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(54) Title: CHIMERIC ANTIBODY IMMUNE EFFCTOR CELL ENGAGERS AND METHODS OF USE THEREOF

(57) Abstract: The present application provides a chimeric antibody immune cell engager comprising a target cell binding domain that specifically binds to an antigen on a target cell, and an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. Pharmaceutical compositions, kits and methods of treatment are also provided.

CHIMERIC ANTIBODY IMMUNE EFFCTOR CELL ENGAGERS AND METHODS OF USE THEREOF

SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0001] The contents of the following submission on ASCII text file are incorporated herein by reference in their entirety: a computer readable form (CRF) of the Sequence Listing (file name: 761422000440SEQLISTING.txt, date recorded: June 14, 2017, size: 257 KB).

FIELD OF THE PRESENT APPLICATION

[0002] The present invention relates to chimeric antibody immune effector cell engagers, and methods of preparation and use thereof. The present application further provides compositions and methods useful for human therapy, such as cancer therapy.

BACKGROUND OF THE PRESENT APPLICATION

[0003] Immunotherapy is a rapidly growing area of cancer research. Antibody therapy is an important medicinal approach to treat human diseases, especially cancer. With high specificity, bispecific antibodies can bring together two distinct antigens, and therefore have great potential as therapeutic agents. Bispecific antibodies have found wide applications in cancer immunotherapy. For example, bispecific antibodies have been engineered to simultaneously bind to a cytotoxic cell (*e.g.*, via the CD3 receptor on a T cell) and a target cell such as a tumor cell for destruction.

[0004] As part of the T cell receptor complex, the CD3 complex is an antigen expressed on mature human T cells, thymocytes and a subset of natural killer cells. In human, the T cell receptor (TCR) complex comprises TCRα and β chains as the central components. Accessory components of the TCR complex include the CD3 complex consisting of a γ chain, a δ chain, two ε chains, and two ξ chains. The intracellular tails of the CD3 molecules and the ξ chain contain immunoreceptor tyrosine-based activation motifs (ITAM), which are essential for signal transduction of the TCR complex. For example, activation of the TCR complex by binding to MHC-presented specific antigen epitopes results in phosphorylation of the ITAMs by Src family kinases, triggering recruitment of downstream kinases which results in T cell activation including Ca^{2+} release. *See*, Lin and Weiss, *Journal of Cell Science* 114, 243-244 (2001). Clustering of CD3 on T cells, *e.g.*, by immobilized anti-CD3 antibodies, leads to similar T cell

activation response as by MHC engagement of the TCR, but independent from its clonal typical specificity to MHC epitopes.

[0005] Due to TCR's central role in modulating T cell activity, there have been attempts to develop molecules capable of specific binding to TCR with much focus on the generation of antibodies specific to the human CD3 antigen. Although T cell engaging bispecific antibodies developed to date have great therapeutic potential for the treatment of malignant diseases, most of these known bispecific molecules have limited usage. For example, some are species specific, and others lack desirable safety profile, or high efficacy in targeting certain cancer antigens.

[0006] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

BRIEF SUMMARY OF THE PRESENT APPLICATION

[0007] The present application provides chimeric antibody immune effector cell engagers, including chimeric antibody T cell engagers (CATE) and chimeric antibody Natural Killer cell engagers (CANKE). Also provides are pharmaceutical compositions, and methods of treating diseases (such as cancer or autoimmune disease) using the chimeric antibody immune effector cell engagers.

[0008] One aspect of the present application provides a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising a single-domain antibody (sdAb) that specifically binds to an antigen on a target cell; and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the sdAb is camelid, chimeric, human or humanized. In some embodiments, the sdAb is a V_HH fragment. In some embodiments, the target cell is a tumor cell. In some embodiments, the target cell is a B cell.

[0009] In some embodiments according to any one of the chimeric antibody immune effector cell engagers described above, the target cell binding domain comprises an anti-BCMA sdAb. In some embodiments, the anti-BCMA sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:1; a CDR2 comprising the amino acid sequence of SEQ ID NO:12; and a CDR3 comprising the amino acid sequence of SEQ ID NO:23; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:2; a CDR2 comprising the amino acid sequence of SEQ ID NO:13; and a CDR3 comprising the amino acid sequence of SEQ ID NO:24; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:3; a CDR2

comprising the amino acid sequence of SEQ ID NO:14; and a CDR3 comprising the amino acid sequence of SEQ ID NO:25; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:4; a CDR2 comprising the amino acid sequence of SEQ ID NO:15; and a CDR3 comprising the amino acid sequence of SEQ ID NO:26; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:5; a CDR2 comprising the amino acid sequence of SEQ ID NO:16; and a CDR3 comprising the amino acid sequence of SEQ ID NO:27; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO:6; a CDR2 comprising the amino acid sequence of SEQ ID NO:17; and a CDR3 comprising the amino acid sequence of SEQ ID NO:28; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:7; a CDR2 comprising the amino acid sequence of SEQ ID NO:18; and a CDR3 comprising the amino acid sequence of SEQ ID NO:29; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:8; a CDR2 comprising the amino acid sequence of SEQ ID NO:19; and a CDR3 comprising the amino acid sequence of SEQ ID NO:30; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:9; a CDR2 comprising the amino acid sequence of SEQ ID NO:20; and a CDR3 comprising the amino acid sequence of SEQ ID NO:31; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:10; a CDR2 comprising the amino acid sequence of SEQ ID NO:21; and a CDR3 comprising the amino acid sequence of SEQ ID NO:32; or (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:11; a CDR2 comprising the amino acid sequence of SEQ ID NO:22; and a CDR3 comprising the amino acid sequence of SEQ ID NO:33. In some embodiments, the anti-BCMA sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-88.

[0010] In some embodiments according to any one of the chimeric antibody immune effector cell engagers described above, the target cell binding domain comprises an anti-CD38 sdAb. In some embodiments, the anti-CD38 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:34; a CDR2 comprising the amino acid sequence of SEQ ID NO:46; and a CDR3 comprising the amino acid sequence of SEQ ID NO:58; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:35; a CDR2 comprising the amino acid sequence of SEQ ID NO:47; and a CDR3 comprising the amino acid sequence of SEQ ID NO:59; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:36; a CDR2 comprising the amino acid sequence of SEQ ID NO:48; and a CDR3 comprising the amino acid sequence of SEQ ID NO:60; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:37;

a CDR2 comprising the amino acid sequence of SEQ ID NO:49; and a CDR3 comprising the amino acid sequence of SEQ ID NO:61; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:38; a CDR2 comprising the amino acid sequence of SEQ ID NO:50; and a CDR3 comprising the amino acid sequence of SEQ ID NO:62; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO:39; a CDR2 comprising the amino acid sequence of SEQ ID NO:51; and a CDR3 comprising the amino acid sequence of SEQ ID NO:63; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:40; a CDR2 comprising the amino acid sequence of SEQ ID NO:52; and a CDR3 comprising the amino acid sequence of SEQ ID NO:64; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:41; a CDR2 comprising the amino acid sequence of SEQ ID NO:53; and a CDR3 comprising the amino acid sequence of SEQ ID NO:65; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:42; a CDR2 comprising the amino acid sequence of SEQ ID NO:54; and a CDR3 comprising the amino acid sequence of SEQ ID NO:66; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:43; a CDR2 comprising the amino acid sequence of SEQ ID NO:55; and a CDR3 comprising the amino acid sequence of SEQ ID NO:67; or (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:44; a CDR2 comprising the amino acid sequence of SEQ ID NO:56; and a CDR3 comprising the amino acid sequence of SEQ ID NO:68. In some embodiments, the anti-CD38 sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-100.

[0011] In some embodiments according to any one of the chimeric antibody immune effector cell engagers described above, the target cell binding domain comprises a first sdAb that specifically binds to a first antigen on a first target cell and a second sdAb that specifically binds to a second antigen on a second target cell. In some embodiments, the first sdAb is fused to the second sdAb via a peptide linker. In some embodiments, the first target cell and the second target cell are the same cell. In some embodiments, the first target cell and the second target cell are different cells. In some embodiments, the first antigen and the second antigen are the same. In some embodiments, the target cell binding domain comprises two, three or more anti-BCMA sdAbs. In some embodiments, the target cell binding domain comprises two, three or more anti-CD38 sdAbs. In some embodiments, the first sdAb and the second sdAb specifically bind to the same epitope. In some embodiments, the first sdAb and the second sdAb specifically bind to different epitopes. In some embodiments, the first antigen and the second antigen are different.

In some embodiments, the target cell binding domain comprises an anti-BCMA sdAb and an anti-CD38 sdAb. In some embodiments, the anti-BCMA sdAb is fused to the N-terminus of the anti-CD38 sdAb. In some embodiments, the anti-BCMA sdAb is fused to the C-terminus of the anti-CD38 sdAb.

[0012] Another aspect of the present application provides a chimeric antibody immune effector cell engager, comprising: (a) a target cell binding domain comprising an anti-BCMA scFv; and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the anti-BCMA scFv comprises the amino acid sequence of SEQ ID NO: 101 or SEQ ID NO: 102.

[0013] In some embodiments according to any one of the chimeric antibody immune effector cell engagers described above, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain.

[0014] In some embodiments according to any one of the chimeric antibody immune effector cell engagers described above, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker.

[0015] In some embodiments according to any one of the chimeric antibody immune effector cell engagers described above, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell.

[0016] In some embodiments according to any one of the chimeric antibody immune effector cell engagers described above, the antigen-binding fragment in the immune effector cell binding domain is a Fab, scFv, or sdAb. In some embodiments, the antigen-binding fragment in the immune effector cell binding domain is murine, camelid, chimeric, human or humanized.

[0017] In some embodiments according to any one of the chimeric antibody immune effector cell engagers described above, the immune effector cell binding domain comprises an anti-CD3 antigen-binding fragment. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2

comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105.

[0018] Further provided by the present application are pharmaceutical compositions, comprising any one of the chimeric antibody immune effector cell engagers described above, and a pharmaceutically acceptable carrier.

[0019] Another aspect of the present application provides a method of treating a disease in an individual, comprising administering to the individual an effective amount of any one of the pharmaceutical compositions described above. In some embodiments, the disease is a B cell-related disorder. In some embodiments, the disease is cancer, such as multiple myeloma. In some embodiments, the disease is an autoimmune disease, such as systemic lupus erythematosus.

[0020] Also provided are methods of use, kits, and articles of manufacture comprising any one of the chimeric antibody immune effector cell engagers, isolated nucleic acids, or vectors described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1 shows expression of exemplary CATEs by 293-6E cells. Antibody expression levels in the supernatants from the cells were determined using an anti-6xHis tag-HRP based ELISA. A recombinant protein, CD123-His, was used to generate a standard curve. On average, the expression levels of the exemplary CATEs were about 1-2 μg/mL.

[0022] FIG. 2A shows dose-dependent T cell-mediated cytotoxicity against tumor cells by exemplary CATEs in a co-culture assay. The exemplary CATEs (also referred herein as "CATE-L2K.07 scFv") each comprising an anti-CD3 scFv derived from L2K.07.

[0023] FIG. 2B shows dose-dependent T cell-mediated cytotoxicity against tumor cells by exemplary CATEs in a co-culture assay. The exemplary CATEs (also referred herein as "CATE-OKT3 scFv") each comprising an anti-CD3 scFv derived from OKT3.

[0024] FIG. 3 shows *in vitro* IFNγ release by primary T cells in response to exemplary CATEs in a co-culture assay with tumor cells.

DETAILED DESCRIPTION OF THE PRESENT APPLICATION

[0025] The present application provides chimeric antibody immune effector cell engagers comprising a target cell binding domain comprising an antigen binding domain such as a single-domain antibody (*e.g.*, a V_HH) that specifically binds to an antigen on a target cell, and an immune effector cell binding domain that specifically binds to an antigen on an immune effector cell. In some embodiments, the target cell binding domain comprises two or more single-domain antibodies each specifically binding to an antigen on a target cell, thereby allowing multivalent or multispecific targeting of one or more target cells. Exemplary chimeric antibody immune effector cell engagers that target tumor cells via specific binding to BCMA and/or CD38 and engage T cells and/or NK cells via specific binding to CD3 epsilon are provided herein. The chimeric antibody immune effector cell engagers can be useful for treating a variety of diseases, including plasma cell disorders, B cell disorders, and autoimmune diseases.

[0026] Accordingly, one aspect of the present application provides a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising a single-domain antibody (sdAb) that specifically binds to an antigen on a target cell; and (b) an immune effector cell binding domain that specifically binds to an antigen on an immune effector cell. In some embodiments, the target cell binding domain comprises an anti-BCMA sdAb (such as V_HH). In some embodiments, the target cell binding domain comprises an anti-CD38 sdAb (such as V_HH). In some embodiments, the immune effector cell binding domain comprises an anti-CD3 antigen-binding fragment.

[0027] In some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a first sdAb that specifically binds to a first antigen on a first target cell

and a second sdAb that specifically binds to a second antigen on a second target cell, and (b) an immune effector cell binding domain that specifically binds to an antigen on an immune effector cell. In some embodiments, the first antigen and the second antigen are the same. In some embodiments, the first antigen and the second antigen are different. In some embodiments, the target cell binding domain comprises an anti-BCMA sdAb and an anti-CD3 sdAb.

[0028] Another aspect of the present application provides chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-BCMA scFv; and (b) an immune effector cell binding domain that specifically binds to an antigen on an immune effector cell. In some embodiments, the immune effector cell binding domain comprises an anti-CD3 antigen-binding fragment.

[0029] Further provided are pharmaceutical compositions, kits, articles of manufacture and methods of treating a disease (such as cancer or autoimmune disease) using the chimeric antibody immune effector cell engagers described herein.

I. Definitions

[0030] The practice of the present invention will employ, unless indicated specifically to the contrary, conventional methods of virology, immunology, microbiology, molecular biology and recombinant DNA techniques within the skill of the art, many of which are described below for the purpose of illustration. Such techniques are explained fully in the literature. See, *e.g.*, Current Protocols in Molecular Biology or Current Protocols in Immunology, John Wiley & Sons, New York, N.Y.(2009); Ausubel *et al*, Short Protocols in Molecular Biology, 3rd ed., Wiley & Sons, 1995; Sambrook and Russell, Molecular Cloning: A Laboratory Manual (3rd Edition, 2001); Maniatis *et al.* Molecular Cloning: A Laboratory Manual (1982); DNA Cloning: A Practical Approach, vol. I & II (D. Glover, ed.); Oligonucleotide Synthesis (N. Gait, ed., 1984); Nucleic Acid Hybridization (B. Hames & S. Higgins, eds., 1985); Transcription and Translation (B. Hames & S. Higgins, eds., 1984); Animal Cell Culture (R. Freshney, ed., 1986); Perbal, A Practical Guide to Molecular Cloning (1984) and other like references.

[0031] The term "chimeric antibody immune effector cell engager" as used herein refers to a multispecific antibody (such as bispecific antibody) that can specifically bind to both an immune effector cell (such as T cell or NK cell) and a target cell (such as tumor cell).

[0032] The term "antibody" includes monoclonal antibodies (including full length 4-chain antibodies or full length heavy-chain only antibodies which have an immunoglobulin Fc region),

antibody compositions with polyepitopic specificity, multispecific antibodies (*e.g.*, bispecific antibodies, diabodies, and single-chain molecules), as well as antibody fragments (*e.g.*, Fab, $F(ab')_2$, and Fv). The term "immunoglobulin" (Ig) is used interchangeably with "antibody" herein. Antibodies contemplated herein include single-domain antibodies, such as heavy chain only antibodies.

[0033] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. An IgM antibody consists of 5 of the basic heterotetramer units along with an additional polypeptide called a J chain, and contains 10 antigen binding sites, while IgA antibodies comprise from 2-5 of the basic 4-chain units which can polymerize to form polyvalent assemblages in combination with the J chain. In the case of IgGs, the 4-chain unit is generally about 150,000 daltons. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. Each H and L chain also has regularly spaced intrachain disulfide bridges. Each H chain has at the N-terminus, a variable domain (V_H) followed by three constant domains (C_H) for each of the α and γ chains and four C_H domains for μ and ε isotypes. Each L chain has at the N-terminus, a variable domain (V_L) followed by a constant domain at its other end. The V_L is aligned with the V_H and the C_L is aligned with the first constant domain of the heavy chain (C_H1). Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains. The pairing of a V_H and V_L together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, see *e.g.*, *Basic and Clinical Immunology*, 8th Edition, Daniel P. Sties, Abba I. Terr and Tristram G. Parsolw (eds), Appleton & Lange, Norwalk, Conn., 1994, page 71 and Chapter 6. The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda, based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (C_H), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG and IgM, having heavy chains designated α, δ, ε, γ and μ, respectively. The γ and α classes are further divided into subclasses on the basis of relatively minor differences in the C_H sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2A, IgG2B, IgG3, IgG4, IgA1 and IgA2.

[0034] The term "heavy chain-only antibody" or "HCAb" refers to a functional antibody, which comprises heavy chains, but lacks the light chains usually found in 4-chain antibodies. Camelid animals (such as camels, llamas, or alpacas) are known to produce HCAbs.

[0035] The term "single-domain antibody" or "sdAb" refers to a single antigen-binding polypeptide having three complementary determining regions (CDRs). The sdAb alone is capable of binding to the antigen without pairing with a corresponding CDR-containing polypeptide. In some cases, single-domain antibodies are engineered from camelid HCAbs, and their heavy chain variable domains are referred herein as "V_HHs". Some V_HHs may also be known as Nanobodies. Camelid sdAb is one of the smallest known antigen-binding antibody fragments (see, *e.g.*, Hamers-Casterman *et al.*, Nature 363:446-8 (1993); Greenberg *et al.*, Nature 374:168-73 (1995); Hassanzadeh-Ghassabeh *et al.*, Nanomedicine (Lond), 8:1013-26 (2013)). A basic V_HH has the following structure from the N-terminus to the C-terminus: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3.

[0036] An "isolated" antibody is one that has been identified, separated and/or recovered from a component of its production environment (*e.g.*, natural or recombinant). Preferably, the isolated polypeptide is free of association with all other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody will be prepared by at least one purification step.

[0037] The "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and

light chain may be referred to as "V_H" and "V_L", respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites. Heavy-chain only antibodies from the Camelid species have a single heavy chain variable region, which is referred to as "V_HH". V_HH is thus a special type of V_H.

[0038] The term "variable" refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen binding site of antibodies (see Kabat *et al.*, *Sequences of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md. (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

[0039] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture or recombinantly, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For

example, the monoclonal antibodies to be used in accordance with the present application may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein., *Nature,* 256:495-97 (1975); Hongo *et al.*, *Hybridoma,* 14 (3): 253-260 (1995), Harlow *et al.*, *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Pat. No. 4,816,567), phage-display technologies (see, *e.g.*, Clackson *et al.*, *Nature,* 352: 624-628 (1991); Marks *et al.*, *J. Mol. Biol.* 222: 581-597 (1992); Sidhu *et al.*, *J. Mol. Biol.* 338(2): 299-310 (2004); Lee *et al.*, *J. Mol. Biol.* 340(5): 1073-1093 (2004); Fellouse, *Proc. Natl. Acad. Sci. USA* 101(34): 12467-12472 (2004); and Lee *et al.*, *J. Immunol. Methods* 284(1-2): 119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits *et al.*, *Proc. Natl. Acad. Sci. USA* 90: 2551 (1993); Jakobovits *et al.*, *Nature* 362: 255-258 (1993); Bruggemann *et al.*, *Year in Immunol.* 7:33 (1993); U.S. Pat. Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks *et al.*, *Bio/Technology* 10: 779-783 (1992); Lonberg *et al.*, *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-813 (1994); Fishwild *et al.*, *Nature Biotechnol.* 14: 845-851 (1996); Neuberger, *Nature Biotechnol.* 14: 826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

[0040] The terms "full-length antibody," "intact antibody" or "whole antibody" are used interchangeably to refer to an antibody in its substantially intact form, as opposed to an antibody fragment. Specifically, full-length 4-chain antibodies include those with heavy and light chains including an Fc region. Full-length heavy-chain only antibodies include the heavy chain (such as V_HH) and an Fc region. The constant domains may be native sequence constant domains (*e.g.*, human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0041] An "antibody fragment" comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', $F(ab')_2$ and Fv fragments; diabodies; linear antibodies (see U.S. Pat. No. 5,641,870, Example 2; Zapata *et al.*, *Protein Eng.* 8(10): 1057-1062 [1995]); single-chain antibody molecules; single-domain antibodies (such as V_HH), and multispecific antibodies

formed from antibody fragments. Papain digestion of antibodies produced two identical antigen-binding fragments, called "Fab" fragments, and a residual "Fc" fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V_H), and the first constant domain of one heavy chain (C_H1). Each Fab fragment is monovalent with respect to antigen binding, *i.e.*, it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large $F(ab')_2$ fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab′ fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C_H1 domain including one or more cysteines from the antibody hinge region. Fab′-SH is the designation herein for Fab′ in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab′ fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0042] The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[0043] "Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0044] "Single-chain Fv" also abbreviated as "sFv" or "scFv" are antibody fragments that comprise the V_H and V_L antibody domains connected into a single polypeptide chain. Preferably, the sFv polypeptide further comprises a polypeptide linker between the V_H and V_L domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0045] "Functional fragments" of the antibodies described herein comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the Fc region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0046] The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; Morrison *et al.*, *Proc. Natl. Acad. Sci. USA,* 81:6851-6855 (1984)). Chimeric antibodies of interest herein include PRIMATTZFD® antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque monkeys with an antigen of interest. As used herein, "humanized antibody" is used a subset of "chimeric antibodies."

[0047] "Humanized" forms of non-human (*e.g.*, camelid) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In some embodiments, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR (hereinafter defined) of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, framework ("FR") residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody

performance, such as binding affinity, isomerization, immunogenicity, etc. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see, *e.g.*, Jones *et al.*, *Nature* 321:522-525 (1986); Riechmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See also, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Pat. Nos. 6,982,321 and 7,087,409.

[0048] A "human antibody" is an antibody that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks *et al.*, *J. Mol. Biol.*, 222:581 (1991). Also available for the preparation of human monoclonal antibodies are methods described in Cole *et al.*, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner *et al.*, *J. Immunol.*, 147(1):86-95 (1991). See also van Dijk and van de Winkel, *Curr. Opin. Pharmacol.*, 5: 368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (see, *e.g.*, U.S. Pat. Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). See also, for example, Li *et al.*, *Proc. Natl. Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

[0049] The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, single-domain antibodies comprise three HVRs (or CDRs): HVR1 (or CDR1), HVR2 (or CDR2), and HVR3 (or CDR3). HVR3 displays the most diversity of the three HVRs, and is believed to play a unique role in conferring fine specificity to

antibodies. See, *e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993); Sheriff *et al.*, *Nature Struct. Biol.* 3:733-736 (1996).

[0050] The term "Complementarity Determining Region" or "CDR" are used to refer to hypervariable regions as defined by the Kabat system. See Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)

[0051] A number of HVR delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk, *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat HVRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below in Table 1.

Table 1. HVR delineations.

Loop	Kabat	AbM	Chothia	Contact
L1	L24-L34	L24-L34	L26-L32	L30-L36
L2	L50-L56	L50-L56	L50-L52	L46-L55
L3	L89-L97	L89-L97	L91-L96	L89-L96
H1	H31-H35B	H26-H35B	H26-H32	H30-H35B
		(Kabat Numbering)		
H1	H31-H35	H26-H35	H26-H32	H30-H35
		(Chothia Numbering)		
H2	H50-H65	H50-H58	H53-H55	H47-H58
H3	H95-H102	H95-H102	H96-H101	H93-H101

[0052] HVRs may comprise "extended HVRs" as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2) and 89-97 or 89-96 (L3) in the V_L and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102, or 95-102 (H3) in the V_H. The variable domain residues are numbered according to Kabat *et al.*, supra, for each of these definitions.

[0053] The amino acid residues of a single-domain antibody (such as V_HH) are numbered according to the general numbering for V_H domains given by Kabat et al. ("Sequence of proteins

of immunological interest", US Public Health Services, NIH Bethesda, Md., Publication No. 91), as applied to V_HH domains from Camelids in the article of Riechmann and Muyldermans, J. Immunol. Methods 2000 Jun. 23; 240 (1-2): 185-195. According to this numbering, FR1 of a V_HH comprises the amino acid residues at positions 1-30, CDR1 of a V_HH comprises the amino acid residues at positions 31-35, FR2 of a V_HH comprises the amino acids at positions 36-49, CDR2 of a V_HH comprises the amino acid residues at positions 50-65, FR3 of a V_HH comprises the amino acid residues at positions 66-94, CDR3 of a V_HH comprises the amino acid residues at positions 95-102, and FR4 of a V_HH comprises the amino acid residues at positions 103-113. In this respect, it should be noted that—as is well known in the art for V_H domains and for V_HH domains—the total number of amino acid residues in each of the CDR's may vary and may not correspond to the total number of amino acid residues indicated by the Kabat numbering (that is, one or more positions according to the Kabat numbering may not be occupied in the actual sequence, or the actual sequence may contain more amino acid residues than the number allowed for by the Kabat numbering).

[0054] The expression "variable-domain residue-numbering as in Kabat" or "amino-acid-position numbering as in Kabat," and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat *et al.*, supra. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.* residues 82a, 82b, and 82c, etc. according to Kabat) after heavy-chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0055] Unless indicated otherwise herein, the numbering of the residues in an immunoglobulin heavy chain is that of the EU index as in Kabat *et al.*, supra. The "EU index as in Kabat" refers to the residue numbering of the human IgG1 EU antibody.

[0056] "Framework" or "FR" residues are those variable-domain residues other than the HVR residues as herein defined.

[0057] A "human consensus framework" or "acceptor human framework" is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin V_L or V_H framework sequences. Generally, the selection of human immunoglobulin V_L or V_H sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat *et al.*, *Sequences of Proteins of Immunological Interest,* 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). Examples include for the V_L, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat *et al.*, supra. Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat *et al.* Alternatively, a human consensus framework can be derived from the above in which particular residues, such as when a human framework residue is selected based on its homology to the donor framework by aligning the donor framework sequence with a collection of various human framework sequences. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less.

[0058] An "amino-acid modification" at a specified position, *e.g.* of the Fc region, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. Insertion "adjacent" to a specified residue means insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue. The preferred amino acid modification herein is a substitution.

[0059] An "affinity-matured" antibody is one with one or more alterations in one or more HVRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does not possess those alteration(s). In some embodiments, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks *et al.*, *Bio/Technology* 10:779-783 (1992) describes affinity maturation by V_H- and V_L -domain shuffling. Random mutagenesis of HVR and/or framework residues is described by, for example: Barbas *et al. Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier *et al. Gene* 169:147-155

(1995); Yelton *et al. J. Immunol.* 155:1994-2004 (1995); Jackson *et al., J. Immunol.* 154(7):3310-9 (1995); and Hawkins *et al, J. Mol. Biol.* 226:889-896 (1992).

[0060] As use herein, the term "specifically binds," "specifically recognizes," or is "specific for" refers to measurable and reproducible interactions such as binding between a target and an antigen binding protein, which is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antigen binding protein that specifically binds a target (which can be an epitope) is an antigen binding protein that binds this target with greater affinity, avidity, more readily, and/or with greater duration than it binds other targets. In some embodiments, the extent of binding of an antigen binding protein to an unrelated target is less than about 10% of the binding of the antigen binding protein to the target as measured, *e.g.*, by a radioimmunoassay (RIA). In some embodiments, an antigen binding protein that specifically binds a target has a dissociation constant (Kd) of ≦1 μM, ≦100 nM, ≦10 nM, ≦1 nM, or ≦0.1 nM. In some embodiments, an antigen binding protein specifically binds an epitope on a protein that is conserved among the protein from different species. In some embodiments, specific binding can include, but does not require exclusive binding.

[0061] The term "specificity" refers to selective recognition of an antigen binding protein for a particular epitope of an antigen. Natural antibodies, for example, are monospecific. The term "multispecific" as used herein denotes that an antigen binding protein has two or more antigen-binding sites of which at least two bind a different antigen or a different epitope of the same antigen. "Bispecific" as used herein denotes that an antigen binding protein has two different antigen-binding specificities. The term "monospecific" as used herein denotes an antigen binding protein that has one or more binding sites each of which bind the same epitope of the same antigen.

[0062] The term "valent" as used herein denotes the presence of a specified number of binding sites in an antigen binding protein. A natural antibody for example or a full length antibody has two binding sites and is bivalent. As such, the terms "trivalent", "tetravalent", "pentavalent" and "hexavalent" denote the presence of two binding site, three binding sites, four binding sites, five binding sites, and six binding sites, respectively, in an antigen binding protein.

[0063] "Binding affinity" generally refers to the strength of the sum total of non-covalent interactions between a single binding site of a molecule (*e.g.*, an antibody or a chimeric antibody

immune effector cell engager) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity that reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein. Low-affinity antibodies generally bind antigen slowly and tend to dissociate readily, whereas high-affinity antibodies generally bind antigen faster and tend to remain bound longer. A variety of methods of measuring binding affinity are known in the art, any of which can be used for purposes of the present application. Specific illustrative and exemplary embodiments for measuring binding affinity are described in the following.

[0064] "Percent (%) amino acid sequence identity" and "homology" with respect to a peptide, polypeptide or antibody sequence are defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN™ (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

[0065] An "isolated" nucleic acid molecule described herein is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. Preferably, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

[0066] The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are

suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[0067] Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0068] The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

[0069] The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells.

[0070] As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, one or more of the following: alleviating one or more symptoms resulting from the disease, diminishing the extent of the disease, stabilizing the disease (*e.g.*, preventing or delaying the worsening of the disease), preventing or delaying the spread (*e.g.*, metastasis) of the disease, preventing or delaying the recurrence of the disease, delay or slowing the progression of the disease, ameliorating the disease state, providing a remission (partial or total) of the disease, decreasing the dose of one or more other medications required to treat the disease, delaying the progression of the disease, increasing the quality of life,

and/or prolonging survival. Also encompassed by "treatment" is a reduction of pathological consequence of the disease. The methods of the present application contemplate any one or more of these aspects of treatment.

[0071] As used herein, an "individual" or a "subject" refers to a mammal, including, but not limited to, human, bovine, horse, feline, canine, rodent, or primate. In some embodiments, the individual is a human.

[0072] The term "effective amount" used herein refers to an amount of an agent, such as a chimeric antibody immune effector cell engager, or a pharmaceutical composition thereof, sufficient to treat a specified disorder, condition or disease such as ameliorate, palliate, lessen, and/or delay one or more of its symptoms. In reference to cancer, an effective amount comprises an amount sufficient to cause a tumor to shrink and/or to decrease the growth rate of the tumor (such as to suppress tumor growth) or to prevent or delay other unwanted cell proliferation. In some embodiments, an effective amount is an amount sufficient to delay development. In some embodiments, an effective amount is an amount sufficient to prevent or delay recurrence. An effective amount can be administered in one or more administrations. The effective amount of the drug or composition may: (i) reduce the number of cancer cells; (ii) reduce tumor size; (iii) inhibit, retard, slow to some extent and preferably stop cancer cell infiltration into peripheral organs; (iv) inhibit (*i.e.*, slow to some extent and preferably stop) tumor metastasis; (v) inhibit tumor growth; (vi) prevent or delay occurrence and/or recurrence of tumor; and/or (vii) relieve to some extent one or more of the symptoms associated with the cancer.

[0073] As used herein, "delaying" the development of cancer means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. A method that "delays" development of cancer is a method that reduces probability of disease development in a given time frame and/or reduces the extent of the disease in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a statistically significant number of individuals. Cancer development can be detectable using standard methods, including, but not limited to, computerized axial tomography (CAT Scan), Magnetic Resonance Imaging (MRI), abdominal ultrasound, clotting tests, arteriography, or biopsy. Development may

also refer to cancer progression that may be initially undetectable and includes occurrence, recurrence, and onset.

[0074] The term “pharmaceutical formulation” refers to a preparation that is in such form as to permit the biological activity of the active ingredient to be effective, and that contains no additional components that are unacceptably toxic to a subject to which the formulation would be administered. Such formulations are sterile. A “sterile” formulation is aseptic or free from all living microorganisms and their spores.

[0075] It is understood that embodiments of the present application described herein include “consisting” and/or “consisting essentially of” embodiments.

[0076] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter *per se*. For example, description referring to "about X" includes description of "X".

[0077] As used herein, reference to "not" a value or parameter generally means and describes "other than" a value or parameter. For example, the method is not used to treat cancer of type X means the method is used to treat cancer of types other than X.

[0078] The term “about X-Y” used herein has the same meaning as “about X to about Y.”

[0079] As used herein and in the appended claims, the singular forms "a," "or," and "the" include plural referents unless the context clearly dictates otherwise.

II. Chimeric antibody immune effector cell engagers

[0080] The present application provides a chimeric antibody immune effector cell engager comprising a target cell binding domain that specifically binds to an antigen on a target cell, and an immune effector cell that specifically binds to an antigen of an immune effector cell. In some embodiments, the target cell binding domain comprises one or more antigen-binding domains derived from single-domain antibodies (sdAb), such as V_HH. In some embodiments, the target cell binding domain comprises one or more antigen-binding fragments derived from four-chain antibodies, such as scFv. In some embodiments, the target cell binding domain comprises an anti-BCMA scFv.

[0081] Thus, in some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising a sdAb (such as V_HH) that specifically binds to an antigen on a target cell; and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune

effector cell. In some embodiments, the target cell is a tumor cell or a B cell. In some embodiments, the antigen-binding fragment in the immune effector cell binding domain is a Fab, scFv, or sdAb. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0082] In some embodiments, the immune effector cell is a T cell, and the chimeric antibody T cell engager is also referred herein as CATE. In some embodiments, the immune effector cell is a T cell, and the chimeric antibody NK cell engager is also referred herein as CANKE. In some embodiments, the immune effector cell binding domain comprises an antigen-binding fragment that specifically binds to CD3, such as CD3ε.

[0083] In some embodiments, there is provided a chimeric antibody T cell engager comprising: (a) a target cell binding domain comprising a sdAb (such as V_HH) that specifically binds to an antigen on a target cell; and (b) a T cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on a T cell. In some embodiments, the target cell is a tumor cell or a B cell. In some embodiments, the antigen-binding fragment in the immune effector cell binding domain is a Fab, scFv, or sdAb. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0084] In some embodiments, there is provided a chimeric antibody NK cell engager comprising: (a) a target cell binding domain comprising a sdAb (such as V_HH) that specifically binds to an antigen on a target cell; and (b) an NK cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an NK cell. In some embodiments, the target cell is a tumor cell or a B cell. In some embodiments, the antigen-binding fragment in the immune effector cell binding domain is a Fab, scFv, or sdAb. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In

some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0085] In some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an sdAb (such as V_HH) that specifically binds to an antigen on a target cell; and (b) an immune effector cell binding domain comprising an anti-CD3 antigen-binding fragment. In some embodiments, the target cell is a tumor cell or a B cell. In some embodiments, the anti-CD3 antigen-binding fragment is a Fab, scFv, or sdAb. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell

binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0086] In some embodiments, the target cell binding domain comprises an sdAb that specifically binds to BCMA (also referred herein as "anti-BCMA sdAb"), such as human BCMA. In some embodiments, the target cell binding domain comprises an anti-BCMA V_HH. The anti-BCMA sdAb can be derived from any single-domain antibodies, such as heavy chain only antibodies, that specifically bind to BCMA. In some embodiments, the anti-BCMA sdAb is camelid, chimeric, human or humanized. In some embodiments, the target cell binding domain comprises an anti-BCMA scFv. In some embodiments, the anti-BCMA scFv is derived from C11D5.3 or J22.9-xi.

[0087] B cell mature antigen (BCMA), also known as CD269, is a member of the tumor necrosis factor receptor superfamily, namely TNFRSF17 (Thompson et al., J. Exp. Medicine, 192 (1):129-135, 2000). Human BCMA is almost exclusively expressed in plasma cells and multiple myeloma cells (see *e.g.* Novak et al., Blood, 103(2): 689-694, 2004; Neri et al., Clinical Cancer Research, 73(19):5903-5909; Felix et al., Mol. Oncology, 9(7):1348-58, 2015). BCMA can bind B-cell activating factor (BAFF) and a proliferation including ligand (APRIL) (*e.g.* Mackay et al., 2003 and Kalled et al., Immunological Review, 204: 43-54, 2005). BCMA can be a suitable tumor antigen target for immunotherapeutic agents against multiple myeloma. Antibodies of high affinity can block the binding between BCMA and its native ligands BAFF and APRIL.

[0088] Thus, in some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-BCMA sdAb (such as anti-BCMA V_HH); and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the anti-BCMA sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:1; a CDR2 comprising the amino acid sequence of SEQ ID NO:12; and a CDR3 comprising the amino acid sequence of SEQ ID NO:23; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:2; a CDR2 comprising the amino acid sequence of SEQ ID NO:13; and a CDR3 comprising the

amino acid sequence of SEQ ID NO:24; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:3; a CDR2 comprising the amino acid sequence of SEQ ID NO:14; and a CDR3 comprising the amino acid sequence of SEQ ID NO:25; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:4; a CDR2 comprising the amino acid sequence of SEQ ID NO:15; and a CDR3 comprising the amino acid sequence of SEQ ID NO:26; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:5; a CDR2 comprising the amino acid sequence of SEQ ID NO:16; and a CDR3 comprising the amino acid sequence of SEQ ID NO:27; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO:6; a CDR2 comprising the amino acid sequence of SEQ ID NO:17; and a CDR3 comprising the amino acid sequence of SEQ ID NO:28; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:7; a CDR2 comprising the amino acid sequence of SEQ ID NO:18; and a CDR3 comprising the amino acid sequence of SEQ ID NO:29; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:8; a CDR2 comprising the amino acid sequence of SEQ ID NO:19; and a CDR3 comprising the amino acid sequence of SEQ ID NO:30; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:9; a CDR2 comprising the amino acid sequence of SEQ ID NO:20; and a CDR3 comprising the amino acid sequence of SEQ ID NO:31; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:10; a CDR2 comprising the amino acid sequence of SEQ ID NO:21; and a CDR3 comprising the amino acid sequence of SEQ ID NO:32; or (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:11; a CDR2 comprising the amino acid sequence of SEQ ID NO:22; and a CDR3 comprising the amino acid sequence of SEQ ID NO:33. In some embodiments, the anti-BCMA sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-88. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0089] In some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-BCMA sdAb (such as anti-BCMA V_HH); and (b) an immune effector cell binding domain comprising an anti-CD3 antigen-binding fragment. In some embodiments, the target cell is a tumor cell or a B cell. In

some embodiments, the anti-CD3 antigen-binding fragment is a Fab, scFv, or sdAb. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105. In some embodiments, the anti-BCMA sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:1; a CDR2 comprising the amino acid sequence of SEQ ID NO:12; and a CDR3 comprising the amino acid sequence of SEQ ID NO:23; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:2; a CDR2 comprising the amino acid sequence of SEQ ID NO:13; and a CDR3 comprising the amino acid sequence of SEQ ID NO:24; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:3; a CDR2 comprising the amino acid sequence of SEQ ID NO:14; and a CDR3 comprising the amino acid sequence of SEQ ID NO:25; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:4; a CDR2 comprising the amino acid sequence of SEQ ID NO:15; and a CDR3 comprising the amino acid sequence of SEQ ID NO:26; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:5; a CDR2 comprising the amino acid sequence of SEQ ID NO:16; and a CDR3 comprising the amino acid sequence of SEQ ID NO:27; (6) a CDR1 comprising the amino acid

sequence of SEQ ID NO:6; a CDR2 comprising the amino acid sequence of SEQ ID NO:17; and a CDR3 comprising the amino acid sequence of SEQ ID NO:28; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:7; a CDR2 comprising the amino acid sequence of SEQ ID NO:18; and a CDR3 comprising the amino acid sequence of SEQ ID NO:29; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:8; a CDR2 comprising the amino acid sequence of SEQ ID NO:19; and a CDR3 comprising the amino acid sequence of SEQ ID NO:30; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:9; a CDR2 comprising the amino acid sequence of SEQ ID NO:20; and a CDR3 comprising the amino acid sequence of SEQ ID NO:31; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:10; a CDR2 comprising the amino acid sequence of SEQ ID NO:21; and a CDR3 comprising the amino acid sequence of SEQ ID NO:32; or (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:11; a CDR2 comprising the amino acid sequence of SEQ ID NO:22; and a CDR3 comprising the amino acid sequence of SEQ ID NO:33. In some embodiments, the anti-BCMA sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-88. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0090] In some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-BCMA scFv; and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the antigen-binding fragment in the immune effector cell binding domain is a Fab, scFv, or sdAb. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the anti-BCMA scFv is derived from C11D5.3. In some embodiments, the anti-BCMA scFv is derived from J22.9-xi. In some embodiments, the anti-BCMA scFv has the format N-terminus-VH-VL-C terminus. In some embodiments, the anti-BCMA scFv has the format N-terminus-VL-VH-C terminus. In some

embodiments, the anti-BCMA scFv comprises the amino acid sequence of SEQ ID NO: 101 or SEQ ID NO: 102. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0091] In some embodiments, the target cell binding domain comprises an sdAb that specifically binds to CD38 (also referred herein as "anti-CD38 sdAb"), such as human CD38. In some embodiments, the target cell binding domain comprises an anti-CD38 V_HH. The anti-CD38 sdAb can be derived from any single-domain antibodies, such as heavy chain only antibodies, that specifically bind to CD38. In some embodiments, the anti-CD38 sdAb is camelid, chimeric, human or humanized.

[0092] CD38 is a type II transmembrane glycoprotein that associates with cell-surface receptors, regulates cytoplasmic Ca^{2+} flux, and mediates signal transduction in lymphoid and myeloid (Konopleva et al., J Immunol, 161:4702-8, 1998; Deaglio et al., Blood, 109:5390-8, 2007). Human CD38 is highly and uniformly expressed on myeloma cells and is expressed at relatively low levels on normal lymphoid and myeloid cells and in some tissues of non-hematopoietic origin, which makes it a potential target in the treatment of myeloma (See, for example, Lin *et al.*, *Am J Clin Pathol*, 2004, 121:482; H. M. Lokhorst *et al.*, *New Eng. J. Med.*, 2015, 373:13).

[0093] Thus, in some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-CD38 sdAb (such as anti-CD38 V_HH); and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the anti-CD38 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:34; a CDR2 comprising the amino acid sequence of SEQ ID NO:46; and a CDR3 comprising the amino acid sequence of SEQ ID NO:58; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:35; a CDR2

comprising the amino acid sequence of SEQ ID NO:47; and a CDR3 comprising the amino acid sequence of SEQ ID NO:59; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:36; a CDR2 comprising the amino acid sequence of SEQ ID NO:48; and a CDR3 comprising the amino acid sequence of SEQ ID NO:60; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:37; a CDR2 comprising the amino acid sequence of SEQ ID NO:49; and a CDR3 comprising the amino acid sequence of SEQ ID NO:61; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:38; a CDR2 comprising the amino acid sequence of SEQ ID NO:50; and a CDR3 comprising the amino acid sequence of SEQ ID NO:62; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO:39; a CDR2 comprising the amino acid sequence of SEQ ID NO:51; and a CDR3 comprising the amino acid sequence of SEQ ID NO:63; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:40; a CDR2 comprising the amino acid sequence of SEQ ID NO:52; and a CDR3 comprising the amino acid sequence of SEQ ID NO:64; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:41; a CDR2 comprising the amino acid sequence of SEQ ID NO:53; and a CDR3 comprising the amino acid sequence of SEQ ID NO:65; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:42; a CDR2 comprising the amino acid sequence of SEQ ID NO:54; and a CDR3 comprising the amino acid sequence of SEQ ID NO:66; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:43; a CDR2 comprising the amino acid sequence of SEQ ID NO:55; and a CDR3 comprising the amino acid sequence of SEQ ID NO:67; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:44; a CDR2 comprising the amino acid sequence of SEQ ID NO:56; and a CDR3 comprising the amino acid sequence of SEQ ID NO:68; or (12) a CDR1 comprising the amino acid sequence of SEQ ID NO:45; a CDR2 comprising the amino acid sequence of SEQ ID NO:57; and a CDR3 comprising the amino acid sequence of SEQ ID NO:69. In some embodiments, the anti-CD38 sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-100. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0094] In some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-CD38 sdAb (such as anti-CD38 V_HH); and (b) an immune effector cell binding domain comprising an anti-CD3 antigen-binding fragment. In some embodiments, the target cell is a tumor cell or a B cell. In some embodiments, the anti-CD3 antigen-binding fragment is a Fab, scFv, or sdAb. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105. In some embodiments, the anti-CD38 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:34; a CDR2 comprising the amino acid sequence of SEQ ID NO:46; and a CDR3 comprising the amino acid sequence of SEQ ID NO:58; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:35; a CDR2 comprising the amino acid sequence of SEQ ID NO:47; and a CDR3 comprising the amino acid sequence of SEQ ID NO:59; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:36; a CDR2 comprising the amino acid sequence of SEQ ID NO:48; and a CDR3 comprising the amino acid sequence of SEQ ID NO:60; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:37;

a CDR2 comprising the amino acid sequence of SEQ ID NO:49; and a CDR3 comprising the amino acid sequence of SEQ ID NO:61; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:38; a CDR2 comprising the amino acid sequence of SEQ ID NO:50; and a CDR3 comprising the amino acid sequence of SEQ ID NO:62; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO:39; a CDR2 comprising the amino acid sequence of SEQ ID NO:51; and a CDR3 comprising the amino acid sequence of SEQ ID NO:63; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:40; a CDR2 comprising the amino acid sequence of SEQ ID NO:52; and a CDR3 comprising the amino acid sequence of SEQ ID NO:64; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:41; a CDR2 comprising the amino acid sequence of SEQ ID NO:53; and a CDR3 comprising the amino acid sequence of SEQ ID NO:65; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:42; a CDR2 comprising the amino acid sequence of SEQ ID NO:54; and a CDR3 comprising the amino acid sequence of SEQ ID NO:66; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:43; a CDR2 comprising the amino acid sequence of SEQ ID NO:55; and a CDR3 comprising the amino acid sequence of SEQ ID NO:67; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:44; a CDR2 comprising the amino acid sequence of SEQ ID NO:56; and a CDR3 comprising the amino acid sequence of SEQ ID NO:68; or (12) a CDR1 comprising the amino acid sequence of SEQ ID NO:45; a CDR2 comprising the amino acid sequence of SEQ ID NO:57; and a CDR3 comprising the amino acid sequence of SEQ ID NO:69. In some embodiments, the anti-CD38 sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-100. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0095] In some embodiments, the target cell binding domain is monospecific. In some embodiments, the target cell binding domain is monovalent. In some embodiments, the target cell binding domain is multivalent, such as bivalent or trivalent. In some embodiments, the target cell binding domain is multispecific, such as bispecific or trispecific.

[0096] In some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising a first sdAb (such as V_HH) that specifically binds to a first antigen on a first target cell and a second sdAb (such as V_HH) that specifically binds to a second antigen on a second target cell; and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the first target cell and the second target cell are the same cell. In some embodiments, the first target cell and the second target cell are different cells. In some embodiments, the first cell and the second cell are tumor cells. In some embodiments, the first sdAb is fused to the second sdAb via a peptide linker. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the immune effector cell binding domain comprises an anti-CD3 antigen-binding fragment. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105. In some embodiments,

the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0097] In some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising a first sdAb (such as V_HH) that specifically binds to a first antigen on a target cell and a second sdAb (such as V_HH) that specifically binds to a second antigen on the target cell; and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the first antigen and the second antigen are the same. In some embodiments, the first sdAb and the second sdAb specifically bind to the same epitope. In some embodiments, the first sdAb and the second sdAb specifically bind to different epitopes. In some embodiments, the first antigen and the second antigen are different. In some embodiments, the first sdAb is fused to the second sdAb via a peptide linker. In some embodiments, the target cell is a tumor cell or a B cell. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the immune effector cell binding domain comprises an anti-CD3 antigen-binding fragment. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH

comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0098] In some embodiments, the target cell binding domain comprises a first anti-BCMA sdAb and a second anti-BCMA sdAb. In some embodiments, the first anti-BCMA sdAb and the second anti-BCMA sdAb recognizes different epitopes on BCMA. In some embodiments, the target cell binding domain comprises a first anti-CD38 sdAb and a second anti-CD38 sdAb. In some embodiments, the first anti-CD38 sdAb and the second anti-CD38 sdAb recognizes different epitopes on CD38. In some embodiments, the target cell binding domain comprises an anti-BCMA sdAb and an anti-CD38 sdAb. In some embodiments, the anti-BCMA sdAb is fused to the N-terminus of the anti-CD38 sdAb. In some embodiments, the anti-BCMA sdAb is fused to the C-terminus of the anti-CD38 sdAb.

[0099] Thus, in some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising a first anti-BCMA sdAb (such as anti-BCMA V_HH) and a second anti-BCMA sdAb (such as anti-BCMA V_HH); and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the first anti-BCMA sdAb is fused to the second anti-BCMA sdAb via a peptide linker. In some embodiments, the first anti-BCMA sdAb and the second anti-BCMA sdAb recognizes different epitopes on BCMA. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the immune effector cell

binding domain comprises an anti-CD3 antigen-binding fragment. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105. In some embodiments, the first anti-BCMA sdAb and/or the second anti-BCMA sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:1; a CDR2 comprising the amino acid sequence of SEQ ID NO:12; and a CDR3 comprising the amino acid sequence of SEQ ID NO:23; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:2; a CDR2 comprising the amino acid sequence of SEQ ID NO:13; and a CDR3 comprising the amino acid sequence of SEQ ID NO:24; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:3; a CDR2 comprising the amino acid sequence of SEQ ID NO:14; and a CDR3 comprising the amino acid sequence of SEQ ID NO:25; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:4; a CDR2 comprising the amino acid sequence of SEQ ID NO:15; and a CDR3 comprising the amino acid sequence of SEQ ID NO:26; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:5; a CDR2 comprising the amino acid sequence of SEQ ID NO:16; and a CDR3 comprising the amino acid sequence of SEQ ID NO:27;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO:6; a CDR2 comprising the amino acid sequence of SEQ ID NO:17; and a CDR3 comprising the amino acid sequence of SEQ ID NO:28; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:7; a CDR2 comprising the amino acid sequence of SEQ ID NO:18; and a CDR3 comprising the amino acid sequence of SEQ ID NO:29; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:8; a CDR2 comprising the amino acid sequence of SEQ ID NO:19; and a CDR3 comprising the amino acid sequence of SEQ ID NO:30; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:9; a CDR2 comprising the amino acid sequence of SEQ ID NO:20; and a CDR3 comprising the amino acid sequence of SEQ ID NO:31; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:10; a CDR2 comprising the amino acid sequence of SEQ ID NO:21; and a CDR3 comprising the amino acid sequence of SEQ ID NO:32; or (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:11; a CDR2 comprising the amino acid sequence of SEQ ID NO:22; and a CDR3 comprising the amino acid sequence of SEQ ID NO:33. In some embodiments, the first anti-BCMA sdAb and/or the second anti-BCMA sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-88. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0100] In some embodiments, there is provided a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-BCMA sdAb (such as anti-BCMA V_HH) and an anti-CD38 sdAb (such as anti-CD38 V_HH); and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the anti-BCMA sdAb is fused to the anti-CD38 sdAb via a peptide linker. In some embodiments, the anti-BCMA sdAb is fused to the N-terminus of the anti-CD38 sdAb. In some embodiments, the anti-BCMA sdAb is fused to the C-terminus of the anti-CD38 sdAb. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the

immune effector cell binding domain comprises an anti-CD3 antigen-binding fragment. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105. In some embodiments, the anti-BCMA sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:1; a CDR2 comprising the amino acid sequence of SEQ ID NO:12; and a CDR3 comprising the amino acid sequence of SEQ ID NO:23; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:2; a CDR2 comprising the amino acid sequence of SEQ ID NO:13; and a CDR3 comprising the amino acid sequence of SEQ ID NO:24; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:3; a CDR2 comprising the amino acid sequence of SEQ ID NO:14; and a CDR3 comprising the amino acid sequence of SEQ ID NO:25; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:4; a CDR2 comprising the amino acid sequence of SEQ ID NO:15; and a CDR3 comprising the amino acid sequence of SEQ ID NO:26; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:5; a CDR2 comprising the amino acid sequence of SEQ ID NO:16; and a CDR3 comprising the amino acid sequence of SEQ ID NO:27; (6) a CDR1 comprising the amino acid

sequence of SEQ ID NO:6; a CDR2 comprising the amino acid sequence of SEQ ID NO:17; and a CDR3 comprising the amino acid sequence of SEQ ID NO:28; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:7; a CDR2 comprising the amino acid sequence of SEQ ID NO:18; and a CDR3 comprising the amino acid sequence of SEQ ID NO:29; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:8; a CDR2 comprising the amino acid sequence of SEQ ID NO:19; and a CDR3 comprising the amino acid sequence of SEQ ID NO:30; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:9; a CDR2 comprising the amino acid sequence of SEQ ID NO:20; and a CDR3 comprising the amino acid sequence of SEQ ID NO:31; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:10; a CDR2 comprising the amino acid sequence of SEQ ID NO:21; and a CDR3 comprising the amino acid sequence of SEQ ID NO:32; or (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:11; a CDR2 comprising the amino acid sequence of SEQ ID NO:22; and a CDR3 comprising the amino acid sequence of SEQ ID NO:33. In some embodiments, the anti-BCMA sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-88. In some embodiments, the anti-CD38 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:34; a CDR2 comprising the amino acid sequence of SEQ ID NO:46; and a CDR3 comprising the amino acid sequence of SEQ ID NO:58; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:35; a CDR2 comprising the amino acid sequence of SEQ ID NO:47; and a CDR3 comprising the amino acid sequence of SEQ ID NO:59; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:36; a CDR2 comprising the amino acid sequence of SEQ ID NO:48; and a CDR3 comprising the amino acid sequence of SEQ ID NO:60; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:37; a CDR2 comprising the amino acid sequence of SEQ ID NO:49; and a CDR3 comprising the amino acid sequence of SEQ ID NO:61; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:38; a CDR2 comprising the amino acid sequence of SEQ ID NO:50; and a CDR3 comprising the amino acid sequence of SEQ ID NO:62; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO:39; a CDR2 comprising the amino acid sequence of SEQ ID NO:51; and a CDR3 comprising the amino acid sequence of SEQ ID NO:63; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:40; a CDR2 comprising the amino acid sequence of SEQ ID NO:52; and a CDR3 comprising the amino acid sequence of SEQ ID NO:64; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:41; a CDR2 comprising the amino acid

sequence of SEQ ID NO:53; and a CDR3 comprising the amino acid sequence of SEQ ID NO:65; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:42; a CDR2 comprising the amino acid sequence of SEQ ID NO:54; and a CDR3 comprising the amino acid sequence of SEQ ID NO:66; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:43; a CDR2 comprising the amino acid sequence of SEQ ID NO:55; and a CDR3 comprising the amino acid sequence of SEQ ID NO:67; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:44; a CDR2 comprising the amino acid sequence of SEQ ID NO:56; and a CDR3 comprising the amino acid sequence of SEQ ID NO:68; or (12) a CDR1 comprising the amino acid sequence of SEQ ID NO:45; a CDR2 comprising the amino acid sequence of SEQ ID NO:57; and a CDR3 comprising the amino acid sequence of SEQ ID NO:69. In some embodiments, the anti-CD38 sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-100. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0101] In some embodiments, there is provided a chimeric antibody immune effector cell engager comprising the amino acid sequence of any one of SEQ ID NOs: 106-163. In some embodiments, there is provided a polypeptide comprising the amino acid of any one of SEQ ID NOs: 106-163.

Target cell binding domain

[0102] The chimeric antibody immune effector cell engagers described herein comprise a target cell binding domain. The target cell binding domain comprises one or more antigen-binding fragments derived from single-domain antibodies or four-chain antibodies.

1. Single-domain antibodies

[0103] In some embodiments, the target cell binding domain comprises one or more (such as any one of 1, 2, 3, 4, or more) single-domain antibodies. The sdAbs may be of the same or different origins, and of the same or different sizes. Exemplary sdAbs include, but are not limited to, heavy chain variable domains from heavy-chain only antibodies (*e.g.*, V_HH or V_{NAR}), binding

molecules naturally devoid of light chains, single domains (such as V_H or V_L) derived from conventional 4-chain antibodies, humanized heavy-chain only antibodies, human single-domain antibodies produced by transgenic mice or rats expressing human heavy chain segments, and engineered domains and single domain scaffolds other than those derived from antibodies. Any sdAbs known in the art or described herein that target an antigen on a target cell (such as tumor cell) may be used to construct the chimeric antibody immune effector cell engagers described herein. The sdAbs may be derived from any species including, but not limited to mouse, rat, human, camel, llama, lamprey, fish, shark, goat, rabbit, and bovine. Single-domain antibodies contemplated herein also include naturally occurring single-domain antibody molecules from species other than *Camelidae* and sharks.

[0104] In some embodiments, the sdAb is derived from a naturally occurring single-domain antigen binding molecule known as heavy chain antibody devoid of light chains (also referred herein as "heavy chain only antibodies"). Such single domain molecules are disclosed in WO 94/04678 and Hamers-Casterman, C. *et al.* (1993) *Nature* 363:446-448, for example. For clarity reasons, the variable domain derived from a heavy chain molecule naturally devoid of light chain is known herein as a V_HH to distinguish it from the conventional V_H of four chain immunoglobulins. Such a V_HH molecule can be derived from antibodies raised in *Camelidae* species, for example, camel, llama, vicuna, dromedary, alpaca and guanaco. Other species besides Camelidae may produce heavy chain molecules naturally devoid of light chain, and such V_HHs are within the scope of the present application.

[0105] V_HH molecules from Camelids are about 10 times smaller than IgG molecules. They are single polypeptides and can be very stable, resisting extreme pH and temperature conditions. Moreover, they can be resistant to the action of proteases which is not the case for conventional 4-chain antibodies. Furthermore, *in vitro* expression of V_HH s produces high yield, properly folded functional V_HHs. In addition, antibodies generated in Camelids can recognize epitopes other than those recognized by antibodies generated *in vitro* through the use of antibody libraries or via immunization of mammals other than Camelids (see, for example, WO9749805). As such, multispecific or multivalent chimeric antibody immune effector cell engagers comprising one or more V_HH domains may interact more efficiently with targets than multispecific or multivalent chimeric antibody immune effector cell engagers comprising antigen binding fragments derived from conventional 4-chain antibodies. Since V_HHs are known to bind into 'unusual' epitopes such

as cavities or grooves, the affinity of chimeric antibody immune effector cell engagers comprising such V_HHs may be more suitable for therapeutic treatment than conventional multispecific polypeptides.

[0106] In some embodiments, the sdAb is derived from a variable region of the immunoglobulin found in cartilaginous fish. For example, the sdAb can be derived from the immunoglobulin isotype known as Novel Antigen Receptor (NAR) found in the serum of shark. Methods of producing single domain molecules derived from a variable region of NAR ("IgNARs") are described in WO 03/014161 and Streltsov (2005) *Protein Sci.* 14:2901-2909.

[0107] In some embodiments, the sdAb is recombinant, CDR-grafted, humanized, camelized, de-immunized and/or *in vitro* generated (*e.g.*, selected by phage display). In some embodiments, the amino acid sequence of the framework regions may be altered by "camelization" of specific amino acid residues in the framework regions. Camelization refers to the replacing or substitution of one or more amino acid residues in the amino acid sequence of a (naturally occurring) V_H domain from a conventional 4-chain antibody by one or more of the amino acid residues that occur at the corresponding position(s) in a V_HH domain of a heavy chain antibody. This can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the further description herein. Such "camelizing" substitutions are preferably inserted at amino acid positions that form and/or are present at the V_H-V_L interface, and/or at the so-called Camelidae hallmark residues, as defined herein (see for example WO 94/04678, Davies and Riechmann FEBS Letters 339: 285-290, 1994; Davies and Riechmann Protein Engineering 9 (6): 531-537, 1996; Riechmann J. Mol. Biol. 259: 957-969, 1996; and Riechmann and Muyldermans J. Immunol. Meth. 231: 25-38, 1999).

[0108] In some embodiments, the sdAb is a human single-domain antibody produced by transgenic mice or rats expressing human heavy chain segments. See, *e.g.*, US20090307787A1, U.S. Pat. No. 8,754,287, US20150289489A1, US20100122358A1, and WO2004049794. In some embodiments, the sdAb is affinity matured.

[0109] In some embodiments, naturally occurring V_HH domains against a particular antigen or target, can be obtained from (naïve or immune) libraries of Camelid V_HH sequences. Such methods may or may not involve screening such a library using said antigen or target, or at least one part, fragment, antigenic determinant or epitope thereof using one or more screening techniques known per se. Such libraries and techniques are for example described in WO

99/37681, WO 01/90190, WO 03/025020 and WO 03/035694. Alternatively, improved synthetic or semi-synthetic libraries derived from (naïve or immune) V_HH libraries may be used, such as V_HH libraries obtained from (naïve or immune) V_HH libraries by techniques such as random mutagenesis and/or CDR shuffling, as for example described in WO 00/43507.

[0110] In some embodiments, the single-domain antibodies are generated from conventional four-chain antibodies. See, for example, EP 0 368 684, Ward et al. (Nature 1989 Oct. 12; 341 (6242): 544-6), Holt et al., Trends Biotechnol., 2003, 21(11):484-490; WO 06/030220; and WO 06/003388.

2. Antigens

[0111] Each sdAb in the target cell binding domain specifically binds to an antigen on a target cell. In some embodiments, the antigen is a cell surface molecule. The single-domain antibodies may be chosen to recognize an antigen that acts as a cell surface marker on target cells associated with a special disease state. In some embodiments, the antigen, such as the first antigen and/or the second antigen on the target cell(s), is a tumor antigen. In some embodiments, the chimeric antibody immune effector cell engager target a single tumor antigen. In some embodiments, the chimeric antibody immune effector cell engager targets two or more tumor antigens. In some embodiments, the tumor antigen is associated with a B cell malignancy. Tumors express a number of proteins that can serve as a target antigen for an immune response, particularly T cell mediated immune responses. The antigens targeted by the chimeric antibody immune effector cell engager may be antigens on a single diseased cell or antigens that are expressed on different cells that each contribute to the disease. The antigens targeted by the chimeric antibody immune effector cell engager may be directly or indirectly involved in the diseases.

[0112] Tumor antigens are proteins that are produced by tumor cells that can elicit an immune response, particularly T cell mediated immune responses. The selection of the targeted antigen of the invention will depend on the particular type of cancer to be treated. Exemplary tumor antigens include, for example, a glioma-associated antigen, carcinoembryonic antigen (CEA), β-human chorionic gonadotropin, alphafetoprotein (AFP), lectin-reactive AFP, thyroglobulin, RAGE-1, MN-CAIX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostase, prostate-specific antigen (PSA), PAP, NY-ESO-1, LAGE-la, p53, prostein, PSMA, HER2/neu, survivin and telomerase, prostate-carcinoma

tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrinB2, CD22, insulin growth factor (IGF)-I, IGF-II, IGF-I receptor and mesothelin.

[0113] In some embodiments, the tumor antigen comprises one or more antigenic cancer epitopes associated with a malignant tumor. Malignant tumors express a number of proteins that can serve as target antigens for an immune attack. These molecules include but are not limited to tissue-specific antigens such as MART-1, tyrosinase and gp100 in melanoma and prostatic acid phosphatase (PAP) and prostate-specific antigen (PSA) in prostate cancer. Other target molecules belong to the group of transformation-related molecules such as the oncogene HER2/Neu/ErbB-2. Yet another group of target antigens are onco-fetal antigens such as carcinoembryonic antigen (CEA). In B-cell lymphoma the tumor-specific idiotype immunoglobulin constitutes a truly tumor-specific immunoglobulin antigen that is unique to the individual tumor. B-cell differentiation antigens such as CD 19, CD20 and CD37 are other candidates for target antigens in B-cell lymphoma.

[0114] In some embodiments, the tumor antigen is a tumor-specific antigen (TSA) or a tumor-associated antigen (TAA). A TSA is unique to tumor cells and does not occur on other cells in the body. A TAA associated antigen is not unique to a tumor cell, and instead is also expressed on a normal cell under conditions that fail to induce a state of immunologic tolerance to the antigen. The expression of the antigen on the tumor may occur under conditions that enable the immune system to respond to the antigen. TAAs may be antigens that are expressed on normal cells during fetal development, when the immune system is immature, and unable to respond or they may be antigens that are normally present at extremely low levels on normal cells, but which are expressed at much higher levels on tumor cells.

[0115] Non-limiting examples of TSA or TAA antigens include the following: Differentiation antigens such as MART-1/MelanA (MART-I), gp 100 (Pmel 17), tyrosinase, TRP-1, TRP-2 and tumor-specific multilineage antigens such as MAGE-1, MAGE-3, BAGE, GAGE-1, GAGE-2, pl5; overexpressed embryonic antigens such as CEA; overexpressed oncogenes and mutated tumor-suppressor genes such as p53, Ras, HER2/neu; unique tumor antigens resulting from chromosomal translocations; such as BCR-ABL, E2A-PRL, H4-RET, IGH-IGK, MYL-RAR; and viral antigens, such as the Epstein Barr virus antigens EBVA and the human papillomavirus (HPV) antigens E6 and E7. Other large, protein-based antigens include TSP-180, MAGE-4, MAGE-5, MAGE-6, RAGE, NY-ESO, pl85erbB2, pl80erbB-3, c-met, nm-23HI, PSA, TAG-72,

CA 19-9, CA 72-4, CAM 17.1, NuMa, K-ras, beta-Catenin, CDK4, Mum-1, p 15, p 16, 43-9F, 5T4, 791Tgp72, alpha-fetoprotein, beta-HCG, BCA225, BTAA, CA 125, CA 15-3\CA 27.29\BCAA, CA 195, CA 242, CA-50, CAM43, CD68\P1, CO-029, FGF-5, G250, Ga733\EpCAM, HTgp-175, M344, MA-50, MG7-Ag, MOV18, NB/70K, NY-CO- 1, RCAS 1, SDCCAG16, TA-90\Mac-2 binding protein\cyclophilin C-associated protein, TAAL6, TAG72, TLP, and TPS.

[0116] In some embodiments, the antigen (such as the first antigen and/or the second antigen) is selected from the group consisting of CD19, CD20, CD22, CD33, CD38, BCMA, CS1, ROR1, GPC3, CD123, IL-13R, CD138, c-Met, EGFRvIII, GD-2, NY-ESO-1, MAGE A3, and glycolipid F77.

[0117] In some embodiments, the antigen is expressed on a B cell. In some embodiments, the antigen (such as the first antigen and/or the second antigen) is BCMA. In some embodiments, the antigen (such as the first antigen and/or the second antigen) is CD38. In some embodiments, the first antigen is BCMA and the second antigen is CD38.

3. Exemplary sdAbs

[0118] Sequences of exemplary single-domain antibodies suitable for use in the target cell binding domain include, but are not limited to the single domain antibodies listed in Table 2 below.

Table 2. Sequences of exemplary single-domain antibodies.

Ab	Ex. AA SEQ ID	CDR1	CDR2	CDR3
Exemplary Anti-BCMA single-domain antibodies				
269A37346	78	SGFTLDYYAIG (SEQ ID NO: 1)	CISRSDGSTYYADSVKG (SEQ ID NO: 12)	AGADCSGYLRDYEF (SEQ ID NO: 23)
269A37348	79	SGRTFSTYGMA (SEQ ID NO: 2)	SKASMNYSGRTYYADSVKG (SEQ ID NO: 13)	AGTGCSTYGCFDAQIIDY (SEQ ID NO: 24)
269A37917	80	SGRTFTMG (SEQ ID NO: 3)	AISLSPTLAYYAESVKG (SEQ ID NO: 14)	ADRKSVMSIRPDY (SEQ ID NO: 25)
269A37355	81	SGGIFVINAMG (SEQ ID NO: 4)	SIRGLGRTNYDDSVKG	VYVTLLGGVNRDY (SEQ ID NO: 26)

			(SEQ ID NO: 15)	
269A37915	82	SGRTFSSIVMG (SEQ ID NO: 5)	AIMWNDGITYLQDSVKG (SEQ ID NO: 16)	ASKGRYSEYEY (SEQ ID NO: 27)
269A37936	83	SGFTFDRAVIV (SEQ ID NO: 6)	FIKPSDGTIYYIDSLKG (SEQ ID NO: 17)	ASPEDWYTDWIDWSIYR (SEQ ID NO: 28)
269A37953	84	STYTVNSDVMG (SEQ ID NO: 7)	AIMWNDGITYLQDSVKG (SEQ ID NO: 18)	ASKGRYSEYEY (SEQ ID NO: 29)
269A37965	85	SGATLTNDHMA (SEQ ID NO: 8)	AIDWSGRTTNYADPVEG (SEQ ID NO: 19)	VLRAWISYDNDY (SEQ ID NO: 30)
269A37972	86	SGGTLSKNTVA (SEQ ID NO: 9)	SITWDGRTTYYADSVKG (SEQ ID NO: 20)	DLGKWPAGPADY (SEQ ID NO: 31)
269A37353	87	SEHTFSSHVMG (SEQ ID NO: 10)	VIGWRDISTSYADSVKG (SEQ ID NO: 21)	ARRIDAADFDS (SEQ ID NO: 32)
269A37948	88	SGRAFSTYFMA (SEQ ID NO: 11)	GIAWSGGSTAYADSVKG (SEQ ID NO: 22)	SRGIEVEEFGA (SEQ ID NO: 33)
Exemplary Anti-CD38 single-domain antibodies				
38A37333	89	SGLTFSSYPMM (SEQ ID NO: 34)	RISDSGGYTNYDDSVKG (SEQ ID NO: 46)	ILGLPT (SEQ ID NO: 58)
38A37336	90	SGFTFSSNWMY (SEQ ID NO: 35)	TISTDGRGTYYKDSVKG (SEQ ID NO: 47)	KEPRVLMAYLRNLGDFGS (SEQ ID NO: 59)
38A37699	91	SGRIFSINAMG (SEQ ID NO: 36)	AISTAGSTNYGDSVKG (SEQ ID NO: 48)	LNFPPYVY (SEQ ID NO: 60)
38A37331	92	SGSIFKVFRVFAMS (SEQ ID NO: 37)	SISSGETTTYADSVKG (SEQ ID NO: 49)	ADHTFTGDF (SEQ ID NO: 61)
38A37717	93	TGKVFSIYDMG (SEQ ID NO: 38)	EITSSGTTHYDDFVSG (SEQ ID NO: 50)	NHVFGGSY (SEQ ID NO: 62)
38A37719	94	SASIFTRLPMG (SEQ ID NO: 39)	GIVPSGRINYADSVKG (SEQ ID NO: 51)	ADTFPLPT (SEQ ID NO: 63)
38A37330	95	SGRAYATMA (SEQ ID NO: 40)	HLRVSGDTTYYTDSVKG (SEQ ID NO: 52)	GPYGILAAARVSNPGNYDY (SEQ ID NO: 64)
38A37334	96	SGLTFSSYIMG (SEQ ID NO: 41)	EISSGGMTSYADSVKG	APERGSIWYSRYEYKY

			(SEQ ID NO: 53)	(SEQ ID NO: 65)
38A37730	97	SQGIFTINAMG (SEQ ID NO: 42)	EVSSGGRTDYADSVKG (SEQ ID NO: 54)	VSGWHVFVGDRIV (SEQ ID NO: 66)
38A37340	98	SGRTFSSYAMA (SEQ ID NO: 43)	SISTSGGITDYADSVKG (SEQ ID NO: 55)	ARTWYLRTSLQYDY (SEQ ID NO: 67)
38A37731	99	SGTIVSISTMG (SEQ ID NO: 44)	TITRRGRTNYTDSVKG (SEQ ID NO: 56)	AEVQLDIWASAYDY (SEQ ID NO: 68)
38A37326	100	SGRTYAMG (SEQ ID NO: 45)	TISGAGNTKYADSVKG (SEQ ID NO: 57)	AGKWFPAANEY (SEQ ID NO: 69)

Anti-BCMA sdAb

[0119] In some embodiments, the target cell binding domain comprises one or more anti-BCMA sdAbs, such as anti-BCMA V_HH. In some embodiments, the anti-BCMA sdAb is affinity matured. In some embodiments, the anti-BCMA sdAb is camelid. In some embodiments, the anti-BCMA sdAb is humanized. In some embodiments, the anti-BCMA sdAb comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework. In some embodiments, the anti-BCMA sdAb modulates BCMA activity. In some embodiments, the anti-BCMA sdAb is an antagonist antibody.

[0120] In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 78. In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 79. In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 80. In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 81. In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 82. In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 83. In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 84. In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 85. In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 86. In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID

NO: 87. In some embodiments, the anti-BCMA sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 88.

[0121] In some embodiments, the anti-BCMA sdAb comprises at least one, at least two, or all three CDRs selected from (a) a CDR1 comprising an amino acid sequence selected from SEQ ID NO: 1-11; (b) a CDR2 comprising an amino acid sequence selected from SEQ ID NO: 12-22; and (c) a CDR3 comprising an amino acid sequence selected from SEQ ID NO: 23-33. In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NO:1-11; (b) a CDR2 having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NO:12-22; and (c) a CDR3 having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NO:23-33. In some embodiments, a CDR having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but the anti-BCMA sdAb comprising that sequence retains the ability to bind to BCMA. In some embodiments, the anti-BCMA sdAb comprises a substitution (*e.g.*, conservative substitutions), insertions, or deletions of one, two, three or more amino acids in any one of the CDRs described herein, but the anti-BCMA sdAb comprising that sequence retains the ability to bind to BCMA.

[0122] In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 1; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 12; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 23.

[0123] In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 2; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 13; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 24.

[0124] In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 3; (b) a CDR2 comprising the amino

acid sequence of SEQ ID NO: 14; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 25.

[0125] In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 4; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 15; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 26.

[0126] In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 5; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 16; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 27.

[0127] In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 6; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 17; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO:28.

[0128] In some embodiments, the anti-BCMA sdAb comprises comprising three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 7; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 18; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 29.

[0129] In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 8; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 19; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 30.

[0130] In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 9; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 20; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 31.

[0131] In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 10; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 21; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 32.

[0132] In some embodiments, the anti-BCMA sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 11; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 22; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 33.

[0133] In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NO: 78-88. In some embodiments, a V_HH sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but the anti-BCMA sdAb comprising that sequence retains the ability to bind to BCMA. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in an amino acid sequence selected from SEQ ID NO: 78-88. In some embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs). Optionally, the anti-BCMA sdAb comprises an amino acid sequence selected from SEQ ID NO: 78-88, including post-translational modifications of that sequence.

[0134] In some embodiments, the anti-BCMA sdAb comprises comprising a V_HH domain having the amino acid sequence of SEQ ID NO: 78. In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 79. In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 80. In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 81. In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 82. In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 83. In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 84. In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 85. In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 86. In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 87. In some embodiments, the anti-BCMA sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 88.

[0135] In some embodiments, functional epitopes can be mapped by combinatorial alanine scanning. In this process, a combinatorial alanine-scanning strategy can be used to identify amino acids in the BCMA protein that are necessary for interaction with anti-BCMA single-domain antibodies. In some embodiments, the epitope is conformational and crystal structure of anti-BCMA single-domain antibody bound to BCMA may be employed to identify the epitopes. In some embodiments, the anti-BCMA sdAb specifically binds to the same epitope as any of the anti-BCMA single-domain antibodies provided herein. For example, in some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 78. In some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 79. In some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 80. In some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 81. In some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 82. In some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 83. In some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 84. In some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 85. In some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 86. In some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 87. In some embodiments, the anti-BCMA sdAb binds to the same epitope as an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO: 88.

[0136] In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with any one of the anti-BCMA single-domain antibodies described herein. In some embodiments, competitive binding may be determined using an ELISA assay. For example, in some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an

anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:78. In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:79. In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:80. In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:81. In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:82. In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:83. In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:84. In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:85. In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:86. In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:87. In some embodiments, the anti-BCMA sdAb specifically binds to BCMA competitively with an anti-BCMA single-domain antibody comprising the amino acid sequence of SEQ ID NO:88.

Anti-CD38 sdAb

[0137] In some embodiments, the target cell binding domain comprises one or more anti-CD38 sdAbs, such as anti-CD38 V_HH. In some embodiments, the anti-CD38 sdAb is affinity matured. In some embodiments, the anti-CD38 sdAb is camelid. In some embodiments, the anti-CD38 sdAb is humanized. In some embodiments, the anti-CD38 sdAb comprises an acceptor human framework, *e.g.*, a human immunoglobulin framework or a human consensus framework. In some embodiments, the anti-CD38 sdAb modulates CD38 activity. In some embodiments, the anti-CD38 sdAb is an antagonist antibody.

[0138] In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 89. In some embodiments, the anti-CD38 sdAb

comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 90. In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 91. In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 92. In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 93. In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 94. In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 95. In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 96. In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 97. In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 98. In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 99. In some embodiments, the anti-CD38 sdAb comprises one, two, or all three CDRs of the amino acid sequence of SEQ ID NO: 100.

[0139] In some embodiments, the anti-CD38 sdAb comprises at least one, at least two, or all three CDRs selected from (a) a CDR1 comprising an amino acid sequence selected from SEQ ID NO: 34-45; (b) a CDR2 comprising an amino acid sequence selected from SEQ ID NO: 46-57; and (c) a CDR3 comprising an amino acid sequence selected from SEQ ID NO:58-69. In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NO:34-45; (b) a CDR2 having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NO:46-57; and (c) a CDR3 having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NO:58-69. In some embodiments, a CDR having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but the anti-CD38 sdAb comprising that sequence retains the ability to bind to CD38. In some embodiments, the anti-CD38 sdAb

comprises a substitution (*e.g.*, conservative substitutions), insertions, or deletions of one, two, three or more amino acids in any one of the CDRs described herein, but the anti-CD38 sdAb comprising that sequence retains the ability to bind to CD38.

[0140] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 34; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 46; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 58.

[0141] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 35; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 47; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 59.

[0142] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 36; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 48; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 60.

[0143] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 37; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 49; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 61.

[0144] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 38; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 50; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 62.

[0145] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 39; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 51; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 63.

[0146] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 40; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 52; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 64.

[0147] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 41; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 53; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 65.

[0148] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 42; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 54; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 66.

[0149] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 43; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 55; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 67.

[0150] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 44; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 56; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 68.

[0151] In some embodiments, the anti-CD38 sdAb comprises three CDRs comprising: (a) a CDR1 comprising the amino acid sequence of SEQ ID NO: 45; (b) a CDR2 comprising the amino acid sequence of SEQ ID NO: 57; and (c) a CDR3 comprising the amino acid sequence of SEQ ID NO: 69.

[0152] In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to an amino acid sequence selected from SEQ ID NO: 89-100. In some embodiments, a V_HH sequence having at least about any one of 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but the anti-CD38 sdAb comprising that sequence retains the ability to bind to CD38. In some embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in an amino acid sequence selected from SEQ ID NO: 89-100. In some embodiments, substitutions, insertions, or deletions occur in regions outside the CDRs (i.e., in the FRs).

Optionally, the anti-CD38 sdAb comprises an amino acid sequence selected from SEQ ID NO: 89-100, including post-translational modifications of that sequence.

[0153] In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 89. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 90. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 91. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 92. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 93. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 94. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 95. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 96. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 97. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 98. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 99. In some embodiments, the anti-CD38 sdAb comprises a V_HH domain having the amino acid sequence of SEQ ID NO: 100.

[0154] In some embodiments, functional epitopes can be mapped by combinatorial alanine scanning. In this process, a combinatorial alanine-scanning strategy can be used to identify amino acids in the CD38 protein that are necessary for interaction with the anti-CD38 single-domain antibodies. In some embodiments, the epitope is conformational and crystal structure of anti-CD38 single-domain antibody bound to CD38 may be employed to identify the epitopes. In some embodiments, the anti-CD38 sdAb specifically binds to the same epitope as any of the anti-CD38 single-domain antibodies provided herein. For example, in some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 89. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 90. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 91. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-

domain antibody comprising the amino acid sequence of SEQ ID NO: 92. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 93. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 94. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 95. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 96. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 97. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 98. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 99. In some embodiments, the anti-CD38 sdAb binds to the same epitope as an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO: 100.

[0155] In some embodiments, the present application provides an anti-CD38 antibody, or antigen binding fragment thereof, that specifically binds to CD38 competitively with any one of the anti-CD38 single-domain antibodies described herein. In some embodiments, competitive binding may be determined using an ELISA assay. For example, in some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:89. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:90. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:91. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:92. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:93. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody

comprising the amino acid sequence of SEQ ID NO:94. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:95. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:96. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:97. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:98. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:99. In some embodiments, the anti-CD38 sdAb specifically binds to CD38 competitively with an anti-CD38 single-domain antibody comprising the amino acid sequence of SEQ ID NO:100.

4. Other antigen-binding fragments

[0156] In some embodiments, the target cell binding domain comprises an antigen-binding fragment derived from a four-chain antibody that specifically binds to an antigen on a target cell. Exemplary antigen-binding fragments include, but are not limited to, Fab, Fab', Fab'-SH, $F(ab')_2$, Fv, and scFv fragments. For a review of certain antibody fragments, see Hudson et al. *Nat. Med.* 9:129-134 (2003). For a review of scFv fragments, see, *e.g.*, Pluckthün, in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and $F(ab')_2$ fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046. In some embodiments, the antibody fragment does not comprise an Fc region.

[0157] Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson et al., *Nat. Med.* 9:129-134 (2003); and Hollinger et al., *Proc. Natl. Acad. Sci. USA* 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson et al., *Nat. Med.* 9:129-134 (2003).

[0158] Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (*e.g. E. coli* or phage), as described herein.

[0159] In some embodiments, the target cell binding domain comprises an anti-BCMA scFv. The anti-BCMA scFv may be derived from any known anti-BCMA four-chain antibodies, including, but not limited to, C11D5.3 and J22.9-xi. In some embodiments, the anti-BCMA comprises the amino acid sequence of SEQ ID NO: 101. In some embodiments, the anti-BCMA comprises the amino acid sequence of SEQ ID NO: 102.

5. Multivalent target cell binding domain

[0160] In some embodiments, the target cell binding domain has two or more (such as about any one of 2, 3, 4, 5, 6, or more) antigen binding fragments such as single-domain antibodies. In some embodiments, the multivalent target cell binding domain targets a single antigen, and comprises two or more antigen binding fragments for the single antigen. In some embodiments, the multivalent target cell binding domain targets more than one antigen, and the multivalent target cell binding domain comprises two or more antigen binding fragments for at least one antigen. The antigen binding fragments specific for the same antigen may bind to the same epitope of the antigen or bind to different epitopes of the antigen. The antigen binding fragments specific for the same antigen may comprise the same or different single-domain antibodies.

[0161] The chimeric antibody immune effector cell engagers comprising multivalent target cell binding domains describe herein may be especially suitable for targeting multimeric antigens via synergistic binding by the different antigen binding sites, or for enhancing binding affinity or avidity to the antigen. Any of the single-domain antibodies described herein as well as other antigen-binding fragments (*e.g.*, scFv), such as the anti-BCMA, or anti-CD38 antibodies, may be used to provide a multivalent target cell binding domain.

[0162] In some embodiments, the target cell binding domain comprises a plurality of anti-BCMA sdAbs. In some embodiments, the plurality of the anti-BCMA sdAb is fused to each other via peptide bonds or peptide linkers. In some embodiments, each peptide linker is no more than about 50 (such as no more than about any one of 35, 25, 20, 15, 10, or 5) amino acids long.

6. Multispecific target cell binding domain

[0163] In some embodiments, the target cell binding domain can specifically bind to two or more (such as about any one of 2, 3, 4, 5, 6, or more) different antigens. In some embodiments, the multispecific target cell binding domain has one antigen binding fragments for each antigen. In some embodiments, the multispecific target cell binding domain has more than two antigen

binding fragments for at least one antigen. Each antigen binding fragment may comprise a single-domain antibody.

[0164] Depending on the desired antigens to be targeted, the target cell binding domain can be engineered to include the appropriate single-domain antibodies that are specific to the desired antigens. Any one or more of the anti-BCMA or anti-CD38 antibodies described herein may be used in the target cell binding domain in the chimeric antibody immune effector engagers of the present application. In some embodiments, the target cell binding domain comprises an anti-BCMA sdAb and an anti-CD-38 sdAb. The antigen binding fragments (such as sdAbs) can be arranged in any suitable order. For example, the first sdAb is fused to the N-terminus or the C-terminus of the second sdAb. A suitable peptide linker may be placed between different sdAbs to avoid steric hindrance between the sdAbs.

Immune effector cell binding domain

[0165] The chimeric antibody immune effector cell engagers described herein comprise an immune effector cell binding domain. The immune effector cell binding domain comprises an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. Immune effector cells include, but are not limited to, T cells and NK cells.

[0166] In some embodiments, the immune effector cell binding domain specifically binds to CD3, such as human CD3. "CD3" is known in the art as a multi-protein complex of six chains (see, Abbas and Lichtman, 2003; Janeway *et al.*, p172 and 178, 1999). In mammals, the complex comprises a CD3 gamma chain, a CD3 delta chain, two CD3 epsilon chains, and a homodimer of CD3 zeta chains. CD3 as used herein may be from various animal species, including human, primate, mouse, rat, or other mammals. In some embodiments, the immune effector cell binding domain comprises an antigen-binding fragment that specifically binds to an individual CD3 chain, such as CD3 gamma chain, CD3 delta chain, or CD3 epsilon chain. In some embodiments, the antigen-binding fragment specifically binds to a complex formed from two or more individual CD3 chains (*e.g.*, a complex of more than one CD3 epsilon chains, a complex of a CD3 gamma and CD3 epsilon chain, a complex of a CD3 delta and CD3 epsilon chain). In some embodiments, the antigen-binding fragment specifically binds to a CD3 epsilon chain.

[0167] The antigen-binding fragment targeting CD3 can be of any suitable antigen-binding fragments, including but not limited to Fab, scFv, and sdAb (*e.g.*, V_HH). In some embodiments, the antigen-binding fragment is murine, camelid, chimeric, human or humanized. The antigen-

binding fragment can be designed based on any known CD3 antibodies in the art, including, but not limited to, SP34 mouse monoclonal antibody, (see, for example, Pressano, S. The EMBO J. 4:337-344, 1985; Alarcon, B. EMBO J. 10:903-912, 1991; Salmeron A. et al., J. Immunol. 147:3047-52, 1991; Yoshino N. et al., Exp. Anim 49:97-110, 2000; Conrad M L. et al., Cytometry 71A:925-33, 2007; and Yang et al., J. Immunol. 137:1097-1100: 1986), Cris-7 monoclonal antibody (Reinherz, E. L. et al. (eds.), Leukocyte typing II, Springer Verlag, New York, (1986)), BC3 monoclonal antibody (Anasetti et al. (1990) J. Exp. Med. 172:1691), OKT3 (Ortho multicenter Transplant Study Group (1985) N. Engl. J. Med. 313:337) and derivatives thereof such as OKT3 ala-ala (Herold et al. (2003) J. Clin. Invest. 11:409), visilizumab (Carpenter et al. (2002) Blood 99:2712), 145-2C11 monoclonal antibody (Hirsch et al. (1988) J. Immunol. 140: 3766), UCHT-1 (Beverley, P C and Callard, R. E. (1981) Eur. J. Immunol. 11: 329-334) and CD3 binding molecules described in WO2004/106380; WO2004/106381; WO2010/037838; WO2008/119567; WO2007/042261; WO2010/0150918. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment is a scFv derived from OKT3, L2K or UCHT. In some embodiments, the anti-CD3 antigen-binding fragment is derived from an antibody that binds to the same epitope as OKT3, L2K or UCHT. In some embodiments, the anti-CD3 antigen-binding fragment is derived from an antibody that specifically binds to CD3 competitively with OKT3, L2K or UCHT. Sequences of exemplary anti-CD3 antigen-binding fragments are shown in Table 3 below.

Table 3. Sequences of exemplary anti-CD3 antibodies.

Ab fragment	CDR1	CDR2	CDR3
OKT3 VH	YTMHW (SEQ ID NO: 164)	INPSRGYTNYNQKFKD KAT (SEQ ID NO: 165)	YYDDHYCLDY (SEQ ID NO: 166)
OKT3 VL	SASSSVSYMN (SEQ ID NO: 167)	YDTSKLA (SEQ ID NO: 168)	CQQWSSNPF (SEQ ID NO: 169)
L2K.07 VH	YTMHW (SEQ ID NO: 170)	INPSRGYTNYNQKFKD KAT (SEQ ID NO: 171)	YYDDHYCLDY (SEQ ID NO: 172)
L2K.07 VL	RASSSVSYMN (SEQ ID NO: 173)	YDTSKVA (SEQ ID NO: 174)	QQWSSNPLTF (SEQ ID NO: 175)
UCHT1 VH	YTMHW (SEQ ID NO: 176)	INPYKGVSTYNQKFKD KAT (SEQ ID NO: 177)	SGYYGDSDWYFDV (SEQ ID NO: 178)
UCHT1 VL	RASQDIRNYLN (SEQ ID NO: 179)	YTSRLHS (SEQ ID NO: 180)	QQGNTLPWT (SEQ ID NO: 181)

[0168] In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3. In some embodiments, the anti-CD3 antigen-binding fragment comprises a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and/or a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169. In some embodiments, the anti-CD3 antigen-binding fragment is a scFv comprising the amino acid sequence of SEQ ID NO: 103.

[0169] In some embodiments, the anti-CD3 antigen-binding fragment is derived from L2K. In some embodiments, the anti-CD3 antigen-binding fragment comprises a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and/or a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175. In some embodiments, the anti-CD3 antigen-binding fragment is a scFv comprising the amino acid sequence of SEQ ID NO: 104.

[0170] In some embodiments, the anti-CD3 antigen-binding fragment is derived from UCHT. In some embodiments, the anti-CD3 antigen-binding fragment comprises a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and/or a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment is a scFv comprising the amino acid sequence of SEQ ID NO: 105.

[0171] In some embodiments, the anti-CD3 antigen binding fragment is an sdAb, such as V_HH.

Peptide linkers

[0172] The target cell binding domain and the immune effector cell binding domain may be fused to each other via a peptide linker. In some embodiments, the target cell binding domain and the immune effector cell binding domain are directly fused to each other without any peptide linker.

[0173] In some embodiments, the various antigen-binding fragments (such as sdAbs) in the multispecific or multivalent target cell binding domain are fused to each other via peptide linker(s). In some embodiments, the antigen-binding fragments (such as sdAbs) are directly fused to each other without any peptide linkers. The peptide linkers connecting different antigen-binding fragments (such as sdAbs) may be the same or different.

[0174] Each peptide linker in a chimeric antibody immune effector cell engager may have the same or different length and/or sequence depending on the structural and/or functional features of the antigen-binding fragments (such as sdAbs) and/or the various domains. Each peptide linker may be selected and optimized independently. The length, the degree of flexibility and/or other properties of the peptide linker(s) used in the chimeric antibody immune effector cell engagers may have some influence on properties, including but not limited to the affinity, specificity or avidity for one or more particular antigens or epitopes. For example, longer peptide linkers may be selected to ensure that two adjacent domains do not sterically interfere with one another. For example, in a multivalent or multispecific target cell binding domain that comprises sdAbs directed against a multimeric antigen, the length and flexibility of the peptide linkers are preferably such that it allows each antigen-binding fragment (such as sdAb) to bind to the antigenic determinant on each of the subunits of the multimer.

[0175] In some embodiment, a peptide linker comprises flexible residues (such as glycine and serine) so that the adjacent domains are free to move relative to each other. For example, a $(GGGGS)_3$ linker (SEQ ID NO: 74) can be a suitable peptide linker between the target cell binding domain and the immune effector cell binding domain. In some embodiments, the peptide linker is no more than about 50 (such as no more than about any one of 35, 25, 20, 15, 10, or 5) amino acids long. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0176] The peptide linker can be of any suitable length. In some embodiments, the peptide linker is at least about any of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, 75, 100 or more amino acids long. In some embodiments, the peptide linker is no more than about any of 100, 75, 50, 40, 35, 30, 25, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5 or fewer amino acids long. In some embodiments, the length of the peptide linker is any of about 1 amino acid to about 10 amino acids, about 1 amino acids to about 20 amino acids,

about 1 amino acid to about 30 amino acids, about 5 amino acids to about 15 amino acids, about 10 amino acids to about 25 amino acids, about 5 amino acids to about 30 amino acids, about 10 amino acids to about 30 amino acids long, about 30 amino acids to about 50 amino acids, about 50 amino acids to about 100 amino acids, or about 1 amino acid to about 100 amino acids.

[0177] The peptide linker may have a naturally occurring sequence, or a non-naturally occurring sequence. For example, a sequence derived from the hinge region of heavy chain only antibodies may be used as the linker. *See,* for example, WO1996/34103. In some embodiments, the peptide linker is a flexible linker. Exemplary flexible linkers include glycine polymers $(G)_n$, glycine-serine polymers (including, for example, $(GS)_n$, $(GSGGS)_n$, $(GGGS)_n$, and $(GGGGS)_n$, where n is an integer of at least one), glycine-alanine polymers, alanine-serine polymers, and other flexible linkers known in the art. In some embodiments, the peptide linker comprises the amino acid sequence GGGGS (SEQ ID NO: 72), $(GGGGS)_2$ (SEQ ID NO: 73), $(GGGGS)_3$ (SEQ ID NO: 74), $(GGGS)_2$ (SEQ ID NO: 75), $(GGGS)_4$ (SEQ ID NO: 76), or GSTSGSGKPGSGEGSTKG(SEQ ID NO: 77).

Signal peptide

[0178] The chimeric antibody immune effector cell engagers of the present application may comprise a signal peptide (also known as a signal sequence) at the N-terminus of the polypeptide. In general, signal peptides are peptide sequences that target a polypeptide to the desired site in a cell. In some embodiments, the signal peptide targets the chimeric antibody immune effector cell engager to the secretory pathway of the cell and will allow secretion of the chimeric antibody immune effector cell engager into the cell culture media. Signal peptides including signal sequences of naturally occurring proteins or synthetic, non-naturally occurring signal sequences. In some embodiments, the signal peptide is derived from a human albumin signal peptide (e.g., MKWVTFISLLFLFSSAYS, SEQ ID NO: 70). In some embodiments, the signal peptide is derived from a human azurocidin secretion signal (e.g., MTRLTVLALLAGLLASSRA, SEQ ID NO: 71).

Exemplary chimeric antibody immune effector engagers

[0179] Exemplary chimeric antibody immune effector engagers are provided herein.

[0180] In some embodiments, there is provided a chimeric antibody immune effector engager comprising at least one anti-BCMA V_HH and an anti-CD3 scFv. In some embodiments, the

chimeric antibody immune effector engager comprises at least one anti-BCMA V_HH domain and an L2K.07 scFv ("BCMA-L2K.07 CATE"). In some embodiments, the chimeric antibody immune effector engager comprises a single anti-BCMA V_HH domain. In some embodiments, the chimeric antibody immune effector engager comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 106-116. In some embodiments, the chimeric antibody immune effector engager comprises two, three or more anti-BCMA V_HH domains. In some embodiments, the chimeric antibody immune effector engager comprises an amino acid sequence of SEQ ID NO: 152 or 154.

[0181] In some embodiments, the chimeric antibody immune effector engager comprises at least one anti-BCMA V_HH and an OKT3 scFv ("BCMA-OKT3 CATE"). In some embodiments, the chimeric antibody immune effector engager comprises a single anti-BCMA V_HH domain. In some embodiments, the chimeric antibody immune effector engager comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 117-127. In some embodiments, the chimeric antibody immune effector engager comprises two, three or more anti-BCMA V_HH domains. In some embodiments, the chimeric antibody immune effector engager comprises an amino acid sequence of SEQ ID NO:153 or 155.

[0182] In some embodiments, the chimeric antibody immune effector engager comprises at least one anti-BCMA VHH domain and an UCHT1 scFv ("BCMA-UCHT1 CATE").

[0183] In some embodiments, the chimeric antibody immune effector engager comprises at least one anti-BCMA V_HH and an anti-CD3 V_HH.

[0184] In some embodiments, the chimeric antibody immune effector engager comprises at least one anti-CD38 V_HH and an anti-CD3 scFv. In some embodiments, the chimeric antibody immune effector engager comprises at least one anti-CD38 V_HH and an L2K.07 scFv ("CD38-L2K.07 CATE"). In some embodiments, the chimeric antibody immune effector engager comprises a single anti-CD38 V_HH domain. In some embodiments, the chimeric antibody immune effector engager comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 128-139. In some embodiments, the chimeric antibody immune effector engager comprises two, three or more anti-CD38 V_HH domains. In some embodiments, the chimeric antibody immune effector engager comprises an amino acid sequence of SEQ ID NO:156 or 158.

[0185] In some embodiments, the chimeric antibody immune effector engager comprises at least one anti-CD38 V_HH and an OKT3 scFv ("CD38-OKT3 CATE"). In some embodiments, the chimeric antibody immune effector engager comprises a single anti-CD38 V_HH domain. In some embodiments, the chimeric antibody immune effector engager comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 140-151. In some embodiments, the chimeric antibody immune effector engager comprises two, three or more anti-CD38 V_HH domains. In some embodiments, the chimeric antibody immune effector engager comprises an amino acid sequence of SEQ ID NO:157 or 159.

[0186] In some embodiments, the chimeric antibody immune effector engager comprises at least one anti-CD38 V_HH and an UCHT1 scFv ("CD38-UCHT1 CATE").

[0187] In some embodiments, the chimeric antibody immune effector engager comprises at least one anti-CD38 V_HH and an anti-CD3 V_HH.

[0188] In some embodiments, the chimeric antibody immune effector engager comprises an anti-BCMA V_HH, an anti-CD38 V_HH, and an anti-CD3 antigen-binding fragment ("BCMA*CD38-CD3 CATE"). In some embodiments, the anti-CD3 antigen-binding fragment is a Fab, scFv or sdAb (such as V_HH). In some embodiments, the anti-CD3 antigen-binding fragment is camelid, chimeric, human or humanized. In some embodiments, the anti-CD3 antigen-binding fragment is derived from of OKT3, L2K or UCHT1. In some embodiments, the chimeric antibody immune effector engager comprises an amino acid sequence selected from SEQ ID NOs: 160-163 are provided.

Sequence Variants

[0189] In some embodiments, amino acid sequence variants of the chimeric antibody immune effector engagers provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of any one of the sdAbs or antigen-binding fragments. Amino acid sequence variants of an antigen-binding fragment may be prepared by introducing appropriate modifications into the nucleic acid sequence encoding the antigen-binding fragment, or by peptide synthesis. Such modifications include, for example, deletions from, insertions into and/or substitutions of residues within the amino acid sequences of the antigen-binding fragments. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding and immune effector cell activation.

[0190] In some embodiments, the chimeric antibody immune effector engager variant has one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 4 under the heading of "Preferred substitutions." More substantial changes are provided in Table 4 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes.

Table 4. Amino Acid Substitutions

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

[0191] Amino acids may be grouped according to common side-chain properties:

(1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

(2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

(3) acidic: Asp, Glu;

(4) basic: His, Lys, Arg;

(5) residues that influence chain orientation: Gly, Pro;

(6) aromatic: Trp, Tyr, Phe.

[0192] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0193] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody or fragment thereof (*e.g.*, a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, *e.g.*, using phage display-based affinity maturation techniques known in the art. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (*e.g.* binding affinity).

[0194] Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antigen-binding affinity. Such alterations may be made in HVR "hotspots," *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (*see, e.g.*, Chowdhury, *Methods Mol. Biol.* 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant V_H or V_L being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom et al. in *Methods in Molecular Biology* 178:1-37 (O'Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using

alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0195] In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody or antigen-binding fragment thereof to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or CDRs. In some embodiments of the variant V_HH sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0196] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) *Science*, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0197] Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an N-terminal methionyl residue. Other insertional variants of the chimeric antibody immune effector engager include the fusion to the N- or C-terminus of the chimeric antibody immune effector engager to an enzyme (*e.g.*, for ADEPT) or a polypeptide which increases the serum half-life of the chimeric antibody immune effector engager. Peptide tags, such as Hisx6 tags, may be added to the chimeric antibody immune effector engager (e.g., at the C-terminus) to facilitate its purification and detection.

III. Methods of preparation

[0198] The chimeric antibody immune effector cell engagers may be prepared using any methods known in the art for recombinant preparation of antibodies or as described herein.

Nucleic acid molecules encoding chimeric antibody immune effector cell engagers

[0199] Nucleic acid molecules comprising polynucleotides that encode one or more chains of the chimeric antibody immune effector cell engagers are provided. In some embodiments, wherein the chimeric antibody immune effector cell engager comprises a single polypeptide, a nucleic acid molecule comprises a polynucleotide that encodes the target cell binding domain fused to the immune cell binding domain is provided.

[0200] In some embodiments, a polynucleotide encoding the chimeric antibody immune effector cell engager comprises a nucleotide sequence that encodes a leader sequence, which, when translated, is located at the N terminus of the chimeric antibody immune effector cell engager. The leader sequence may be the native heavy or light chain leader sequence, or may be another heterologous leader sequence.

[0201] Nucleic acid molecules may be constructed using recombinant DNA techniques conventional in the art. In some embodiments, a nucleic acid molecule is an expression vector that is suitable for expression in a selected host cell.

[0202] Vectors comprising polynucleotides that encode chimeric antibody immune effector cell engagers are provided. Such vectors include, but are not limited to, DNA vectors, phage vectors, viral vectors, retroviral vectors, *etc.*

[0203] In some embodiments, a vector is selected that is optimized for expression of polypeptides in CHO or CHO-derived cells, or in other mammalian cells. Exemplary such vectors are described, *e.g.*, in Running Deer *et al.*, *Biotechnol. Prog.* 20:880-889 (2004).

Host Cells

[0204] In various embodiments, the chimeric antibody immune effector cell engagers may be expressed in prokaryotic cells, such as bacterial cells; or in eukaryotic cells, such as fungal cells (such as yeast), plant cells, insect cells, and mammalian cells. Such expression may be carried out, for example, according to procedures known in the art. Exemplary eukaryotic cells that may be used to express polypeptides include, but are not limited to, COS cells, including COS 7 cells; 293 cells, including 293-6E cells; CHO cells, including CHO-S, DG44. Lec13 CHO cells, and FUT8 CHO cells; PER.C6® cells (Crucell); and NSO cells. In some embodiments, the chimeric

antibody immune effector cell engagers may be expressed in yeast. See, *e.g.*, U.S. Publication No. US 2006/0270045 A1. In some embodiments, a particular eukaryotic host cell is selected based on its ability to make desired post-translational modifications to the chimeric antibody immune effector cell engagers.

[0205] Introduction of one or more nucleic acids into a desired host cell may be accomplished by any method, including but not limited to, calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, *etc.* Nonlimiting exemplary methods are described, *e.g.*, in Sambrook *et al.*, Molecular Cloning, A Laboratory Manual, 3rd ed. Cold Spring Harbor Laboratory Press (2001). Nucleic acids may be transiently or stably transfected in the desired host cells, according to any suitable method.

[0206] The present application also provides host cells comprising any of the polynucleotides or vectors described herein. In some embodiments, the present application provides a host cell comprising a chimeric antibody immune effector cell engager. Any host cells capable of over-expressing heterologous DNAs can be used for the purpose of isolating the genes encoding the antibody, polypeptide or protein of interest. Non-limiting examples of mammalian host cells include but not limited to COS, HeLa, and CHO cells. See also PCT Publication No. WO 87/04462. Suitable non-mammalian host cells include prokaryotes (such as *E. coli* or *B. subtillis*) and yeast (such as *S. cerevisae*, *S. pombe*; or *K. lactis*).

Purification of chimeric antibody immune effector cell engagers

[0207] The chimeric antibody immune effector cell engagers may be purified by any suitable method. Such methods include, but are not limited to, the use of affinity matrices or hydrophobic interaction chromatography. Hydrophobic interactive chromatography, for example, a butyl or phenyl column, may be suitable for purifying some chimeric antibody immune effector cell engagers. Ion exchange chromatography (*e.g.* anion exchange chromatography and/or cation exchange chromatography) may also be suitable for purifying some chimeric antibody immune effector cell engagers. Mixed-mode chromatography (*e.g.* reversed phase/anion exchange, reversed phase/cation exchange, hydrophilic interaction/anion exchange, hydrophilic interaction/cation exchange, *etc.*) may also be suitable for purifying chimeric antibody immune effector cell engagers. Many methods of purifying polypeptides are known in the art.

Cell-free production of chimeric antibody immune effector cell engagers

[0208] In some embodiments, the chimeric antibody immune effector cell engager is produced in a cell-free system. Nonlimiting exemplary cell-free systems are described, *e.g.*, in Sitaraman *et al.*, *Methods Mol. Biol.* 498: 229-44 (2009); Spirin, *Trends Biotechnol.* 22: 538-45 (2004); Endo *et al.*, *Biotechnol. Adv.* 21: 695-713 (2003).

IV. Pharmaceutical compositions

[0209] Further provided by the present application are pharmaceutical compositions comprising any one of the chimeric antibody immune effector cell engagers described herein, and a pharmaceutically acceptable carrier. Pharmaceutical compositions can be prepared by mixing a chimeric antibody immune effector cell engager having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions.

[0210] Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers, antioxidants including ascorbic acid, methionine, Vitamin E, sodium metabisulfite; preservatives, isotonicifiers, stabilizers, metal complexes (*e.g.* Zn-protein complexes); chelating agents such as EDTA and/or non-ionic surfactants.

[0211] Buffers are used to control the pH in a range which optimizes the therapeutic effectiveness, especially if stability is pH dependent. Buffers are preferably present at concentrations ranging from about 50 mM to about 250 mM. Suitable buffering agents for use with the present invention include both organic and inorganic acids and salts thereof. For example, citrate, phosphate, succinate, tartrate, fumarate, gluconate, oxalate, lactate, acetate. Additionally, buffers may comprise histidine and trimethylamine salts such as Tris.

[0212] Preservatives are added to retard microbial growth, and are typically present in a range from 0.2%-1.0% (w/v). Suitable preservatives for use with the present invention include octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium halides (*e.g.*, chloride, bromide, iodide), benzethonium chloride; thimerosal, phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol, 3-pentanol, and m-cresol.

[0213] Tonicity agents, sometimes known as “stabilizers” are present to adjust or maintain the tonicity of liquid in a composition. When used with large, charged biomolecules such as proteins

and antibodies, they are often termed "stabilizers" because they can interact with the charged groups of the amino acid side chains, thereby lessening the potential for inter and intra-molecular interactions. Tonicity agents can be present in any amount between 0.1% to 25% by weight, preferably 1 to 5%, taking into account the relative amounts of the other ingredients. Preferred tonicity agents include polyhydric sugar alcohols, preferably trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol.

[0214] Additional excipients include agents which can serve as one or more of the following: (1) bulking agents, (2) solubility enhancers, (3) stabilizers and (4) and agents preventing denaturation or adherence to the container wall. Such excipients include: polyhydric sugar alcohols (enumerated above); amino acids such as alanine, glycine, glutamine, asparagine, histidine, arginine, lysine, ornithine, leucine, 2-phenylalanine, glutamic acid, threonine, etc.; organic sugars or sugar alcohols such as sucrose, lactose, lactitol, trehalose, stachyose, mannose, sorbose, xylose, ribose, ribitol, myoinisitose, myoinisitol, galactose, galactitol, glycerol, cyclitols (*e.g.*, inositol), polyethylene glycol; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α-monothioglycerol and sodium thio sulfate; low molecular weight proteins such as human serum albumin, bovine serum albumin, gelatin or other immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; monosaccharides (*e.g.*, xylose, mannose, fructose, glucose; disaccharides (*e.g.*, lactose, maltose, sucrose); trisaccharides such as raffinose; and polysaccharides such as dextrin or dextran.

[0215] Non-ionic surfactants or detergents (also known as "wetting agents") are present to help solubilize the therapeutic agent as well as to protect the therapeutic protein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the active therapeutic protein or antibody. Non-ionic surfactants are present in a range of about 0.05 mg/ml to about 1.0 mg/ml, preferably about 0.07 mg/ml to about 0.2 mg/ml.

[0216] Suitable non-ionic surfactants include polysorbates (20, 40, 60, 65, 80, etc.), polyoxamers (184, 188, etc.), PLURONIC® polyols, TRITON®, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.), lauromacrogol 400, polyoxyl 40 stearate, polyoxyethylene hydrogenated castor oil 10, 50 and 60, glycerol monostearate, sucrose fatty acid ester, methyl celluose and carboxymethyl cellulose. Anionic detergents that can be used include sodium lauryl

sulfate, dioctyle sodium sulfosuccinate and dioctyl sodium sulfonate. Cationic detergents include benzalkonium chloride or benzethonium chloride.

[0217] In order for the pharmaceutical compositions to be used for in vivo administration, they must be sterile. The pharmaceutical composition may be rendered sterile by filtration through sterile filtration membranes. The pharmaceutical compositions herein generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[0218] The route of administration is in accordance with known and accepted methods, such as by single or multiple bolus or infusion over a long period of time in a suitable manner, *e.g.*, injection or infusion by subcutaneous, intravenous, intraperitoneal, intramuscular, intraarterial, intralesional or intraarticular routes, topical administration, inhalation or by sustained release or extended-release means.

[0219] Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semi-permeable matrices of solid hydrophobic polymers containing the antagonist, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly (2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and. ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid.

[0220] The pharmaceutical compositions described herein may also contain more than one active compound or agent as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. Alternatively, or in addition, the composition may comprise a cytotoxic agent, chemotherapeutic agent, cytokine, immunosuppressive agent, or growth inhibitory agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

[0221] The active ingredients may also be entrapped in microcapsules prepared, for example, by coascervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-

particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in *Remington's Pharmaceutical Sciences* 18th edition.

V. Methods of treatment

[0222] The present application further provides methods of treating a disease (such as cancer or autoimmune disease) in an individual comprising administering to the individual an effective amount of any one of the pharmaceutical compositions or the chimeric antibody immune effector cell engagers described herein. In some embodiments, the disease is a B cell-related disorder. In some embodiments, the disease is a cancer. In some embodiments the disease is an autoimmune disease.

[0223] In some embodiments, there is provided a method of treating a disease (such as a B cell-related disorder) in an individual (such as a human individual), comprising administering to the individual an effective amount of a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising a sdAb (such as V_HH) that specifically binds to an antigen on a target cell; and (b) an immune effector cell binding domain comprising an anti-CD3 antigen-binding fragment. In some embodiments, the target cell is a tumor cell or a B cell. In some embodiments, the anti-CD3 antigen-binding fragment is a Fab, scFv, or sdAb. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence

of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0224] The methods described herein are suitable for treating various B cell-related disorders, including, but not limited to, plasmacytoma, Hodgkins' lymphoma, follicular lymphomas, small non-cleaved cell lymphomas, endemic Burkitt's lymphoma, sporadic Burkitt's lymphoma, marginal zone lymphoma, extranodal mucosa-associated lymphoid tissue lymphoma , nodal monocytoid B cell lymphoma, splenic lymphoma, mantle cell lymphoma, large cell lymphoma, diffuse mixed cell lymphoma, immunoblastic lymphoma, primary mediastinal B cell lymphoma, pulmonary B cell angiocentric lymphoma, small lymphocytic lymphoma, B cell proliferations of uncertain malignant potential, lymphomatoid granulomatosis, post-transplant lymphoproliferative disorder, an immunoregulatory disorder, rheumatoid arthritis, myasthenia gravis, idiopathic thrombocytopenia purpura, anti-phospholipid syndrome, Chagas1 disease, Grave's disease, Wegener's granulomatosis, poly-arteritis nodosa, Sjogren's syndrome, pemphigus vulgaris, scleroderma, multiple sclerosis, anti-phospholipid syndrome, ANCA associated vasculitis, Goodpasture's disease, Kawasaki disease, autoimmune hemolytic anemia, and rapidly progressive glomerulonephritis, heavy-chain disease, primary or immunocyte-associated amyloidosis, or monoclonal gammopathy of undetermined significance. In some embodiments, the plasma cell malignancy is multiple myeloma. In some embodiments, the autoimmune disease is systemic lupus erythematosus.

[0225] The methods described herein are suitable for treating various cancers, including solid cancers and liquid cancers. Exemplary cancers include, but are not limited to, plasmacytoma, Hodgkins' lymphoma, follicular lymphomas, small non-cleaved cell lymphomas, endemic Burkitt's lymphoma, sporadic Burkitt's lymphoma, marginal zone lymphoma, extranodal mucosa-associated lymphoid tissue lymphoma , nodal monocytoid B cell lymphoma, splenic

lymphoma, mantle cell lymphoma, large cell lymphoma, diffuse mixed cell lymphoma, immunoblastic lymphoma, primary mediastinal B cell lymphoma, pulmonary B cell angiocentric lymphoma, and small lymphocytic lymphoma. In some embodiments, the method is applicable to cancers of at a particular stage, such as early stage, advanced stage and/or metastatic cancer. In some embodiments, the method is used as a first therapy, second therapy, third therapy, or combination therapy with other types of cancer therapies known in the art, such as chemotherapy, surgery, radiation, gene therapy, immunotherapy, bone marrow transplantation, stem cell transplantation, targeted therapy, cryotherapy, ultrasound therapy, photodynamic therapy, radio-frequency ablation or the like, in an adjuvant setting or a neoadjuvant setting. In some embodiments, the method is used for treating a plasma cell disorder, such as multiple myeloma, plasmacytoma or plasma cell leukemia. In some embodiments, the method is used for treating a B cell disorder, such as Non-Hodgkin Lymphoma (NHL) or Chronic Lymphocytic Leukemia (CLL).

[0226] Thus, in some embodiments, there is provided a method of treating a cancer (such as multiple myeloma) in an individual (such as a human individual), comprising administering to the individual an effective amount of a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-BCMA sdAb (such as anti-BCMA V_HH); and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the anti-BCMA sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:1; a CDR2 comprising the amino acid sequence of SEQ ID NO:12; and a CDR3 comprising the amino acid sequence of SEQ ID NO:23; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:2; a CDR2 comprising the amino acid sequence of SEQ ID NO:13; and a CDR3 comprising the amino acid sequence of SEQ ID NO:24; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:3; a CDR2 comprising the amino acid sequence of SEQ ID NO:14; and a CDR3 comprising the amino acid sequence of SEQ ID NO:25; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:4; a CDR2 comprising the amino acid sequence of SEQ ID NO:15; and a CDR3 comprising the amino acid sequence of SEQ ID NO:26; (5) a CDR1 comprising the amino acid

sequence of SEQ ID NO:5; a CDR2 comprising the amino acid sequence of SEQ ID NO:16; and a CDR3 comprising the amino acid sequence of SEQ ID NO:27; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO:6; a CDR2 comprising the amino acid sequence of SEQ ID NO:17; and a CDR3 comprising the amino acid sequence of SEQ ID NO:28; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:7; a CDR2 comprising the amino acid sequence of SEQ ID NO:18; and a CDR3 comprising the amino acid sequence of SEQ ID NO:29; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:8; a CDR2 comprising the amino acid sequence of SEQ ID NO:19; and a CDR3 comprising the amino acid sequence of SEQ ID NO:30; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:9; a CDR2 comprising the amino acid sequence of SEQ ID NO:20; and a CDR3 comprising the amino acid sequence of SEQ ID NO:31; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:10; a CDR2 comprising the amino acid sequence of SEQ ID NO:21; and a CDR3 comprising the amino acid sequence of SEQ ID NO:32; or (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:11; a CDR2 comprising the amino acid sequence of SEQ ID NO:22; and a CDR3 comprising the amino acid sequence of SEQ ID NO:33. In some embodiments, the anti-BCMA sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-88. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0227] In some embodiments, there is provided a method of treating a cancer (such as multiple myeloma) in an individual (such as a human individual), comprising administering to the individual an effective amount of a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-CD38 sdAb (such as anti-CD38 V_HH); and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the anti-CD38 sdAb comprises any one of the following: (1) a CDR1

comprising the amino acid sequence of SEQ ID NO:34; a CDR2 comprising the amino acid sequence of SEQ ID NO:46; and a CDR3 comprising the amino acid sequence of SEQ ID NO:58; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:35; a CDR2 comprising the amino acid sequence of SEQ ID NO:47; and a CDR3 comprising the amino acid sequence of SEQ ID NO:59; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:36; a CDR2 comprising the amino acid sequence of SEQ ID NO:48; and a CDR3 comprising the amino acid sequence of SEQ ID NO:60; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:37; a CDR2 comprising the amino acid sequence of SEQ ID NO:49; and a CDR3 comprising the amino acid sequence of SEQ ID NO:61; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:38; a CDR2 comprising the amino acid sequence of SEQ ID NO:50; and a CDR3 comprising the amino acid sequence of SEQ ID NO:62; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO:39; a CDR2 comprising the amino acid sequence of SEQ ID NO:51; and a CDR3 comprising the amino acid sequence of SEQ ID NO:63; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:40; a CDR2 comprising the amino acid sequence of SEQ ID NO:52; and a CDR3 comprising the amino acid sequence of SEQ ID NO:64; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:41; a CDR2 comprising the amino acid sequence of SEQ ID NO:53; and a CDR3 comprising the amino acid sequence of SEQ ID NO:65; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:42; a CDR2 comprising the amino acid sequence of SEQ ID NO:54; and a CDR3 comprising the amino acid sequence of SEQ ID NO:66; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:43; a CDR2 comprising the amino acid sequence of SEQ ID NO:55; and a CDR3 comprising the amino acid sequence of SEQ ID NO:67; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:44; a CDR2 comprising the amino acid sequence of SEQ ID NO:56; and a CDR3 comprising the amino acid sequence of SEQ ID NO:68; or (12) a CDR1 comprising the amino acid sequence of SEQ ID NO:45; a CDR2 comprising the amino acid sequence of SEQ ID NO:57; and a CDR3 comprising the amino acid sequence of SEQ ID NO:69. In some embodiments, the anti-CD38 sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-100. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell

binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0228] In some embodiments, there is provided a method of treating a cancer (such as multiple myeloma) in an individual (such as a human individual), comprising administering to the individual an effective amount of a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising a first sdAb (such as V_HH) that specifically binds to a first antigen on a first target cell and a second sdAb (such as V_HH) that specifically binds to a second antigen on a second target cell; and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the first target cell and the second target cell are the same cell. In some embodiments, the first target cell and the second target cell are different cells. In some embodiments, the first cell and the second cell are tumor cells. In some embodiments, the first sdAb is fused to the second sdAb via a peptide linker. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the immune effector cell binding domain comprises an anti-CD3 antigen-binding fragment. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence

of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77. In some embodiments, the first sdAb is an anti-BCMA sdAb and the second sdAb is an anti-CD38 sdAb. In some embodiments, the first sdAb and the second sdAb are anti-BCMA sdAbs.

[0229] The methods described herein are also suitable for treating various autoimmune diseases, including, but not limited to, rheumatoid arthritis, myasthenia gravis, idiopathic thrombocytopenia purpura, anti-phospholipid syndrome, Chagas1 disease, Grave's disease, Wegener's granulomatosis, poly-arteritis nodosa, Sjogren's syndrome, pemphigus vulgaris, scleroderma, multiple sclerosis, anti-phospholipid syndrome, ANCA associated vasculitis, Goodpasture's disease, Kawasaki disease, autoimmune hemolytic anemia, and rapidly progressive glomerulonephritis, heavy-chain disease, primary or immunocyte-associated amyloidosis, or monoclonal gammopathy of undetermined significance. In some embodiments, the method is used for treating Systemic Lupus Erythematosus (SLE) or Multiple Sclerosis (MS).

[0230] Thus, in some embodiments, there is provided a method of treating an autoimmune disease (such as systemic lupus erythematosus) in an individual (such as a human individual), comprising administering to the individual an effective amount of a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-BCMA sdAb (such as anti-BCMA V_HH); and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the anti-BCMA sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:1; a CDR2 comprising the amino acid sequence of SEQ ID NO:12; and a CDR3 comprising the amino acid

sequence of SEQ ID NO:23; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:2; a CDR2 comprising the amino acid sequence of SEQ ID NO:13; and a CDR3 comprising the amino acid sequence of SEQ ID NO:24; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:3; a CDR2 comprising the amino acid sequence of SEQ ID NO:14; and a CDR3 comprising the amino acid sequence of SEQ ID NO:25; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:4; a CDR2 comprising the amino acid sequence of SEQ ID NO:15; and a CDR3 comprising the amino acid sequence of SEQ ID NO:26; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:5; a CDR2 comprising the amino acid sequence of SEQ ID NO:16; and a CDR3 comprising the amino acid sequence of SEQ ID NO:27; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO:6; a CDR2 comprising the amino acid sequence of SEQ ID NO:17; and a CDR3 comprising the amino acid sequence of SEQ ID NO:28; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:7; a CDR2 comprising the amino acid sequence of SEQ ID NO:18; and a CDR3 comprising the amino acid sequence of SEQ ID NO:29; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:8; a CDR2 comprising the amino acid sequence of SEQ ID NO:19; and a CDR3 comprising the amino acid sequence of SEQ ID NO:30; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:9; a CDR2 comprising the amino acid sequence of SEQ ID NO:20; and a CDR3 comprising the amino acid sequence of SEQ ID NO:31; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:10; a CDR2 comprising the amino acid sequence of SEQ ID NO:21; and a CDR3 comprising the amino acid sequence of SEQ ID NO:32; or (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:11; a CDR2 comprising the amino acid sequence of SEQ ID NO:22; and a CDR3 comprising the amino acid sequence of SEQ ID NO:33. In some embodiments, the anti-BCMA sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-88. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0231] In some embodiments, there is provided a method of treating an autoimmune disease (such as systemic lupus erythematosus) in an individual (such as a human individual),

comprising administering to the individual an effective amount of a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising an anti-CD38 sdAb (such as anti-CD38 V_HH); and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the anti-CD38 sdAb comprises any one of the following: (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:34; a CDR2 comprising the amino acid sequence of SEQ ID NO:46; and a CDR3 comprising the amino acid sequence of SEQ ID NO:58; (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:35; a CDR2 comprising the amino acid sequence of SEQ ID NO:47; and a CDR3 comprising the amino acid sequence of SEQ ID NO:59; (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:36; a CDR2 comprising the amino acid sequence of SEQ ID NO:48; and a CDR3 comprising the amino acid sequence of SEQ ID NO:60; (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:37; a CDR2 comprising the amino acid sequence of SEQ ID NO:49; and a CDR3 comprising the amino acid sequence of SEQ ID NO:61; (5) a CDR1 comprising the amino acid sequence of SEQ ID NO:38; a CDR2 comprising the amino acid sequence of SEQ ID NO:50; and a CDR3 comprising the amino acid sequence of SEQ ID NO:62; (6) a CDR1 comprising the amino acid sequence of SEQ ID NO:39; a CDR2 comprising the amino acid sequence of SEQ ID NO:51; and a CDR3 comprising the amino acid sequence of SEQ ID NO:63; (7) a CDR1 comprising the amino acid sequence of SEQ ID NO:40; a CDR2 comprising the amino acid sequence of SEQ ID NO:52; and a CDR3 comprising the amino acid sequence of SEQ ID NO:64; (8) a CDR1 comprising the amino acid sequence of SEQ ID NO:41; a CDR2 comprising the amino acid sequence of SEQ ID NO:53; and a CDR3 comprising the amino acid sequence of SEQ ID NO:65; (9) a CDR1 comprising the amino acid sequence of SEQ ID NO:42; a CDR2 comprising the amino acid sequence of SEQ ID NO:54; and a CDR3 comprising the amino acid sequence of SEQ ID NO:66; (10) a CDR1 comprising the amino acid sequence of SEQ ID NO:43; a CDR2 comprising the amino acid sequence of SEQ ID NO:55; and a CDR3 comprising the amino acid sequence of SEQ ID NO:67; (11) a CDR1 comprising the amino acid sequence of SEQ ID NO:44; a CDR2 comprising the amino acid sequence of SEQ ID NO:56; and a CDR3 comprising the amino acid sequence of SEQ ID NO:68; or (12) a

CDR1 comprising the amino acid sequence of SEQ ID NO:45; a CDR2 comprising the amino acid sequence of SEQ ID NO:57; and a CDR3 comprising the amino acid sequence of SEQ ID NO:69. In some embodiments, the anti-CD38 sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-100. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77.

[0232] In some embodiments, there is provided a method of treating an autoimmune disease (such as systemic lupus erythematosus) in an individual (such as a human individual), comprising administering to the individual an effective amount of a chimeric antibody immune effector cell engager comprising: (a) a target cell binding domain comprising a first sdAb (such as V_HH) that specifically binds to a first antigen on a first target cell and a second sdAb (such as V_HH) that specifically binds to a second antigen on a second target cell; and (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell. In some embodiments, the first target cell and the second target cell are the same cell. In some embodiments, the first target cell and the second target cell are different cells. In some embodiments, the first cell and the second cell are tumor cells. In some embodiments, the first sdAb is fused to the second sdAb via a peptide linker. In some embodiments, the immune effector cell is a T cell. In some embodiments, the immune effector cell is an NK cell. In some embodiments, the antigen-binding fragment of the immune effector cell is a Fab, scFv, or sdAb. In some embodiments, the immune effector cell binding domain comprises an anti-CD3 antigen-binding fragment. In some embodiments, the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1. In some embodiments, the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino

acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181. In some embodiments, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105. In some embodiments, the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain. In some embodiments, the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker, such as a peptide linker comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 72-77. In some embodiments, the first sdAb is an anti-BCMA sdAb and the second sdAb is an anti-CD38 sdAb. In some embodiments, the first sdAb and the second sdAb are anti-BCMA sdAbs.

[0233] Administration of the pharmaceutical compositions may be carried out in any convenient manner, including by injection, ingestion, transfusion, implantation or transplantation. The compositions may be administered to a patient transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, intravenously, or intraperitoneally. In some embodiments, the pharmaceutical composition is administered systemically. In some embodiments, the pharmaceutical composition is administered to an individual by infusion, such as intravenous infusion. Infusion techniques for immunotherapy are known in the art (see, *e.g.*, Rosenberg *et al.*, New Eng. J. of Med. 319: 1676 (1988)). In some embodiments, the pharmaceutical composition is administered to an individual by intradermal or subcutaneous injection. In some embodiments, the compositions are administered by intravenous injection. In some embodiments, the compositions are injected directly into a tumor, or a lymph node. In some embodiments, the pharmaceutical composition is administered locally to a site of tumor, such as directly into tumor cells, or to a tissue having tumor cells.

[0234] Dosages and desired drug concentration of pharmaceutical compositions of the present invention may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles laid down by Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in Toxicokinetics," In *Toxicokinetics and New Drug Development*, Yacobi *et al.*, Eds, Pergamon Press, New York 1989, pp. 42-46. It is within the scope of the present application that different formulations will be effective for different treatments and different disorders, and that administration intended to treat a specific organ or tissue may necessitate delivery in a manner different from that to another organ or tissue.

[0235] In some embodiments, the pharmaceutical composition or the chimeric antibody immune effector cell engager is administered at ta dosage of about 10 ng/kg up to about 100 mg/kg of body weight of the individual or more per day, for example, at about 1 mg/kg/day to 10 mg/kg/day, depending upon the route of administration. In some embodiments, the pharmaceutical composition is administered for a single time. In some embodiments, the pharmaceutical composition is administered for multiple times (such as any of 2, 3, 4, 5, 6, or more times). In some embodiments, the pharmaceutical composition is administered at a frequency of once per week to once per year. In some embodiments, the interval between administrations is from about 1 week to about a year. The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

[0236] In some embodiments, the pharmaceutical composition or the chimeric antibody immune effector cell engager is administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

VI. Kits and articles of manufacture

[0237] Further provided are kits, unit dosages, and articles of manufacture comprising any one of the chimeric antibody immune effector cell engagers described herein. In some embodiments,

a kit is provided which contains any one of the pharmaceutical compositions described herein and preferably provides instructions for its use.

[0238] The kits of the present application are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Kits may optionally provide additional components such as buffers and interpretative information. The present application thus also provides articles of manufacture, which include vials (such as sealed vials), bottles, jars, flexible packaging, and the like.

[0239] The article of manufacture can comprise a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, *etc*. The containers may be formed from a variety of materials such as glass or plastic. Generally, the container holds a composition which is effective for treating a disease or disorder (such as cancer or autoimmune disease) as described herein, and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The label or package insert indicates that the composition is used for treating the particular condition in an individual. The label or package insert will further comprise instructions for administering the composition to the individual. The label may indicate directions for reconstitution and/or use. The container holding the pharmaceutical composition may be a multi-use vial, which allows for repeat administrations (*e.g.* from 2-6 administrations) of the reconstituted formulation. Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. Additionally, the article of manufacture may further comprise a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[0240] The kits or article of manufacture may include multiple unit doses of the pharmaceutical composition and instructions for use, packaged in quantities sufficient for storage and use in pharmacies, for example, hospital pharmacies and compounding pharmacies.

EXAMPLES

[0241] The examples and exemplary embodiments below are intended to be purely exemplary of the invention and should therefore not be considered to limit the invention in any way. The following examples and detailed description are offered by way of illustration and not by way of limitation.

Example 1. Preparation of chimeric antibody immune cell engagers

[0242] This example describes the design and preparation of exemplary chimeric antibody T cell engagers (CATEs) using antigen-binding fragments from previously identified anti-BCMA, anti-CD38 and anti-CD3 antibodies. The exemplary CATEs are referred herein as BCMA CATEs, CD38 CATEs and BCMA*CD38 CATEs respectively.

1. Single domain antibody generation

[0243] Single-domain antibody or V_HH repertoires obtained from llamas and cloned as a phage library were used for selection. Methods of sdAb generation have been described, for example, in PCT/CN2017/072723. Briefly, to develop single-domain antibodies with high binding affinity to specified antigens, llamas were immunized and a phage display library was constructed to identify V_HH leads. Distinct clones were picked at random and were classified according to the sequence of the heavy chain complementarity determining region 3 (CDR3), a region that plays a major role in antigen binding. Through library screening for particular characteristics, several anti-BCMA sdAbs and anti-CD38 sdAbs with excellent properties were obtained. The nucleotide sequences of the heavy chain variable domains of anti-BCMA sdAbs and anti-CD38 sdAbs were obtained by gene sequencing. Some of the sdAbs were chosen to design the exemplary CATEs.

2. Design of exemplary chimeric antibody T cell engagers

[0244] Exemplary CATE molecules were designed, each comprising a target cell binding domain targeting human antigen BCMA or CD38, and a T-cell binding domain targeting CD3 epsilon.

[0245] Any methods known in the art can be used to prepare an antibody against a given antigen, which comprises immunoglobulin light and/or heavy chain variable regions. Antigen-binding fragments of various formats can be designed based on the full-length antibodies. For example, the antigen-binding fragment of a single domain antibody can be a single heavy chain variable

domain (V_HH), which can have high affinity to an antigen without the aid of a light chain. Suitable antigen-binding fragments based on four-chain antibodies include scFv and Fab.

[0246] In this example, the immune effector cell binding domain is an anti-CD3 scFv derived from known anti-CD3 antibodies, OKT3, L2K and UCHT1. The VH and VL of the anti-CD3 antibodies were gene edited to prepare the corresponding scFvs, which were fused to a target cell binding domain comprising an anti-BCMA sdAb and/or an anti-CD38 sdAb to provide sdAb-based CATEs. Additionally, BCMA scFv-based CATEs comprising an anti-BCMA scFv derived from anti-BCMA antibody J22.9-xi (PDB database: 4ZFO) or C11D5.3 (*see*, WO2010104949) were prepared.

3. Vectors encoding CATEs

[0247] Three CATE backbone plasmids carrying three different anti-CD3 scFv nucleotide sequences were designed based on the mammalian protein expression vector pTT5 (NRC Biotechnology Research Institute). These three CATE backbone plasmids were named pTT5-LIB-BB1, pTT5-LIB-BB2, and pTT5-LIB-BB3 respectively. The CATE backbone vectors were prepared as follows.

[0248] Construction of pTT5-LIB-BB1 vector: nucleotide sequence encoding anti-CD3 scFv (with 6xHis tag at the C-terminus) from the clone L2K-O7 (Drugbank access NO. DB09052) was codon optimized for expression in human and CHO cell systems. The codon-optimized nucleotide sequence was chemically synthesized and subcloned into a pTT5 vector via *Xba*I and *Hind*III cloning sites using known molecular cloning techniques in the art.

[0249] Construction of pTT5-LIB-BB2 vector: nucleotide sequence encoding anti-CD3 scFv (with 6xHis tag at the C-terminus) from the clone mOKT3 (PDB access NO. 1SY6) was codon optimized for expression in human and CHO cell systems. The codon-optimized nucleotide sequence was chemically synthesized and subcloned into a pTT5 vector via *Xba*I and *Hind*III cloning sites using known molecular cloning techniques in the art.

[0250] Construction of pTT5-LIB-BB3 vector: nucleotide sequence encoding anti-CD3 scFv (with 6xHis tag at the C-terminus) from the clone mUCHT1 (PDB access NO. 1XIW) were codon optimized for better expression in human and CHO cell systems. The codon-optimized nucleotide sequence was chemically synthesized and subcloned to a pTT5 vector via *Xba*I and *Hind*III cloning sites using known molecular cloning techniques in the art.

[0251] Two different signal peptides, human albumin secretion signal peptide (*e.g.*, SEQ ID NO: 70) and human azurocidin secretion signal (*e.g.*, SEQ ID NO: 71) were selected. See, Biotechnol Bioeng. 2013 Apr; 110(4):1164-73.

[0252] To construct the CATE expression plasmids, nucleotides encoding a signal peptide, at least one anti-BCMA V_HH and/or an anti-CD38 V_HH, and a peptide linker were fused together in sequence. The nucleotide sequences were codon optimized for expression in human and CHO cell systems. The codon-optimized nucleotide sequences having a Kozak sequence at 5' end were chemically synthesized and subcloned into each CATE backbone vector (pTT5-LIB-BB1, pTT5-LIB-BB2, and pTT5-LIB-BB3), via *EcoR*I and *Xba*I restriction endounclease sites known molecular cloning techniques in the art. The exemplary CATE constructs of this example are shown in Table 5 below.

Table 5. Exemplary CATE constructs.

CATE code	Signal peptide	First antibody	Linker	CATE backbone plasmids
CATE#001	Human albumin SP	BCMA V_HH1	$(G4S)_3$ (SEQ ID NO: 74)	pTT5-LIB-BB1
CATE#002	Human albumin SP			pTT5-LIB-BB2
CATE#003	Human albumin SP			pTT5-LIB-BB3
CATE#004	Human azurocidin SP			pTT5-LIB-BB1
CATE#005	Human azurocidin SP			pTT5-LIB-BB2
CATE#006	Human azurocidin SP			pTT5-LIB-BB3
CATE#007	Human albumin SP	BCMA V_HH2	$(G4S)_3$ (SEQ ID NO: 74)	pTT5-LIB-BB1
CATE#008	Human albumin SP			pTT5-LIB-BB2
CATE#009	Human albumin SP			pTT5-LIB-BB3
CATE#010	Human albumin SP	BCMA V_HH1*BCMA V_HH2		pTT5-LIB-BB1
CATE#011	Human albumin SP			pTT5-LIB-BB2
CATE#012	Human albumin SP			pTT5-LIB-BB3
CATE#019	Human albumin SP	BCMA scFv (C11D5.3)VL-VH	$(G4S)_3$ (SEQ ID NO: 74)	pTT5-LIB-BB1
CATE#020	Human albumin SP			pTT5-LIB-BB2
CATE#021	Human albumin SP			pTT5-LIB-BB3
CATE#022	Human albumin SP	BCMA scFv (J22.9-xi)VH-VL	$(G4S)_3$ (SEQ ID NO: 74)	pTT5-LIB-BB1
CATE#023	Human albumin SP			pTT5-LIB-BB2
CATE#024	Human albumin SP			pTT5-LIB-BB3
CATE#025	Human albumin SP	BCMA V_HH*CD38 V_HH	$(G4S)_3$ (SEQ ID NO: 74)	pTT5-LIB-BB1
CATE#026	Human albumin SP			pTT5-LIB-BB2
CATE#027	Human albumin SP			pTT5-LIB-BB3
CATE#028	Human albumin SP	CD38 V_HH	$(G4S)_3$ (SEQ ID	pTT5-LIB-BB1
CATE#029	Human albumin SP			pTT5-LIB-BB2

CATE#030	Human albumin SP		NO: 74)	pTT5-LIB-BB3

Example 2. Expression of exemplary CATEs in mammalian host cells

[0253] We established cell lines to express the exemplary CATEs.

[0254] Briefly, plasmids encoding exemplary CATEs were transiently transfected into 293-6E cells respectively. Briefly, $3x10^5$ 293-6E cells were seeded into 12-well plates. 2 μg of each ready-to-transfect CATE plasmid was mixed at a pre-optimized ratio with polyetherimide (PEI), then mixed thoroughly and incubated at room temperature for 5 minutes. The transfection mix was then added dropwise to the 293-6E cells and mixed gently. Afterwards, cells were incubated overnight in a 37°C and 5% CO_2 cell incubator.

[0255] On the next day, the wells containing transfected cells were refreshed with fresh freestyle medium, and then the transfected cells were allowed to grow in a cell incubator for 4 additional days. On the last day, supernatants from the wells were collected and centrifuged at 500 g 4°C for 10 min. Supernatants were then aliquoted and stored at -80°C. The pMAX-GFP plasmids (Lonza) were used as controls with identical transfection protocol to monitor the transfection efficiency on each plate. The expression level of GFP in each transfection was analyzed using an ATTUNE™ Nxt flow cytometer (Themorfisher Scientific). The GFP expression level in each control well was over 95% indicating successful transfections.

[0256] The expression levels of the exemplary CATEs in the supernatants of the 293-6E cells were determined using an anti-6xHis tag-HRP based ELISA. FIG. 1 shows the expression levels of the exemplary CATEs, which were about 1-2 μg/mL on average.

Example 3. *In vitro* cytotoxicity of exemplary CATEs against multiple myeloma cells

1. Primary human T cell preparation

[0257] Leukocytes were collected from healthy donors by apheresis, and cell concentration was adjusted to 5×10^6 cells /ml in R10 medium. Leukocytes were then mixed with 0.9% NaCl solution at 1:1 (v/v) ratio. 3 mL lymphoprep medium was added to a 15 mL centrifuge tube, and 6 ml of diluted lymphocyte mix was slowly layered on top of the lymphoprep medium. The lymphocyte mix was centrifuged at 800 g for 30 minutes without break at 20°C. Lymphocyte buffy coat was then collected with a 200 μL pipette. The harvested fraction was diluted at least 6 folds with 0.9% NaCl or R10 to reduce density of the solution. The harvested fraction was then

centrifuged at 250g for 10 minutes at 20°C. The supernatant was aspirated completely, and 10 mL of R10 was added for re-suspending the cell pellet. The mixture was further centrifuged at 250 g for 10 minutes at 20°C. The supernatant was again aspirated. 2 mL of 37°C pre-warmed R10 with 100IU/mL IL-2 was added to the cell pellet, and the cell pellet was re-suspended softly. The cell number was determined following Trypan Blue staining. The obtained PBMC sample was ready for later experiments.

[0258] Human T cells were purified from PBMCs using Miltenyi Pan T cell isolation kit (Cat#130-096-535), following the manufacturer's protocol. Briefly, cell number was first determined and the cell suspension was centrifuged at 300 g for 10 minutes. The supernatant was then aspirated completely, and the cell pellets were re-suspended in 40 μL buffer per 10^7 total cells. 10 μL of Pan T Cell Biotin-Antibody Cocktail was added per 10^7 total cells, mixed thoroughly and incubated for about 5 minutes in the refrigerator (2~8°C). 30 μL of buffer was then added per 10^7 cells. 20 μL of Pan T Cell MicroBead Cocktail was added per 10^7 cells. The cell suspension mixture was mixed well and incubated for an additional 10 minutes in the refrigerator (2~8°C). A minimum of 500 μL is required for magnetic separation. For magnetic separation, an LS column was placed in the magnetic field of a suitable MACS™ Separator. The column was prepared by rinsing with 3 ml buffer. The cell suspension was then applied onto the column, and flow-through containing the unlabeled cells was collected, which represented the enriched T cell fractions. Additional T cells were collected by washing the column with 3 ml buffer and collecting unlabeled cells that passed through. These unlabeled cells again represented the enriched T cells, and were combined with the flow-through from the previous step. The pooled enriched T cells were then centrifuged and re-suspended in R10 supplemented with 100IU/mL IL-2.

[0259] The prepared T cells were subsequently pre-activated for 48-96 hours with human T cell activation/expansion kit (Miltenyi#130-091-441) according to the manufacturer's protocol, in which anti-CD3/CD28 MACSI™ Beads were added to the T cells at a bead-to-cell ratio of 1:2.

2. In vitro T cell-mediated cytotoxicity assays

[0260] Human multiple myeloma cell line RPMI8226.Luc was developed by transduction of firefly luciferase gene into RPMI8226 host cells. *In vitro* T cell-mediated cytotoxicity assays were performed on RPMI8226.Luc cells either cultured alone or co-cultured with pre-stimulated T cells.

[0261] To assess the T cell-mediated cytotoxicity of exemplary CATEs against tumor cells, ONE-GLO™ luminescent luciferase assay reagents (Promega#E6110) were prepared according to the manufacturer's protocol and added to the cell samples (*i.e.*, RPMI8226.Luc alone, or RPMI8226.Luc+pre-stimulated T cells). The remaining luciferase activity in each well was determined. Since luciferase is expressed only in RPMI8226.Luc cells, the remaining luciferase activity in each well correlates directly with the number of viable RPMI8226.Luc cells (*i.e.*, target cells) in the well. The maximum luciferase activity was obtained by adding culture media to target cells in absence of the pre-stimulated T cells (*i.e.*, effector cells). The minimum luciferase activity was determined by adding Triton X-100 at a final concentration of 1% at the beginning of the cytotoxicity assay to lyse all target cells. Specific cytotoxicity was calculated using the formula: Specific Cytotoxicity%= 100% * (1-(RLUsample-RLUmin)/(RLUmax-RLUmin)).

[0262] First, a preliminary screen of exemplary CATEs was performed using the *in vitro* T cell-mediated cytotoxicity assay as described above. The supernatant containing each exemplary CATE was diluted with assay medium (RPMI1640+10%FBS) with a dilution factor of 1:5 or 1:50 and incubated with 2000 RPMI8226.Luc cells in a 384-well format microplate. Pre-stimulated T cells were added to each well at an effector to target cell ratio of 5:1 to initiate the T cell-mediated cytotoxicity of CATE against RPMI8226.Luc cells. Freestyle medium from pMax-GFP transfected 293-6E cells were used as negative controls. After incubation overnight in a cell culture incubator, ONE-GLO™ Luciferase Assay System reagents were added to each well, and the remaining luciferase activities were measured to assess the number of remaining viable tumor cells. From the preliminary screen, several CATEs with high *in vitro* T-cell mediated cytotoxicity were picked for further experiments.

[0263] A dose-dependent T cell-mediated cytotoxicity curve of each selected CATE was determined. The supernatant containing each selected CATE was diluted with assay medium (RPMI1640+10%FBS) and incubated with 2000 RPMI8226.Luc cells in a 384-well format microplate. In group 1, the pre-stimulated T cells were added to each well at an effector to target cell ratio of 5:1 to initiate the T cell-mediated cytotoxicity of CATE against RPMI8226.Luc cells. In group 2, assay medium (RPMI1640+10%FBS) instead of pre-stimulated T cells was added to each well containing the exemplary CATE and RPMI8226.Luc cells to determine T cell-independent cytotoxicity of CATE against RPMI8226.Luc cells. After overnight incubation, the

assay plates were briefly centrifuged at 300g for 1 min, and then10 μL of supernatant in each well was collected to detect the level of secreted IFNgamma. The remaining cells in the co-culture assay were added with ONE-GLO™ Luciferase Assay System reagents, and the remaining luciferase activities were measured to assess the number of remaining viable tumor cells.

[0264] FIG. 2A shows dose-dependent T cell-mediated cytotoxicity of CATEs based on L2K-07-derived anti-CD3 scFv (CATE-L2K-07 scFv). FIG. 2B shows dose-dependent T cell-mediated cytotoxicity of CATEs based on OKT3-derived anti-CD3 scFv (CATE-OKT3 scFv). EC50 values of the exemplary CATEs are listed in Table 6 below. Lower EC50 values correspond to higher T-cell mediated cytotoxicity by CATE. The results demonstrate that CATEs comprising VHH domains targeting BCMA and/or CD38 have potent T-cell mediated cytotoxic effects against multiple myeloma cells. Additionally, CATEs comprising anti-BCMA V_HH domains have higher T-cell mediated cytotoxic effects against multiple myeloma cells, compared to CATEs comprising anti-BCMA scFv domains.

Table 6. EC50 values of exemplary CATEs.

	CATE#001	CATE#007	CATE#010	CATE#019	CATE#022	CATE#025	CATE#028
Bottom	39.3	43.54	45.95	36.37	45.89	38.79	48.82
Top	97.02	99.25	98.34	99.46	~ 100.0	~ 100.0	~ 100.0
LogEC50	1.406	1.857	1.398	1.948	2.154	2.373	1.923
HillSlope	1.582	1.116	1.22	0.9966	1.253	0.96	0.9471
EC50, pg/ml	**25.45**	**72.02**	**25.03**	**88.72**	**142.4**	**235.8**	**83.81**

	CATE#002	CATE#008	CATE#011	CATE#020	CATE#023	CATE#026	CATE#029
Bottom	~ 0	~ 0	~ 0	4.789	~ 0	~ 0	~ 0
Top	70.29	72.53	78.15	82.66	82.44	66.42	78.55
LogEC50	2.174	2.629	2.55	3.201	3.26	3.419	3.201
HillSlope	1.056	2.046	0.7657	1.344	0.9659	2.753	1.704
EC50, pg/ml	**149.3**	**425.9**	**354.6**	**1588**	**1822**	**2623**	**1589**

3. In vitro IFN-γ release assay

[0265] According to the procedures described above, in group 2, 10 μL of supernatant from each well was transferred to a small-volume round bottom white wall 384 well-plate to determine secreted IFNγ level in each co-culture using a HTRF kit (Cisbio, catalog number

62IFNPEB) according to the manual. FIG. 3 shows the IFN-γ levels released by T cells co-cultured with in response to exemplary CATEs. IFNγ release is a sign of T cell activation. The data shows that CATEs comprising anti-BCMA V_HH triggered higher level of IFNγ release from T cells co-incubated with RPMI8226.Luc cells compared to CATEs comprising anti-BCMA scFvs.

CLAIMS

1. A chimeric antibody immune effector cell engager comprising:
   (a) a target cell binding domain comprising a single-domain antibody (sdAb) that specifically binds to an antigen on a target cell; and
   (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell.
2. The chimeric antibody immune effector cell engager of claim 1, wherein the sdAb is camelid, chimeric, human or humanized.
3. The chimeric antibody immune effector cell engager of claim 1 or claim 2, wherein the sdAb is a V_HH fragment.
4. The chimeric antibody immune effector cell engager of any one of claims 1-3, wherein the target cell is a tumor cell.
5. The chimeric antibody immune effector cell engager of any one of claims 1-4, wherein the target cell is a B cell.
6. The chimeric antibody immune effector cell engager of any one of claims 1-5, wherein the target cell binding domain comprises an anti-BCMA sdAb.
7. The chimeric antibody immune effector cell engager of claim 6, wherein the anti-BCMA sdAb comprises any one of the following:
   (1) a CDR1 comprising the amino acid sequence of SEQ ID NO:1; a CDR2 comprising the amino acid sequence of SEQ ID NO:12; and a CDR3 comprising the amino acid sequence of SEQ ID NO:23;
   (2) a CDR1 comprising the amino acid sequence of SEQ ID NO:2; a CDR2 comprising the amino acid sequence of SEQ ID NO:13; and a CDR3 comprising the amino acid sequence of SEQ ID NO:24;
   (3) a CDR1 comprising the amino acid sequence of SEQ ID NO:3; a CDR2 comprising the amino acid sequence of SEQ ID NO:14; and a CDR3 comprising the amino acid sequence of SEQ ID NO:25;
   (4) a CDR1 comprising the amino acid sequence of SEQ ID NO:4; a CDR2 comprising the amino acid sequence of SEQ ID NO:15; and a CDR3 comprising the amino acid sequence of SEQ ID NO:26;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO:5; a CDR2 comprising the amino acid sequence of SEQ ID NO:16; and a CDR3 comprising the amino acid sequence of SEQ ID NO:27;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO:6; a CDR2 comprising the amino acid sequence of SEQ ID NO:17; and a CDR3 comprising the amino acid sequence of SEQ ID NO:28;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO:7; a CDR2 comprising the amino acid sequence of SEQ ID NO:18; and a CDR3 comprising the amino acid sequence of SEQ ID NO:29;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO:8; a CDR2 comprising the amino acid sequence of SEQ ID NO:19; and a CDR3 comprising the amino acid sequence of SEQ ID NO:30;

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO:9; a CDR2 comprising the amino acid sequence of SEQ ID NO:20; and a CDR3 comprising the amino acid sequence of SEQ ID NO:31;

(10) a CDR1 comprising the amino acid sequence of SEQ ID NO:10; a CDR2 comprising the amino acid sequence of SEQ ID NO:21; and a CDR3 comprising the amino acid sequence of SEQ ID NO:32; or

(11) a CDR1 comprising the amino acid sequence of SEQ ID NO:11; a CDR2 comprising the amino acid sequence of SEQ ID NO:22; and a CDR3 comprising the amino acid sequence of SEQ ID NO:33.

8. The chimeric antibody immune effector cell engager of claim 7, wherein the anti-BCMA sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 78-88.
9. The chimeric antibody immune effector cell engager of any one of claims 1-5, wherein the target cell binding domain comprises an anti-CD38 sdAb.
10. The chimeric antibody immune effector cell engager of claim 9, wherein the anti-CD38 sdAb comprises any one of the following:

(1) a CDR1 comprising the amino acid sequence of SEQ ID NO:34; a CDR2 comprising the amino acid sequence of SEQ ID NO:46; and a CDR3 comprising the amino acid sequence of SEQ ID NO:58;

(2) a CDR1 comprising the amino acid sequence of SEQ ID NO:35; a CDR2 comprising the amino acid sequence of SEQ ID NO:47; and a CDR3 comprising the amino acid sequence of SEQ ID NO:59;

(3) a CDR1 comprising the amino acid sequence of SEQ ID NO:36; a CDR2 comprising the amino acid sequence of SEQ ID NO:48; and a CDR3 comprising the amino acid sequence of SEQ ID NO:60;

(4) a CDR1 comprising the amino acid sequence of SEQ ID NO:37; a CDR2 comprising the amino acid sequence of SEQ ID NO:49; and a CDR3 comprising the amino acid sequence of SEQ ID NO:61;

(5) a CDR1 comprising the amino acid sequence of SEQ ID NO:38; a CDR2 comprising the amino acid sequence of SEQ ID NO:50; and a CDR3 comprising the amino acid sequence of SEQ ID NO:62;

(6) a CDR1 comprising the amino acid sequence of SEQ ID NO:39; a CDR2 comprising the amino acid sequence of SEQ ID NO:51; and a CDR3 comprising the amino acid sequence of SEQ ID NO:63;

(7) a CDR1 comprising the amino acid sequence of SEQ ID NO:40; a CDR2 comprising the amino acid sequence of SEQ ID NO:52; and a CDR3 comprising the amino acid sequence of SEQ ID NO:64;

(8) a CDR1 comprising the amino acid sequence of SEQ ID NO:41; a CDR2 comprising the amino acid sequence of SEQ ID NO:53; and a CDR3 comprising the amino acid sequence of SEQ ID NO:65;

(9) a CDR1 comprising the amino acid sequence of SEQ ID NO:42; a CDR2 comprising the amino acid sequence of SEQ ID NO:54; and a CDR3 comprising the amino acid sequence of SEQ ID NO:66;

(10) a CDR1 comprising the amino acid sequence of SEQ ID NO:43; a CDR2 comprising the amino acid sequence of SEQ ID NO:55; and a CDR3 comprising the amino acid sequence of SEQ ID NO:67; or

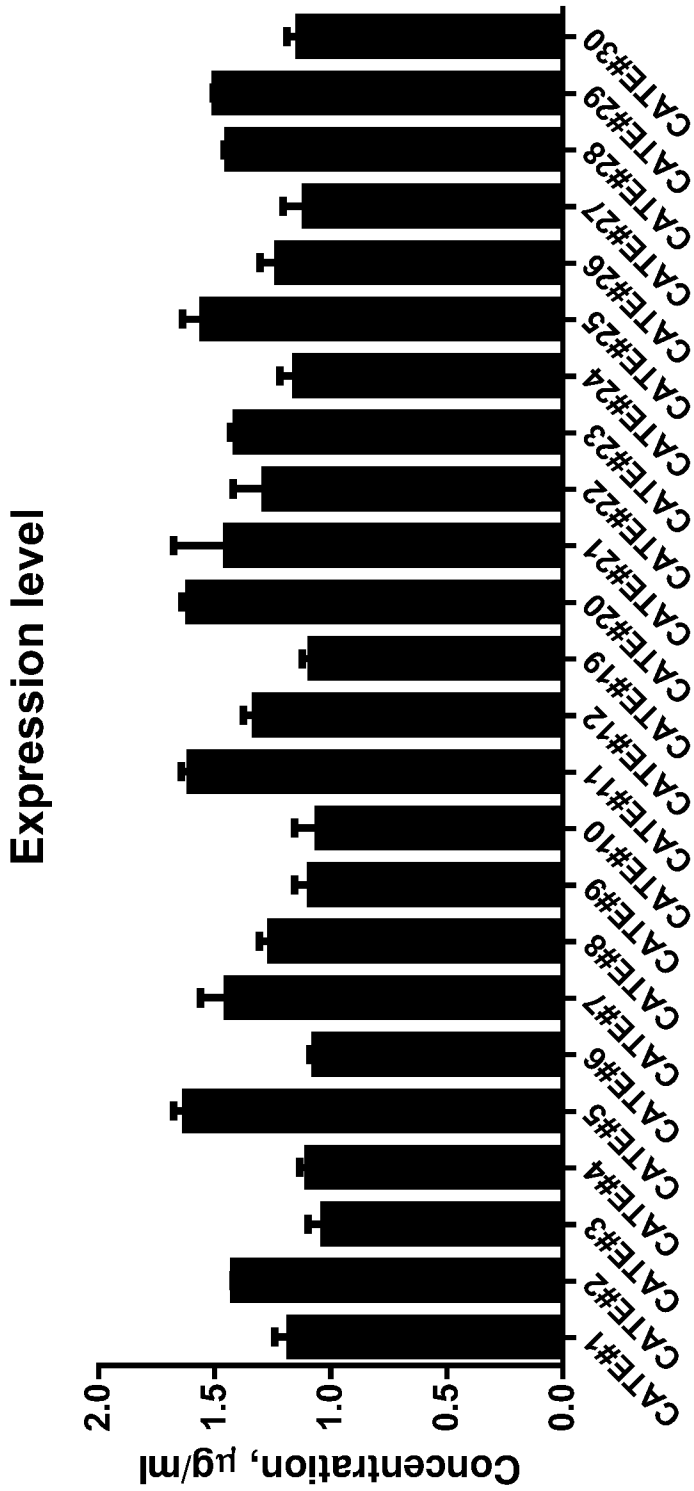
(11) a CDR1 comprising the amino acid sequence of SEQ ID NO:44; a CDR2 comprising the amino acid sequence of SEQ ID NO:56; and a CDR3 comprising the amino acid sequence of SEQ ID NO:68.

11. The chimeric antibody immune effector cell engager of claim 10, wherein the anti-CD38 sdAb comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 89-100.
12. The chimeric antibody immune effector cell engager of any one of claims 1-11, wherein the target cell binding domain comprises a first sdAb that specifically binds to a first antigen on a first target cell and a second sdAb that specifically binds to a second antigen on a second target cell.
13. The chimeric antibody immune effector cell engager of claim 12, wherein the first sdAb is fused to the second sdAb via a peptide linker.
14. The chimeric antibody immune effector cell engager of claim 12 or claim 13, wherein the first target cell and the second target cell are the same cell.
15. The chimeric antibody immune effector cell engager of claim 12 or claim 13, wherein the first target cell and the second target cell are different cells.
16. The chimeric antibody immune effector cell engager of any one of claims 12-15, wherein the first antigen and the second antigen are the same.
17. The chimeric antibody immune effector cell engager of claim 16, wherein the first sdAb and the second sdAb specifically bind to the same epitope.
18. The chimeric antibody immune effector cell engager of claim 16, wherein the first sdAb and the second sdAb specifically bind to different epitopes.
19. The chimeric antibody immune effector cell engager of any one of claims 12-15, wherein the first antigen and the second antigen are different.
20. The chimeric antibody immune effector cell engager of claim 19, wherein the target cell binding domain comprises an anti-BCMA sdAb and an anti-CD38 sdAb.
21. The chimeric antibody immune effector cell engager of claim 20, wherein the anti-BCMA sdAb is fused to the N-terminus of the anti-CD38 sdAb.
22. The chimeric antibody immune effector cell engager of claim 20, wherein the anti-BCMA sdAb is fused to the C-terminus of the anti-CD38 sdAb.
23. A chimeric antibody immune effector cell engager, comprising:
    (a) a target cell binding domain comprising an anti-BCMA scFv; and
    (b) an immune effector cell binding domain comprising an antigen-binding fragment that specifically binds to an antigen on an immune effector cell.

24. The chimeric antibody immune effector cell engager of claim 23, wherein the anti-BCMA scFv comprises the amino acid sequence of SEQ ID NO: 101 or SEQ ID NO: 102.
25. The chimeric antibody immune effector cell engager of any one of claims 1-24, wherein the target cell binding domain is fused to the N-terminus of the immune effector cell binding domain.
26. The chimeric antibody immune effector cell engager of any one of claims 1-24, wherein the target cell binding domain is fused to the C-terminus of the immune effector cell binding domain.
27. The chimeric antibody immune effector cell engager of any one of claims 1-26, wherein the target cell binding domain is fused to the immune effector cell binding domain via a peptide linker.
28. The chimeric antibody immune effector cell engager of any one of claims 1-27, wherein the immune effector cell is a T cell.
29. The chimeric antibody immune effector cell engager of any one of claims 1-27, wherein the immune effector cell is an NK cell.
30. The chimeric antibody immune effector cell engager of any one of claims 1-29, wherein the antigen-binding fragment in the immune effector cell binding domain is a Fab, scFv, or sdAb.
31. The chimeric antibody immune effector cell engager of any one of claims 1-30, wherein the antigen-binding fragment in the immune effector cell binding domain is murine, camelid, chimeric, human or humanized.
32. The chimeric antibody immune effector cell engager of any one of claims 1-31, wherein the immune effector cell binding domain comprises an anti-CD3 antigen-binding fragment.
33. The chimeric antibody immune effector cell engager of claim 32, wherein the anti-CD3 antigen-binding fragment is derived from OKT3, L2K or UCHT1.
34. The chimeric antibody immune effector cell engager of claim 33, wherein the anti-CD3 antigen-binding fragment comprises any one of the following: (1) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:164; a CDR2 comprising the amino acid sequence of SEQ ID NO:165; and a CDR3 comprising the amino acid sequence of SEQ ID NO:166; and a VL comprising a CDR1 comprising the amino acid

sequence of SEQ ID NO:167; a CDR2 comprising the amino acid sequence of SEQ ID NO:168; and a CDR3 comprising the amino acid sequence of SEQ ID NO:169; (2) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:170; a CDR2 comprising the amino acid sequence of SEQ ID NO:171; and a CDR3 comprising the amino acid sequence of SEQ ID NO:172; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:173; a CDR2 comprising the amino acid sequence of SEQ ID NO:174; and a CDR3 comprising the amino acid sequence of SEQ ID NO:175; or (3) a VH comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:176; a CDR2 comprising the amino acid sequence of SEQ ID NO:177; and a CDR3 comprising the amino acid sequence of SEQ ID NO:178; and a VL comprising a CDR1 comprising the amino acid sequence of SEQ ID NO:179; a CDR2 comprising the amino acid sequence of SEQ ID NO:180; and a CDR3 comprising the amino acid sequence of SEQ ID NO:181.

35. The chimeric antibody immune effector cell engager of claim 34, the anti-CD3 antigen-binding fragment comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 103-105.
36. An isolated nucleic acid comprising a nucleic acid sequence encoding the chimeric antibody immune effector cell engager of any one of claims1-35.
37. A vector comprising the isolated nucleic acid of claim 36.
38. A pharmaceutical composition, comprising the chimeric antibody immune effector cell engager of any one of claims 1-35, and a pharmaceutically acceptable carrier.
39. A method of treating a disease in an individual, comprising administering to the individual an effective amount of the pharmaceutical composition of claim 38.
40. The method of claim 39, wherien the disease is a B cell-related disorder.
41. The method of claim 39 or claim 40, wherein the disease is cancer.
42. The method of claim 41, wherein the cancer is multiple myeloma.
43. The method of claim 39 or claim 40, wherein the disease is an autoimmune disease.
44. The method of claim 43, wherein the disease is systemic lupus erythematosus.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CATE#6
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CATE#8
CATE#9
CATE#10
CATE#11
CATE#12
CATE#19
CATE#20
CATE#21
CATE#22
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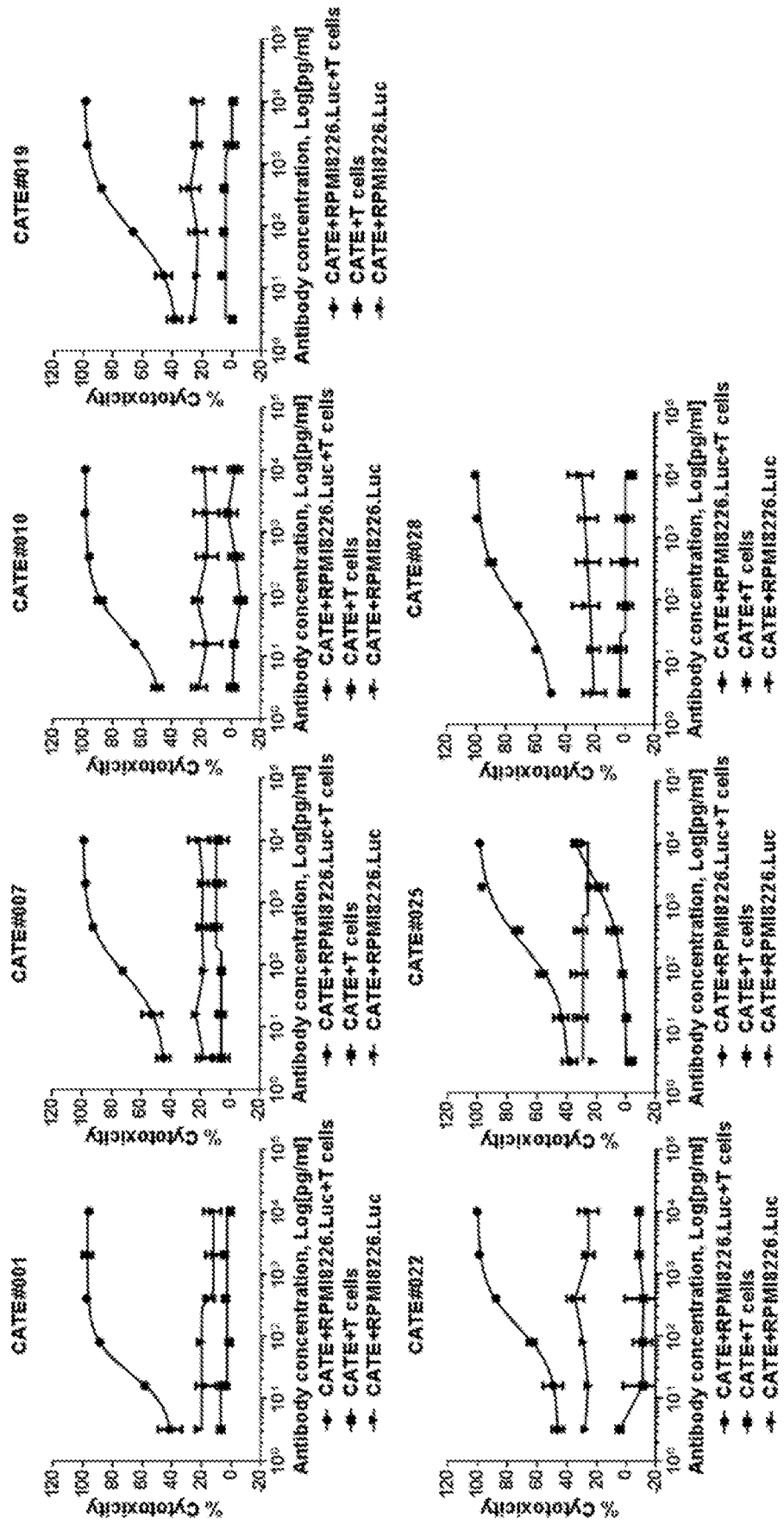
CATE#29
CATE#30

FIG. 1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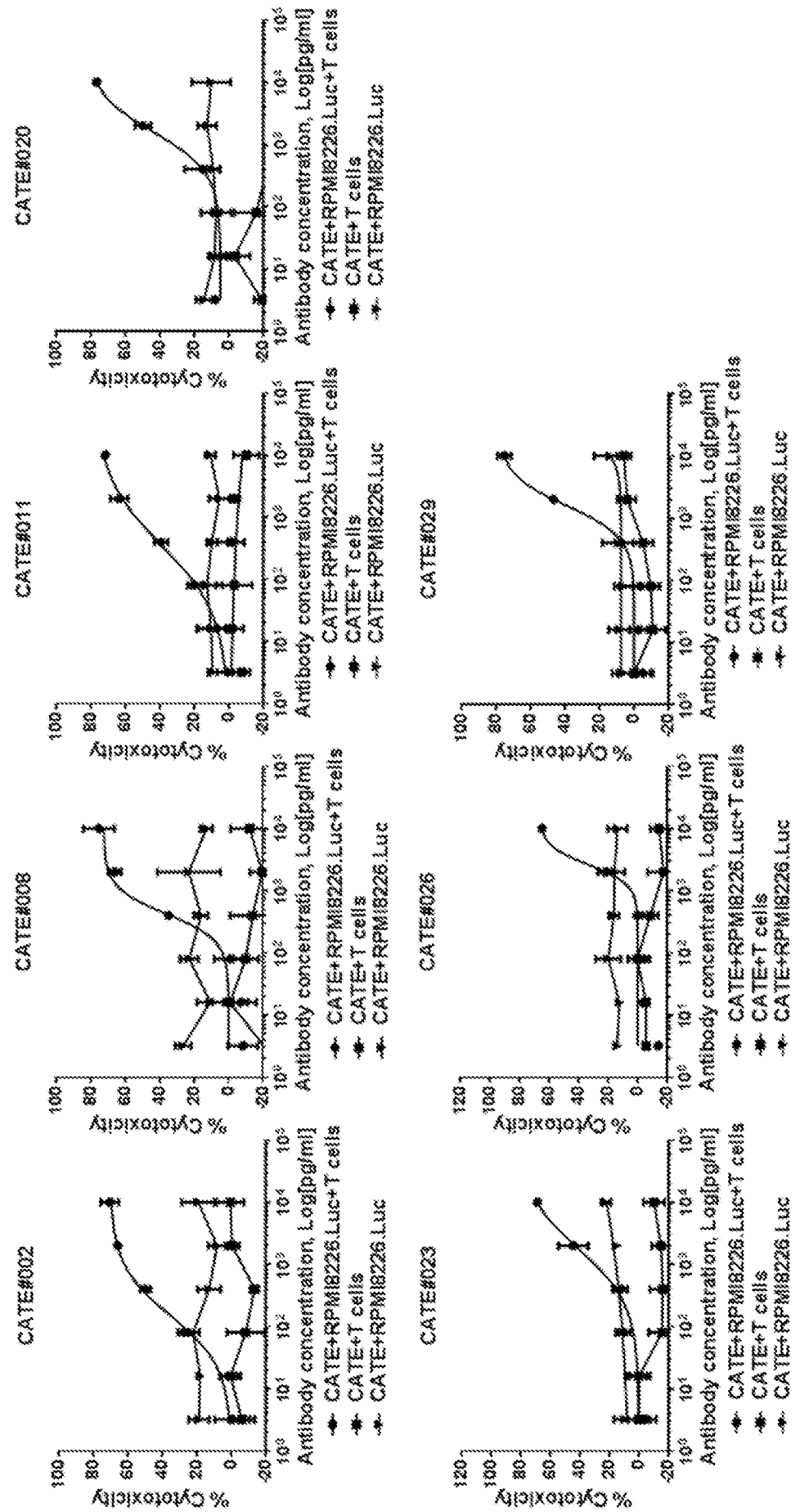
CATE#007
CATE#010
CATE#019
CATE#022
CATE#025
CATE#028
% Cytotoxicity
Antibody concentration, Log[pg/ml]
CATE+RPMI8226.Luc+T cells
CATE+T cells
CATE+RPMI8226.Luc

FIG. 2A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CATE#008
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CATE#023
CATE#026
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% Cytotoxicity
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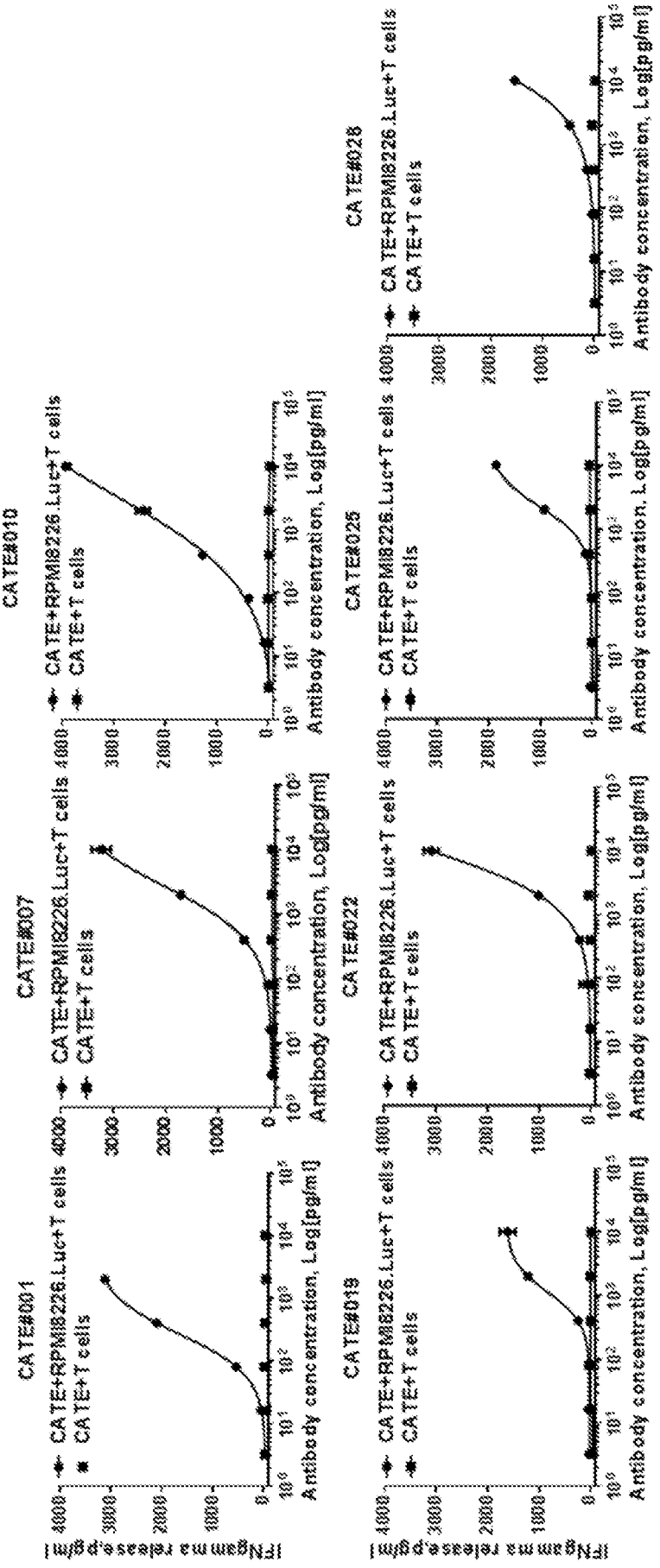
CATE+T cells
CATE+RPMI8226.Luc

FIG. 2B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CATE#007
CATE#010
CATE#019
CATE#022
CATE#025
CATE#028
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CATE+T cells
IFNgam ma release,pg/ml
Antibody concentration, Log[pg/ml]

FIG. 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/090343

A. CLASSIFICATION OF SUBJECT MATTER

C07K 16/28(2006.01)i; C07K 16/18(2006.01)i; C07K 16/30(2006.01)i; C07K 14/005(2006.01)i; C07K 14/705(2006.01)i; C12N 15/09(2006.01)i; C12N 15/62(2006.01)i; C12N 5/16(2006.01)i; A61K 48/00(2006.01)i; A61K 39/395(2006.01)i; A61P 35/00(2006.01)i

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K, C12N, A61K, A61P

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)

DWPI, SIPOABS, EPTXT, USTXT, WOTXT, JPTXT, ISI web of Knowledge, PubMed, Genbank, EMBL, Retrieving System for Biological Sequence of Chinese Patent and searched items: anti-BCMA, CD269, B cell maturation antigen, anti-cd3, B cell, T cell, NK cell, sdAb, single domain antibody, nanobody, bispecific T-cell engager, BiTE, VHH, variable domain of heavy chain, dual, bispecific, bivalent, diabody, SEQ ID Nos:1-44, 46-56, 58-68, 78-105, 164-181

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2017031104 A1 (JANSSEN PHARMACEUTICA NV) 23 February 2017 (2017-02-23) see description, pages 8, 11, 25, 26, 29, 30, 45 and claims 4-16	1-6, 23-38
Y	WO 2017031104 A1 (JANSSEN PHARMACEUTICA NV) 23 February 2017 (2017-02-23) see description, pages 8, 11, 25, 26, 29, 30, 45 and claims 4-16	9, 12-22
A	WO 2017031104 A1 (JANSSEN PHARMACEUTICA NV) 23 February 2017 (2017-02-23) the whole document	7, 8, 10, 11
Y	US 2014302064 A1 (XENCOR INC) 09 October 2014 (2014-10-09) see description, paragraphs [165], [166] and [168]	9, 12-22
A	US 2014302064 A1 (XENCOR INC) 09 October 2014 (2014-10-09) the whole document	1-8, 10, 11, 23-38

☐ 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
06 March 2018	**27 March 2018**

Name and mailing address of the ISA/CN	Authorized officer
STATE INTELLECTUAL PROPERTY OFFICE OF THE P.R.CHINA **6, Xitucheng Rd., Jimen Bridge, Haidian District, Beijing 100088** **China**	**WANG,Xiangyu**
Facsimile No. **(86-10)62019451**	Telephone No. **(86-10)62411992**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/090343

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 filed or furnished:

   a. (means)

   ☐ on paper

   ☑ in electronic form

   b. (time)

   ☑ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/090343

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.: **39-44**
because they relate to subject matter not required to be searched by this Authority, namely:

[1] Rule 39.1(iv) PCT- Method for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

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

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2017/090343

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