, ABM proprotein, MBM or MBM proprotein encompasses an ABS chain unless the context dictates otherwise. Thus, when describing an ABM, ABM proprotein, MBM or MBM proprotein in which an Fc domain is operably linked to an ABS, the Fc domain may be covalently linked directly or indirectly (*e.g.*, via a linker) through a peptide bond to, *e.g.*, (1) a first ABS chain of a Fab (with the other components of the Fab on a second, associated ABS chain) or (2) the single ABS chain containing the scFv.

[0031] <u>About, Approximately</u>: 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.

[0032] **Activate, activation**: The terms "activation", "activation", and the like in conjunction with an MBM proprotein of the disclosure refers to the protease-mediated enzymatic cleavage of a protease-cleavable linker that results in the unmasking of a TCE ABS and thus the production of an ABM or MBM with increased ability of the TCE ABS to bind to its target, for example through the release or separation of the TCE ABS from an anti-TCE ABS or an Fc domain that sterically hinders the binding of the TCE ABS to its target when the protease-cleavable linker is intact. Sometimes, activation is referred herein as "release" of the MBM, the masking moiety or the TCE ABS.

[0033] **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.

[0034] **Anti-idiotype antibody, anti-idiotypic antibody**: The terms "anti-idiotype antibody", "anti-idiotypic antibody" and the like refer to an antibody that recognizes the idiotype of an antigen-binding site, *e.g.*, an antigen-binding site specific for a TCR component such as CD3. The anti-idiotype antibody is capable of specifically binding to the variable region of the antigen-binding site and thereby reducing or preventing specific binding of the antigen-binding site to its cognate antigen. When associated with a molecule that comprises the antigen-binding site, the anti-idiotype antibody can function as a masking moiety of the molecule. The antigen-binding component of an anti-idiotypic antibody that recognizes the variable region of a T-cell engaging antibody, *e.g.*, an antibody that recognizes CD3 or another component of the T-cell receptor, is often referred to herein as a "TCE ABS".

[0035] **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 site 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 sites and/or antigen-binding sites having non-native configurations, *e.g.*, a molecule that has a Fab or scFv domain C-terminal to the CH3 domain.

[0036] **<u>Antigen-binding molecule, ABM</u>:** The terms “antigen-binding molecule” and “ABM” as used herein refer to a molecule (e.g., an assembly of multiple polypeptide chains) comprising one or more antigen-binding sites. The ABMs of the disclosure can be monospecific or multispecific (e.g., bispecific). The antigen-binding sites in monospecific binding molecules all bind to the same epitope whereas multispecific binding molecules have

at least two antigen-binding sites that bind to different epitopes, which can be one the same or different target molecules. In some embodiments, an antigen-binding molecule is an antibody.

[0037] **Antigen-binding site:** The term "antigen-binding site" or "ABS" as used herein refers to a portion of an antibody, ABM or MBM proprotein that has the ability to bind to an antigen non-covalently, reversibly and specifically in the absence of a masking. For example, in some embodiments, an ABS can be masked in the context of an ABM or MBM proprotein but has the ability to bind to an antigen non-covalently, reversibly and specifically when an ABM or MBM is produced by cleavage of a protease cleavable linker in the ABM or MBM proprotein. Examples of antigen-binding sites include antibody fragments such as, but not limited to, single-chain Fvs (scFv), Fab fragments, monovalent fragments consisting of the VL, VH, CL and CH1 domains; F(ab)2 fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; Fd fragments consisting of the VH and CH1 domains; Fv fragments consisting of the VL and VH domains of a single arm of an antibody; dAb fragments (Ward *et al.*, 1989, Nature 341:544-546), which consist of a VH domain; VHH antibodies (also "VHH" or "Nanobody®", Vincke *et al.*, 2012, Methods Mol Biol. 911:15-26); and isolated complementarity determining regions (CDRs). Thus, the term "antibody fragment" encompasses both proteolytic fragments of antibodies (*e.g.*, Fab and $F(ab)_2$ fragments) and engineered proteins comprising one or more portions of an antibody (*e.g.*, an scFv). Antibody fragments can also be incorporated into single domain antibodies, maxibodies, minibodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, *e.g.*, Hollinger and Hudson, 2005, Nature Biotechnology 23: 1126-1136).

[0038] **Associated:** The term "associated" in the context of an ABM or MBM proprotein refers to a functional relationship between two or more polypeptide chains. 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 MBM proprotein. Examples of associations that might be present in an MBM proprotein of the disclosure include (but are not limited to) associations between Fc regions in an Fc domain (homodimeric or, more preferably, heterodimeric as described in Section 6.10.2, associations between VH and VL regions in a Fab or Fv, and associations between CH1 and CL in a Fab.

[0039] **Bivalent:** The term "bivalent" as used herein in the context of an antigen-binding molecule refers to an antigen-binding molecule that has two antigen-binding sites. The domains can be the same or different. Accordingly, a bivalent antigen-binding molecule can be monospecific or bispecific.

[0040] **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, *e.g.*, any TAA-positive cancers of any of the foregoing types.

[0041] **CD3:** The term "CD3" refers to the cluster of differentiation 3 co-receptor (or co-receptor complex, or polypeptide chain of the co-receptor complex) of the T cell receptor. The amino acid sequence of the polypeptide chains of human CD3 are provided in NCBI Accession P04234, P07766 and P09693.

[0042] **Complementarity Determining Region:** 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. For example, in general, there are three CDRs in each heavy chain variable region (*e.g.*, CDR-H1, CDR-H2, and CDR- H3) and three CDRs in each light chain variable region (CDR-L1, CDR-L2, and CDR-L3). The precise amino acid sequence boundaries of a given CDR can be determined using any of a number of well-known schemes, including those described by Kabat *et al.*, 1991, "Sequences of Proteins of Immunological Interest," 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD ("Kabat" numbering scheme), Al-Lazikani *et al.*, 1997, JMB 273:927-948 ("Chothia" numbering scheme) and ImMunoGenTics (IMGT) numbering (Lefranc, 1999, The Immunologist 7:132-136; 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.

[0043] **Effector Function:** The term "effector function" refers to an activity of an antibody molecule that is mediated by binding through a domain of the antibody other than the antigen-binding site, usually mediated by binding of effector molecules. Effector function includes complement-mediated effector function, which is mediated by, for example, binding of the C1 component of the complement to the antibody. Activation of complement is important in the opsonization and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and may also be involved in autoimmune hypersensitivity. Effector function also includes Fc receptor (FcR)-mediated effector function, which may be triggered upon binding of the constant domain of an antibody to an Fc receptor (FcR). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody- dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer and control of immunoglobulin production. An effector function of an antibody may be altered by altering, *e.g.*, enhancing or reducing, the affinity of the antibody for an effector molecule such as an Fc receptor or a complement component. Binding affinity will generally be varied by modifying the effector molecule binding site, and in this case it is appropriate to locate the site of interest and modify at least part of the site in a suitable way. It is also envisaged that an alteration in the binding site on the antibody for the effector molecule need not alter significantly the overall binding affinity but may alter the geometry of the interaction rendering the effector mechanism ineffective as in non-productive binding. It is further envisaged that an effector function may also be altered by modifying a site not directly involved in effector molecule binding, but otherwise involved in performance of the effector function.

[0044] **Epitope:** An epitope, or antigenic determinant, is a portion of an antigen recognized by an antibody or other antigen-binding moiety as described herein. An epitope can be linear or conformational.

[0045] **Fab:** The term "Fab" refers to a pair of polypeptide chains, the first comprising a variable heavy (VH) domain of an antibody operably linked (typically N-terminal to) to a first

constant domain (referred to herein as C1), and the second comprising variable light (VL) domain of an antibody N-terminal operably linked (typically 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. The term "Fab" encompasses single chain Fabs.

[0046] **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.

[0047] **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. By way of example and not limitation, the VH and VL can come together in any configuration described herein to form a half antibody, or can each be present on a separate half antibody and come together to form an antigen binding domain when the separate half antibodies associate, for example to form a ABM or MBM proprotein of the disclosure. When present on a single polypeptide chain (*e.g.*, a scFv), the VH and be N- terminal or C-terminal to the VL.

[0048] **Half Antibody:** The term "half antibody" refers to a molecule that comprises at least one ABS or ABS chain and can associate with another molecule comprising an ABS or ABS chain through, *e.g.*, a disulfide bridge or molecular interactions (*e.g.*, knob-in-hole interactions between Fc heterodimers). 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). In a preferred embodiment, a half-antibody comprises an Fc region. 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 ABS. Yet another example of a half antibody is a polypeptide comprising an scFv domain, a CH2 domain and a CH3 domain.

[0049] A half antibody might include more than one ABS, for example a half-antibody comprising (in N- to C-terminal order) an VH1 domain, a CH1 domain, a CH2 domain, a CH3 domain, and another VH1 domain or an scFv domain. Half antibodies might also include an ABS chain that when associated with another ABS chain in another half antibody forms a complete ABS.

[0050] Thus, an ABM or MBM proprotein can comprise one, more typically two, or even more than two half antibodies, and a half antibody can comprise one or more ABSs or ABS chains.

[0051] In some ABM or MBM proproteins, a first half antibody will associate, *e.g.*, heterodimerize, with a second half antibody. In other ABM or MBM proproteins, a first half antibody will be covalently linked to a second half antibody, for example through disulfide bridges or chemical crosslinking. In yet other ABM or MBM proproteins, a first half antibody will associate with a second half antibody through both covalent attachments and non-covalent interactions, for example disulfide bridges and knob-in-hole interactions.

[0052] 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.

[0053] **Host cell or recombinant host cell:** The terms “host cell” or “recombinant host cell” 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 may 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 may 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 an ABM or MBM proprotein of the disclosure, a host cell is preferably 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.*, derivatives that grow at higher density than the original cell lines and/or glycan profile modified derivatives and and/or site- specific integration site derivatives.

[0054] **Linker:** The term "linker" as used herein refers to a protease-cleavable linker or a non-cleavable linker.

[0055] **Masking Moiety:** The term "masking moiety" as used herein in relation to an MBM proprotein refers an amino acid sequence in an MBM proprotein that inhibits a TCE ABS's ability to specifically bind its target, either through a specific interaction with the TCE ABS (*e.g.*, where the masking moiety is an anti-idiotype of the TCE ABS or "anti-TCE ABS") or through positioning of the TCE ABS relative to another component of the MBM proprotein that sterically hinders the binding of the TCE ABS to its target (*e.g.*, by connecting the TCE ABS to the Fc region through short linkers). The masking moiety and the TCE ABS are arranged in the MBM proprotein such that cleavage of a protease cleavable linker reduces the inhibition of the TCE ABS's interaction with its target, either through the generation of an MBM that lacks the masking moiety or an MBM in which spatial constrains on the TCE ABS's ability to interact with its target are alleviated. The term "masking moiety" when used in relation to an antigen binding-molecule more generally refers to an amino acid sequence in the antigen-binding molecule proprotein that inhibits the ability of an antigen binding site (ABS) in the antigen-binding molecule to specifically bind its target, either through a specific interaction with the ABS (*e.g.*, where the masking moiety is an anti-idiotype of the ABS) or through positioning of the ABS relative to another component of the antigen-binding molecule that sterically hinders the binding of the ABS to its target (*e.g.*, by connecting the ABS to an Fc region through short linkers). Generally, the masking moiety and the ABS are arranged in the antigen-binding molecule such that cleavage of a protease cleavable linker reduces the inhibition of the ABS's interaction with its target, either through the generation of an antigen-binding molecule that lacks the masking moiety or an antigen-binding molecule in which spatial constrains on the ABS's ability to interact with its target are alleviated.

[0056] **MBM proprotein:** The term "MBM proprotein" as used herein refers to an MBM having reduced or abrogated ability to bind to a target recognized by at least one of its ABSs, *e.g.*, due to the presence of a masking moiety. The masking moiety hinders binding of

an ABS to its target is separated from the MBM by proteolytic cleavage, *e.g.*, by proteolytic cleavage of a protease cleavable linker connecting the masking moiety to the MBM. Typically, in the MBM proproteins of the disclosure, the TCE ABS is masked while a TAA ABS is not. The TAA ABS can serve as a targeting moiety to direct the MBM proprotein to the site of a tumor, wherein in the presence of proteases that recognize a substrate in the protease cleavable linker results in cleavage of the linker, restoring the binding of the TCE ABS to its target and the ability of the MBM to activate the T-cell against tumor cells that express the TAA.

[0057] **Monovalent:** The term "monovalent" as used herein in the context of an antigen-binding molecule refers to an antigen-binding molecule that has a single antigen-binding site.

[0058] **Multispecific binding molecule, MBM:** The terms "multispecific binding molecule" and "MBM" refer to a molecule that comprises two or more antigen binding sites. Typically, the MBMs comprise a TAA ABS and a TCE ABS.

[0059] **Non-cleavable linker:** A non-cleavable linker refers to a peptide whose amino acid sequence lacks a substrate sequence for a protease, *e.g.*, a protease as described in Section 6.4.1, that recognizes and cleaves a specific sequence motif, *e.g.*, a substrate as described in Section 6.4.2.

[0060] **Operably linked:** The term "operably linked" refers to a functional relationship between two or more peptide or polypeptide domains or nucleic acid (*e.g.*, DNA) segments. 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. For example, in the context of an ABM or MBM proprotein of the disclosure, separate ABSs (or chains of an ABS) can be operably linked through peptide linker sequences. In the context of a nucleic acid encoding a fusion protein, such as a polypeptide chain of an ABM or MBM 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.

[0061] **Polypeptide, Peptide and Protein:** The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues.

[0062] **Proprotein:** A "proprotein" is a protein precursor that is inactive and which can be activated by proteolysis by a protease. Thus, proproteins are "protease activatable".

[0063] Protease: The term "protease" as used herein refers to any enzyme that that catalyzes hydrolysis of a peptide bond. Generally, the proteases useful in the present disclosure, *e.g.*, the proteases described in Section 6.4.1, recognize and cleaves a specific sequence motif, *e.g.*, a substrate as described in Section 6.4.2. Preferably, the proteases are expressed at higher levels in cancer tissues as compared to normal tissues.

[0064] Protease cleavable linker: As used herein, the term "protease cleavable linker" or "PCL" refers to a peptide whose amino acid sequence contains one or more (*e.g.*, two or three) substrate sequences for one or more proteases.

[0065] Recognize: The term "recognize" as used herein refers to an ABS that finds and interacts (*e.g.*, binds) with its epitope.

[0066] Single Chain Fab or scFab: The term "single chain Fab" or "scFab" as used herein refers an ABS 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).

[0067] Single Chain Fv or scFv: The term "single-chain Fv" or "scFv" as used herein refers to ABSs comprising the VH and VL domains of an antibody, wherein 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. The VH and VL and be arranged in the N- to C-terminal order VH-VL or VL-VH, typically separated by a linkers, for example a linker as set forth in Table E.

[0068] Spacer: As used herein, the term "spacer" refers to a peptide, the amino acid sequence of which is not a substrate for a protease, incorporated into a linker containing a substrate. A spacer can be used to separate the substrate from other domains in a molecule, for example an ABS. In some aspects, residues in the spacer minimize aminopeptidase and/or exopeptidase action to prevent cleavage of N-terminal amino acids.

[0069] **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 site (*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 site as a "specific" binder. In certain embodiments, an antigen-binding site of the disclosure (*e.g.*, a "TAA ABS") 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*.

[0070] **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 preferred embodiments, the subject is human.

[0071] **Substrate:** The term "substrate" refers to peptide sequence on which a protease will act and within which the protease will cleave a peptide bond.

[0072] **TAA ABS:** The term "TAA ABS" refers to an ABS that recognizes or specifically binds to an extracellular matrix ("ECM") protein, a tumor reactive lymphocyte antigen, a cell surface molecule of tumor or viral lymphocytes, a T-cell antigen ("TCA"), a checkpoint inhibitor, or a TAA. 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, and other molecules may be considered both TCAs and checkpoint inhibitors.

[0073] **T-Cell Antigen, TCA:** The term "T-cell antigen" or "TCA" refers to a molecule (typically a protein, carbohydrate, lipid or some combination thereof) that is expressed on the surface of a T-lymphocyte and is useful for the preferential targeting of a pharmacological agent to a particular site. In some embodiments, the site is cancer tissue and/or the T-cell antigen is a tumor reactive lymphocyte antigen, a cell surface molecule of tumor or viral lymphocytes, or a checkpoint inhibitor expressed on a T-lymphocyte.

[0074] **TCE ABS:** The term "TCE ABS" refers to an ABS that recognizes or specifically binds to a T-cell antigen in a manner that can initiate signaling through the TCR complex.

Exemplary T-cell antigens recognized or specifically bound by a TCE ABS are CD3 and other components of the TCR complex.

[0075] **T-cell Receptor, TCR**: The "T-cell receptor" or "TCR" is the component of the T-cell that is responsible for interacting with and sensing the targets of T-cell adaptive immunity. In general terms, the TCR is comprised of a heterodimeric protein complex presented on the cell surface. T cells may be broadly classified as αβ or γδ according to the somatically rearranged TCR form they express at their surface. There exist two TCR chain pair forms; TCRα and TCRβ pairs TCRγ and TCRδ. Mature αβ and γδ TCR chain pairs are presented at the cell surface in a complex with a number of accessory CD3 subunits, denoted ε, γ, δ and ζ. These subunits associate with αβ or γδ TCRs as three dimers (εγ, εδ, ζζ). This TCR complex forms the unit for initiation of cellular signaling responses upon engagement of a TCR αβ or TCRγδ with cognate antigen. The terms "T-cell receptor complex" and "TCR complex" refer to complexes of TCR αβ or TCRγδ and CD3.

[0076] **Trivalent**: The term "trivalent" as used herein in the context of an antigen-binding molecule refers to an antigen-binding molecule that has three antigen-binding sites. The antigen-binding sites can all be the same, all be different, or include two of the same antigen-binding site and a third, different antigen-binding site.

[0077] **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.

[0078] **Tumor-Associated Antigen**: The term "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.*, MHC/peptide), 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. 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.*, MHC/peptide), 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").

[0079] **TAA ABS:** The term "TAA ABS" as used herein refers to an antigen-binding site that recognizes or specifically binds a TAA.

[0080] **Treat, Treatment, Treating:** As used herein, the terms "treat", "treatment" and "treating" refer to the reduction or amelioration of the progression, severity and/or duration of a proliferative disorder, or the amelioration of one or more symptoms (preferably, one or more discernible symptoms) of a proliferative disorder resulting from the administration of one or more ABM or MBM proproteins of the disclosure. In specific embodiments, the terms "treat", "treatment" and "treating" refer to the amelioration of at least one measurable physical parameter of a proliferative disorder, such as growth of a tumor, not necessarily discernible by the patient. In other embodiments the terms "treat", "treatment" and "treating" refer to the inhibition of the progression of a proliferative disorder, either physically by, *e.g.*, stabilization of a discernible symptom, physiologically by, *e.g.*, stabilization of a physical parameter, or both. In other embodiments the terms "treat", "treatment" and "treating" refer to the reduction or stabilization of tumor size or cancerous cell count.

[0081] **Universal Light Chain, UCL**: The term "universal light chain" or "ULC" as used herein refers to a light chain variable region (VL) that can pair with more than on heavy chain variable region (VL). In the context of an ABS, the term "universal light chain" or "ULC" refers to a light chain polypeptide capable of pairing with the heavy chain region of the ABS and also capable of pairing with other heavy chain regions. ULCs can also include constant domains, *e.g.*, a CL domain of an antibody. Universal light chains are also known as "common light chains.

[0082] **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.

[0083] **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. Antigen-Binding Molecule Proproteins

[0084] The present disclosure relates to antigen-binding molecule proproteins comprising an ABS, a masking moiety, and a protease-cleavable linker (PCL), arranged so that the masking moiety diminishes or blocks the ABS from binding to its target and configured such that upon encountering a protease, e.g., a protease that is overexpressed in the tumor environment, the masking moiety is cleaved and a binding molecule is produced having enhanced target binding. Typically, the masking moiety of an antigen-binding molecule

proprotein masks the ABS via steric hindrance (*e.g.*, an Fc domain masking moiety) and/or via binding of a targeting moiety to the ABS (*e.g.*, an anti-ABS binding moiety).

[0085] Accordingly, the antigen-binding molecule proproteins of the disclosure generally comprise: (a) a masking moiety, and (b) a an ABS or component thereof connected to the masking moiety via a PCL, where the ABS is hindered (*e.g.*, sterically hindered) from binding its target. Exemplary masking moieties include Fc domains and anti-ABS binding moieties. Use of an Fc domain as a masking moiety provides for steric hindrance of the ABS, which steric hindrance is released following protease cleavage of the PCL (*e.g.*, by a tumor-specific protease). Particular example Fc domain-masked antigen-binding molecule proproteins include Type IIA and Type IIB MBM proproteins (*e.g.*, as described in Section 6.3.2). Use of an anti-ABS binding moiety as a masking moiety blocks binding of the ABS to its target, which blockage is released following protease cleavage of the PCL (*e.g.*, by a tumor-specific protease). Particular example anti-ABS binding moiety-masked antigen-binding molecule proproteins include Type IA and Type IB MBM proproteins (*e.g.*, as described in Section 6.3.1.1). An antigen-binding molecule proprotein may comprise multiple masking moieties, for example an Fc domain and also an anti-ABS binding moiety, where one or more of the masking moieties are released following protease cleavage of the PCL.

[0086] The PCL comprises one or more substrates that are recognized and cleaved by one or more proteases, *e.g.*, one or more of the proteases described in Section 6.4.1. Exemplary substrate sequences are described in Section 6.4.2. The PCL may further include spacer sequences, *e.g.*, as described in Section 6.4.3. Examples of entire protease-cleavable linkers comprising substrate and spacer sequences are provided in Section 6.4.4.

[0087] Without being bound by theory, it is believed that a PCL connecting an ABS and a masking moiety (particularly a masking moiety that sterically hinders binding of the ABS to its target such as an Fc domain) being of small length (*e.g.*, 30 or fewer) provides for maximal hindrance of the ABS binding prior to protease cleavage of the PCL, thereby minimizing off-target effects of the ABS. Accordingly, in certain aspects, the PCL is 30 or fewer, 29 or fewer, 28 or fewer, 27 or fewer, 26 or fewer, 25 or fewer, 24 or fewer, 23 or fewer, 22 or fewer, 21 or fewer, 20 or fewer, 19 or fewer, 18 or fewer, 17 or fewer, 16 or fewer, 15 or fewer, 14 or fewer, 13 or fewer, 12 or fewer, 11 or fewer, 10 or fewer, 9 or fewer, or 8 or fewer amino acids in length.

[0088] Antigen-binding molecule proproteins generally comprise at least two polypeptide chains, where a first polypeptide chain comprises the masking moiety, the PCL, and a first component of the ABS (*e.g.*, a VH), and a second polypeptide chain comprises a second component of the ABS that pairs with the first component to form the ABS (*e.g.*, a VL). In

cases where the masking moiety is an Fc domain, the second polypeptide may further comprise an additional Fc domain that pairs with the first Fc domain to form an Fc region (*e.g.*, an Fc heterodimer). The ABS may be, for example, a TAA ABS (*e.g.*, as described in Section 6.6) or a TCE ABS (*e.g.*, as described in Section 6.7).

5 **[0089]** In some aspects, the antigen-binding molecule proprotein further comprises an additional ABS which is not masked by the masking moiety. In such cases, the first ABS may be a TCE ABS while the additional ABS is a TAA ABS; alternatively the first ABS may be a TAA ABS while the additional ABS is a TCE ABS. An antigen-binding molecule proprotein comprising two or more ABSs may, upon protease cleavage of the PCL, release a
10 multispecific binding molecule (*e.g.*, bispecific or trispecific binding molecule). Certain examples of antigen-binding molecule proproteins having multiple ABSs are described in Section 6.3, *infra*.

6.3. MBM Proproteins

[0090] In some embodiments, the present disclosure relates to MBM proproteins comprising
15 a TCE ABS, a TAA ABS, a masking moiety, and a protease-cleavable linker, arranged so that the masking moiety diminishes or blocks the TCE ABS from binding to its target. The MBM proprotein is configured such that upon encountering a protease, *e.g.*, a protease that is overexpressed in the tumor environment, the masking moiety is cleaved and an MBM is produced in which the inhibition of TCE ABS binding is reversed. Typically, the MBM
20 proproteins of the disclosure further comprise two Fc domains that associate for form an Fc region, and the TCE ABS is C-terminal to the Fc region.

[0091] Accordingly, the MBM proproteins of the disclosure generally comprise: (a) a first Fc domain and a second Fc domain capable of associating to form an Fc region; (b) a TCE ABS C-terminal to the first Fc domain and/or the second Fc domain; (c) a TAA ABS; and (d)
25 a protease-cleavable linker (PCL) C-terminal to the first Fc domain and/or the second Fc domain. Typically, the TAA ABS in the MBM proprotein is capable of binding to its target whether or not the protease-cleavable linker is in the uncleaved state while the binding of the TCE ABS to its target is significantly enhanced following protease cleavage of the protease-cleavable linker. Following cleavage of the protease-cleavable linker, *e.g.*, in the tumor
30 environment, an MBM is released comprising the TAA ABS and the TCE ABS.

[0092] In Type I MBM proproteins, the TCE ABS is masked by an anti-idiotypic antibody. In Type II MBM proproteins, the Fc region acts as the masking moiety by sterically hindering the TCE ABS from binding to its target.

[0093] The protease-cleavable linkers in Type IA, Type IIA, Type IB and Type II MBM
35 proproteins comprise one or more substrates that are recognized and cleaved by one or

more proteases, *e.g.*, one or more of the proteases described in Section 6.4.1. Exemplary substrate sequences are described in Section 6.4.2. The protease-cleavable linkers typically further include spacer sequences, *e.g.*, as described in Section 6.4.3. Examples of entire protease-cleavable linkers comprising substrate and spacer sequences are provided in Section 6.4.4. Depending on the configuration of the MBM proprotein, the Fc region may be retained in the resulting MBM following cleavage of the protease-cleavable linker, *e.g.*, following cleavage of the protease-cleavable linker in the Type IA and Type IIA MBM proproteins, or may be released from the resulting MBM following cleavage of the protease-cleavable linker, *e.g.*, following cleavage of the protease-cleavable linker in Type IB and Type IIB MBM proproteins.

[0094] Generally, the MBM proproteins of the disclosure contain multiple linkers. Preferably, other than the protease-cleavable linker whose cleavage releases the MBM, the remaining linkers are non-cleavable. Examples of non-cleavable linkers are set forth in Section 6.5.

[0095] Exemplary TAA ABSs that can be used in the MBM proproteins of the disclosure (*e.g.*, Type IA, Type IIA, Type IB and Type II MBM proproteins of the disclosure) are disclosed in Section 6.6. Exemplary TCE ABSs that can be used in the MBM proproteins of the disclosure (*e.g.*, Type IA, Type IIA, Type IB and Type II MBM proproteins of the disclosure) are disclosed in Section 6.7. Exemplary anti-TCE ABSs that can be used in the Type I MBM proproteins of the disclosure are disclosed Section 6.8. Section 6.9 describes suitable formats for the ABSs that are incorporated in the MBM proproteins of the disclosure. Section 6.10 describes suitable Fc domains that can be incorporated in the MBM proproteins of the disclosure (and which can be used as masking moieties in the Type II MBM proproteins of the disclosure).

6.3.1. Type I MBM proproteins (with anti-idiotypic masking moieties)

[0096] The present disclosure provides MBM proproteins comprising a TCE ABS masked by an anti-TCE ABS, referred to herein as "Type I" MBM proproteins. Typically, both the TCE ABS and the anti-TCE ABS are arranged C-terminal to an Fc region, with each operably linked to a separate Fc domain. The TCE ABS or the anti-TCE ABS can be separated from one of the Fc domains of the Fc region by a protease-cleavable linker, *e.g.*, as described in Section 6.4. In the Type IA MBM proproteins, the Fc region is retained in the MBM activated by cleavage of the protease-cleavable linker. In the Type IB MBM proproteins, the Fc region is separated from the MBM activated by cleavage of the protease-cleavable linker.

6.3.1.1. Type IA MBM Proproteins

[0097] Generally, Type IA MBM proproteins comprise first polypeptide chain comprising, in an N-terminal to C-terminal orientation, an Fc domain (with an optional hinge domain at its

N-terminus), a non-cleavable linker, and a TCE ABS (or TCE ABS chain), and a second polypeptide chain comprising, in an N-terminal to C-terminal orientation, an Fc domain (with an optional hinge domain at its N-terminus), a protease-cleavable linker, and an anti-TCE ABS (or anti-TCE ABS chain). One or both Fc domains preferably further comprise a TAA ABS (or TAA ABS chain) at its N-terminus. When the TCE ABS, TAA ABS and/or anti-TCE ABS are composed of multiple polypeptide chains, the MBM proprotein will further comprise the other chain(s), for example the light chain of a Fab.

[0098] Following cleavage of the protease-cleavable linker the anti-TCE ABS is released, producing an MBM comprising first polypeptide chain comprising, in an N-terminal to C-terminal orientation, an Fc domain (with an optional hinge domain at its N-terminus), a non-cleavable linker, and a TCE ABS, and a second polypeptide chain comprising, in an N-terminal to C-terminal orientation, an Fc domain (with an optional hinge domain at its N-terminus), and a portion of the protease-cleavable linker. One or both Fc domains preferably further comprise a TAA ABS at its N-terminus. When the TCE ABS and/or TAA ABS are composed of multiple polypeptide chains, the resulting MBM will further comprise the other chain(s), for example the light chain of a Fab.

[0099] Preferably, the resulting MBM is bispecific for the TAA and the TCE ABS's target (*e.g.*, CD3).

[0100] FIGS. 1A to 1C illustrate exemplary embodiments of Type IA MBM proproteins. As shown in FIGS. 1A and 1B, the Type IA MBM proproteins can include only a single TAA ABS. Alternatively, as shown in FIG. 1C, Type IA MBM proproteins can include two TAA ABSs. Although depicted as a Fab for illustrative purposes, the Anti-TCE ABS of FIGs. 1A to 1C may be an scFv, a VHH, a VH, or other fragment or region from an anti-idiotypic antibody. The depiction of a single substrate and two spacers in the protease-cleavable linker of FIGS. 1A-1C is for illustrative purposes only; as described in Section 6.4, protease-cleavable linkers can include multiple substrates and more than two spacer sequences.

6.3.1.2. Type IB MBM Proproteins

[0101] Generally, Type IB MBM proproteins comprise first polypeptide chain comprising, in an N-terminal to C-terminal orientation, an Fc domain (with an optional hinge domain at its N-terminus), a non-cleavable linker, and an anti-TCE ABS (or anti-TCE ABS chain), and a second polypeptide chain comprising, in an N-terminal to C-terminal orientation, an Fc domain (with an optional hinge domain at its N-terminus), a protease-cleavable linker, a TCE ABS (or TCE ABS chain) and a TAA ABS (or TAA ABS chain), generally with the TCE ABS and the TAA ABS separated by a non-cleavable linker. When the TCE ABS, TAA ABS

and/or anti-TCE ABS are composed of multiple polypeptide chains, the MBM proprotein will further comprise the other chain(s), for example the light chain of a Fab.

[0102] Following cleavage of the protease-cleavable linker an MBM is released comprising the TCE ABS, optional linker and the TAA ABS. When the TCE ABS and/or TAA ABS are composed of multiple polypeptide chains, the resulting MBM will further comprise the other chain(s), for example the light chain of a Fab.

[0103] Preferably, the resulting MBM is bispecific for the TAA and the TCE ABS's target (*e.g.*, CD3).

[0104] FIGS. 5A-5B illustrate an exemplary embodiment of a Type IB MBM proprotein. Although depicted as a Fab for illustrative purposes, the Anti-TCE ABS of FIG. 5A may be an scFv, a VHH, a VH, or other fragment or region from an anti-idiotypic antibody. The depiction of a single substrate and two spacers in the protease-cleavable linker of FIGS. 5A-5B is for illustrative purposes only; as described in Section 6.4, protease-cleavable linkers can include multiple substrates and more than two spacer sequences.

6.3.2. Type II MBM proproteins (with sterically hindering masking moieties)

6.3.2.1. Type IIA MBM Proproteins

[0105] Generally, Type IIA MBM proproteins comprise first polypeptide chain comprising, in an N-terminal to C-terminal orientation, an Fc domain (with an optional hinge domain at its N-terminus), a non-cleavable linker, and a first TCE ABS chain, and a second polypeptide chain comprising, in an N-terminal to C-terminal orientation, an Fc domain (with an optional hinge domain at its N-terminus), a protease-cleavable linker, and the second TCE ABS chain. The TCE ABS chains may include a VH and a VL without corresponding CH1 and CL domain domains, such that a TCE ABS in the form of an Fv is formed by the association of the TCE ABS chains. The TCE ABS chains may include a VH and a VL with corresponding CH1 and CL domain domains, such that a TCE ABS in the form of a Fab is formed by the association of the TCE ABS chains. In some embodiments, the VH and/or VL of the TCE ABS chains attached to the Fc domains comprise a deletion of one or more amino acids at or near their N-terminus, *e.g.*, an N-terminal truncation. A VH or VL may have such a deletion precisely at the N-terminus (i.e., may be a truncation), or in some cases within 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids of the N-terminus. In some embodiments, the VH of a TCE ABS of a Type IIA MBM proprotein of the present disclosure comprises a deletion (*e.g.*, a truncation) or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids at or near (*e.g.*, within 10 amino acids of) the N-terminus of a reference VH, *e.g.*, a wild-type VH or the VH upon which the TCE ABS is based, such as the VH of an antibody or antibody sequence of Table G. In some embodiments, the VL of a TCE ABS of a Type IIA MBM proprotein of the present

disclosure comprises a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids at or near (*e.g.*, within 10 amino acids of) the N-terminus of a reference VL, *e.g.*, a wild-type VL or the VL upon which the TCE ABS is based, such as the VL of an antibody or antibody sequence of Table G.

[0106] The length of the linkers separating the Fc domains and the TCE ABS chains is chosen so as to optimize the ability of the Fc domains to sterically hinder the TCE ABS from binding its target. Preferably, the length of non-cleavable linker typically ranges between 5 and 30 amino acids, and in various embodiments is 25 amino acids or less (*e.g.*, ranges between 5 and 25 amino acids), 20 amino acids or less (*e.g.*, ranges between 5 and 20 amino acids), 15 amino acids or less (*e.g.*, ranges between 5 and 15 amino acids), or 10 amino acids or less (*e.g.*, ranges between 5 and 10 amino acids). In particular embodiments, the non-cleavable linker is 5, 6, 7, 8, 9 or 10 amino acids in length.

[0107] One or both Fc domains preferably further comprise a TAA ABS (or TAA ABS chain) at its N-terminus. When the TAA ABS is composed of multiple polypeptide chains, the MBM proprotein will further comprise the other chain, for example the light chain of a Fab.

[0108] One of both Fc domains may, in some embodiments, comprise a deletion of one or more amino acids at or near the C-terminus (*e.g.*, a C-terminal truncation) relative to a wild-type Fc domain. In some embodiments, one or both Fc domains of a Type IIA MBM proprotein of the present disclosure comprise a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids at or near the C-terminus relative to a wild-type Fc domain. An Fc domain may have such a deletion precisely at the C-terminus (i.e., may be a truncation), or in some cases within 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids of the C-terminus.

[0109] In particular embodiments, a Type IIA MBM proprotein of the disclosure comprises (1) Fc domains having a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, or 6 amino acids at or near the C-terminus relative to a wild-type Fc domain; and (2) TCE ABS chains having VH and VL domains with a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, or 6 amino acids at or near the N-terminus. For example, a Type IIA MBM proprotein may comprise (1) two Fc domains, each having a deletion of 4, 5, or 6 amino acids at or near the C-terminus; and (2) a TCE ABS comprising a VH and VL, each having a deletion of 1, 2, or 3 amino acids at or near the N-terminus.

[0110] Following cleavage of the protease-cleavable linker the steric hindrance constraining the ability of the TCE ABS to bind to its target is released.

[0111] Preferably, the resulting MBM is bispecific for the TAA and the TCE ABS's target (*e.g.*, CD3).

[0112] FIGS. 3A to 3F illustrate exemplary embodiments of Type IIA MBM proproteins. As shown in FIGS. 3A and 3B, the Type IIA MBM proproteins can include only a single TAA ABS. Alternatively, as shown in FIGS. 3C through 3F, Type IIA MBM proproteins can include two TAA ABSs. The depiction of a single substrate and two spacers in the protease-cleavable linker of FIGS. 3A-3G is for illustrative purposes only; as described in Section 6.4, protease-cleavable linkers can include multiple substrates and more than two spacer sequences.

6.3.2.2. Type IIB MBM Proproteins

[0113] Generally, Type IIB MBM proproteins comprise first polypeptide chain comprising, in an N-terminal to C-terminal orientation, an Fc domain (with an optional hinge domain at its N-terminus), a first protease cleavable linker, an anti-TCE ABS chain, an optional non-cleavable linker and a TAA ABS (or TAA ABS chain) and a second polypeptide chain comprising, in an N-terminal to C-terminal orientation, an Fc domain (with an optional hinge domain at its N-terminus), a protease-cleavable linker, and a TCE ABS chain. When the TAA ABS is composed of multiple polypeptide chains, the MBM proprotein will further comprise the other chain(s), for example the light chain of a Fab. The TCE ABS chains may include a VH and a VL with corresponding CH1 and CL domain domains, such that a TCE ABS in the form of a Fab is formed by the association of the TCE ABS chains. In some embodiments, the VH and/or VL of the TCE ABS chains attached to the Fc domains comprise a deletion of one or more amino acids at or near their N-terminus, *e.g.*, an N-terminal truncation. A VH or VL may have such a deletion precisely at the N-terminus (i.e., may be a truncation), or in some cases within 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids of the N-terminus. In some embodiments, the VH of a TCE ABS of a Type IIB MBM proprotein of the present disclosure comprises a deletion (*e.g.*, a truncation) or 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids at or near (*e.g.*, within 10 amino acids of) the N-terminus of a reference VH, *e.g.*, a wild-type VH or the VH upon which the TCE ABS is based, such as the VH of an antibody or antibody sequence of Table G. In some embodiments, the VL of a TCE ABS of a Type IIB MBM proprotein of the present disclosure comprises a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids at or near (*e.g.*, within 10 amino acids of) the N-terminus of a reference VL, *e.g.*, a wild-type VL or the VL upon which the TCE ABS is based, such as the VL of an antibody or antibody sequence of Table G.

[0114] The length of the linkers separating the Fc domains and the TCE ABS chains is chosen so as to optimize the ability of the Fc domains to sterically hinder the TCE ABS from binding its target. Preferably, the length of linkers typically ranges between 5 and 30 amino

acids, and in various embodiments is 25 amino acids or less (*e.g.*, ranges between 5 and 25 amino acids), 20 amino acids or less (*e.g.*, ranges between 5 and 20 amino acids), 15 amino acids or less (*e.g.*, ranges between 5 and 15 amino acids), or 10 amino acids or less (*e.g.*, ranges between 5 and 10 amino acids).

5 **[0115]** One of both Fc domains may, in some embodiments, comprise a deletion of one or more amino acids at or near the C-terminus (*e.g.*, a C-terminal truncation) relative to a wild-type Fc domain. In some embodiments, one or both Fc domains of a Type IIB MBM proprotein of the present disclosure comprise a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids at or near the C-terminus relative to a wild-type Fc domain. An Fc
10 domain may have such a deletion precisely at the C-terminus (i.e., may be a truncation), or in some cases within 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids of the C-terminus.

[0116] In particular embodiments, a Type IIB MBM proprotein of the disclosure comprises (1) Fc domains having a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, or 6 amino acids at or near the C-terminus relative to a wild-type Fc domain; and (2) TCE ABS chains having VH
15 and VL domains with a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, or 6 amino acids at or near the N-terminus. For example, a Type IIB MBM proprotein may comprise (1) two Fc domains, each having a deletion of 4, 5, or 6 amino acids at or near the C-terminus; and (2) a TCE ABS comprising a VH and VL, each having a deletion of 1, 2, or 3 amino acids at or near the N-terminus.

20 **[0117]** Following cleavage of the protease-cleavable linker an MBM is released comprising the TCE ABS, optional linker and the TAA ABS. When the TAA ABS is composed of multiple polypeptide chains, the resulting MBM will further comprise the other TAA ABS chain, for example the light chain of a Fab.

[0118] Preferably, the resulting MBM is bispecific for the TAA and the TCE ABS's target
25 (*e.g.*, CD3).

[0119] FIG. 7 illustrates an exemplary embodiment of a Type IIB MBM proprotein. The depiction of a single substrate and two spacers in the protease-cleavable linkers of FIG. 7 is for illustrative purposes only; as described in Section 6.4, protease-cleavable linkers can include multiple substrates and more than two spacer sequences.

30 **6.4. Protease Cleavable Linkers**

[0120] The MBM proproteins of the disclosure typically comprise at least components that are connected to one another by a linker with one or more substrates for a protease and whose cleavage results in activation of the MBM.

[0121] A protease-cleavable linker can range from 20 amino acids to 80 or more amino acids, and in certain aspects a non-cleavable peptide linker ranges from 20 amino acids to 60 amino acids, 20 amino acids to 40 amino acids, from 30 amino acids to 50 amino acids, from 20 amino acids to 80 amino acids, or from 30 amino acids to 70 amino acids in length.

[0122] The protease cleavable linkers comprise one or more substrate sequences for one or more proteases, for example one or more of the proteases set forth in Section 6.4.1. The one or more substrate sequences, *e.g.*, one or more of the substrate sequences set forth in Section 6.4.2, are typically flanked by one or more spacer sequences, *e.g.*, spacer sequences as described in Section 6.4.3. Each protease-cleavable linker can include one, two, three or more substrate sequences. The spacer sequences can be adjoining, overlapping, or separated by spacer sequences. Preferably, the C- and N-termini of the protease-cleavable linkers contain spacer sequences.

[0123] Exemplary protease-cleavable linker sequences ae set forth in Section 6.4.4

6.4.1. Proteases

[0124] Exemplary protease whose substrate sequences can be incorporated into the protease-cleavable linkers are set forth in Table A below.

TABLE A
Exemplary Proteases for Substrate Cleavage

ADAMS, ADAMTS, *e.g.*	Caspases, *e.g.,*	MMP24
ADAM8	Caspase 1	MMP26
ADAM9	Caspase 2	MMP27
ADAM10	Caspase 3	
ADAM12	Caspase 4	
ADAM15	Caspase 5	
ADAM17/TACE	Caspase 6	
ADAMDEC1	Caspase 7	
ADAMTS1	Caspase 8	Cysteine proteinases, *e.g.*,
ADAMTS4	Caspase 9	Cruzipain
ADAMTS5	Caspase 10	Legumain
	Caspase 14	Otubain-2
Aspartate proteases, *e.g.,*		
BACE	Cysteine cathepsins, *e.g.,*	KLKs, *e.g.*,
Renin	Cathepsin B	KLK4
	Cathepsin C	KLK5
Aspartic cathepsins, *e.g.*,	Cathepsin K	KLK6
Cathepsin D	Cathepsin L	KLK7
Cathepsin E	Cathepsin S	KLK8
	Cathepsin V/L2	KLK10
NS3/4A	Cathepsin X/Z/P	KLK11
PACE4		KLK13
Plasmin	MMPs, *e.g.*,	KLK14
PSA	MMP1	

TABLE A Exemplary Proteases for Substrate Cleavage		
tPA Thrombin Tryptase uPA	MMP2 MMP3 MMP7 MMP8 MMP9 MMP10	Metallo proteinases, *e.g.*, Meprin Neprilysin PSMA BMP-1
Type II Transmembrane Serine Proteases (TTSPs), *e.g.*, DESC1 DPP-4 FAP Hepsin Matriptase-2 MT/SP1/Matriptase TMPRSS2 TMPRSS3 TMPRSS4	MMP11 MMP12 MMP13 MMP14 MMP15 MMP16 MMP17 MMP19 MMP20 MMP23	Serine proteases, *e.g.*, activated protein C Cathepsin A Cathepsin G Chymase coagulation factor proteases (e.g., FVIIa, FIXa, FXa, FXIa, FXIIa) Human Neutrophil Elastase Lactoferrin

[0125] In particular embodiments, the protease is matrix metalloprotease (MMP)-2, MMP-9, legumain asparaginyl endopeptidase, thrombin, fibroblast activation protease (FAP), MMP-1, MMP-3, MMP-7, MMP-8, MMP-12, MMP-13, MMP-14, membrane type 1 matrix metalloprotease (MT1-MMP), plasmin, transmembrane protease, serine (TMPRSS-3/4), cathepsin A, cathepsin B, cathepsin D, cathepsin E, cathepsin F, cathepsin H, cathepsin K, cathepsin L, cathepsin L2, cathepsin O, cathepsin S, caspase 1, caspase 2, caspase 3, caspase 4, caspase 5, caspase 6, caspase 7, caspase 8, caspase 9, caspase 10, caspase 11, caspase 12, caspase 13, caspase 14, human neutrophil elastase, urokinase/urokinase-type plasminogen activator (uPA), a disintegrin and metalloprotease (ADAM)10, ADAM12, ADAM17, ADAM with thrombospondin motifs (ADAMTS), ADAMTS5, beta secretase (BACE), granzyme A, granzyme B, guanidinobenzoatase, hepsin, matriptase, matriptase 2, meprin, neprilysin, prostate-specific membrane antigen (PSMA), tumor necrosis factor-converting enzyme (TACE), kallikrein-related peptidase (KLK)3, KLK5, KLK7, KLK11, NS3/4 protease of hepatitis C virus (HCV-NS3/4), tissue plasminogen activator (tPA), calpain, calpain 2, glutamate carboxypeptidase II, plasma kallikrein, AMSH-like protease, AMSH, γ-secretase component, antiplasmin cleaving enzyme (APCE), decysin 1, apoptosis-related cysteine peptidase, or N-acetylated alpha-linked acidic dipeptidase-like 1.

6.4.2. Substrates

[0126] Exemplary substrate sequences that are cleavable by a tumor protease and can be incorporated into the protease-cleavable linkers are set forth in Table B below.

TABLE B Substrate Sequences for Protease Cleavable Linkers		
Substrate Sequence	**Designation**	**Cleaving Protease**
$(DE)_8$RPLALWRS$(DR)_8$ (SEQ ID NO: 2)	SU1	MMP7
AARGPAIH (SEQ ID NO: 3)	SU2	
AAYHLVSQ (SEQ ID NO: 4)	SU3	Collagenase
AGLGISST (SEQ ID NO: 5)	SU4	Collagenase
AGLGVVER (SEQ ID NO: 6)	SU5	Collagenase
ALAL (SEQ ID NO: 7)	SU6	Lysosomal Enzyme
ALFFSSPP (SEQ ID NO: 8)	SU7	
ALFKSSFP (SEQ ID NO: 9)	SU8	
ALLLALL (SEQ ID NO: 10)	SU9	TOP
AQFVLTEG (SEQ ID NO: 11)	SU10	Collagenase
AQNLLGMV (SEQ ID NO: 12)	SU11	
AVGLLAPP (SEQ ID NO: 13)	SU12	Serine protease
DAFK (SEQ ID NO: 14)	SU13	Urokinase plasminogen activator (uPA)
DEVD (SEQ ID NO: 15)	SU14	Caspase-3
DEVDP (SEQ ID NO: 16)	SU15	Caspase-3
DPRSFL (SEQ ID NO: 17)	SU16	Thrombin
DVAQFVLT (SEQ ID NO: 18)	SU17	Collagenase
DVLK (SEQ ID NO: 19)	SU18	Plasmin
DWLYWPGI (SEQ ID NO: 20)	SU19	
EDDDDKA (SEQ ID NO: 21)	SU20	Enterokinase
EP(Cit)G(Hof)YL (SEQ ID NO: 22)	SU21	MMP2, MMP9, MMP14
EPQALAMS (SEQ ID NO: 23)	SU22	Collagenase
ESLPVVAV (SEQ ID NO: 24)	SU23	Collagenase
ESPAYYTA (SEQ ID NO: 25)	SU24	MMP
F(Pip)RS	SU25	Thrombin
FK	SU26	Lysosomal Enzyme
FPRPLGITGL (SEQ ID NO: 27)	SU27	
FRLLDWQW (SEQ ID NO: 28)	SU28	
GFLG (SEQ ID NO: 29)	SU29	Lysosomal Enzyme
GGAANLVRGG (SEQ ID NO: 30)	SU30	MMP11
GGGRR (SEQ ID NO: 31)	SU31	Urokinase plasminogen activator (uPA)
GGPRGLPG (SEQ ID NO: 32)	SU32	Cathepsin K
GGQPSGMWGW (SEQ ID NO: 33)	SU33	
GGSIDGR (SEQ ID NO: 34)	SU34	Factor Xa
GGWHTGRN (SEQ ID NO: 35)	SU35	
GIAGQ (SEQ ID NO: 36)	SU36	Collagenase
GKAFRR (SEQ ID NO: 37)	SU37	Kallikrein 2
GPAGLYAQ (SEQ ID NO: 38)	SU38	
GPAGMKGL (SEQ ID NO: 39)	SU39	
GPEGLRVG (SEQ ID NO: 40)	SU40	Collagenase
GPLGIAGI (SEQ ID NO: 41)	SU41	Collagenase
GPLGVRG (SEQ ID NO: 42)	SU42	
GPQGIAGQ (SEQ ID NO: 43)	SU43	Collagenase
GPQGLLGA (SEQ ID NO: 44)	SU44	Collagenase

TABLE B Substrate Sequences for Protease Cleavable Linkers		
Substrate Sequence	**Designation**	**Cleaving Protease**
GPRSFG (SEQ ID NO: 45)	SU45	
GPRSFGL (SEQ ID NO: 46)	SU46	
GPSHLVLT (SEQ ID NO: 47)	SU47	
GVSQNYPIVG (SEQ ID NO: 48)	SU48	HIV Protease
GVVQASCRLA (SEQ ID NO: 49)	SU49	CMV Protease
GWEHDG (SEQ ID NO: 50)	SU50	Interleukin 1β converting enzyme
HSSKLQ (SEQ ID NO: 51)	SU51	Prostate Specific Antigen
HSSKLQEDA (SEQ ID NO: 52)	SU52	Prostate Specific Antigen
HSSKLQL (SEQ ID NO: 53)	SU53	Prostate Specific Antigen
HTGRSGAL (SEQ ID NO: 54)	SU54	
IDGR (SEQ ID NO: 55)	SU55	Factor Xa
IEGR (SEQ ID NO: 56)	SU56	Factor Xa
ILPRSPAF (SEQ ID NO: 57)	SU57	
IPVSLRSG (SEQ ID NO: 58)	SU58	MMP
ISSGL (SEQ ID NO: 59)	SU59	MMP
ISSGLL (SEQ ID NO: 60)	SU60	MMP
ISSGLLS (SEQ ID NO: 61)	SU61	MMP
ISSGLLSS (SEQ ID NO: 62)	SU62	MMP
ISSGLSS (SEQ ID NO: 63)	SU63	MMP
KGSGDVEG (SEQ ID NO: 64)	SU64	Caspase-3
KQEQNPGST (SEQ ID NO: 65)	SU65	FAP
KRALGLPG (SEQ ID NO: 66)	SU66	MMP7
LAAPLGLL (SEQ ID NO: 67)	SU67	
LAPLGLQRR (SEQ ID NO: 68)	SU68	
LAQKLKSS (SEQ ID NO: 69)	SU69	
LAQRLRSS (SEQ ID NO: 70)	SU70	
LEATA (SEQ ID NO: 71)	SU71	MMP9
LKAAPRWA (SEQ ID NO: 72)	SU72	
LLAPSHRA (SEQ ID NO: 73)	SU73	
LPGGLSPW (SEQ ID NO: 74)	SU74	
LSGRSANI (SEQ ID NO: 75)	SU75	Serine protease
LSGRSANP (SEQ ID NO: 76)	SU76	Serine protease
LSGRSDDH (SEQ ID NO: 77)	SU77	Serine protease
LSGRSDIH (SEQ ID NO: 78)	SU78	Serine protease
LSGRSDNH (SEQ ID NO: 79)	SU79	Serine protease
LSGRSDNI (SEQ ID NO: 80)	SU80	Serine protease
LSGRSDNP (SEQ ID NO: 81)	SU81	Serine protease
LSGRSDQG (SEQ ID NO: 82)	SU82	Serine protease
LSGRSDQH (SEQ ID NO: 83)	SU83	Serine protease
LSGRSDTH (SEQ ID NO: 84)	SU84	Serine protease
LSGRSDYH (SEQ ID NO: 85)	SU85	Serine protease
LSGRSGNH (SEQ ID NO: 86)	SU86	Serine protease
LVLASSSFGY (SEQ ID NO: 87)	SU87	Herpes Simplex Virus Protease
MDAFLESS (SEQ ID NO: 88)	SU88	Collagenase

TABLE B Substrate Sequences for Protease Cleavable Linkers		
Substrate Sequence	**Designation**	**Cleaving Protease**
MGLFSEAG (SEQ ID NO: 89)	SU89	
MIAPVAYR (SEQ ID NO: 90)	SU90	
MVLGRSLL (SEQ ID NO: 91)	SU91	
NLL	SU92	Cathepsin B
NTLSGRSENHSG (SEQ ID NO: 92)	SU93	
NTLSGRSGNHGS (SEQ ID NO: 93)	SU94	
PAGLWLDP (SEQ ID NO: 94)	SU95	
PGGPAGIG (SEQ ID NO: 95)	SU96	
PIC(Et)FF (SEQ ID NO: 96)	SU97	Cathepsin D
PLGC(me)AG (SEQ ID NO: 97)	SU98	MMP
PLGL (SEQ ID NO: 98)	SU99	
PLGLAG (SEQ ID NO: 99)	SU100	MMP
PLGLAX (SEQ ID NO: 100)	SU101	MMP
PLGLWA (SEQ ID NO: 101)	SU102	MMP
PLGLWSQ (SEQ ID NO: 102)	SU103	MMP
PLTGRSGG (SEQ ID NO: 103)	SU104	
PMAKK (SEQ ID NO: 104)	SU105	
PPRSFL (SEQ ID NO: 105)	SU106	Thrombin
PR(S/T)(L/I)(S/T)	SU107	MMP9
PRFRIIGG (SEQ ID NO: 107)	SU108	Plasmin
PVGYTSSL (SEQ ID NO: 108)	SU109	
PVQPIGPQ (SEQ ID NO: 109)	SU110	Collagenase
QALAMSAI (SEQ ID NO: 110)	SU111	Collagenase
QGRAITFI (SEQ ID NO: 111)	SU112	
QNQALRMA (SEQ ID NO: 112)	SU113	
RGPA (SEQ ID NO: 113)	SU114	
RGPAFNPM (SEQ ID NO: 114)	SU115	
RGPATPIM (SEQ ID NO: 115)	SU116	
RKSSIIIRMRDVVL (SEQ ID NO: 116)	SU117	Plasmin
RLQLKAC (SEQ ID NO: 117)	SU118	MMP
RLQLKL (SEQ ID NO: 118)	SU119	MMP
RMHLRSLG (SEQ ID NO: 119)	SU120	
RPSPMWAY (SEQ ID NO: 120)	SU121	
RQARVVNG (SEQ ID NO: 121)	SU122	Matripase
SAGFSLPA (SEQ ID NO: 122)	SU123	
SAPAVESE (SEQ ID NO: 123)	SU124	Collagenase
SARGPSRW (SEQ ID NO: 124)	SU125	
SGEPAYYTA (SEQ ID NO: 125)	SU126	
SGGPLGVR (SEQ ID NO: 126)	SU127	
SGRIGFLRTA (SEQ ID NO: 127)	SU128	MMP14
SGRSA (SEQ ID NO: 128)	SU129	Urokinase plasminogen activator (uPA)
SGRSANPRG (SEQ ID NO: 129)	SU130	
SMLRSMPL (SEQ ID NO: 130)	SU131	
SPLPLRVP (SEQ ID NO: 131)	SU132	
SPLTGRSG (SEQ ID NO: 132)	SU133	

TABLE B Substrate Sequences for Protease Cleavable Linkers		
Substrate Sequence	**Designation**	**Cleaving Protease**
SPRSIMLA (SEQ ID NO: 133)	SU134	
SSRGPAYL (SEQ ID NO: 134)	SU135	
SSRHRRALD (SEQ ID NO: 135)	SU136	Plasmin
SSSFDKGKYKKGDDA (SEQ ID NO: 136)	SU137	Plasmin
SSSFDKGKYKRGDDA (SEQ ID NO: 137)	SU138	Plasmin
STFPFGMF (SEQ ID NO: 138)	SU139	
TARGPSFK (SEQ ID NO: 139)	SU140	
TGRGPSWV (SEQ ID NO: 140)	SU141	
TSGRSANP (SEQ ID NO: 141)	SU142	
TSTSGRSANPRG (SEQ ID NO: 142)	SU143	
VAGRSMRP (SEQ ID NO: 143)	SU144	
VAQFVLTE (SEQ ID NO: 144)	SU145	Collagenase
VHMPLGFLGP (SEQ ID NO: 145)	SU146	
VPLSLYSG (SEQ ID NO: 146)	SU147	MMP9
VVPEGRRS (SEQ ID NO: 147)	SU148	
WATPRPMR (SEQ ID NO: 148)	SU149	
YGAGLGVV (SEQ ID NO: 149)	SU150	Collagenase
HPVGLLAR (SEQ ID NO:260)	SU151	

6.4.3. Spacers

[0127] Exemplary spacer sequences that can be incorporated into the protease-cleavable linkers are set forth in Table C below. In addition to the spacer sequences set forth in Table C, any of the non-cleavable linker sequences described in Section 6.5, *e.g.*, the non-cleavable linker sequences set forth in Table E, or portions thereof can be used as spacer sequences.

TABLE C Spacer Sequences for Protease Cleavable Linkers	
Spacer Sequence	**Designation**
$(GGGGS)_n$ (SEQ ID NO: 150)	SP1
$(GGGS)_n$ (SEQ ID NO: 151)	SP2
$(GGS)_n$	SP3
$(GS)_n$	SP4
$(GSGGS)_n$ (SEQ ID NO: 154)	SP5
GGGGSGGGGS (SEQ ID NO: 155)	SP6
GGGGSGGGGSGGGGS (SEQ ID NO: 156)	SP7
GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 157)	SP8
GGGKSGGGKSGGGKS (SEQ ID NO: 158)	SP9

TABLE C Spacer Sequences for Protease Cleavable Linkers	
Spacer Sequence	**Designation**
GGGKSGGKGSGKGGS (SEQ ID NO: 159)	SP10
GGGS (SEQ ID NO: 160)	SP11
GGGSG (SEQ ID NO: 161)	SP12
GGKGSGGKGSGGKGS (SEQ ID NO: 162)	SP13
GGSGGGGSGGGGS (SEQ ID NO: 163)	SP14
GGSGGS (SEQ ID NO: 164)	SP15
GGSGGSGGSGS (SEQ ID NO: 165)	SP16
GSGGG (SEQ ID NO: 166)	SP17
GSGSG (SEQ ID NO: 167)	SP18
GSS	SP19
GSSG (SEQ ID NO: 168)	SP20
GSSGGSGGSG (SEQ ID NO: 169)	SP21
GSSGGSGGSGG (SEQ ID NO: 170)	SP22
GSSGGSGGSGGS (SEQ ID NO: 171)	SP23
GSSGGSGGSGGSG (SEQ ID NO: 172)	SP24
GSSGGSGGSGGSGGGS (SEQ ID NO: 173)	SP25
GSSGGSGGSGS (SEQ ID NO: 174)	SP26
GSSGT (SEQ ID NO: 175)	SP27
GSSSG (SEQ ID NO: 176)	SP28
QGQSGQ (SEQ ID NO: 177)	SP29
QGQSGQG (SEQ ID NO: 178)	SP30
QGQSGS (SEQ ID NO: 179)	SP31
QSGQ (SEQ ID NO: 180)	SP32
QSGQG (SEQ ID NO: 181)	SP33
QSGS (SEQ ID NO: 182)	SP34
SGQ	SP35
SGQG (SEQ ID NO: 183)	SP36
SGS	SP37
$(G)_n$	SP38

[0128] In some embodiments, as used in Table C above, n is an integer from 1 to 10, *e.g.,* 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10.

6.4.4. Exemplary Protease Cleavable Linkers

[0129] Exemplary protease-cleavable linkers comprising one or more substrate sequences as well as spacer sequences are set forth in Table D below.

TABLE D Protease Cleavable Linker Sequences		
Linker Sequence	**Designation**	**Cleaving Protease(s)**
GGGISSGLLSGRSDNHGGGISSGLLSGRSDNHGGS (SEQ ID NO: 184)	PCL1	
GGGISSGLLSGRSDNHGGGISSGLLSGRSDNHGGS GGGISSGLLSGRSDNHGGGISSGLLSGRSDNHGGS (SEQ ID NO: 185)	PCL2	
GGSGGSIPVSLRSGGGISSGLLSGRSDNHGGSGGS (SEQ ID NO: 186)	PCL3	
GGSGGSVPLSLYSGGGISSGLLSGRSDNHGGSGGS (SEQ ID NO: 187)	PCL4	
GGSHPVGLLARGGGHPVGLLARGGGHPVGLLARGS (SEQ ID NO: 188)	PCL5	
GGSHPVGLLARGGGHPVGLLARGGSGRSAGGSGRSA (SEQ ID NO: 189)	PCL6	
AVGLLAPPGGLSGRSANI (SEQ ID NO: 190)	PCL7	ADAM17_2, FAPa_1, CTSL1_1
AVGLLAPPGGLSGRSANP (SEQ ID NO: 191)	PCL8	FAPa_1, ADAM17_2, CTSL1_1
AVGLLAPPGGLSGRSDDH (SEQ ID NO: 192)	PCL9	MMP14_1, MMP14_1, MMP14_1
AVGLLAPPGGLSGRSDIH (SEQ ID NO: 193)	PCL10	MMP14_1, MMP14_1, MMP14_1
AVGLLAPPGGLSGRSDNH (SEQ ID NO: 194)	PCL11	MMP14_1, MMP14_1
AVGLLAPPGGLSGRSDNI (SEQ ID NO: 195)	PCL12	MMP14_1, CTSL1_1, ADAM17_2
AVGLLAPPGGLSGRSDNP (SEQ ID NO: 196)	PCL13	CTSL1_1, ADAM17_2, FAPa_1
AVGLLAPPGGLSGRSDQH (SEQ ID NO: 197)	PCL14	
AVGLLAPPGGLSGRSDTH (SEQ ID NO: 198)	PCL15	FAPa_1, CTSL1_1, ADAM17_2
AVGLLAPPGGLSGRSDYH (SEQ ID NO: 199)	PCL16	
AVGLLAPPGGTSTSGRSANPRG (SEQ ID NO: 200)	PCL17	
AVGLLAPPSGRSANPRG (SEQ ID NO: 201)	PCL18	
AVGLLAPPTSGRSANPRG (SEQ ID NO: 202)	PCL19	

TABLE D Protease Cleavable Linker Sequences		
Linker Sequence	**Designation**	**Cleaving Protease(s)**
GGALFKSSFPGPAGLYAQPLAQKLKSSGGK (SEQ ID NO: 203)	PCL20	CTSL1_1, MMP14_1, ADAM17_2
GGGGSGGGGSGGGGSFVGGTGGGGSGGGGSGGS (SEQ ID NO: 204)	PCL21	
GGGGSGGGGSGGGGSISSGLLSGRSDNHGGSGGS (SEQ ID NO: 205)	PCL22	
GGGGSGGGGSGGGGSVPLSLYSGGGSGGSGGSGS (SEQ ID NO: 206)	PCL23	
GGGGSGGGGSGPLGLWSQGGGGSGGGGSGGGGSGG (SEQ ID NO: 207)	PCL24	
GGGGSGGGGSKKAAPGGGGSGGGGSGGGGSGGS (SEQ ID NO: 208)	PCL25	
GGGGSGGGGSKKAAPVNGGGGGSGGGGSGGGGS (SEQ ID NO: 209)	PCL26	
GGGGSGGGGSPMAKKGGGGSGGGGSGGGGSGGS (SEQ ID NO: 210)	PCL27	
GGGGSGGGGSPMAKKVNGGGGGSGGGGSGGGGS (SEQ ID NO: 211)	PCL28	
GGGGSGGGGSQARAKGGGGSGGGGSGGGGSGGS (SEQ ID NO: 212)	PCL29	
GGGGSGGGGSQARAKVNGGGGGSGGGGSGGGGS (SEQ ID NO: 213)	PCL30	
GGGGSGGGGSRQARVVNGGGGGSGGGGSGGGGS (SEQ ID NO: 214)	PCL31	
GGGGSGGGGSRQARVVNGGGGGSVPLSLYSGGGGGSGGGGS (SEQ ID NO: 215)	PCL32	
GGGGSGGGGSRQARVVNSVPLSLYSGGGGGSGGGGS (SEQ ID NO: 216)	PCL33	
GGGGSGGGGSVHMPLGFLGPGGGGSGGGGSGGS (SEQ ID NO: 217)	PCL34	
GGGGSVHMPLGFLGPGRSRGSFPGGGGS (SEQ ID NO: 218)	PCL35	

TABLE D Protease Cleavable Linker Sequences		
Linker Sequence	**Designation**	**Cleaving Protease(s)**
GGGGSVHMPLGFLGPPMAKKGG GGSGGGGSGGS (SEQ ID NO: 219)	PCL36	
GGGGSVHMPLGFLGPRQARVVN GGGGSGGGGS (SEQ ID NO: 220)	PCL37	
GGGGSVHMPLGFLGPRQARVVN GGGGSGGGGSGG (SEQ ID NO: 221)	PCL38	
GGPLAQKLKSSALFKSSFPGPAG LYAQGGR (SEQ ID NO: 222)	PCL39	ADAM17_2, CTSL1_1, MMP14_1
GLSGRSDNHGGAVGLLAPP (SEQ ID NO: 223)	PCL40	
GLSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 224)	PCL41	
ISSGLLSGRSANI (SEQ ID NO: 225)	PCL42	MMP, Serine protease
ISSGLLSGRSANP (SEQ ID NO: 226)	PCL43	MMP, Serine protease
ISSGLLSGRSANPRG (SEQ ID NO: 227)	PCL44	MMP, Serine protease
ISSGLLSGRSDDH (SEQ ID NO: 228)	PCL45	MMP, Serine protease
ISSGLLSGRSDIH (SEQ ID NO: 229)	PCL46	MMP, Serine protease
ISSGLLSGRSDNH (SEQ ID NO: 230)	PCL47	MMP, Serine protease
ISSGLLSGRSDNI (SEQ ID NO: 231)	PCL48	CTSL1_1, MMP14_1
ISSGLLSGRSDNP (SEQ ID NO: 232)	PCL49	MMP, Serine protease
ISSGLLSGRSDQH (SEQ ID NO: 233)	PCL50	MMP, Serine protease
ISSGLLSGRSDTH (SEQ ID NO: 234)	PCL51	MMP, Serine protease
ISSGLLSGRSDYH (SEQ ID NO: 235)	PCL52	MMP, Serine protease
ISSGLLSGRSGNH (SEQ ID NO: 236)	PCL53	MMP, Serine protease
ISSGLLSSGGSGGSLSGRSDNH (SEQ ID NO: 237)	PCL54	
ISSGLLSSGGSGGSLSGRSGNH (SEQ ID NO: 238)	PCL55	
KGGPGGPAGIGPLAQRLRSSALF KSSFPGR (SEQ ID NO: 239)	PCL56	FAPa_1, ADAM17_1, CTSL1_1
KSGPGGPAGIGALFFSSPPLAQKL KSSGGR (SEQ ID NO: 240)	PCL57	FAPa_1, CTSL1_2, ADAM17_2

TABLE D Protease Cleavable Linker Sequences		
Linker Sequence	**Designation**	**Cleaving Protease(s)**
LSGRSDNHGGAVGLLAPP (SEQ ID NO: 241)	PCL58	
LSGRSDNHGGSGGSISSGLLSS (SEQ ID NO: 242)	PCL59	
LSGRSDNHGGSGGSQNQALRMA (SEQ ID NO: 243)	PCL60	
LSGRSDNHGGVHMPLGFLGP (SEQ ID NO: 244)	PCL61	
LSGRSGNHGGSGGSISSGLLSS (SEQ ID NO: 245)	PCL62	
LSGRSGNHGGSGGSQNQALRMA (SEQ ID NO: 246)	PCL63	
QNQALRMAGGSGGSLSGRSDNH (SEQ ID NO: 247)	PCL64	
QNQALRMAGGSGGSLSGRSGNH (SEQ ID NO: 248)	PCL65	
RGGALFKSSFPLAQKLKSSGPAG LYAQGGK (SEQ ID NO: 249)	PCL66	CTSL1_1, ADAM17_2, MMP14_1
RGGGPAGLYAQPLAQKLKSSALF KSSFPGG (SEQ ID NO: 250)	PCL67	MMP14_1, ADAM17_2, CTSL1_1
SGGFPRSGGSFNPRTFGSKRKR RGSRGGGG (SEQ ID NO: 251)	PCL68	thrombin, factor Xa, hepsin
SGPLAQKLKSSGPAGLYAQALFK SSFPGSK (SEQ ID NO: 252)	PCL69	ADAM17_2, MMP14_1, CTSL1_1
TSTSGRSANPRGGGAVGLLAPP (SEQ ID NO: 253)	PCL70	
TSTSGRSANPRGGGVHMPLGFL GP (SEQ ID NO: 254)	PCL71	
VHMPLGFLGPGGLSGRSDNH (SEQ ID NO: 255)	PCL72	
VHMPLGFLGPGGTSTSGRSANP RG (SEQ ID NO: 256)	PCL73	
SGRSAGGGSGRSAGGGSGRSA (SEQ ID NO: 257)	PCL74	uPA
HPVGLLARGGGHPVGLLARGGG SGRSAGGGSGRSA (SEQ ID NO: 258)	PCL75	MPA (MMP-2 and uPA)
GPLGVRGK (SEQ ID NO: 259)	PCL76	MMP-2
HPVGLLAR (SEQ ID NO: 260)	PCL77	MMP-2
GPQGIAGQ (SEQ ID NO: 43)	PCL78	MMP-2, MMP-9, and to some degree MT1-MMP
VPMSMRGG (SEQ ID NO: 261)	PCL79	MMP-9 and MMP-2
IPVSLRSG (SEQ ID NO: 58)	PCL80	MMP-2, and to some degree MMP-9 or MMP-7
RPFSMIMG (SEQ ID NO: 262)	PCL81	MMP-9 and MMP-7, to some degree MMP-3

TABLE D Protease Cleavable Linker Sequences		
Linker Sequence	**Designation**	**Cleaving Protease(s)**
VPLSLTMG (SEQ ID NO: 263)	PCL82	MMP-7, to some degree MMP-9, MMP-2, MPT-1-MMP
VPLSLYSG (SEQ ID NO: 146)	PCL83	MMP-2, MMP-9, MMP-7
IPESLRAG (SEQ ID NO: 264)	PCL84	MMP-2, MMP-7, MMP-9, to some degree MPT-1-MMP
GGGISSGLLSGRSDNHGGGS (SEQ ID NO: 339)	PCL85	
GGGHPVGLLARGGGS (SEQ ID NO: 340)	PCL86	
GGGSGGGSGGGGISSGLLSGRSDNHGGGSGGGSGGS (SEQ ID NO: 341)	PCL87	
GGGGISSGLLSGRSDNHGGGISSGLLSGRSDNHGGS (SEQ ID NO: 342)	PCL88	
GGGSGGSIPVSLRSGGGISSGLLSGRSDNHGGSGGS (SEQ ID NO: 343)	PCL89	
GGGSGGSVPLSLYSGGGISSGLLSGRSDNHGGSGGS (SEQ ID NO: 344)	PCL90	
GGGSHPVGLLARGGGHPVGLLARGGGHPVGLLARGS (SEQ ID NO: 345)	PCL91	
GGGSHPVGLLARGGGHPVGLLARGGSGRSAGGSGRS (SEQ ID NO: 346)	PCL92	

[0130] In certain aspects, the protease-cleavable linker comprises an amino acid sequence having up to 5, up to 4, up to 3, up to 2 or up to 1 amino acid substitution(s) as compared to the sequence set forth in Table D. Thus, in some embodiments, the protease cleavable linker comprises or consists of any amino acid sequence in Table D with 1-5 amino acid substitutions as compared to the sequence set forth in Table D.

6.5. Non-Cleavable Linkers

[0131] In certain aspects, the present disclosure provides MBM proproteins in which two or more components of an MBM proprotein are connected to one another by a peptide linker. By way of example and not limitation, linkers can be used to connect (a) an ABS and an Fc domain; (b) two ABSs; or (c) different domains within an ABS (*e.g.,* VH and VL domains in an scFv).

[0132] Preferably, all linkers in the MBM proprotein other than the protease-cleavable linker whose cleavage results in activation of the MBM are non-cleavable linkers (NCLs).

[0133] A non-cleavable linker can range from 2 amino acids to 60 or more amino acids, and in certain aspects a non-cleavable 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.

[0134] In particular aspects, a non-cleavable 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.

[0135] In some embodiments of the foregoing, the non-cleavable 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 non-cleavable 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 non-cleavable 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.

[0136] Charged (*e.g.,* charged hydrophilic linkers) and/or flexible non-cleavable linkers are particularly preferred.

[0137] Examples of flexible non-cleavable linkers that can be used in the MBM proproteins 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 non-cleavable linkers are or comprise repeats of glycines and serines, *e.g.,* a monomer or multimer of G_nS (SEQ ID NO: 265) or SG_n (SEQ ID NO: 266), 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 non-cleavable linker is or comprises a monomer or multimer of repeat of G_4S (SEQ ID NO: 1) *e.g.,* $(GGGGS)_n$ (SEQ ID NO: 150).

[0138] Polyglycine non-cleavable linkers can suitably be used in the MBM proproteins of the disclosure. In some embodiments, a peptide non-cleavable linker comprises two consecutive glycines (2Gly), three consecutive glycines (3Gly), four consecutive glycines (4Gly) (SEQ ID NO: 267), five consecutive glycines (5Gly) (SEQ ID NO: 268), six consecutive glycines (6Gly) (SEQ ID NO: 269), seven consecutive glycines (7Gly) (SEQ ID NO: 270), eight

consecutive glycines (8Gly) (SEQ ID NO: 271) or nine consecutive glycines (9Gly) (SEQ ID NO: 272).

[0139] Exemplary non-cleavable linker sequences are set forth in Table E below.

TABLE E Non-Cleavable Linker Sequences		
Linker Sequence	**Designation**	**SEQ ID NO:**
$(GGGGS)_n$ (SEQ ID NO: 150)	NCL1	
$(GGGS)_n$ (SEQ ID NO: 151)	NCL2	
$(GGS)_n$	NCL3	
$(GS)_n$	NCL4	
$(GSGGS)_n$ (SEQ ID NO: 154)	NCL5	
ADAAP (SEQ ID NO: 273)	NCL6	
ADAAPTVSIFP (SEQ ID NO: 274)	NCL7	
ADAAPTVSIFPP (SEQ ID NO: 275)	NCL8	
AKTTAP (SEQ ID NO: 276)	NCL9	
AKTTAPSVYPLAP (SEQ ID NO: 277)	NCL10	
AKTTPKLEEGEFSEARV (SEQ ID NO: 278)	NCL11	
AKTTPKLGG (SEQ ID NO: 279)	NCL12	
AKTTPP (SEQ ID NO: 280)	NCL13	
AKTTPPSVTPLAP (SEQ ID NO: 281)	NCL14	
ASTKGP (SEQ ID NO: 282)	NCL15	
ASTKGPSVFPLAPASTKGPSVFPLAP (SEQ ID NO: 283)	NCL16	
EGKSSGSGSESKST (SEQ ID NO: 284)	NCL17	
GEGESGEGESGEGES (SEQ ID NO: 285)	NCL18	
GEGESGEGESGEGESGEGES (SEQ ID NO: 286)	NCL19	
GEGGSGEGGSGEGGS (SEQ ID NO: 287)	NCL20	
GENKVEYAPALMALS (SEQ ID NO: 288)	NCL21	
GGEGSGGEGSGGEGS (SEQ ID NO: 289)	NCL22	
GGGESGGEGSGEGGS (SEQ ID NO: 290)	NCL23	
GGGESGGGESGGGES (SEQ ID NO: 291)	NCL24	
GGGGSGGGGS (SEQ ID NO: 155)	NCL25	
GGGGSGGGGSGGGGS (SEQ ID NO: 156)	NCL26	
GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 157)	NCL27	
GGGKSGGGKSGGGKS (SEQ ID NO: 158)	NCL28	
GGGKSGGKGSGKGGS (SEQ ID NO: 159)	NCL29	

TABLE E Non-Cleavable Linker Sequences		
Linker Sequence	**Designation**	**SEQ ID NO:**
GGGS (SEQ ID NO: 160)	NCL30	
GGGSG (SEQ ID NO: 161)	NCL31	
GGKGSGGKGSGGKGS (SEQ ID NO: 162)	NCL32	
GGS	NCL33	
GGSG (SEQ ID NO: 292)	NCL34	
GGSGG (SEQ ID NO: 293)	NCL35	
GGSGG (SEQ ID NO: 293)	NCL36	
GGSGGGGSG (SEQ ID NO: 294)	NCL37	
GGSGGGGSGGGGS (SEQ ID NO: 163)	NCL38	
GHEAAAVMQVQYPAS (SEQ ID NO: 295)	NCL39	
GKGGSGKGGSGKGGS (SEQ ID NO: 296)	NCL40	
GKGKSGKGKSGKGKS (SEQ ID NO: 297)	NCL41	
GKGKSGKGKSGKGKSGKGKS (SEQ ID NO: 298)	NCL42	
GKPGSGKPGSGKPGS (SEQ ID NO: 299)	NCL43	
GKPGSGKPGSGKPGSGKPSGS (SEQ ID NO: 300)	NCL44	
GPAKELTPLKEAKVS (SEQ ID NO: 301)	NCL45	
GSAGSAAGSGEF (SEQ ID NO: 302)	NCL46	
GSGGG (SEQ ID NO: 166)	NCL47	
GSGSG (SEQ ID NO: 167)	NCL48	
GSS	NCL49	
GSSG (SEQ ID NO: 168)	NCL50	
GSSGGSGGSG (SEQ ID NO: 169)	NCL51	
GSSGGSGGSGG (SEQ ID NO: 170)	NCL52	
GSSGGSGGSGGS (SEQ ID NO: 171)	NCL53	
GSSGGSGGSGGSG (SEQ ID NO: 172)	NCL54	
GSSGGSGGSGGSGGGS (SEQ ID NO: 173)	NCL55	
GSSGGSGGSGS (SEQ ID NO: 174)	NCL56	
GSSGT (SEQ ID NO: 175)	NCL57	
GSSSG (SEQ ID NO: 176)	NCL58	
GSTSGSGKPGSGEGSTKG (SEQ ID NO: 303)	NCL59	
GTAAAGAGAAGGAAAGAAG (SEQ ID NO: 304)	NCL60	
GTSGSSGSGSGGSGSGGGG (SEQ ID NO: 305)	NCL61	
IRPRAIGGSKPRVA (SEQ ID NO: 306)	NCL62	
KESGSVSSEQLAQFRSLD (SEQ ID NO: 307)	NCL63	

TABLE E Non-Cleavable Linker Sequences		
Linker Sequence	**Designation**	**SEQ ID NO:**
KTTPKLEEGEFSEAR (SEQ ID NO: 308)	NCL64	
PRGASKSGSASQTGSAPGS (SEQ ID NO: 309)	NCL65	
QPKAAP (SEQ ID NO: 310)	NCL66	
QPKAAPSVTLFPP (SEQ ID NO: 311)	NCL67	
RADAAAA$(G4S)_4$ (SEQ ID NO: 312)	NCL68	
RADAAAAGGPGS (SEQ ID NO: 313)	NCL69	
RADAAP (SEQ ID NO: 314)	NCL70	
RADAAPTVS (SEQ ID NO: 315)	NCL71	
SAKTTP (SEQ ID NO: 316)	NCL72	
SAKTTPKLEEGEFSEARV (SEQ ID NO: 317)	NCL73	
SAKTTPKLGG (SEQ ID NO: 318)	NCL74	
STAGDTHLGGEDFD (SEQ ID NO: 319)	NCL75	
TVAAP (SEQ ID NO: 320)	NCL76	
TVAAPSVFIFPP (SEQ ID NO: 321)	NCL77	
TVAAPSVFIFPPTVAAPSVFIFPP (SEQ ID NO: 322)	NCL78	

[0140] In certain aspects, the MBM proprotein of the disclosure may comprise a polypeptide chain comprising, in an N- to C-terminal orientation, an ABS (or ABS chain), a hinge domain and a CH2 domain, and a CH3 domain. Thus, the hinge domain connects the ABS with the CH2 domain and can be said to constitute a type of linker. Exemplary hinge domains are set forth in Section 6.10.3.

6.6. TAA ABSs

[0141] The MBM proproteins of the disclosure comprise at least one ABS that binds specifically to an extracellular matrix ("ECM") antigen, a tumor reactive lymphocyte antigen, a cell surface molecule of tumor or viral lymphocytes, a T-cell antigen ("TCA"), a checkpoint inhibitor, or a tumor-associated antigen (TAA), referred to herein as "TAA ABS". 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, and other molecules may be considered both TCAs and checkpoint inhibitors. Preferably, the ECM antigen, tumor reactive lymphocyte antigen, cell surface molecule of tumor or viral lymphocytes, TCA, checkpoint inhibitor, or TAA is a human antigen. The antigen may or may not be present on normal cells. Particular aspects are directed to MBM proproteins comprising at least one ABS that binds specifically to a TAA. In

certain embodiments, the TAA is preferentially expressed or upregulated on tumor cells as compared to normal cells. In other embodiments, the TAA is a lineage marker.

[0142] 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, TCA, checkpoint inhibitor, or TAA may be targeted by the MBM proproteins 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.

[0143] 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.

[0144] Other target molecules are cell surface molecules of tumor or viral lymphocytes, for example T-cell co-stimulatory proteins such as CD27, CD28, 4-1BB (CD137), OX40, CD30, CD40, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, and B7-H3.

[0145] 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 TAA ABS 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.

[0146] In certain embodiments, the target molecules are TAAs. Exemplary TAAs are set forth in Table F below, together with references to exemplary antibodies or antibody sequences upon which the TAA ABS can be based.

TABLE F
Exemplary Anti-TAAs

Target	**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)
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)
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 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)

TABLE F Exemplary Anti-TAAs	
Target	**Antibody Name and/or Binding Sequences**
	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)
CD40	Lukatumumab
CD40	Dacetuzumab
CD40L	Hu5c8 (ruplizumab)
CD44v6	vibatuzumab mertansine
CD52	Campath™ (alemtuzumab) 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
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

TABLE F Exemplary Anti-TAAs	
Target	**Antibody Name and/or Binding Sequences**
	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
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)
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 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

TABLE F Exemplary Anti-TAAs	
Target	**Antibody Name and/or Binding Sequences**
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
GUCY2C	PF-07062119
Her2	Herceptin™ (trastuzumab)
Her2	Aldesleukin (proleukine)
Her2	Sargramustim (leukine)
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/IL23	Stelara™ (ustekinumab)

TABLE F Exemplary Anti-TAAs	
Target	**Antibody Name and/or Binding Sequences**
IL1Ra	Antril™, Kineret™ (ankinra)
IL2R	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/IL23 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
Integrinα4	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
Integrinα4 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.
Mesothelin	Amatuximab
Mesothelin	HPN536
MUC1	civatuzumab tetraxetane
MUC1	Pankomab ™ (gatipotuzumab)
MUC1	femtumumab
MUC1	Cantuzumab ravtansine
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
NGF	(tanezumab)
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, Abgemx 4.219.3, Abgenix 4.288.1, Abgenix 4.333.1, Abgemx 4.54.1, Abgenix 4.153.1, Abgenix 4.232.3, Abgenix 4.292.3, Abgenix 4.304.1,

TABLE F Exemplary Anti-TAAs	
Target	**Antibody Name and/or Binding Sequences**
	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 Abgemx 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
PMSA	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.
PMSA	An anti-PMSA 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.
PMSA	ES414
PMSA	BAY2010112 (pasotuxizumab)
PMSA	CCW702
PMSA	JNJ-63898081
PMSA	CC-1
PMSA	Acapatamab
PSMA	HPN424
RAAG12	RAV12
RANKL	Prolia™ (denosumab) VH: SEQ ID NO:51 of US Patent Pub. 2017/0002060

TABLE F Exemplary Anti-TAAs	
Target	**Antibody Name and/or Binding Sequences**
	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. 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;

TABLE F Exemplary Anti-TAAs	
Target	**Antibody Name and/or Binding Sequences**
	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.
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

[0147] In some aspects, the TAA ABS competes with an antibody set forth in Table F for binding to the TAA. In further aspects, the TAA ABS comprises CDRs having CDR sequences of an anti-TAA antibody set forth in Table F. In some embodiments, the TAA ABS comprises all 6 CDR sequences of the anti-TAA antibody set forth in Table F. In other embodiments, the TAA ABS 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 TAA ABS comprises a VH comprising the amino acid sequence of the VH of an anti-TAA antibody set forth in Table F. In some embodiments, the TAA ABS further comprises a VL comprising the amino acid sequence of the VL of the anti-TAA antibody set

forth in Table F. In other embodiments, the TAA ABS further comprises a universal light chain VL sequence.

[0148] Additional TAAs that can be targeted by the MBM proproteins are disclosed in Table K below and 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.

[0149] Yet 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, prostase 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).

6.7. TCE ABSs

[0150] The MBM proproteins of the disclosure comprise at least a T-cell engaging ABS that binds specifically to a T-cell receptor complex component, referred to herein as “TCE ABS”. Exemplary targets for the TCE ABS are CD3 and the T-cell receptor (*e.g.*, TCRαβ or TCRγδ). Preferably, the TCE ABS target is a human T-cell receptor complex component. The epitope of the TCE ABS can be an individual polypeptide (*e.g.*, CD3 epsilon) or a multimeric component of the T-cell receptor complex (*e.g.*, the TCRαβ dimer or the TCRγδ dimer).

[0151] Exemplary CD3 and TCR antibodies or antibody sequences are set forth in Table G below, upon which the TAA ABS can be based.

TABLE G
Exemplary T-Cell Engaging Antibodies

Target	Antibody Name and/or Binding Sequences
CD3	Catumaxomab
CD3	(ertumaxomab)
CD3	The CD3 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
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	MT110
CD3	Acapatamab (AMG160)
CD3	AMG199
CD3	AMG330
CD3	AMG427
CD3	AMG562
CD3	AMG596

TABLE G Exemplary T-Cell Engaging Antibodies	
Target	**Antibody Name and/or Binding Sequences**
CD3	AMG673
CD3	AMG701
CD3	Tarlatamab (AMG757)
CD3	AMG910
CD3	BAY2010112
CD3	AMG420
CD3	AMG424
CD3	AMG509
CD3	AMV564
CD3	APVO436
CD3	Alnuctamab (CC-93269)
CD3	ERY974
CD3	A-319
CD3	GEM333
CD3	GEM3PSCA
CD3	Cevostamab
CD3	Runimotamab
CD3	GEN1044
CD3	Epcoritamab
CD3	HPN424
CD3	ISB1302
CD3	ISB1342
CD3	IGM-2323
CD3	IMC-F106C
CD3	IMC-C103C
CD3	IMCnyeso
CD3	JNJ-63709178
CD3	JNJ-63898081
CD3	Teclistamab
CD3	Talquetamab
CD3	JNJ-67571244
CD3	MGD007
CD3	Orlotamab
CD3	Duvortuxizumab

TABLE G Exemplary T-Cell Engaging Antibodies	
Target	**Antibody Name and/or Binding Sequences**
CD3	Flotetuzumab
CD3	MCLA-117
CD3	PF-06671008
CD3	Elranatamab
CD3	Odronextamab
CD3	REGN5458
CD3	REGN5459
CD3	REGN4018
CD3	Glofitamab
CD3	RO6958688
CD3	SAR440234
CD3	TNB-383B
CD3	M802
CD3	Xmab13676
CD3	Xmab18087
CD3	Vibecotamab
CD3	Nivatrotamab
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

TABLE G Exemplary T-Cell Engaging Antibodies	
Target	**Antibody Name and/or Binding Sequences**
	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
TCRαβ	BMA031 sequences disclosed in US 2012/0034221
TCRγδ	6TCS1 antibody disclosed in US 5,980,892

[0152] In some aspects, the TCE ABS competes with a T-cell engaging (TCE) antibody set forth in Table G for binding to the TCE antibody's target (*e.g.*, CD3 or a T-cell receptor). In further aspects, the TCE ABS comprises CDRs having CDR sequences of a TCE antibody set forth in Table G. In some embodiments, the TCE ABS comprises all 6 CDR sequences of the TCE antibody set forth in Table G. In other embodiments, the TCE ABS 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 TCE ABS comprises a VH comprising the amino acid sequence of the VH of a TCE antibody set forth in Table G. In some embodiments, the TCE ABS further comprises a VL comprising the amino acid sequence of the VL of the TCE antibody set forth in Table G. In other embodiments, the TCE ABS further comprises a universal light chain VL sequence.

6.8. Anti-TCE ABSs

[0153] In certain embodiments, the MBM proproteins of the disclosure comprise an anti-TCE ABS. An anti-TCE ABS is a fragment of an anti-idiotypic antibody that is capable of specifically binding the TCE-ABS. Various types of antibody fragments may be used in the context of an anti-TCE ABS of the present disclosure including, for example, an scFv, a Fab, a VHH, and a VH. In some embodiments, the anti-TCE ABS is an scFv of an anti-idiotypic antibody. In some embodiments, the anti-TCE ABS is a VHH derived from an anti-idiotypic antibody. In some embodiments, the anti-TCE ABS is a VH derived from an anti-idiotypic antibody.

[0154] An anti-TCE ABS can be derived from a single domain antibody composed of a single VH or VL domain which exhibits sufficient affinity to the target. In a specific embodiment, the single domain antibody is a camelid VHH domain (see, *e.g.*, Riechmann, 1999, Journal of Immunological Methods 231:25-38; WO 94/04678).

[0155] Anti-idiotypic antibodies are antibodies directed against the antigen-combining region or variable region (called the idiotype or Id) of another antibody molecule. In principle, immunization with an antibody molecule expressing a paratope (antigen-combining site) for a given antigen should produce a group of anti-antibodies, some of which share with the antigen a complementary structure to the paratope.

[0156] Exemplary methods of producing anti-idiotypic antibodies US Patent No. 10,150,817 B2 and in Example 1 of WO 2017/162587 A1.

[0157] Exemplary anti-CD3 anti-idiotypic ABSs that can be used as the anti-TCE ABSs of the disclosure are the scFvs designated as 4.15.64 and 4.32.63, which are anti-idiotypes of an anti-CD3 ABS designated as CH2527. The sequences of 4.15.64, 4.32.63 and CH2527 are disclosed in WO 2017/162587 A1.

6.9. ABS formats

[0158] In certain aspects, an ABS in an MBM proprotein of the disclosure can be any type of antibody or fragment thereof that retains specific binding to an antigenic determinant. In one embodiment the ABS is an immunoglobulin molecule or fragment thereof, particularly an IgG class immunoglobulin molecule, more particularly an IgG_1 or IgG_4 immunoglobulin molecule. Antibody fragments include, but are not limited to, VH (or V_H) fragments, VL (or V_L) fragments, Fab fragments, $F(ab')_2$ fragments, scFv fragments, Fv fragments, minibodies, diabodies, triabodies, and tetrabodies.

6.9.1. Fab

[0159] Fab domains were traditionally produced by proteolytic cleavage of immunoglobulin molecules using enzymes such as papain. The Fab domains can comprise constant domain and variable region sequences from any suitable species, and thus can be murine, chimeric, human or humanized.

[0160] 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 site. A disulfide bond between the two constant domains can further stabilize the Fab domain.

[0161] For the MBM proproteins of the disclosure, particularly when the light chains of the ABSs are not common or universal light chains, it is advantageous to use Fab heterodimerization strategies to permit the correct association of Fab domains belonging to the same ABS and minimize aberrant pairing of Fab domains belonging to different ABSs. 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; 20:472-86; PMID:22014573.
orthogonal Fab VHVRD1CH1CRD2 - VLVRD1CλCRD2	39K, 62E	H172A, F174G	1R, 38D, (36F)	L135Y, S176W	Lewis *et al.*, 2014, Nat Biotechnol 32:191-8
orthogonal Fab VHVRD2CH1wt - VLVRD2Cλwt	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):e0195442

[0162] 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.

[0163] 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.

[0164] 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.

[0165] 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.

[0166] 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.

[0167] 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).

[0168] 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).

[0169] 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.

[0170] 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).

[0171] 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 the 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.

[0172] 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 unique ABS in the MBM proproteins of the disclosure. In various embodiments, employing a common light chain as described herein reduces the number of inappropriate species in the MBM proproteins as compared to employing original cognate VLs. In various embodiments, the VL domains of ABSs are identified from monospecific antibodies comprising a common light chain. In various embodiments, the VH regions of the ABSs in the MBM proproteins 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.

6.9.2. scFv

[0173] 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 non-cleavable linkers identified in Section 6.5.

[0174] 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.

[0175] The scFv can comprise VH and VL sequences from any suitable species, such as murine, human or humanized VH and VL sequences.

[0176] 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 6.5 (typically a repeat of a sequence containing the amino acids glycine

and serine, such as the amino acid sequence (Gly4~Ser)3 (SEQ ID NO: 156), 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).

6.10. Fc Regions

[0177] The MBM proproteins of the disclosure typically include 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.

[0178] The Fc domains can be derived from any suitable species operably linked to an ABS or component thereof. In one embodiment the Fc domain is derived from a human Fc domain. In preferred embodiments, the ABS or component thereof is fused to an IgG Fc molecule. An ABS or component thereof may be fused to the N-terminus or the C-terminus of the IgG Fc domain or both.

[0179] 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. Exemplary sequences of Fc domains from IgG1, IgG2, IgG3, and IgG4 are provided in Table Y, below.

TABLE Y
Fc Sequences

Fc	Sequence	SEQ ID NO
hIgG1 Fc (amino acids 99-330 of UniprotKB P01857-1)	EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVT CVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT QKSLSLSPGK	335
hIgG2 Fc (amino acids 99-326 of	ERKCCVECPPCPAPPVAGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTFRVVSVLTV	336

TABLE Y Fc Sequences		
Fc	**Sequence**	**SEQ ID NO**
UniprotKB P01859-1)	VHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTLPP SREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPM LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK	
hIgG3 Fc (amino acids 99-377 of UniprotKB P01860-1)	ELKTPLGDTTHTCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCP RCPEPKSCDTPPPCPRCPAPELLGGPSVFLFPPKPKDTLMISRTP EVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYNSTF RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPRE PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPEN NYNTTPPMLDSDGSFFLYSKLTVDKSRWQQGNIFSCSVMHEALH NRFTQKSLSLSPGK	337
hIgG4 Fc (amino acids 99-327 of UniprotKB P01861-1)	ESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTL PPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTP PVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQ KSLSLSLGK	338

[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%, about at least 93%, at least about 94%, at eat 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:336. In cases where an Fc domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:336 (*e.g.,* between 90% and 99% sequence identity to SEQ ID NO:336), 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 6.10.1) and/or one or more substitutions that promote Fc heterodimerization (*e.g.*, as described in Section 6.10.2).

[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%, about at least 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:337. In cases where an Fc domain

comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:337 (*e.g.,* between 90% and 99% sequence identity to SEQ ID NO:337), 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 6.10.1) and/or one or more substitutions that promote Fc heterodimerization (*e.g.*, as described in Section 6.10.2).

[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%, about at least 93%, at least about 94%, at eat 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:338. In cases where an Fc domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:338 (*e.g.,,* between 90% and 99% sequence identity to SEQ ID NO:338), 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 6.10.1) and/or one or more substitutions that promote Fc heterodimerization (*e.g.*, as described in Section 6.10.2).

[0183] In some aspects, an Fc domain comprises an amino acid sequence having at least about 90%, at least about 91%, at least about 92%, about at least 93%, at least about 94%, at eat 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:339. In cases where an Fc domain comprises at least 90% sequence identity and less than 100% sequence identity to SEQ ID NO:339 (*e.g.,* between 90% and 99% sequence identity to SEQ ID NO:339), 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 6.10.1) and/or one or more substitutions that promote Fc heterodimerization (*e.g.*, as described in Section 6.10.2).

[0184] 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.,* the MBM proproteins of the disclosure and MBMs produced by their activation, the Fc domains might advantageously be different to allow for heterodimerization, as described in Section 6.10.2 below.

[0185] 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.

[0186] In MBM proproteins 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.

[0187] In one embodiment the Fc region comprises CH2 and CH3 domains derived from IgG1. One or both of the Fc domains of the Fc region may have a deletion of one or more amino acids relative to a naturally occurring IgG1 CH3 sequence. In some embodiments, an Fc domain of the disclosure has a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acids at or near the C-terminus of the CH3 domain relative to a naturally occurring IgG1.

[0188] In one embodiment the Fc region comprises CH2 and CH3 domains derived from IgG2. One or both of the Fc domains of the Fc region may have a deletion of one or more amino acids relative to a naturally occurring IgG2 CH3 sequence. In some embodiments, an Fc domain of the disclosure has a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acids at or near the C-terminus of the CH3 domain relative to a naturally occurring IgG2.

[0189] In one embodiment the Fc region comprises CH2 and CH3 domains derived from IgG3. One or both of the Fc domains of the Fc region may have a deletion of one or more amino acids relative to a naturally occurring IgG3 CH3 sequence. In some embodiments, an Fc domain of the disclosure has a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acids at or near the C-terminus of the CH3 domain relative to a naturally occurring IgG3.

[0190] In one embodiment the Fc region comprises CH2 and CH3 domains derived from IgG4. One or both of the Fc domains of the Fc region may have a deletion of one or more amino acids relative to a naturally occurring IgG4 CH3 sequence. In some embodiments, an Fc domain of the disclosure has a deletion (*e.g.*, a truncation) of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more amino acids at or near the C-terminus of the CH3 domain relative to a naturally occurring IgG4.

[0191] 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.

[0192] In one embodiment the Fc region comprises CH2 and CH3 domains derived from IgG and a CH4 domain derived from IgM.

[0193] It will be appreciated that the heavy chain constant domains for use in producing an Fc region for the MBM proproteins 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.

[0194] Certain variant constant domains contemplated herein include those having a deletion (*e.g.*, truncation) of one or more amino acids at or near (*e.g.*, within 10 amino acids of) the C-terminus relative to a wild-type constant domain. In some embodiments, a constant domain of the present disclosure comprises a deletion of 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids at or near the C-terminus relative to a wild-type constant domain.

[0195] 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 MBM proproteins of the present disclosure do not comprise a tailpiece.

[0196] The Fc domains that are incorporated into the MBM proproteins 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 6.10.1.

[0197] The Fc domains can also be altered to include modifications that improve manufacturability of asymmetric MBM proproteins, for example by allowing heterodimerization, which is the preferential pairing of non-identical Fc domains over identical Fc domains. Heterodimerization permits the production of MBM proproteins 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 6.10.2.

[0198] 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 MBM proproteins.

6.10.1. Fc Domains with Altered Effector Function

[0199] In some embodiments, the Fc domain comprises one or more amino acid substitutions that reduces binding to an Fc receptor and/or effector function.

[0200] 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.

[0201] In one embodiment, the Fc domain or the Fc region (*e.g.*, one or both Fc domains of an MBM proprotein 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 Igd Fc domain or region, particularly a human Igd 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").

[0202] 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).

[0203] 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.

[0204] 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 I below: In some embodiments, the Fc domain includes only the bolded portion of the sequences shown below:

TABLE I	
Fc Domain	**Sequence**
SEQ ID NO: 323 (SEQ ID NO:1 of WO2014/121087)	Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
SEQ ID NO: 324 (SEQ ID NO:2 of WO2014/121087)	Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro

TABLE I	
Fc Domain	**Sequence**
	Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
SEQ ID NO: 325 (SEQ ID NO:30 of WO2014/121087)	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val **Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn** **Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys**
SEQ ID NO: 326 (SEQ ID NO:31 of WO2014/121087)	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val **Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn** **Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp**

TABLE I	
Fc Domain	**Sequence**
	Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys
SEQ ID NO: 327 (SEQ ID NO:37 of WO2014/121087)	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val **Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn** **Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys**
SEQ ID NO: 328 (SEQ ID NO:38 of WO2014/121087)	Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val **Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn** **Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp**

TABLE I	
Fc Domain	**Sequence**
	Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn Arg Phe Thr Gln Lys Ser Leu Ser Leu Ser Leu Gly Lys

[0205] 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.

[0206] 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).

6.10.2. Fc Heterodimerization Variants

[0207] Certain MBM proproteins entail dimerization between two Fc domains that, unlike a native immunoglobulin, are operably linked to non-identical N-terminal regions, *e.g.*, one Fc domain connected to a Fab and the other Fc domain connected to an ABS or component thereof. Inadequate heterodimerization of two Fc domains to form an Fc region has 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 MBM proproteins 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.

[0208] The present disclosure provides MBM proproteins 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.

[0209] Heterodimerization of the two different heavy chains at CH3 domains give rise to the desired MBM proprotein, while homodimerization of identical heavy chains will reduce yield

of the desired MBM proprotein. Thus, in a preferred embodiment, the polypeptides that associate to form an MBM proprotein of the disclosure will contain CH3 domains with modifications that favor heterodimeric association relative to unmodified Fc domains.

[0210] 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).

[0211] 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 (R), 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.

[0212] 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).

[0213] 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.

[0214] 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 MBM proproteins 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 MBM proprotein to Protein A as compared to a corresponding MBM proprotein 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.

[0215] 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.

6.10.3. Hinge Domains

[0216] The MBM proproteins 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 MBM proprotein 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 reion".

5 **[0217]** 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 10 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 15 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.

[0218] A number of modified hinge regions have already been described for example, in 20 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.

[0219] In one embodiment, an MBM proprotein of the disclosure comprises an Fc region in which one or both Fc domains possesses an intact hinge domain at its N-terminus.

25 **[0220]** 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.

[0221] In some embodiments, the MBM proproteins of the disclosure comprise a modified hinge region that reduces binding affinity for an Fcγ receptor relative to a wild-type hinge 30 region of the same isotype (*e.g.*, human IgG1 or human IgG4).

[0222] In one embodiment, the MBM proproteins 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: 329). The core hinge region of human IgG4 contains 35 the sequence CPSC (SEQ ID NO: 330) compared to IgG1 that contains the sequence CPPC

(SEQ ID NO: 334). 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.

6.10.3.1. Chimeric Hinge Sequences

[0223] The hinge domain can be a chimeric hinge domain.

[0224] For example, a chimeric hinge 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.

[0225] In particular embodiments, a chimeric hinge region comprises the amino acid sequence EPKSCDKTHTCPPCPAPPVA (SEQ ID NO: 331) (previously disclosed as SEQ ID NO:8 of WO2014/121087, which is incorporated by reference in its entirety herein) or ESKYGPPCPPCPAPPVA (SEQ ID NO: 332) (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 6.10.1).

6.10.3.2. Hinge Sequences with Reduced Effector Function

[0226] 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.

[0227] 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).

[0228] 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 ("CPPC" disclosed as SEQ ID NO: 329).

[0229] 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: 333) (previously disclosed as SEQ ID NO:1 of WO2016161010A2), CPPCPAPGG--GPSVF (SEQ ID NO: 26) (previously disclosed as SEQ ID NO:2 of WO2016161010A2), CPPCPAPG---GPSVF (SEQ ID NO: 106) (previously disclosed as SEQ ID NO:3 of WO2016161010A2), or CPPCPAP----GPSVF (SEQ ID NO: 152) (previously disclosed as SEQ ID NO:4 of WO2016161010A2).

[0230] The modified hinge regions described above can be incorporated into a heavy chain constant region, which typically include 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.

[0231] 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 6.10.1).

6.11. Tandem Fabs

[0232] Certain aspects of the disclosure relate to tandem Fab MBMs. The tandem Fabs MBMs comprise a TCE ABS, *e.g.*, a TCE ABS as described in Section 6.7, and a TAA ABS, *e.g.*, a TAA ABS as described in Section 6.6, both in the form of a Fab, *e.g.*, a Fab domain as described in Section 6.9.1. The TCE ABS and TAA ABS can be separated by a non-cleavable linker, for example as described in Section 6.5.

[0233] In some aspects, the disclosure provides a tandem Fab MBM comprising (1) a first polypeptide chain comprising from N- to C-terminal orientation a VH of a TCE ABS operably linked to a CH1 domain; an optional linker, *e.g.*, non-cleavable linker; and a VH of a TAA ABS operably linked to a CH1 domain; (2) a second polypeptide chain comprising a VL of the TAA ABS operably linked to a CL domain; and (3) a third polypeptide chain comprising a VL of the TCE ABS operably linked to a CL domain. In some embodiments, the tandem Fab MBM comprises (1) a first polypeptide chain comprising from N- to C-terminal orientation (a)

a VH of a TCE ABS; (b) a CH1 domain; (c) a non-cleavable linker; (d) a VH of a TAA ABS; and (c) a CH1 domain; (2) a second polypeptide chain comprising (a) a VL of the TAA ABS and (b) a CL domain; and (3) a third polypeptide chain comprising (a) a VL of the TCE ABS and (b) a CL domain.

[0234] In other aspects, the disclosure provides a tandem Fab MBM comprising (1) a first polypeptide chain comprising from N- to C-terminal orientation a VH of a TAA ABS operably linked to a CH1 domain; an optional linker, *e.g.*, non-cleavable linker; and a VH of a TCE ABS operably linked to a CH1 domain; (2) a second polypeptide chain comprising a VL of the TAA ABS operably linked to a CL domain; and (3) a third polypeptide chain comprising a VL of the TCE ABS operably linked to a CL domain. In some embodiments, the tandem Fab MBM comprises (1) a first polypeptide chain comprising from N- to C-terminal orientation (a) a VH of a TAA ABS; (b) a CH1 domain; (c) a non-cleavable linker; (d) a VH of a TCE ABS; and (c) a CH1 domain; (2) a second polypeptide chain comprising (a) a VL of the TAA ABS and (b) a CL domain; and (3) a third polypeptide chain comprising (a) a VL of the TCE ABS and (b) a CL domain.

[0235] In some embodiments, the CH1 of the TCE ABS and the CH1 of the TAA ABS are the same. In further embodiments the CL of the TCE ABS and the CL of the TAA ABS are the same. In yet further embodiments, the VL of the TAA ABS and the TCE ABS are the same and are universal light chain VLs. Exemplary universal light chains that can be incorporated into the TAA ABS and TCE ABS are set forth in Table J below.

TABLE J
Exemplary Universal Light Chains

Reference	Universal Light Chain Name and/or SEQ ID NO(s)
WO 2021/244522 A1	SEQ ID NOs: 38, 39, and 40
WO 2021/173844 A1	VL of anti-CD38003
WO 2021/123090 A1	SEQ ID NOs:101 and 103
US 2021/0206865 A1	SEQ ID NO: 129
US 2021/0206870 A1	SEQ ID NO: 3
WO 2020/229621 A1	0325L and 0128L
US 2020/0199250 A1	SEQ ID NO: 405 and SEQ ID NO: 414
US 2022/0090125 A1	SEQ ID NO:1 and SEQ ID NO:2
US 11,254,736 B2	SEQ ID NOS: 1 through 9
US 2021/0179731 A1	SEQ ID NOS: 17, 36, 200 and 204
WO 2019/190327 A2	SEQ ID NOS: 29, 35, 37 and 40
US 10,986,820 B2	ULCs encoded by nucleotides 2362 through 2686 of SEQ ID NO: 1 and nucleotides 2373 through 2697 of SEQ ID NO: 11.
US 2019/0100771 A1	SEQ ID NO:01

TABLE J Exemplary Universal Light Chains	
WO 2016/079081 A1	SEQ ID NO:31 and SEQ ID NO:35
US 2021/0206865 A1	VL of SEQ ID NO: 82 CDR-L1 of SEQ ID NO: 84 CDR-L2 of SEQ ID NO: 86 CDR-L3 of SEQ ID NO: 88

[0236] The optional linkers are preferably selected from the linkers disclosed in Section [0112]. In some embodiments, the linkers are less than 50 amino acids in length amino acids in length (*e.g.*, are 5 to 45 amino acids in length, 10 to 35 amino acids in length, or 5 to 25 amino acids in length) and comprise a glycine-serine sequence, *e.g.*, G4S (SEQ ID NO: 1) or a multimer thereof.

6.12. Nucleic Acids and Host Cells

[0237] In another aspect, the disclosure provides nucleic acids encoding the MBM proproteins and tandem Fab MBMs of the disclosure. In some embodiments, the MBM proproteins and tandem Fab MBMs are encoded by a single nucleic acid. In other embodiments, the MBM proproteins and tandem Fab MBMs can be encoded by a plurality (*e.g.*, two, three, four or more) nucleic acids.

[0238] A single nucleic acid can encode an MBM proprotein or tandem Fab MBM that comprises a single polypeptide chain, an MBM proprotein or tandem Fab MBM that comprises two or more polypeptide chains, or a portion of an MBM proprotein or tandem Fab MBM that comprises more than two polypeptide chains (for example, a single nucleic acid can encode two polypeptide chains of an MBM proprotein or tandem Fab MBM comprising three, four or more polypeptide chains, or three polypeptide chains of an MBM proprotein 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.

[0239] In some embodiments, an MBM proprotein or tandem Fab MBM comprising two or more polypeptide chains is encoded by two or more nucleic acids. The number of nucleic acids encoding an MBM proprotein or tandem Fab MBM can be equal to or less than the number of polypeptide chains in the MBM proprotein or tandem Fab MBM (for example, when more than one polypeptide chains are encoded by a single nucleic acid).

[0240] The nucleic acids of the disclosure can be DNA or RNA (*e.g.*, mRNA).

[0241] 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.

6.12.1. Vectors

[0242] The disclosure provides vectors comprising nucleotide sequences encoding an MBM proprotein, a tandem Fab MBM or a component thereof described herein, for example one or two of the polypeptide chains of a half antibody of an MBM proprotein. The vectors include, but are not limited to, a virus, plasmid, cosmid, lambda phage or a yeast artificial chromosome (YAC).

[0243] Numerous vector systems can be employed. For example, one class of vectors utilizes DNA elements which are derived from animal viruses such as, for example, bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (Rous Sarcoma Virus, MMTV or MOMLV) or SV40 virus. Another class of vectors utilizes RNA elements derived from RNA viruses such as Semliki Forest virus, Eastern Equine Encephalitis virus and Flaviviruses.

[0244] Additionally, cells which have stably integrated the DNA into their chromosomes can be selected by introducing one or more markers which allow for the selection of transfected host cells. The marker may provide, for example, prototropy to an auxotrophic host, biocide resistance (*e.g.*, antibiotics), or resistance to heavy metals such as copper, or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced into the same cell by co-transformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals.

[0245] Once the expression vector or DNA sequence containing the constructs has been prepared for expression, the expression vectors can be transfected or introduced into an appropriate host cell. Various techniques may be employed to achieve this, such as, for example, protoplast fusion, calcium phosphate precipitation, electroporation, retroviral transduction, viral transfection, gene gun, lipid-based transfection or other conventional techniques. Methods and conditions for culturing the resulting transfected cells and for recovering the expressed polypeptides are known to those skilled in the art, and may be varied or optimized depending upon the specific expression vector and mammalian host cell employed, based upon the present description.

6.12.2. Cells

[0246] The disclosure also provides host cells comprising a nucleic acid of the disclosure.

[0247] In one embodiment, the host cells are genetically engineered to comprise one or more nucleic acids described herein.

[0248] In one embodiment, the host cells are genetically engineered by using an expression cassette. The phrase "expression cassette," refers to nucleotide sequences, which are capable of affecting expression of a gene in hosts compatible with such sequences. Such cassettes may include a promoter, an open reading frame with or without introns, and a termination signal. Additional factors necessary or helpful in effecting expression may also be used, such as, for example, an inducible promoter.

[0249] The disclosure also provides host cells comprising the vectors described herein.

[0250] The cell can be, but is not limited to, a eukaryotic cell, a bacterial cell, an insect cell, or a human cell. Suitable eukaryotic cells include, but are not limited to, Vero cells, HeLa cells, COS cells, CHO cells, HEK293 cells, BHK cells and MDCKII cells. Suitable insect cells include, but are not limited to, Sf9 cells.

6.13. Pharmaceutical Compositions

[0251] The MBM proproteins and tandem Fab MBMs of the disclosure may be in the form of compositions comprising the MBM proprotein or tandem Fab 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 MBM proprotein and tandem Fab MBM and, for therapeutic uses, the mode of administration.

[0252] 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 MBM proprotein or tandem Fab MBM, 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.

[0253] Pharmaceutical compositions can be conveniently presented in unit dosage forms containing a predetermined amount of an MBM proprotein or tandem Fab MBM of the disclosure per dose. The quantity of MBM proprotein or tandem Fab 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 MBM proprotein or tandem Fab MBM 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 MBM proprotein suitable for a single administration.

[0254] The pharmaceutical compositions may also be supplied in bulk from containing quantities of MBM proprotein or tandem Fab MBM suitable for multiple administrations.

[0255] Pharmaceutical compositions may be prepared for storage as lyophilized formulations or aqueous solutions by mixing an MBM proprotein or tandem Fab MBM 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.

[0256] 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 glyconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium glyconate 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.

[0257] 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, benzalconium 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 MBM proprotein or tandem Fab MBM.

[0258] 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.

[0259] Additional miscellaneous excipients include bulking agents (*e.g.*, starch), chelating agents (*e.g.*, EDTA), antioxidants (*e.g.*, ascorbic acid, methionine, vitamin E), and cosolvents.

[0260] The MBM proproteins and tandem Fab MBMs of the disclosure can be formulated as pharmaceutical compositions comprising the MBM proproteins or tandem Fab MBMs, for example containing one or more pharmaceutically acceptable excipients or carriers. To prepare pharmaceutical or sterile compositions comprising the MBM proproteins and tandem Fab MBMs of the present disclosure, a MBM proprotein or tandem Fab MBM preparation can be combined with one or more pharmaceutically acceptable excipient or carrier.

[0261] For example, formulations of MBM proproteins and tandem Fab MBMs can be prepared by mixing MBM proproteins or tandem Fab MBMs with physiologically acceptable carriers, excipients, or stabilizers in the form of, *e.g.*, lyophilized powders, slurries, aqueous solutions, lotions, or suspensions (see, *e.g.*, Hardman *et al.,* 2001 , Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y.; Gennaro, 2000, Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wlkins, New York, N.Y.; Avis, *et al.* (eds.),1993, Pharmaceutical Dosage Forms: General Medications, Marcel Dekker, NY; Lieberman, *et al.* (eds.), 1990, Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY; Lieberman, *et al.* (eds.), 1990, Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY; Weiner and Kotkoskie, 2000, Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y.).

[0262] An effective amount for a particular subject may vary depending on factors such as the condition being treated, the overall health of the subject, the method route and dose of administration and the severity of side effects (see, *e.g.*, Maynard, et al. (1996) A Handbook of SOPs for Good Clinical Practice, Interpharm Press, Boca Raton, Fla.; Dent (2001) Good Laboratory and Good Clinical Practice, Urch Publ., London, UK).

[0263] A composition of the present disclosure may also be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Selected routes of administration for MBM proproteins and tandem Fab MBMs include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other general routes of administration, for example by injection or infusion. General administration may represent modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular,

subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, a composition of the disclosure can be administered via a non-general route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. In one embodiment, the MBM proproteins and tandem Fab MBMs are administered by infusion. In another embodiment, the MBM proprotein or tandem Fab MBM of the disclosure is administered subcutaneously.

6.14. Therapeutic Indications and Methods of Treatment

[0264] The MBM proproteins and tandem Fab MBMs of the disclosure can be used in the treatment of any proliferative disorder (*e.g.*, cancer) that expresses a TAA. In particular embodiments, the cancer is acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), adrenocortical carcinoma, anal cancer, appendix cancer, astrocytoma, basal cell carcinoma, brain tumor, bile duct cancer, bladder cancer, bone cancer, breast cancer, bronchial tumor, Burkitt Lymphoma, carcinoma of unknown primary origin, cardiac tumor, cervical cancer, chordoma, chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), chronic myeloproliferative neoplasm, colon cancer, colorectal cancer, craniopharyngioma, cutaneous T-cell lymphoma, ductal carcinoma, embryonal tumor, endometrial cancer, ependymoma, esophageal cancer, esthesioneuroblastoma, fibrous histiocytoma, Ewing sarcoma, eye cancer, germ cell tumor, gallbladder cancer, gastric cancer, gastrointestinal carcinoid tumor, gastrointestinal stromal tumor, gestational trophoblastic disease, glioma, head and neck cancer, hairy cell leukemia, hepatocellular cancer, histiocytosis, Hodgkin lymphoma, hypopharyngeal cancer, intraocular melanoma, islet cell tumor, Kaposi sarcoma, kidney cancer, Langerhans cell histiocytosis, laryngeal cancer, leukemia, lip and oral cavity cancer, liver cancer, lobular carcinoma in situ, lung cancer, lymphoma, macroglobulinemia, malignant fibrous histiocytoma, melanoma, Merkel cell carcinoma, mesothelioma, metastatic squamous neck cancer with occult primary, midline tract carcinoma involving NUT gene, mouth cancer, multiple endocrine neoplasia syndrome, multiple myeloma, mycosis fungoides, myelodysplastic syndrome, myelodysplastic/myeloproliferative neoplasm, nasal cavity and para-nasal sinus cancer, nasopharyngeal cancer, neuroblastoma, non-Hodgkin lymphoma, non-small cell lung cancer, oropharyngeal cancer, osteosarcoma, ovarian cancer, pancreatic cancer, papillomatosis, paraganglioma, parathyroid cancer, penile cancer, pharyngeal cancer, pheochromocytomas, pituitary tumor, pleuropulmonary blastoma, primary central nervous system lymphoma, prostate cancer, rectal cancer, renal cell cancer, renal pelvis and ureter cancer, retinoblastoma, rhabdoid tumor, salivary gland cancer, Sezary syndrome, skin cancer, small cell lung cancer, small intestine cancer, soft tissue sarcoma, spinal cord tumor, stomach cancer, T-cell lymphoma, teratoid tumor, testicular cancer, throat cancer, thymoma

and thymic carcinoma, thyroid cancer, urethral cancer, uterine cancer, vaginal cancer, vulvar cancer, or Wilms tumor.

[0265] Table K below shows exemplary indications for which MBM proproteins and tandem Fab MBMs targeting particular TAAs can be used.

TABLE K Examples of Tumor-Associated Antigen Indications	
Target	**Exemplary Indication(s)**
ADRB3	Ewing sarcoma
ALK	NSCLC, ALCL, IMT, neuroblastoma
B7H3	melanoma, osteosarcoma, leukemia, breast, prostate, ovarian, pancreatic, colorectal cancers
BCMA	multiple myeloma, leukemia (*e.g.*, acute lymphoblastic leukemia ("ALL"), acute myeloid leukemia ("AML"), chronic lymphocytic leukemia ("CLL"), chronic myeloid leukemia ("CML") and hairy cell leukemia ("HCL")); lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, including diffuse large B-cell lymphoma ("DLBCL"))
Cadherin 17	gastric, pancreatic, and colorectal adenocarcinomas
CAIX	clear-cell renal cell carcinoma, hypoxic solid tumors, head and neck squamous carcinoma
CD123	leukemia (*e.g.*, ALL, CLL, AML, CML, HCL); lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, *e.g.*, DLBCL); multiple myeloma. In a preferred embodiment, the indication is AML.
CD171	neuroblastoma, paraganglioma
CD179a	B cell malignancies
CD19	leukemia (*e.g.*, ALL, CLL, AML, CML, HCL); lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, *e.g.*, DLBCL); multiple myeloma.
CD20	leukemia (*e.g.*, ALL, CLL, AML, CML, HCL); lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, *e.g.*, DLBCL); multiple myeloma.
CD22	leukemia (*e.g.*, ALL, CLL, AML, CML, HCL); lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, *e.g.*, DLBCL); multiple myeloma; lung cancer
CD24	ovarian, breast, prostate, bladder, renal, non-small cell carcinomas
CD30	anaplastic large cell lymphoma, embryonal carcinoma, Hodgkin Lymphoma
CD32b	B cell malignancies, gastric, pancreatic, esophageal, glioblastoma, breast, colorectal
CD33	leukemia (*e.g.*, ALL, CLL, AML, CML, HCL); lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, *e.g.*, DLBCL); multiple myeloma. In a preferred embodiment, the indication is AML.
CD38	leukemia (*e.g.*, ALL, CLL, AML, CML, HCL); lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, *e.g.*, DLBCL); multiple myeloma
CD44v6	colon cancer, head and neck small cell carcinoma
CD97	B cell malignancies, gastric, pancreatic, esophageal, glioblastoma, breast, colorectal
CEA (CEACAM5)	colorectal carcinoma, gastric carcinoma, pancreatic carcinoma, lung cancer, breast cancer, medullary thyroid carcinoma
CLDN6	ovarian, breast, lung cancer
CLL-1	leukemia (*e.g.*, ALL, CLL, AML, CML, HCL); lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, *e.g.*, DLBCL); multiple myeloma. In a preferred embodiment, the indication is AML.
CS1 (SLAMF7)	multiple myeloma

TABLE K Examples of Tumor-Associated Antigen Indications	
Target	**Exemplary Indication(s)**
EGFR	squamous cell carcinoma of lung, anal cancer, glioblastoma, epithelial tumors of head and neck, colon cancer
EGFRvIII	Glioblastoma
EPCAM	gastrointestestinal carcinoma, colorectal cancer
EphA2	kaposi's sarcoma, glioblastoma, solid tumors, glioma
Ephrin B2	thyroid cancer, breast cancer, malignant melanoma
ERBB2 (Her2/neu)	breast, ovarian, gastric cancers, lung adenocarcinoma, non-small cell lung cancer, uterine cancer, uterine serous endometrial carcinoma, salivary duct carcinoma
FAP	pancreatic cancer, colorectal cancer, metastasis, epithelial cancers, soft tissue sarcomas
FCRL5	multiple myeloma
FLT3	leukemia (*e.g.*, ALL, CLL, AML, CML, HCL), lymphoma (*e.g.*, Hodgkin's lymphoma, non-Hodgkin's lymphoma, *e.g.*, DLBCL), multiple myeloma
Folate receptor alpha	ovarian, breast, renal, lung, colorectal, brain cancers
Folate receptor beta	ovarian cancer
Fucosyl GM1	AML, myeloma
GD2	malignant melanoma, neuroblastoma
GD3	Melanoma
GloboH	ovarian, gastric, prostate, lung, breast, and pancreatic cancers
gp100	Melanoma
GPNMB	breast cancer, head and neck cancers
GPR20	GIST
GPR64	Ewing sarcoma, prostate, kidney and lung sarcomas
GPRC5D	multiple myeloma
HAVCR1	renal cancer
HER2	HER-2 (+) adenocarcinoma of gastroesophageal junction, HER-2 positive gastric adenocarcinoma, HER2 positive carcinoma of breast
HER3	colon and gastric cancers
HMWMAA	melanoma, glioblastoma, breast cancer
IGF-I receptor	breast, prostate, lung cancers
IL11Rα	papillary thyroid cancer, osteosarcoma, colorectal adenocarcinoma, lymphocytic leukemia
IL13Rα2	renal cell carcinoma, prostate cancer, gliomas, head and neck cancer, astrocytoma
KIT	myeloid leukemia, kaposi's sarcoma, erythroleukemia, gastrointestinal stromal tumors
KLRG2	breast cancers, lung cancers and ovarian cancers.
LewisY	squamous cell lung carcinoma, lung adenocarcinoma, ovarian carcinoma, and colorectal adenocarcinoma
LMP2	prostate cancer, Hodgkin's lymphoma, nasopharyngeal carcinoma
LRP6	breast cancer
LY6K	breast, lung, ovarian, and cervical cancer
LYPD8	colorectal and gastric cancers
Mesothelin	mesothelioma, pancreatic cancer, ovarian cancer, stomach cancer, lung cancer, endometrial cancer
MUC1	breast and ovarian cancers, lung, stomach, pancreatic, prostate cancers

TABLE K Examples of Tumor-Associated Antigen Indications	
Target	**Exemplary Indication(s)**
NCAM	melanoma, Wilms' tumor, small cell lung cancer, neuroblastoma, myeloma, paraganglioma, pancreatic acinar cell carcinoma, myeloid leukemia
NY-BR-1	breast cancer
o-acetyl GD2	neuroblastoma, melanoma
OR51E2	prostate cancer
PANX3	Osteosarcoma
PLAC1	hepatocellular carcinoma
Polysialic acid	small cell lung cancer
PDGFR-beta	myelomonocytic leukemia, chronic myeloid leukemia, acute myelogenous leukemia, acute lymphoblastic leukemia
PRSS21	colon cancer, testicular cancer, ovarian cancer
PSCA	prostate cancer, gastric and bladder cancers
PSMA	prostate cancer
ROR1	metastatic cancers, chronic lymphocytic leukemia, solid tumors in lung, breast, ovarian, colon, pancreatic, sarcoma
SLC34A2	bladder cancer
SLC39A6	breast cancer, esophageal cancer
SLITRK6	breast cancer, urothelial cancer, lung cancer
SSEA-4	breast cancer, cancer stem cells, epithelial ovarian carcinoma
STEAP1	prostate cancer
STEAP2	prostate cancer (including castrate-resistant prostate cancer), bladder cancer, cervical cancer, lung cancer, colon cancer, kidney cancer, breast cancer, pancreatic cancer, stomach cancer, uterine cancer, ovarian cancer, preferably prostate cancer
TACSTD2	carcinomas, *e.g.*, non-small-cell lung cancer
TAG72	ovarian, breast, colon, lung, pancreatic cancers, gastric cancer
TEM1/CD248	colorectal cancer
TEM7R	colorectal cancer
Tn	colorectal, breast cancers, cervical, lung, stomach cancers
TSHR	thyroid cancer, multiple myeloma
Tyrosinase	prostate cancer, melanoma
UPK2	bladder cancer
VEGFR2	ovarian and pancreatic cancers, renal cell carcinoma, colorectal cancer, medullary thyroid carcinoma

[0266] Additional TAAs and corresponding indications are disclosed in, *e.g.*, Hafeez *et al.*, 2020, Molecules 25:4764, doi:10.3390/molecules25204764, particularly in Table 1. Table 1 is incorporated by reference in its entirety here.

7. NUMBERED EMBODIMENTS

[0267] 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.

[0268] In the numbered embodiments that follow, the TAA ABS preferably binds to a mammalian TAA, the TCE ABS preferably binds to a mammalian TCE, the Fc domains are preferably derived from a mammalian antibody, and the subjects preferably mammals. More preferably, the mammal is human.

[0269] The present disclosure is exemplified by the Group A and Group B numbered embodiments set forth below. Unless otherwise specified, features of any of the numbered embodiments of a particular group are applicable mutatis mutandis to the numbered embodiments of the other groups.

Group A Numbered Embodiments

1. A binding molecule comprising:

(a) a first Fc domain;

(b) a second Fc domain; and

(c) a first component of a first antigen-binding site (ABS) connected to the first Fc domain via a protease-cleavable linker (PCL),

wherein (i) the PCL is 25 or fewer amino acids in length, and/or (ii) the first ABS is sterically hindered from binding its target.

2. The binding molecule of embodiment 1, wherein the binding of the first ABS to its target is enhanced following protease cleavage of the PCL.

3. The binding molecule of embodiment 1, wherein the first ABS is sterically hindered from binding its target prior to protease cleavage of the PCL and the steric hindrance is released following protease cleavage of the PCL.

4. The binding molecule of any one of embodiments 1 to 3, wherein the PCL is 20 or fewer amino acids in length.

5. The binding molecule of any one of embodiments 1 to 3, wherein the PCL is 15 or fewer amino acids in length.

6. The binding molecule of any one of embodiments 1 to 3, wherein the PCL is 10 or fewer amino acids in length.

7. The binding molecule of any one of embodiments 1 to 6, wherein, following protease cleavage of the PCL, the first ABS has enhanced binding to its target by at least 10-fold.

8. The binding molecule of any one of embodiments 1 to 6, wherein, following protease cleavage of the PCL, the first ABS has enhanced binding to its target by at least 100-fold.

9. The binding molecule of any one of embodiments 1 to 8, wherein the first ABS is a Fab.

10. The binding molecule of any one of embodiments 1 to 8, wherein the first ABS is a Fv.

11. The binding molecule of any one of embodiments 1 to 10, wherein the first component is a VH.

12. The binding molecule of any one of embodiments 1 to 10, wherein the first component is a VL.

13. The binding molecule of any one of embodiments 1 to 12, wherein the binding molecule comprises at least two polypeptide chains, where the first polypeptide chain comprises the first Fc domain and the first component of the first ABS and the second polypeptide chain comprises the second Fc domain.

14. The binding molecule of embodiment 13, wherein the second polypeptide chain comprises a second component of the first ABS.

15. The binding molecule of embodiment 14, wherein the first component and the second component associate to form the first ABS.

16. The binding molecule of embodiment 14 or 15, wherein the first component is a VH and the second component is a VL.

17. The binding molecule of embodiment 16, wherein (1) the first polypeptide chain further comprises a CH1 C-terminal to the VH, and (2) the second polypeptide chain further comprises a CL C-terminal to the VL.

18. The binding molecule of any one of embodiments 13 to 15, which further comprises a third polypeptide chain comprising a third component of the first ABS.

19. The binding molecule of any one of embodiments 1 to 18, which is a multispecific binding molecule proprotein comprising a second ABS.

20. The binding molecule of embodiment 19, wherein (a) the first ABS is a TCE ABS and the second ABS is a TAA ABS or (b) the first ABS is a TAA ABS and the second ABS is a TCE ABS.

21. The binding molecule of embodiment 20, wherein the first ABS is the TCE ABS.

22. The binding molecule of embodiment 21, wherein the TCE ABS is capable of binding to CD3, TCRαβ, or TCRγδ.

23. The binding molecule any one of embodiments 20 to 22, wherein the TAA ABS is capable of binding to:

(a) any TAA identified in Section 6.6; or

(b) AFP, ALK, a BAGE protein, BIRC5 (survivin), BIRC7, β-catenin, brc-abl, BRCA1, BORIS, CA9, carbonic anhydrase IX, caspase-8, CALR, CEACAM5 (also known as carcinoembryonic antigen or CEA), CCR5, CD19, CD20 (MS4A1), CD22, CD30, CD40, CDK4, CEA, CTLA4, cyclin-B1, CYP1B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EpCAM, EphA2, Fra-1, FOLR1, a GAGE protein (*e.g.*, GAGE-1 or -2), GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, LMP2, MAGE proteins (*e.g.*, MAGE-1, -2, -3, -4, -6, and -12), MART-1, mesothelin, ML-IAP, Muc1, Muc2, Muc3, Muc4, Muc5, Muc16 (CA-125), MUM1, NA17, NY-BR1, NY-BR62, NY-BR85, NY-ESO1, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PLAC1, PRLR, PRAME, PSMA (FOLH1), RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, STEAP1, STEAP2, TAG-72, TGF-β, TMPRSS2, Thompson-nouvelle antigen (Tn), TRP-1, TRP-2, tyrosinase, or uroplakin-3.

24. The binding molecule of any one of embodiments 1 to 23, wherein the first Fc domain and the second Fc domain associate to form a Fc heterodimer.

25. The binding molecule of embodiment 24, wherein the Fc heterodimer comprises knob-in-hole mutations, e.g., wherein (i) the first Fc domain comprises one or more knob mutations and the second Fc domain comprises one or more hole mutations or (ii) the first Fc domain comprises one or more hole mutations and the second Fc domain comprises one or more knob mutations.

26. The binding molecule of any one of embodiments 1 to 25, wherein the first Fc domain and/or the second Fc domain comprise a star mutation.

27. The binding molecule of any one of embodiments 1 to 26, wherein a tandem Fab is produced following protease cleavage of the PCL.

28. The binding molecule of any one of embodiments 1 to 26, wherein a multispecific binding molecule comprising the first Fc domain and the second Fc domain is produced following protease cleavage of the PCL.

29. The binding molecule of any one of embodiments 1 to 28, wherein the PCL comprises a substrate sequence cleavable by any protease set forth in Table A.

30. The binding molecule of any one of embodiments 1 to 29, wherein the PCL comprises one or more substrate sequences selected from the substrate sequences set forth in Table B.

31. The binding molecule of any one of embodiments 1 to 30, wherein the PCL comprises the amino acid sequence of any of the PCL sequences selected from the sequences set forth in Table D.

32. The binding molecule of any one of embodiments 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3A.

33. The binding molecule of any one of embodiments 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3B.

34. The binding molecule of any one of embodiments 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3C.

35. The binding molecule of any one of embodiments 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3D.

36. The binding molecule of any one of embodiments 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3E.

37. The binding molecule of any one of embodiments 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3F.

38. The binding molecule of any one of embodiments 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 7.

39. The binding molecule of any one of embodiments 1 to 38, wherein the first Fc domain and/or the second Fc domain have at least about 90% sequence identity to any one of SEQ ID NOs: 335, 336, 337, and 338.

40. The binding molecule of any one of embodiments 1 to 38, wherein the first Fc domain and/or the second Fc domain have at least about 95% sequence identity to any one of SEQ ID NOs: 335, 336, 337, and 338.

41. The binding molecule of any one of embodiments 1 to 38, wherein the first Fc domain and/or the second Fc domain have at least about 95% sequence identity to any one of SEQ ID NOs: 335, 336, 337, and 338.

42. The binding molecule of any one of embodiments 1 to 41, wherein the first Fc domain and/or the second Fc domain comprise one or more amino acid substitutions that reduce effector function (*e.g.*, as described in Section 6.10.1).

43. The binding molecule of any one of embodiments 1 to 41, wherein the first Fc domain and/or the second Fc domain comprise one or more amino acid substitutions that promote heterodimerization (*e.g.*, as described in Section 6.10.2).

44. A pharmaceutical composition comprising the binding molecule of any one of embodiments 1 to 43 and an excipient.

45. A method of treating cancer, comprising administering to a subject suffering from cancer an effective amount of the binding molecule of any one of embodiments 1 to 43 or the pharmaceutical composition of embodiment 44.

46. The method of embodiment 45, in which the cancer is associated with expression of an epitope bound by the TAA ABS, *e.g.*, as set forth in Table K.

47. A nucleic acid or plurality of nucleic acids encoding the binding molecule of any one of embodiments 1 to 43.

48. A cell transfected with one or more expression vectors comprising one or more nucleic acid sequences encoding the binding molecule of any one of embodiments 1 to 43 under the control of one or more promoters.

49. A method of producing a binding molecule, comprising:

(a) culturing the cell of embodiment 48 in conditions under which the binding molecule is expressed; and

(b) recovering the binding molecule from the cell culture.

50. The method of embodiment 49, which further comprises enriching for the binding molecule and/or purifying the binding molecule.

Group B Numbered Embodiments

1. A multispecific binding molecule (MBM) proprotein, comprising

(a) a first Fc domain and a second Fc domain capable of associating to form an Fc region;

(b) a T-cell engaging antigen-binding site ("TCE ABS") C-terminal to the first Fc domain and/or the second Fc domain;

(c) at least one antigen-binding site that is capable of binding to a tumor-associated antigen ("TAA ABS"); and

(d) a protease-cleavable linker (PCL) C-terminal to the first Fc domain or the second Fc domain,

wherein the TAA ABS in the MBM proprotein is capable of binding to its target when the PCL is in the uncleaved state and the binding of the TCE ABS to its target is enhanced following protease cleavage of the PCL.

2. The MBM proprotein of embodiment 1, wherein cleavage of the PCL releases an MBM comprising the TAA ABS and the TCE ABS.

3. The MBM proprotein of embodiment 1 or embodiment 2, wherein the TCE ABS in the MBM proprotein is masked by an anti-idiotypic antibody.

4. The MBM proprotein of embodiment 1 or embodiment 2, wherein the TCE ABS in the MBM proprotein is sterically hindered from binding to its target by the Fc domain.

5. The MBM proprotein of any one of embodiments 1 to 4, wherein the MBM comprises the first and second Fc domains.

6. The MBM proprotein of embodiment 5, which has the configuration depicted in FIG. 1A.

7. The MBM proprotein of embodiment 5, which has the configuration depicted in FIG. 1B.

8. The MBM proprotein of embodiment 5, which has the configuration depicted in FIG. 1C.

9. The MBM proprotein of embodiment 5, which has the configuration depicted in FIG. 3A.

10. The MBM proprotein of embodiment 5, which has the configuration depicted in FIG. 3B.

11. The MBM proprotein of embodiment 5, which has the configuration depicted in FIG. 3C.

12. The MBM proprotein of embodiment 5, which has the configuration depicted in FIG. 3D.

13. The MBM proprotein of embodiment 5, which has the configuration depicted in FIG. 3E.

14. The MBM proprotein of embodiment 5, which has the configuration depicted in FIG. 3F.

15. The MBM proprotein of any one of embodiments 1 to 4, wherein the MBM lacks the first and second Fc domains.

16. The MBM proprotein of embodiment 15, which has the configuration depicted in FIG. 5.

17. The MBM proprotein of embodiment 15, which has the configuration depicted in FIG. 7.

18. A multispecific binding molecule (MBM) proprotein, which is optionally an MBM of any one of embodiments 1 to 8, comprising:

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

(i) an optional first linker;

(ii) a first Fc domain;

(iii) a second linker; and

(iv) a T-cell engaging antigen-binding site ("TCE ABS") or component thereof; and

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

(i) an optional third linker;

(ii) a second Fc domain capable of heterodimerizing with the first Fc domain to form an Fc heterodimer;

(iii) a fourth linker which is a protease-cleavable linker ("PCL"); and

(iv) an antigen-binding site which is an anti-idiotype of the TCE ABS ("anti-TCE ABS") or component thereof,

wherein the first polypeptide chain and/or second polypeptide chain comprises an antigen-binding site that is capable of binding to a tumor-associated antigen ("TAA ABS") or component thereof.

19. The MBM proprotein of embodiment 18, wherein the TAA ABS(s), TCE ABS and anti-TCE ABS are in the form of Fabs and wherein the TAA ABS chain(s), the TCE ABS chain and anti-TCE ABS chain comprise VH domains associated with their respective VL domains on separate polypeptide chains.

20. The MBM proprotein of embodiment 18, wherein the VL domains are universal light chain VL domains.

21. The MBM proprotein of any one of embodiments 1 to 18, wherein the anti-TCE ABS is in the form of an scFv.

22. The MBM proprotein of any one of embodiments 1 to 18, wherein the anti-TCE ABS is in the form of a VHH.

23. The MBM proprotein of any one of embodiments 1 to 18, wherein the anti-TCE ABS is in the form of a VH.

24. The MBM proprotein of any one of embodiments 18 to 20, wherein the linkers other than the PCL are non-cleavable linkers ("NCLs").

25. A multispecific binding molecule (MBM) proprotein which is optionally an MBM of any one of embodiments 1 to 5and 9 to 14, comprising:

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

(i) an optional first linker;

(ii) a first Fc domain;

(iii) a second linker; and

(iv) a T-cell engaging antigen binding site ("TCE ABS") component comprising a VH;

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

(i) an optional third linker;

(ii) a second Fc domain capable of heterodimerizing with the first Fc domain to form an Fc heterodimer;

(iii) a fourth linker; and

(iv) a T-cell engaging antigen binding site ("TCE ABS") component comprising a VL,

wherein (A) the second linker or fourth linker is a protease-cleavable linker ("PCL"), (B) the first polypeptide chain and/or second polypeptide chain comprises an antigen-binding site that is capable of binding to a tumor-associated antigen ("TAA ABS"), and (C) the TCE ABS is sterically hindered from binding to its target.

26. The MBM proprotein of embodiment 25, wherein the TCE ABS is a Fab.

27. The MBM proprotein of embodiment 26, wherein the first polypeptide chain comprises a CH1 domain C-terminal to the VH of the TCE ABS and the second polypeptide chain comprises a CL domain C-terminal to the VL of the TCE ABS.

28. The MBM proprotein of embodiment 25, wherein the TCE ABS is an Fv.

29. The MBM proprotein of embodiment 28, wherein the first polypeptide chain lacks a CH1 domain C-terminal to the VH of the TCE ABS and the second polypeptide chain lacks a CL domain C-terminal to the VL of the TCE ABS.

30. The MBM proprotein of any one of embodiments 25 to 29, wherein the TAA ABS(s) is (are) in the form of a Fabs and wherein the TAA ABS chain(s) each comprises a VH domain associated with it respective VL domain on a separate polypeptide chain.

31. The MBM proprotein of embodiment 30, wherein the VL domain on a separate polypeptide chain is universal light chain VL domain.

32. The MBM proprotein of any one of embodiments 25 to 31, wherein the linkers other than the PCL are non-cleavable linkers ("NCLs").

33. The MBM proprotein of any one of embodiments 25 to 32, wherein the second linker or fourth linker are 25 or fewer amino acids, 20 or fewer amino acids, 15 or fewer amino acids, or 10 or fewer amino acids.

34. A multispecific binding molecule (MBM) proprotein, which is optionally an MBM of any one of embodiments 1 to 8, 15, and 16, comprising:

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

(i) an optional first linker;

(ii) a first Fc domain;

(iii) a second linker; and

(iv) an antigen-binding site which is an anti-idiotype of the TCE ABS ("anti-TCE ABS") of the second polypeptide chain or component thereof; and

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

(i) an optional third linker;

(ii) a second Fc domain capable of heterodimerizing with the first Fc domain to form an Fc heterodimer;

(iii) a fourth linker which is a protease-cleavable linker ("PCL");

(iv) a T-cell engaging antigen-binding site ("TCE ABS") or component thereof;

(v) an optional fifth linker; and

(vi) an antigen-binding site that is capable of binding to a tumor-associated antigen ("TAA ABS") or component thereof.

35. The MBM proprotein of embodiment 34, wherein the TAA ABS, TCE ABS and anti-TCE ABS are in the form of Fabs and wherein the TAA ABS chain, the TCE ABS chain and anti-TCE ABS chain comprise VH domains associated with their respective VL domains on separate polypeptide chains.

36. The MBM proprotein of embodiment 35, wherein the VL domains are universal light chain VL domains.

37. The MBM proprotein of embodiment 34, wherein the anti-TCE ABS is in the form of an scFv.

38. The MBM proprotein of embodiment 34, wherein the anti-TCE ABS is in the form of a VHH.

39. The MBM proprotein of embodiment 34, wherein the anti-TCE ABS is in the form of a VH.

40. The MBM proprotein of any one of embodiments 34 to 39, wherein the linkers other than the PCL are non-cleavable linkers ("NCLs").

41. A multispecific binding molecule (MBM) proprotein which is optionally an MBM of any one of embodiments 1 to 4, 15and 17, comprising:

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

(i) an optional first linker;

(ii) a first Fc domain;

(iii) a second linker which is a protease cleavable linker ("PCL"); and

(iv) a T-cell engaging antigen binding site ("TCE ABS") component comprising a VH;

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

(i) an optional third linker;

(ii) a second Fc domain capable of heterodimerizing with the first Fc domain to form an Fc heterodimer;

(iii) a fourth linker which is a protease cleavable linker ("PCL"); and

(iv) a T-cell engaging antigen binding site ("TCE ABS") component comprising a VL,

wherein the first polypeptide chain or second polypeptide chain comprises an antigen-binding site that is capable of binding to a tumor-associated antigen ("TAA ABS") or a component thereof C-terminal to the TCE ABS chain.

42. The MBM proprotein of embodiment 41, wherein the TCE ABS is a Fab.

43. The MBM proprotein of embodiment 42, wherein the first polypeptide chain comprises a CH1 domain C-terminal to the VH of the TCE ABS and the second polypeptide chain comprises a CL domain C-terminal to the VL of the TCE ABS.

44. The MBM proprotein of embodiment 41, wherein the TCE ABS is an Fv.

45. The MBM proprotein of embodiment 44, wherein the first polypeptide chain lacks a CH1 domain C-terminal to the VH of the TCE ABS and the second polypeptide chain lacks a CL domain C-terminal to the VL of the TCE ABS.

46. The MBM proprotein of any one of embodiments 41 to 45, wherein the TAA ABS is in the form of a Fab and wherein the TAA ABS chain comprises a VH domain associated with a VL domain on a separate polypeptide chain.

47. The MBM proprotein of embodiment 46, wherein the VL domain on a separate polypeptide chain is universal light chain VL domain.

48. The MBM proprotein of any one of embodiments 41 to 47, wherein the linkers other than the PCLs are non-cleavable linkers ("NCLs").

49. The MBM proprotein of any one of embodiments 41 to 48, wherein the second linker or fourth linker is 25 or fewer amino acids, 20 or fewer amino acids, 15 or fewer amino acids, or 10 or fewer amino acids.

50. The MBM proprotein of any one of embodiments 1 to 49, wherein each PCL comprises a substrate sequence cleavable by any protease set forth in Table A.

51. The MBM proprotein of any one of embodiments 1 to 50, wherein each PCL comprises one or more substrate sequences selected from the substrate sequences set forth in Table B.

52. The MBM proprotein of any one of embodiments 1 to 51, wherein each PCL comprises one or more spacer sequences selected from the substrate sequences set forth in Table C, optionally wherein the PCL comprises or consists of any one of the following amino acid sequences:

(a) GGGGSGGGGSGGGGSISSGLLSGRSDNHGGSGGS (SEQ ID NO: 205);

(b) GGGGSGGGGSGGGGSVPLSLYSGGGSGGSGGSGS (SEQ ID NO: 206);

(c) ISSGLLSGRSDNH (SEQ ID NO: 230); and

(d) a variant of any one of the foregoing with 1-5 amino acid substitutions.

53. The MBM proprotein of any one of embodiments 1 to 52, wherein each PCL comprises the amino acid sequence of any of the PCL sequences selected from the substrate sequences set forth in Table D.

54. The MBM proprotein of any one of embodiments 1 to 53, wherein all linkers in the molecule other than the PCL(s) are non-cleavable linkers ("NCLs").

55. The MBM proprotein of embodiment 54, wherein the NCLs are selected from Table E.

56. The MBM proprotein of any one of embodiments 1 to 55, wherein the TAA ABS is capable of binding to:

(a) any TAA identified in Section 4.5; or

(b) AFP, ALK, a BAGE protein, BIRC5 (survivin), BIRC7, β-catenin, brc-abl, BRCA1, BORIS, CA9, carbonic anhydrase IX, caspase-8, CALR, CEACAM5 (also known as carcinoembryonic antigen or CEA), CCR5, CD19, CD20 (MS4A1), CD22, CD30, CD40, CDK4, CEA, CTLA4, cyclin-B1, CYP1B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EpCAM, EphA2, Fra-1, FOLR1, a GAGE protein (*e.g.*, GAGE-1 or -2), GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, LMP2, MAGE proteins (*e.g.*, MAGE-1, -2, -3, -4, -6, and -12), MART-1, mesothelin, ML-IAP, Muc1, Muc2, Muc3, Muc4, Muc5, Muc16 (CA-125), MUM1, NA17, NY-BR1, NY-BR62, NY-BR85, NY-ESO1, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PLAC1, PRLR, PRAME, PSMA (FOLH1), RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, STEAP1, STEAP2, TAG-72, TGF-β, TMPRSS2, Thompson-nouvelle antigen (Tn), TRP-1, TRP-2, tyrosinase, or uroplakin-3.

57. The MBM proprotein of any one of embodiments 1 to 56, wherein the TAA ABS (a) comprises the (i) CDR or (ii) VH and VL sequences of antibody set forth in Table F or (b) competes with the antibody set forth in Table F for binding to the TAA.

58. The MBM proprotein of any one of embodiments 1 to 57, wherein the TAA is CEACAM5.

59. The MBM proprotein of any one of embodiments 1 to 57, wherein the TAA is EpCAM.

60. The MBM proprotein of any one of embodiments 1 to 57, wherein the TAA is HER2.

61. The MBM proprotein of any one of embodiments 1 to 5571, wherein the TAA is PMSA.

62. The MBM proprotein of any one of embodiments 1 to 57, wherein the TAA is STEAP2.

63. The MBM proprotein of any one of embodiments 1 to 57, wherein the TAA is EGFR.

64. The MBM proprotein of any one of embodiments 1 to 57, wherein the TCE ABS is capable of binding to a component of the T-cell receptor (TCR) complex.

65. The MBM proprotein of embodiment 64, wherein the component of the TCR complex is CD3.

66. The MBM proprotein of embodiment 64, wherein the component of the TCR complex is TCRαβ.

67. The MBM proprotein of embodiment 64, wherein the component of the TCR complex is TCR γδ.

68. The MBM proprotein of any one of embodiments 1 to 67, wherein the TCE ABS (a) comprises the (i) CDR or (ii) VH and VL sequences of antibody set forth in Table G or (b) competes with the antibody set forth in Table G for binding to its target.

69. The MBM proprotein of any one of embodiments 1 to 68, which produces a bispecific binding molecule ("BBM") following protease cleavage of PCL.

70. The MBM proprotein of embodiment 69, wherein the BBM is bivalent.

71. The MBM proprotein of embodiment 69, wherein the BBM is trivalent.

72. The MBM proprotein of any one of embodiments 1 to 71, wherein the Fc region is heterodimeric.

73. The MBM proprotein of embodiment 72, wherein the Fc heterodimer comprises knob-in-hole mutations.

74. The MBM proprotein of any one of embodiments 1 to 72, wherein at least one Fc domain comprises a star mutation as compared to a wild type Fc domain.

75. The MBM proprotein of any one of embodiments 1 to 74, wherein the TCE ABS comprises a VL having a deletion of one or more amino acids at the N-terminus.

76. The MBM proprotein of any one of embodiments 1 to 74, wherein the TCE ABS comprises a VH having a deletion of one or more amino acids at the N-terminus.

77. The MBM proprotein of any one of embodiments 1 to 76, wherein the first Fc domain comprises a deletion of one or more amino acids at the C-terminus relative to a wild type Fc domain.

78. The MBM proprotein of embodiment 77, wherein the first Fc domain comprises a deletion of 1 amino acid at the C-terminus relative to a wild type Fc domain.

79. The MBM proprotein of embodiment 77, wherein the first Fc domain comprises a deletion of 2 amino acids at the C-terminus relative to a wild type Fc domain.

80. The MBM proprotein of embodiment 77, wherein the first Fc domain comprises a deletion of 3 amino acids at the C-terminus relative to a wild type Fc domain.

81. The MBM proprotein of embodiment 77, wherein the first Fc domain comprises a deletion of 4 amino acids at the C-terminus relative to a wild type Fc domain.

82. The MBM proprotein of embodiment 77, wherein the first Fc domain comprises a deletion of 5 amino acids at the C-terminus relative to a wild type Fc domain.

83. The MBM proprotein of embodiment 77, wherein the first Fc domain comprises a deletion of 6 amino acids at the C-terminus relative to a wild type Fc domain.

84. The MBM proprotein of any one of embodiments 1 to 83, wherein the second Fc domain comprises a deletion of one or more amino acids at the C-terminus relative to a wild type Fc domain.

85. The MBM proprotein of embodiment 84, wherein the second Fc domain comprises a deletion of 1 amino acid at the C-terminus relative to a wild type Fc domain.

86. The MBM proprotein of embodiment 84, wherein the second Fc domain comprises a deletion of 2 amino acids at the C-terminus relative to a wild type Fc domain.

87. The MBM proprotein of embodiment 84, wherein the second Fc domain comprises a deletion of 3 amino acids at the C-terminus relative to a wild type Fc domain.

88. The MBM proprotein of embodiment 84, wherein the second Fc domain comprises a deletion of 4 amino acids at the C-terminus relative to a wild type Fc domain.

89. The MBM proprotein of embodiment 84, wherein the second Fc domain comprises a deletion of 5 amino acids at the C-terminus relative to a wild type Fc domain.

90. The MBM proprotein of embodiment 84, wherein the second Fc domain comprises a deletion of 6 amino acids at the C-terminus relative to a wild type Fc domain.

91. A tandem Fab MBM comprising (1) a first polypeptide chain comprising from N- to C-terminal orientation a VH of a TCE ABS operably linked to a CH1 domain; an optional linker, e.g., non-cleavable linker; and a VH of a TAA ABS operably linked to a CH1 domain; (2) a second polypeptide chain comprising a VL of the TAA ABS operably linked to a CL domain; and (3) a third polypeptide chain comprising a VL of the TCE ABS operably linked to a CL domain.

92. A tandem Fab MBM comprising (1) a first polypeptide chain comprising from N- to C-terminal orientation (a) a VH of a TCE ABS; (b) a CH1 domain; (c) a non-

cleavable linker; (d) a VH of a TAA ABS; and (c) a CH1 domain; (2) a second polypeptide chain comprising (a) a VL of the TAA ABS and (b) a CL domain; and (3) a third polypeptide chain comprising (a) a VL of the TCE ABS and (b) a CL domain.

93. A tandem Fab MBM comprising tandem Fab MBM comprising (1) a first polypeptide chain comprising from N- to C-terminal orientation a VH of a TAA ABS operably linked to a CH1 domain; an optional linker, e.g., non-cleavable linker; and a VH of a TCE ABS operably linked to a CH1 domain; (2) a second polypeptide chain comprising a VL of the TAA ABS operably linked to a CL domain; and (3) a third polypeptide chain comprising a VL of the TCE ABS operably linked to a CL domain.

94. A tandem Fab MBM comprising (1) a first polypeptide chain comprising from N- to C-terminal orientation (a) a VH of a TAA ABS; (b) a CH1 domain; (c) a non-cleavable linker; (d) a VH of a TCE ABS; and (c) a CH1 domain; (2) a second polypeptide chain comprising (a) a VL of the TAA ABS and (b) a CL domain; and (3) a third polypeptide chain comprising (a) a VL of the TCE ABS and (b) a CL domain.

95. The tandem Fab MBM of any one of embodiments 91 to 94, wherein the CH1 of the TCE ABS and the CH1 of the TAA ABS are the same.

96. The tandem Fab MBM of any one of embodiments 91 to 95, wherein the CL of the TCE ABS and the CL of the TAA ABS are the same.

97. The tandem Fab MBM of any one of embodiments 91 to 9674, wherein the VL of the TAA ABS and the TCE ABS are the same and are universal light chain VLs.

98. The tandem Fab MBM of any one of embodiments 91 to 97, wherein the TAA ABS is as defined in any one of embodiments 50 to 57.

99. The tandem Fab MBM of any one of embodiments 91 to 98, wherein the TCE ABS is as defined in any one of embodiments 64 to 68.

100. A pharmaceutical composition comprising the MBM proprotein of any one of embodiments 1 to 90 or the tandem Fab MBM of any one of embodiments 91 to 99 and an excipient.

101. A method of treating cancer, comprising administering to a subject suffering from cancer an effective amount of the MBM proprotein of any one of embodiments 1 to 90, the tandem Fab MBM of any one of embodiments 91 to 99, or the pharmaceutical composition of embodiment 100.

102. The method of embodiment 101, in which the cancer is associated with expression of an epitope bound by the TAA ABS, *e.g.*, as set forth in Table K.

103. A nucleic acid or plurality of nucleic acids encoding the MBM proprotein of any one of embodiments 1 to 90 or the tandem Fab MBM of any one of embodiments 91 to 99.

104. A cell engineered to express the MBM proprotein of any one of embodiments 1 to 90 or the tandem Fab MBM of any one of embodiments 91 to 99.

105. A cell transfected with one or more expression vectors comprising one or more nucleic acid sequences encoding the MBM proprotein of any one of embodiments 1 to 90 or the tandem Fab MBM of any one of embodiments 91 to 99 under the control of one or more promoters.

106. A method of producing a MBM proprotein or tandem Fab MBM, comprising:

(a) culturing the cell of embodiment 104 or embodiment 105 in conditions under which the MBM is expressed; and

(b) recovering the MBM proprotein or tandem Fab MBM, as applicable, from the cell culture

107. The method of embodiment 106, which further comprises enriching for the MBM proprotein or tandem Fab MBM, as applicable.

108. The method of embodiment 106 or embodiment 107, which further comprises purifying the MBM proprotein or tandem Fab MBM, as applicable.

8. EXAMPLES

8.1. Example 1: Effect of linker length on Fc-masking of C-terminal antigen binding sites

[0270] Anti-TAAxCD3 bispecific antibodies were generated having the configuration depicted in FIG. 3B with different linker lengths (5, 15 and 25 amino acids) C-terminal to the Fc domains, except that (in reference to FIG. 3B) Linker B was a non-cleavable linker. The goal of the study was to determine the effect of the linker separating the Fc domain from the C-terminal ABS or ABS chain on ABS's ability to bind its target, in this case CD3, on JURKAT cells. A traditional bispecific anti-TAAxCD3 bispecific antibody was used as a control for CD3 binding.

[0271] Flow cytometry analysis was utilized to determine binding of TAAxCD3 bispecific antibodies to JURKAT cells, followed by detection with an allophycocyanin (APC)-labeled anti-human IgG antibody. Briefly, 1x 10^5 cells/well were incubated for 30 minutes at 4°C with a serial dilution of anti-TAAxCD3 bispecific antibodies. After incubation, the cells were washed twice with cold PBS containing 1% filtered FBS and an APC-labeled anti-human secondary antibody was added to the cells and incubated for an additional 30 minutes. Wells containing secondary only were used as a control.

[0272] After incubation, cells were washed, re-suspended in 200 µL cold PBS containing 1% filtered FBS and analyzed by flow cytometry on a BD FACS Canto II.

[0273] The results are shown in FIG. 9. As evident in FIG. 9, the ability of the CD3 ABS to bind its target correlated with linker length, with shorter linker lengths maximizing the steric hindrance by the Fc and resulting in the greatest inhibition of the CD3 binding, and longer linker lengths being more permissive for CD3 binding (but still reduced as compared to a traditional bispecific antibody).

8.2. Example 2: Fc steric hindrance reduces anti-TAAxCD3 bispecific antibody target binding

[0274] Anti-TAAxCD3 bispecific antibodies were generated having the configuration depicted in FIG. 7, wherein linkers A and B were either cleavable linkers as illustrated or replaced by noncleavable G4S linkers (SEQ ID NO: 1), by transient transfection in Expi293 cells. FIG. 10 shows the expression of an anti-TAAxCD3 bispecific antibody with cleavable linkers A and B (Fc-PCL-CD3-TAA) and an anti-TAAxCD3 bispecific antibody without cleavable linkers A and B (Fc-G4S-CD3-TAA) after single step Pro A purification.

[0275] Flow cytometry analysis was utilized as described in Section 8.1, whereby binding of TAAxCD3 bispecific antibodies to JURKAT cells was assessed.

[0276] Relative to the anti-TAAxCD3 bispecific antibody in IgG configuration, all anti-TAAxCD3 bispecific antibodies with Fc steric hindrance displayed inhibition of CD3 binding on JURKAT cells (FIG. 11A). Similar constructs with truncations at the C-terminus of the Fc by 4 or 6 amino acids and/or the N-terminus of the VH/VL by 1 or 2 amino acids similarly inhibited binding of CD3 to JURKAT cells (data not shown).

9. CITATION OF REFERENCES

[0277] All publications, patents, patent applications and other documents cited in this application are hereby incorporated by reference in their entireties for all purposes to the same extent as if each individual publication, patent, patent application or other document were individually indicated to be incorporated by reference for all purposes. In the event that there is an inconsistency between the teachings of one or more of the references incorporated herein and the present disclosure, the teachings of the present specification are intended.

CLAIMS

1. A binding molecule comprising:

(a) a first Fc domain;

(b) a second Fc domain; and

(c) a first component of a first antigen-binding site (ABS) connected to the first Fc domain via a protease-cleavable linker (PCL),

wherein (i) the PCL is 25 or fewer amino acids in length, and/or (ii) the first ABS is sterically hindered from binding its target.

2. The binding molecule of claim 1, wherein the binding of the first ABS to its target is enhanced following protease cleavage of the PCL.

3. The binding molecule of claim 1, wherein the first ABS is sterically hindered from binding its target prior to protease cleavage of the PCL and the steric hindrance is released following protease cleavage of the PCL.

4. The binding molecule of any one of claims 1 to 3, wherein the PCL is 20 or fewer amino acids in length.

5. The binding molecule of any one of claims 1 to 3, wherein the PCL is 15 or fewer amino acids in length.

6. The binding molecule of any one of claims 1 to 3, wherein the PCL is 10 or fewer amino acids in length.

7. The binding molecule of any one of claims 1 to 6, wherein, following protease cleavage of the PCL, the first ABS has enhanced binding to its target by at least 10-fold.

8. The binding molecule of any one of claims 1 to 6, wherein, following protease cleavage of the PCL, the first ABS has enhanced binding to its target by at least 100-fold.

9. The binding molecule of any one of claims 1 to 8, wherein the first ABS is a Fab.

10. The binding molecule of any one of claims 1 to 8, wherein the first ABS is a Fv.

11. The binding molecule of any one of claims 1 to 10, wherein the first component is a VH.

12. The binding molecule of any one of claims 1 to 10, wherein the first component is a VL.

13. The binding molecule of any one of claims 1 to 12, wherein the binding molecule comprises at least two polypeptide chains, where the first polypeptide chain comprises the first Fc domain and the first component of the first ABS and the second polypeptide chain comprises the second Fc domain.

14. The binding molecule of claim 13, wherein the second polypeptide chain comprises a second component of the first ABS.

15. The binding molecule of claim 14, wherein the first component and the second component associate to form the first ABS.

16. The binding molecule of claim 14 or 15, wherein the first component is a VH and the second component is a VL.

17. The binding molecule of claim 16, wherein (1) the first polypeptide chain further comprises a CH1 C-terminal to the VH, and (2) the second polypeptide chain further comprises a CL C-terminal to the VL.

18. The binding molecule of any one of claims 13 to 15, which further comprises a third polypeptide chain comprising a third component of the first ABS.

19. The binding molecule of any one of claims 1 to 18, which is a multispecific binding molecule comprising a second ABS.

20. The binding molecule of claim 19, wherein (a) the first ABS is a TCE ABS and the second ABS is a TAA ABS or (b) the first ABS is a TAA ABS and the second ABS is a TCE ABS.

21. The binding molecule of claim 20, wherein the first ABS is the TCE ABS.

22. The binding molecule of claim 21, wherein the TCE ABS is capable of binding to CD3, TCRαβ, or TCRγδ.

23. The binding molecule any one of claims 20 to 22, wherein the TAA ABS is capable of binding to:

(a) any TAA identified in Section 6.6; or

(b) AFP, ALK, a BAGE protein, BIRC5 (survivin), BIRC7, β-catenin, brc-abl, BRCA1, BORIS, CA9, carbonic anhydrase IX, caspase-8, CALR, CEACAM5 (also known as carcinoembryonic antigen or CEA), CCR5, CD19, CD20 (MS4A1), CD22, CD30, CD40, CDK4, CEA, CTLA4, cyclin-B1, CYP1B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EpCAM, EphA2, Fra-1, FOLR1, a GAGE protein (*e.g.*, GAGE-1 or -2), GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, LMP2, MAGE proteins (*e.g.*, MAGE-1, -2, -3, -4, -6, and -12), MART-1, mesothelin, ML-IAP, Muc1, Muc2, Muc3, Muc4, Muc5, Muc16 (CA-125), MUM1, NA17, NY-BR1, NY-BR62, NY-BR85, NY-ESO1, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PLAC1, PRLR, PRAME, PSMA (FOLH1), RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, STEAP1, STEAP2, TAG-72, TGF-β, TMPRSS2, Thompson-nouvelle antigen (Tn), TRP-1, TRP-2, tyrosinase, or uroplakin-3.

24. The binding molecule of any one of claims 1 to 23, wherein the first Fc domain and the second Fc domain associate to form a Fc heterodimer.

25. The binding molecule of claim 24, wherein the Fc heterodimer comprises knob-in-hole mutations, e.g., wherein (i) the first Fc domain comprises one or more knob mutations and the second Fc domain comprises one or more hole mutations or (ii) the first Fc domain comprises one or more hole mutations and the second Fc domain comprises one or more knob mutations.

26. The binding molecule of any one of claims 1 to 25, wherein the first Fc domain and/or the second Fc domain comprise a star mutation.

27. The binding molecule of any one of claims 1 to 26, wherein a tandem Fab is produced following protease cleavage of the PCL.

28. The binding molecule of any one of claims 1 to 26, wherein a multispecific binding molecule comprising the first Fc domain and the second Fc domain is produced following protease cleavage of the PCL.

29. The binding molecule of any one of claims 1 to 28, wherein the PCL comprises a substrate sequence cleavable by any protease set forth in Table A.

30. The binding molecule of any one of claims 1 to 29, wherein the PCL comprises one or more substrate sequences selected from the substrate sequences set forth in Table B.

31. The binding molecule of any one of claims 1 to 30, wherein the PCL comprises the amino acid sequence of any of the PCL sequences selected from the sequences set forth in Table D.

32. The binding molecule of any one of claims 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3A.

33. The binding molecule of any one of claims 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3B.

34. The binding molecule of any one of claims 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3C.

35. The binding molecule of any one of claims 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3D.

36. The binding molecule of any one of claims 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3E.

37. The binding molecule of any one of claims 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 3F.

38. The binding molecule of any one of claims 1 to 31, wherein the binding molecule has the configuration depicted in FIG. 7.

39. The binding molecule of any one of claims 1 to 38, wherein the first Fc domain and/or the second Fc domain have at least about 90% sequence identity to any one of SEQ ID NOs: 335, 336, 337, and 338.

40. The binding molecule of any one of claims 1 to 38, wherein the first Fc domain and/or the second Fc domain have at least about 95% sequence identity to any one of SEQ ID NOs: 335, 336, 337, and 338.

41. The binding molecule of any one of claims 1 to 38, wherein the first Fc domain and/or the second Fc domain have at least about 95% sequence identity to any one of SEQ ID NOs: 335, 336, 337, and 338.

42. The binding molecule of any one of claims 1 to 41, wherein the first Fc domain and/or the second Fc domain comprise one or more amino acid substitutions that reduce effector function (*e.g.*, as described in Section 6.10.1).

43. The binding molecule of any one of claims 1 to 41, wherein the first Fc domain and/or the second Fc domain comprise one or more amino acid substitutions that promote heterodimerization (*e.g.*, as described in Section 6.10.2).

44. A pharmaceutical composition comprising the binding molecule of any one of claims 1 to 43 and an excipient.

45. A method of treating cancer, comprising administering to a subject suffering from cancer an effective amount of the binding molecule of any one of claims 1 to 43 or the pharmaceutical composition of claim 44.

46. The method of claim 45, in which the cancer is associated with expression of an epitope bound by the TAA ABS, *e.g.*, as set forth in Table K.

47. A nucleic acid or plurality of nucleic acids encoding the binding molecule of any one of claims 1 to 43.

48. A cell transfected with one or more expression vectors comprising one or more nucleic acid sequences encoding the binding molecule of any one of claims 1 to 43 under the control of one or more promoters.

49. A method of producing a binding molecule, comprising:

(a) culturing the cell of claim 48 in conditions under which the binding molecule is expressed; and

(b) recovering the binding molecule from the cell culture.

50. The method of claim 49, which further comprises enriching for the binding molecule and/or purifying the binding molecule.

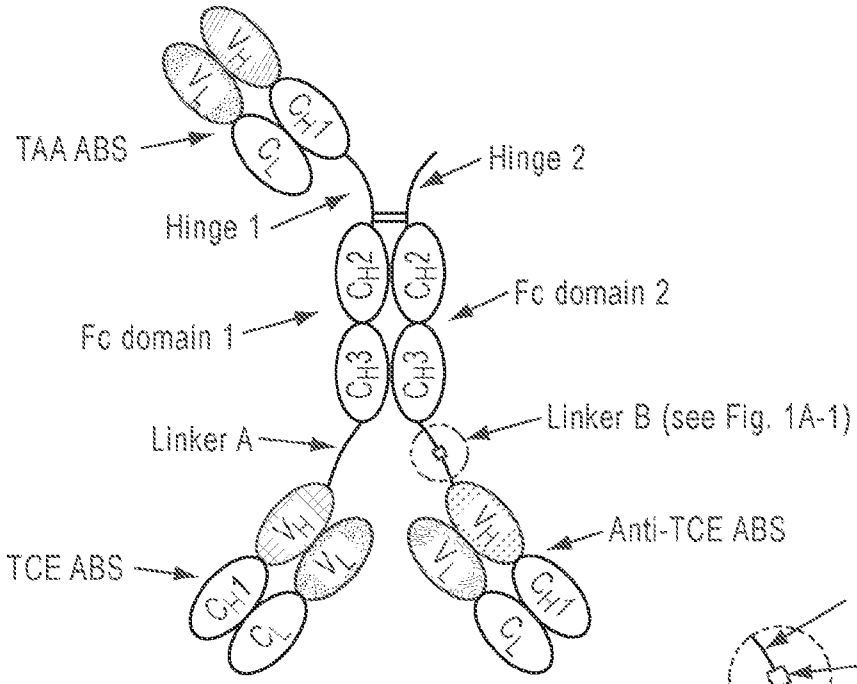


FIG. 1A

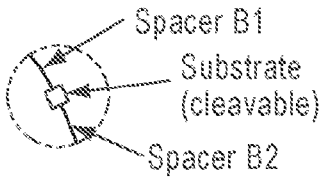


FIG. 1A-1

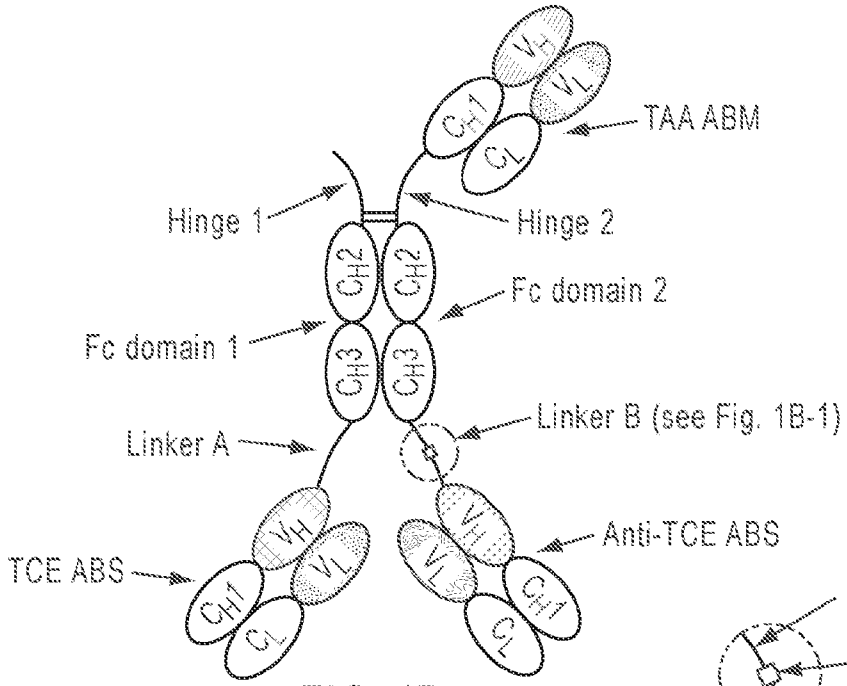


FIG. 1B

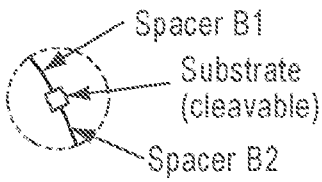


FIG. 1B-1

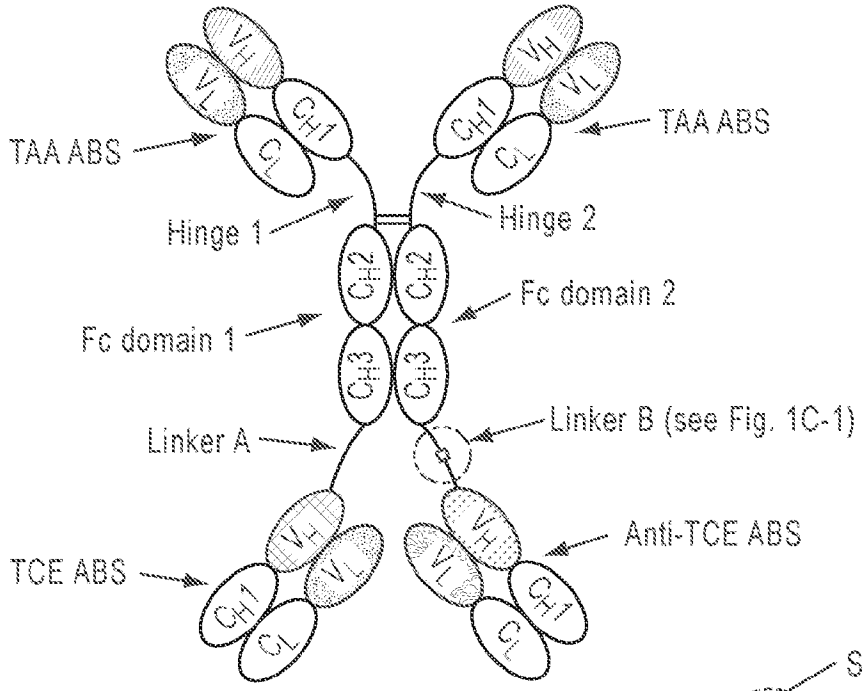


FIG. 1C

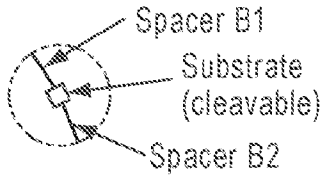


FIG. 1C-1

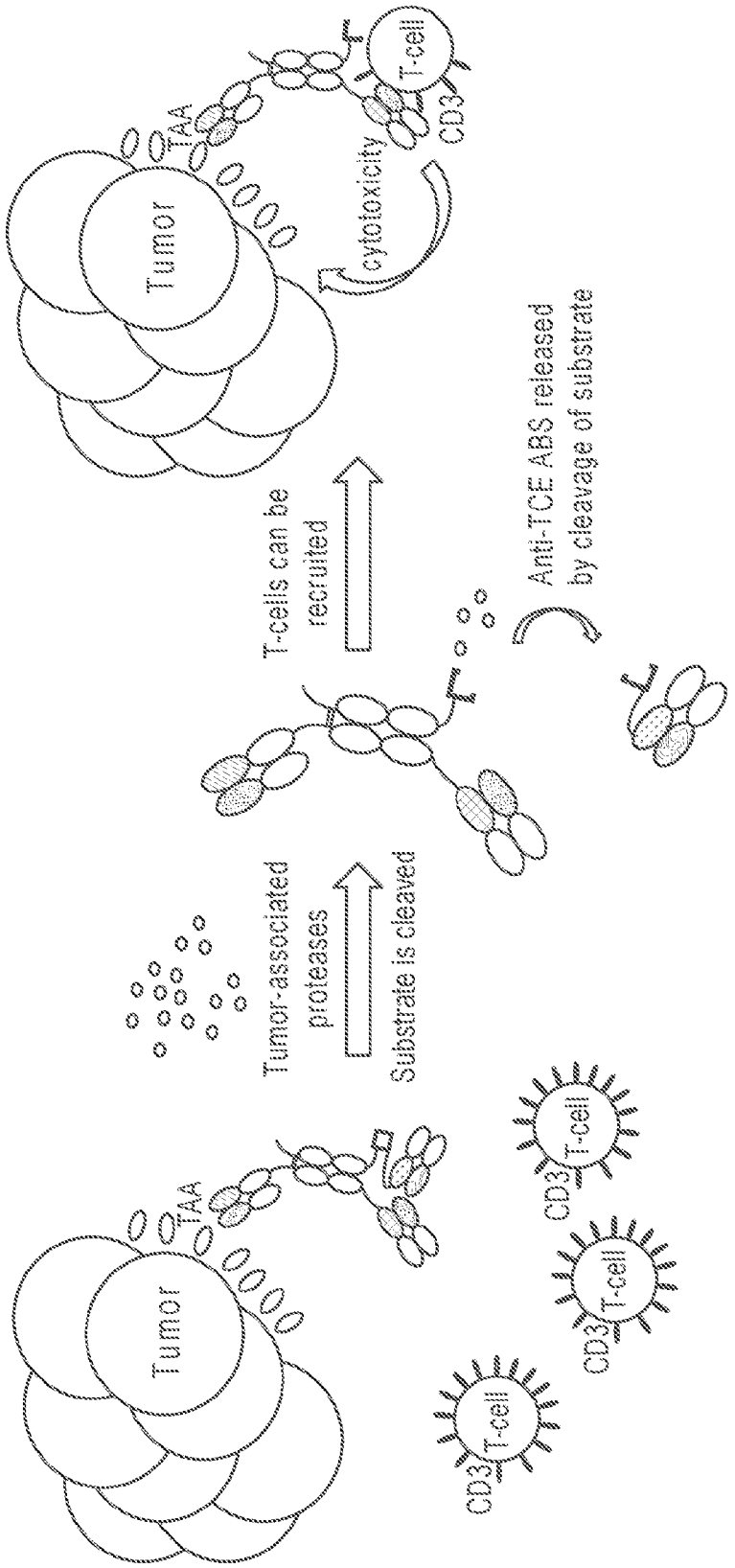

Tumor
TAA
T-cell
CD3
cytotoxicity
T-cells can be recruited
Anti-TCE ABS released by cleavage of substrate
Tumor-associated proteases
Substrate is cleaved
Tumor
TAA
T-cell
CD3
T-cell
CD3
T-cell
CD3

FIG. 2

FIG. 3A

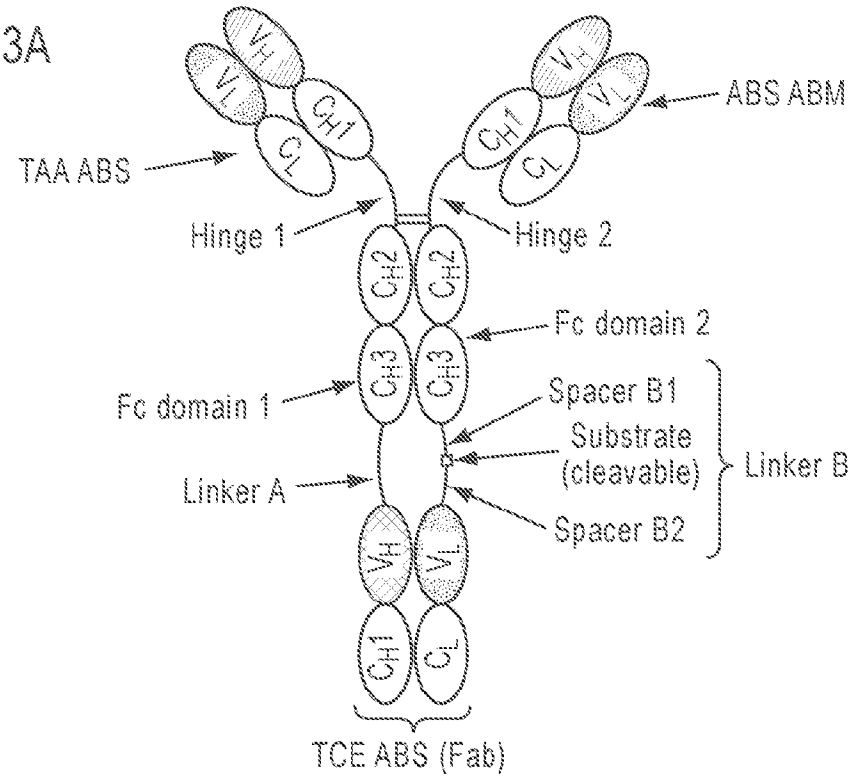

VH
VL
CH1
CL
TAA ABS
ABS ABM
Hinge 1
Hinge 2
CH2
CH2
Fc domain 2
CH3
CH3
Fc domain 1
Spacer B1
Substrate
(cleavable)
Linker B
Linker A
Spacer B2
TCE ABS (Fab)

FIG. 3B

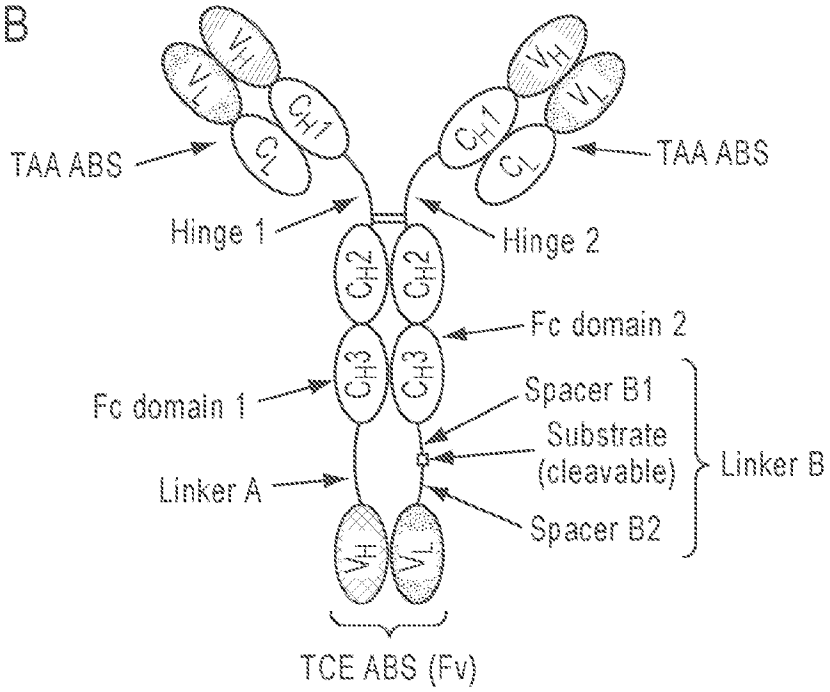

VH
VL
CH1
CL
TAA ABS
TAA ABS
Hinge 1
Hinge 2
CH2
CH2
Fc domain 2
CH3
CH3
Fc domain 1
Spacer B1
Substrate
(cleavable)
Linker B
Linker A
Spacer B2
TCE ABS (Fv)

FIG. 3C

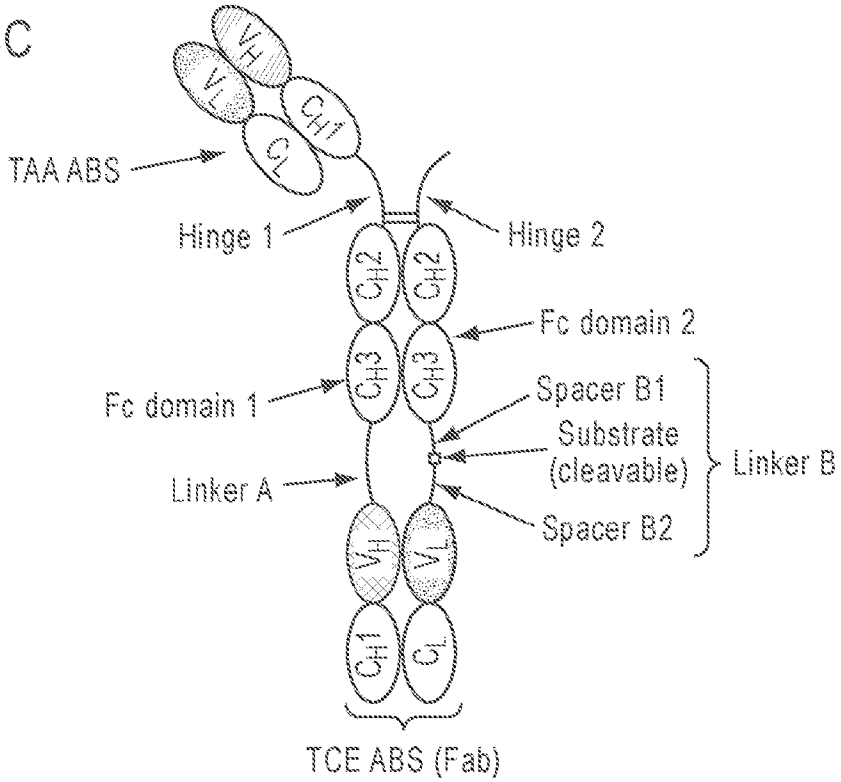

VH
VL
CH1
CL
TAA ABS
Hinge 1
Hinge 2
CH2
CH2
Fc domain 2
CH3
CH3
Fc domain 1
Spacer B1
Substrate
(cleavable)
Linker B
Linker A
Spacer B2
VH
VL
CH1
CL
TCE ABS (Fab)

FIG. 3D

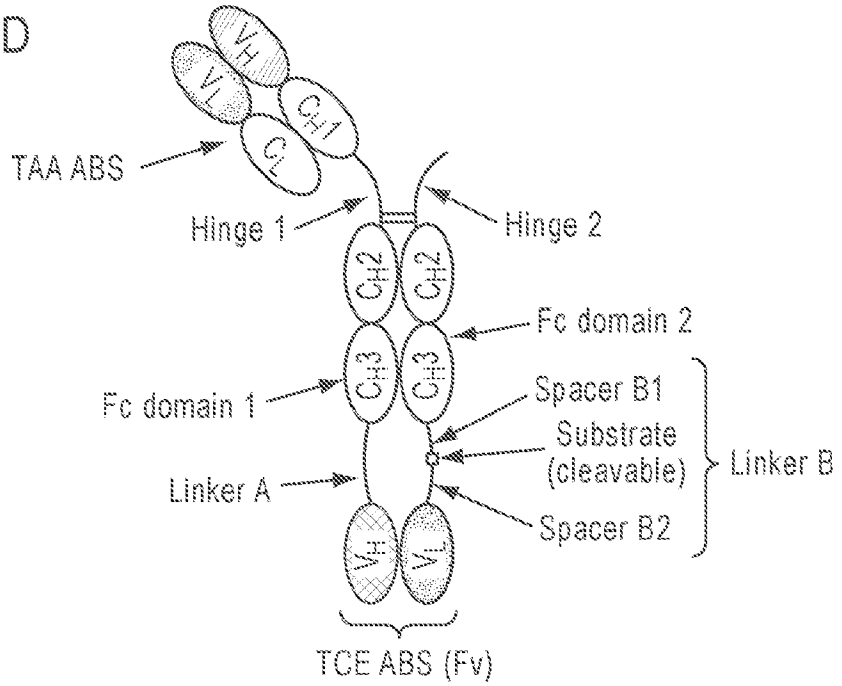

VH
VL
CH1
CL
TAA ABS
Hinge 1
Hinge 2
CH2
CH2
Fc domain 2
CH3
CH3
Fc domain 1
Spacer B1
Substrate
(cleavable)
Linker B
Linker A
Spacer B2
VH
VL
TCE ABS (Fv)

FIG. 3E

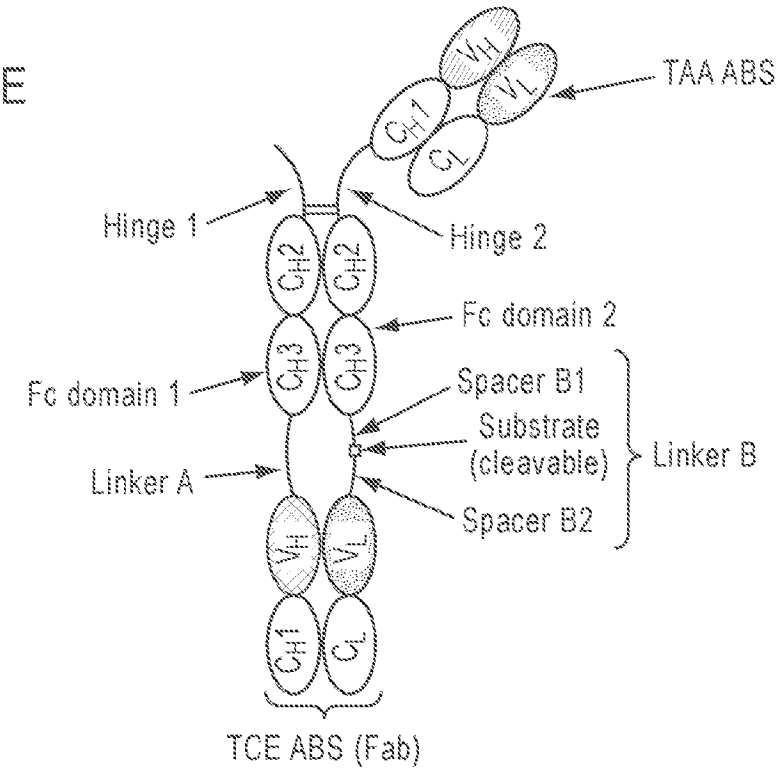
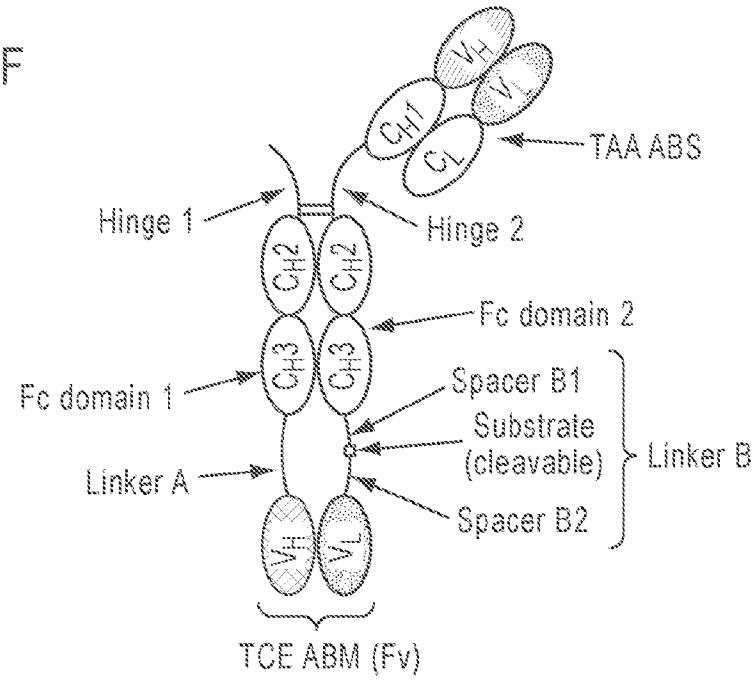

TAA ABS
VH
VL
CH1
CL
Hinge 1
Hinge 2
CH2
CH2
Fc domain 2
CH3
CH3
Fc domain 1
Spacer B1
Substrate
(cleavable)
Linker B
Linker A
Spacer B2
VH
VL
CH1
CL
TCE ABS (Fab)

FIG. 3F


VH
VL
CH1
CL
TAA ABS
Hinge 1
Hinge 2
CH2
CH2
Fc domain 2
CH3
CH3
Fc domain 1
Spacer B1
Substrate
(cleavable)
Linker B
Linker A
Spacer B2
VH
VL
TCE ABM (Fv)

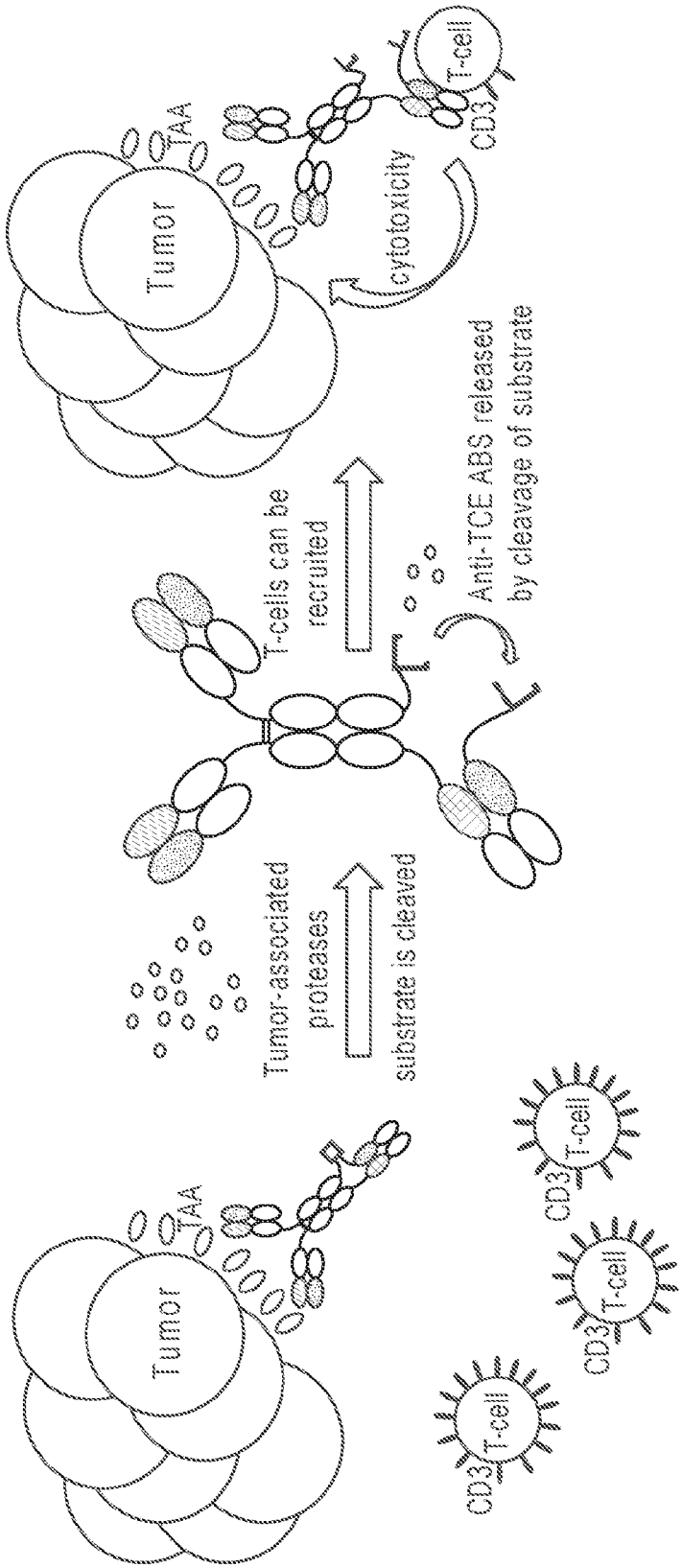

Tumor
TAA
T-cell
CD3
cytotoxicity
T-cells can be recruited
Anti-TCE ABS released by cleavage of substrate
Tumor-associated proteases
substrate is cleaved
Tumor
TAA
T-cell
CD3
T-cell
CD3
T-cell
CD3

FIG. 4

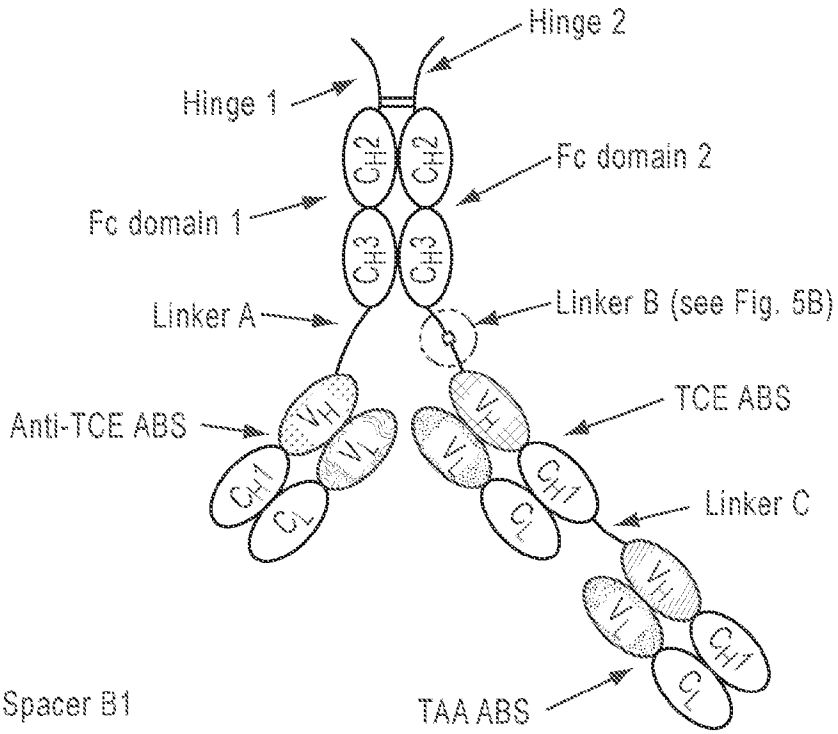

Hinge 2
Hinge 1
Fc domain 2
Fc domain 1
Linker A
Linker B (see Fig. 5B)
Anti-TCE ABS
TCE ABS
Linker C
TAA ABS

FIG. 5A

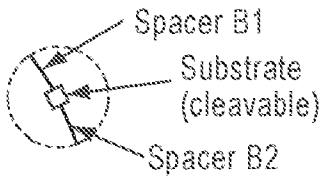

Spacer B1
Substrate
(cleavable)
Spacer B2

FIG. 5B

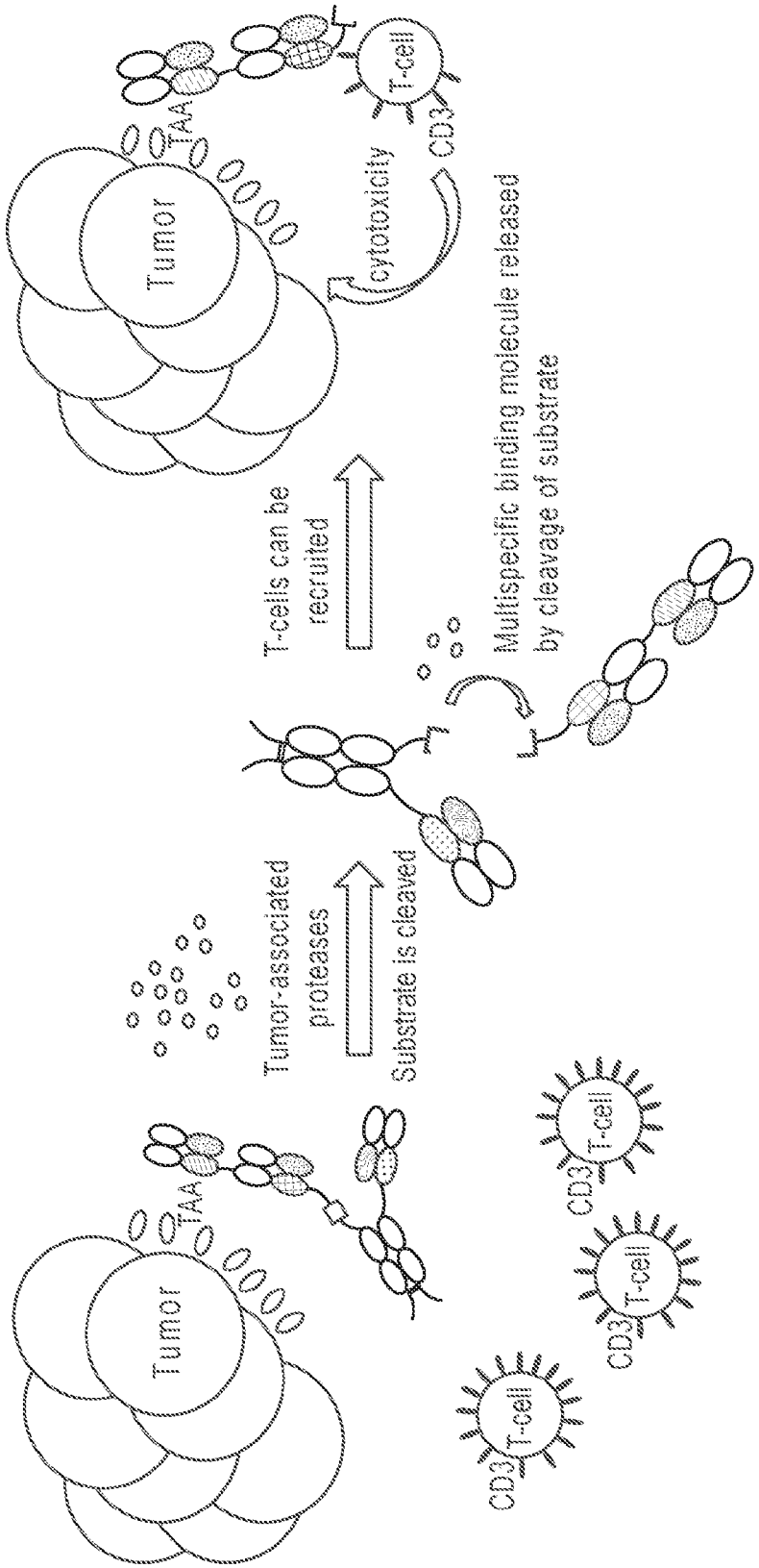

Tumor
TAA
T-cell
CD3
cytotoxicity
T-cells can be recruited
Multispecific binding molecule released by cleavage of substrate
Tumor-associated proteases
Substrate is cleaved
Tumor
TAA
CD3
T-cell
CD3
T-cell
CD3
T-cell

FIG. 6

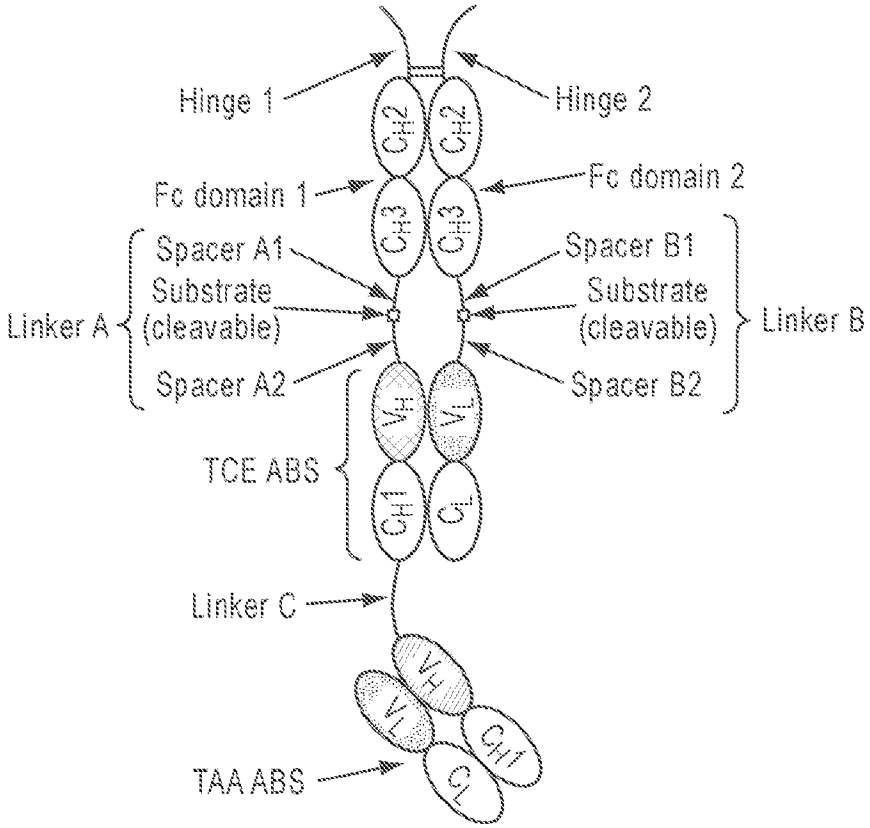

Hinge 1
Hinge 2
CH2
CH2
Fc domain 1
Fc domain 2
CH3
CH3
Spacer A1
Substrate
(cleavable)
Spacer A2
Linker A
Spacer B1
Substrate
(cleavable)
Spacer B2
Linker B
VH
VL
TCE ABS
CH1
CL
Linker C
VH
VL
CH1
CL
TAA ABS

FIG. 7

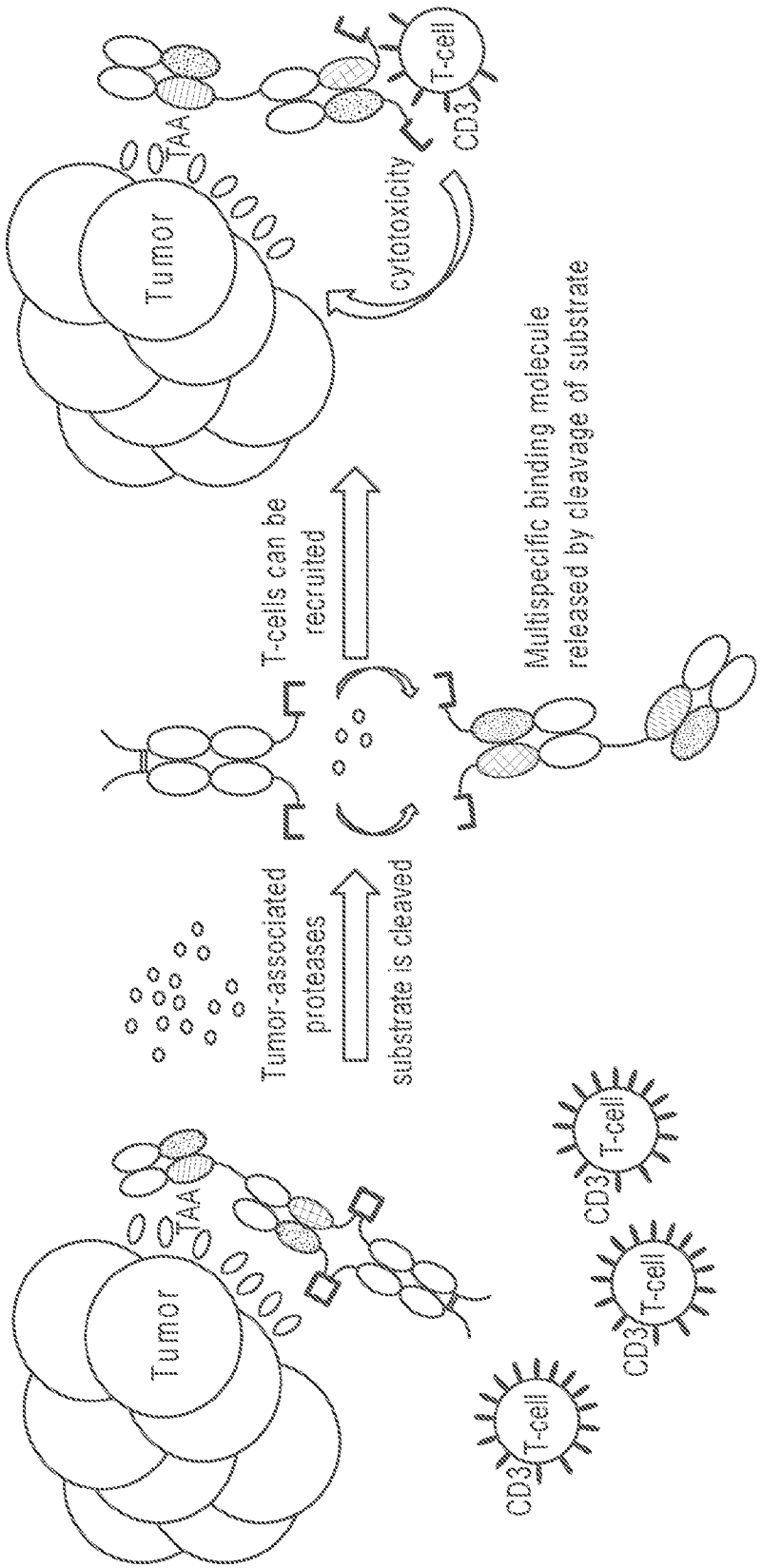

Tumor
TAA
T-cell
CD3
cytotoxicity
T-cells can be recruited
Multispecific binding molecule released by cleavage of substrate
Tumor-associated proteases
substrate is cleaved
Tumor
TAA
T-cell
CD3
T-cell
CD3
T-cell
CD3

FIG. 8

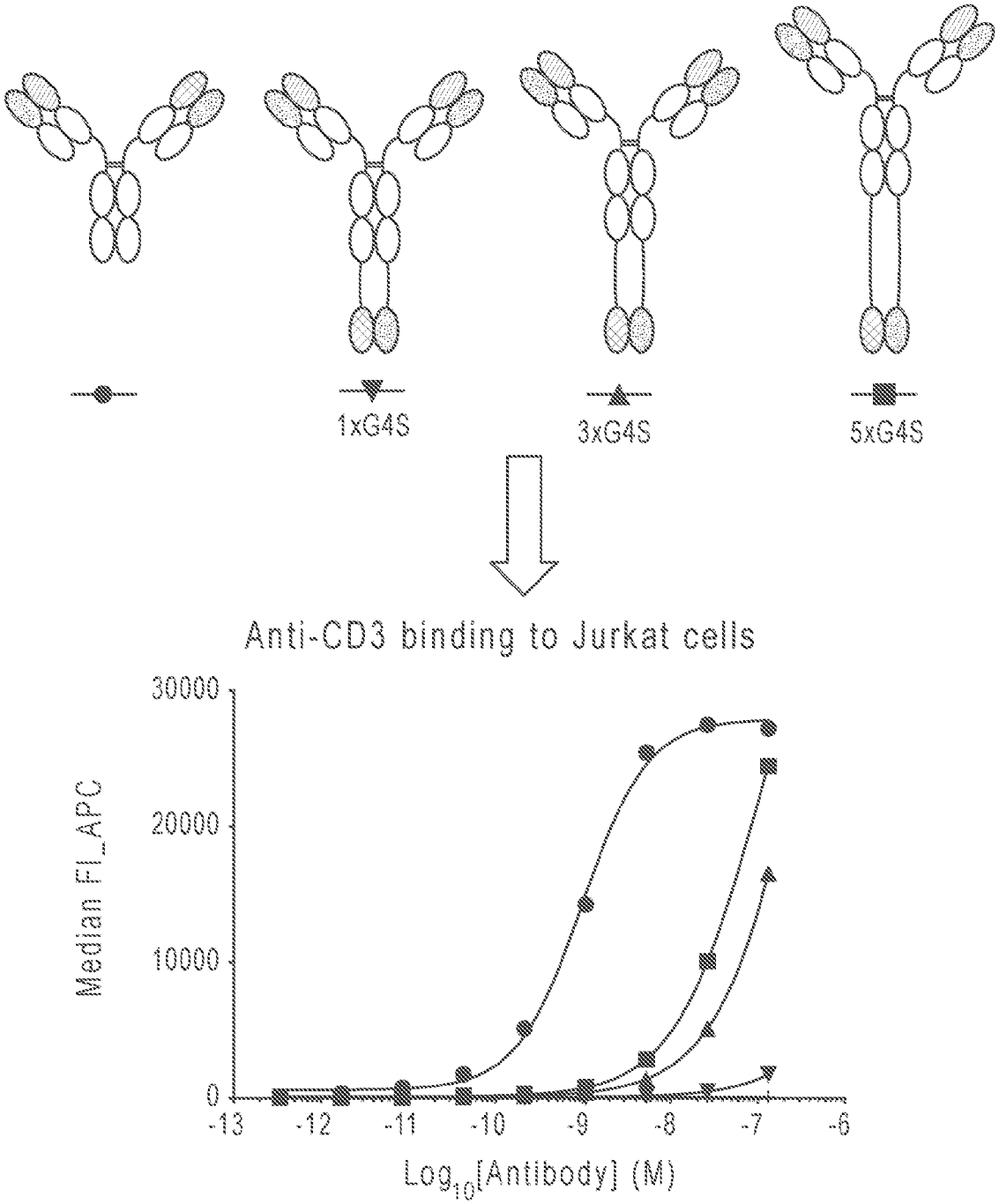
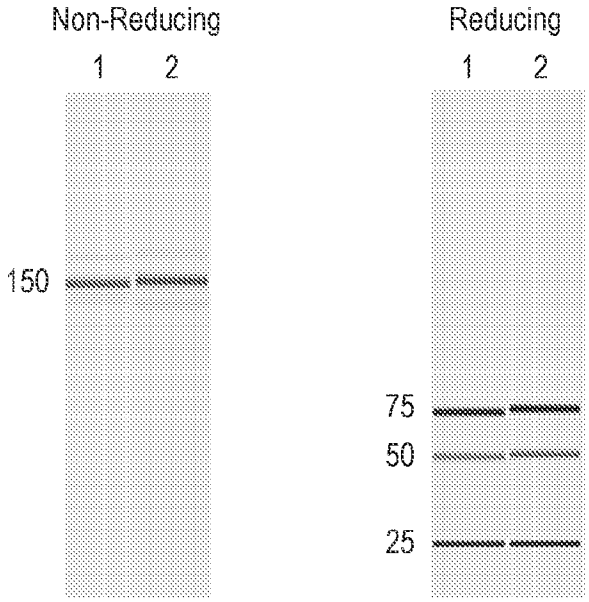

1xG4S
3xG4S
5xG4S
Anti-CD3 binding to Jurkat cells
Median FI_APC
30000
20000
10000
0
-13
-12
-11
-10
-9
-8
-7
-6
Log10[Antibody] (M)

FIG. 9



Lane	Construct
1	Fc-PCL-CD3-TAA
2	Fc-G4S-CD3-TAA

FIG. 10

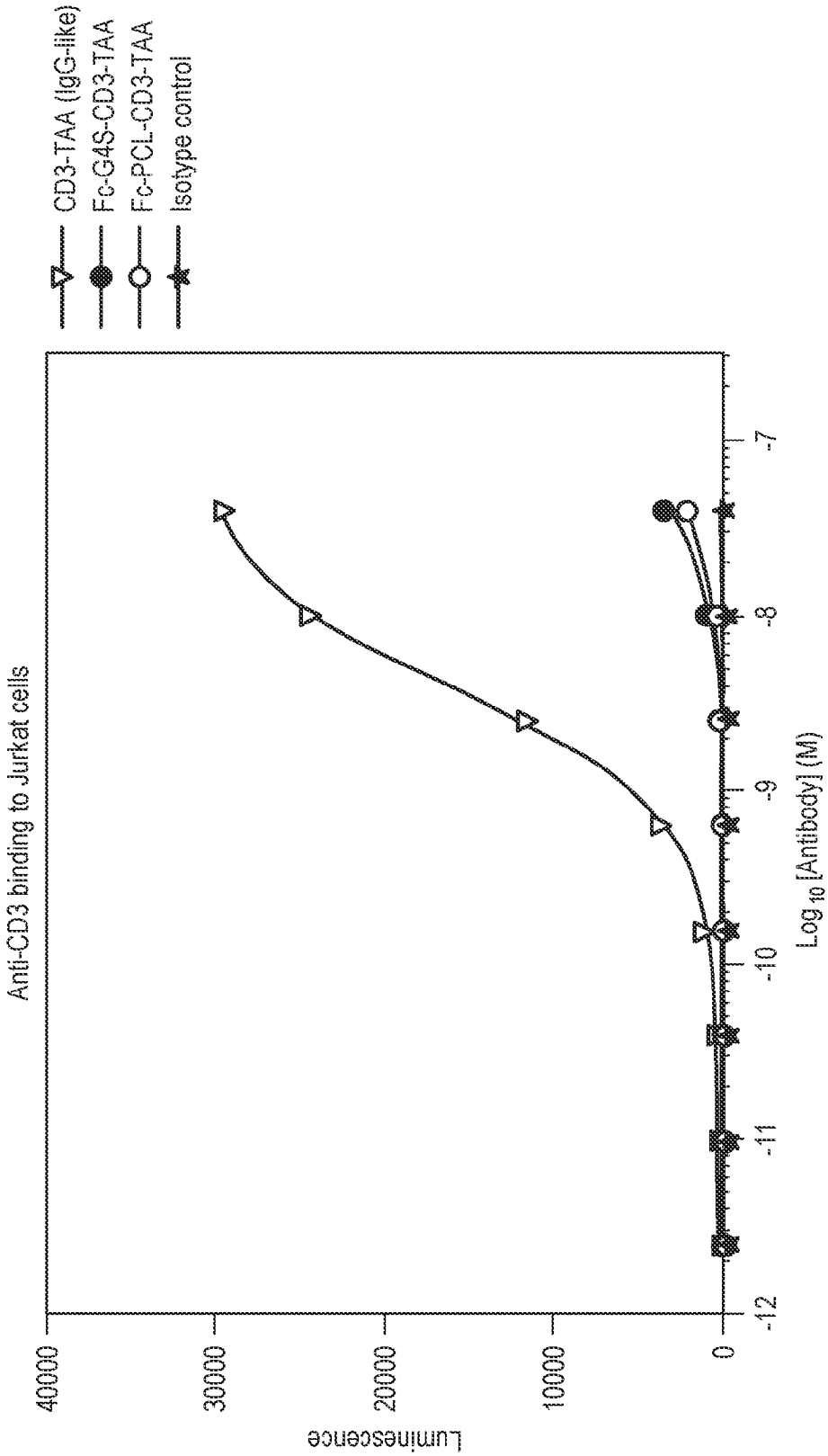

Anti-CD3 binding to Jurkat cells
CD3-TAA (IgG-like)
Fc-G4S-CD3-TAA
Fc-PCL-CD3-TAA
Isotype control
Luminescence
40000
30000
20000
10000
0
-12
-11
-10
-9
-8
-7
Log10 [Antibody] (M)

FIG. 11

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/066841

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07K16/00 C07K16/28 C07K16/30
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K

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

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

EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	S. METZ ET AL: "Bispecific antibody derivatives with restricted binding functionalities that are activated by proteolytic processing", PROTEIN ENGINEERING DESIGN AND SELECTION, vol. 25, no. 10, 1 October 2012 (2012-10-01), pages 571-580, XP055069824, ISSN: 1741-0126, DOI: 10.1093/protein/gzs064 The whole document, in particular, Fig.1; Table 1	1-28, 39-50

-/--

[x] Further documents are listed in the continuation of Box C.

[ ] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 July 2023	08/08/2023

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

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

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2023/066841

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2023/066841

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 29-38
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2023/066841

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WEN-WEI LIN ET AL: "Ab locks for improving the selectivity and safety of antibody drugs", JOURNAL OF BIOMEDICAL SCIENCE, vol. 27, no. 1, 25 June 2020 (2020-06-25), pages 1-20, XP055751564, Do ISSN: 1423-0127, DOI: 10.1186/s12929-020-00652-z The whole document, in particular, Fig.5 -----	1-28, 39-50
X	XUEJUN FAN ET AL: "A single proteolytic cleavage within the lower hinge of trastuzumab reduces immune effector function and in vivo efficacy", BREAST CANCER RESEARCH, CURRENT MEDICINE GROUP LTD, GB, vol. 14, no. 4, 8 August 2012 (2012-08-08) , page R116, XP021123342, ISSN: 1465-5411, DOI: 10.1186/BCR3240 The whole document, in particular, Fig.2 -----	1
A	ELTER ADRIAN ET AL: "Protease-Activation of Fc-Masked Therapeutic Antibodies to Alleviate Off-Tumor Cytotoxicity", FRONTIERS IN IMMUNOLOGY, vol. 12, 3 August 2021 (2021-08-03), XP093048541, DOI: 10.3389/fimmu.2021.715719 The whole document, in particular, Fig.1 -----	1

1

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: 29-38

Present claims 29 - 38 encompass compounds by reference only to the description contrary to Rule 6.2(a) PCT, or defined only by their desired function, contrary to the requirements of clarity of Article 6 PCT, because the result-to-be-achieved type of definition does not allow the scope of the claim to be ascertained. The fact that any compound could be screened does not overcome this objection, as the skilled person would not have knowledge beforehand as to whether it would fall within the scope claimed, except for the compounds disclosed in the description. Undue experimentation would be required to screen compounds randomly. The non-compliance with the substantive provisions is to such an extent that no meaningful search of claims 29- 38 could be carried out at all (Article 17(2) PCT).

The applicant/representative was informed that the search is the responsibility of the ISA under Chapter I of the PCT, the procedure before the ISA is closed and that there is no provision in the PCT for a review of or an appeal against the findings of the ISA by the IPEA.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guidelines C-IV, 7.2), should the problems which led to the Article 17(2) PCT declaration be overcome.