

BCMA-TARGETING ANTIBODY AND USE THEREOF

Technical field

The present invention belongs to the field of tumor immunotherapy or diagnosis;
5 and in particular, the present invention relates to an antibody that targets BCMA and uses thereof.

Background

Multiple myeloma (MM) is a common hematological malignancy, accounting for
10 2% of all deaths resulted from cancer. MM is a heterogeneous disease and mainly caused by chromosomal translocation of t(11;14), t(4;14), t(8;14), del(13), del(17) (among others) (Drach et al., (1998) Blood 92(3): 802-809; Gertz et al., (2005) Blood 106 (8): 2837-2840; Facon et al., (2001) Blood 97 (6): 1566 -1571). The main condition of multiple myeloma (MM) is the infinite expansion and enrichment of plasma cells in bone marrow, thereby
15 leading to osteonecrosis. MM-affected patients may experience a variety of disease-related symptoms due to bone marrow infiltration, bone destruction, renal failure, immunodeficiency, and the psychological burden of cancer diagnosis. At present, the main treatments are chemotherapy and stem cell transplantation. The mainly used chemotherapy drugs are steroid, thalidomide, lenalidomide, bortezomib or a combination
20 of various cytotoxic agents. For younger patients, high-dose chemotherapy can be used in combination with autologous stem cell transplantation.

BCMA (B-cell maturation antigen) is B-cell maturation antigen, a type III transmembrane protein consisting of 185 amino acid residues, and belongs to TNF receptor superfamily. The ligand of BCMA belongs to TNF superfamily, such as
25 proliferation-inducing ligand (APRIL), B lymphocyte stimulating factor (BAFF). After binding to its ligand, BCMA activates B cell proliferation and survival. BCMA is specifically and highly expressed on the surface of plasma cells and multiple myeloma cells, but not expressed in hematopoietic stem cells and other normal tissue cells, therefore BCMA can be an ideal target for targeted therapy of MM.

30 Summing up, there is an urgent need in the art for antibodies specific to BCMA and immune effector cells targeting BCMA.

Summary of invention

It is an object of the present invention to provide antibodies specific to BCMA and

immune effector cells that target BCMA.

In a first aspect, an antibody that targets BCMA is provided in the invention, and the antibody is selected from the group consisting of:

(1) an antibody, comprising a heavy chain variable region comprising HCDR1 as shown in SEQ ID NO: 1, 60 or 62, and/or comprising HCDR2 as shown in SEQ ID NO: 2, 61 or 63, and/or HCDR3 as shown in any one of SEQ ID NO: 3, SEQ ID NO: 4 or SEQ ID NO: 5;

(2) an antibody, comprising a light chain variable region comprising LCDR1 as shown in SEQ ID NO: 6, and/or comprising LCDR2 as shown in SEQ ID NO: 7, and/or comprising LCDR3 as shown in any one of SEQ ID NO: 8, SEQ ID NO: 9 or SEQ ID NO: 10;

(3) an antibody, comprising a heavy chain variable region of the antibody of (1) and a light chain variable region of the antibody of (2);

(4) an antibody, which is a variant of the antibody of any one of (1) to (3) and has the same or similar activity as the antibody of any one of (1) to (3).

In a specific embodiment, the antibody is selected from the group consisting of:

(1) an antibody, comprising HCDR1 as shown in SEQ ID NO: 1, HCDR 2 as shown in SEQ ID NO: 2, HCDR3 as shown in SEQ ID NO: 3, and LCDR1 as shown in SEQ ID NO: 6, LCDR2 as shown in SEQ ID NO: 7 and LCDR3 as shown in SEQ ID NO: 8;

(2) an antibody, comprising HCDR1 as shown in SEQ ID NO: 1, HCDR 2 as shown in SEQ ID NO: 2, HCDR3 as shown in SEQ ID NO: 4, LCDR1 as shown in SEQ ID NO: 6, LCDR2 as shown in SEQ ID NO: 7 and LCDR3 as shown in SEQ ID NO: 9;

(3) an antibody, comprising HCDR1 as shown in SEQ ID NO: 1, HCDR2 as shown in SEQ ID NO: 2, HCDR3 as shown in SEQ ID NO: 5, LCDR1 as shown in SEQ ID NO: 6, LCDR2 as shown in SEQ ID NO: 7 and LCDR3 as shown in SEQ ID NO: 10;

(4) an antibody, comprising HCDR1 as shown in SEQ ID NO: 60, HCDR2 as shown in SEQ ID NO: 61, HCDR3 as shown in SEQ ID NO: 5, LCDR1 as shown in SEQ ID NO: 6, LCDR2 as shown in SEQ ID NO: 7 and LCDR3 as shown in SEQ ID NO: 10;

(5) an antibody, comprising HCDR1 as shown in SEQ ID NO: 62, HCDR2 as shown in SEQ ID NO: 63, HCDR3 as shown in SEQ ID NO: 5, LCDR1 as shown in SEQ ID NO: 6, LCDR2 as shown in SEQ ID NO: 7 and LCDR3 as shown in SEQ ID NO: 10;

(6) an antibody, which is a variant of any one of (1) to (5) and has the same or similar activity as the antibody of any one of (1) to (5).

In a specific embodiment, the antibody is selected from the group consisting of:

(1) an antibody, wherein the heavy chain variable region of the antibody has the

amino acid sequence of SEQ ID NO: 13, the amino acid sequence of SEQ ID NO: 17, the amino acid sequence of SEQ ID NO: 21, or the amino acid sequence of SEQ ID NO: 56 or the amino acid sequence of SEQ ID NO: 58;

(2) an antibody, wherein the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 11, the amino acid sequence of SEQ ID NO: 15, or the amino acid sequence of SEQ ID NO: 19;

(3) an antibody, comprising a heavy chain variable region of the antibody of (1) and a light chain variable region of the antibody of (2);

(4) an antibody, which is a variant of any one of (1) to (3) and has the same or similar activity as the antibody of any one of (1) to (3).

In a specific embodiment, the antibody is selected from the group consisting of:

(1) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 13 and the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 11;

(2) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 17 and the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 15;

(3) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 21 and the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 19;

(4) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 56 and the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 19;

(5) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 58 and the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 19;

(6) an antibody, which is a variant of any one of (1) to (5) and has the same or similar activity as the antibody of any one of (1) to (5).

In a second aspect, an antibody is provided in the present invention, recognizing the same antigenic determinant as the antibody of the first aspect of the invention.

In a third aspect, a nucleic acid is provided in the present invention, encoding the antibody of the first or second aspect of the invention.

In a fourth aspect, an expression vector is provided in the present invention, comprising the nucleic acid of the third aspect of the invention.

5 In a fifth aspect, a host cell is provided in the present invention, comprising the expression vector of the fourth aspect of the invention or has the nucleic acid of the third aspect of the invention integrated in the genome.

In a sixth aspect, the use of the antibody of the first or second aspect of the present invention is provided in the present invention, for the preparation of a targeting drug, an antibody drug conjugate or a multifunctional antibody which specifically targets tumor cells expressing BCMA; or

for the preparation of an agent for diagnosis of a tumor expressing BCMA; or

for the preparation of a chimeric antigen receptor-modified immune cell; and preferably, the immune cell includes T lymphocyte, NK cell or NKT lymphocyte.

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In a seventh aspect, a multifunctional immunoconjugate is provided in the present invention, comprising:

an antibody of the first or second aspect of the invention; and

20 a functional molecule linked thereto; said functional molecule being selected from the group consisting of a molecule that targets a tumor surface marker, a molecule that inhibits tumors, a molecule that targets a surface marker of an immune cell, and a detectable label.

In a specific embodiment, the molecule that inhibits tumors is an antitumor cytokine or an antitumor toxin. Preferably, the cytokine comprises: IL-12, IL-15, type I interferon, 25 TNF-alpha.

In a specific embodiment, the molecule that targets a surface marker of an immune cell is an antibody or a ligand that binds to a surface marker of an immune cell; and preferably, the surface marker of an immune cell comprises: CD3, CD16, CD28, and more preferably, the antibody that binds to a surface marker of an immune cell is an anti-CD3 antibody. 30

In a specific embodiment, the molecule that targets a surface marker of an immune cell is an antibody that binds to a surface marker of a T cell.

In an eighth aspect, a nucleic acid is provided in the present invention, encoding the multifunctional immunoconjugate of the seventh aspect of the invention. 35

In a ninth aspect, the use of the multifunctional immunoconjugate of the seventh aspect of the present invention is provided in the present invention, for the preparation of an antitumor drug, or

- 5 for the preparation of an agent for diagnosis of a tumor expressing BCMA; or
 for the preparation of a chimeric antigen receptor-modified immune cell; and preferably, the immune cell comprises: T lymphocytes, NK cells or NKT lymphocytes.

10 In a tenth aspect, a chimeric antigen receptor is provided in the present invention, comprising an extracellular domain, a transmembrane domain and an intracellular signal domain, the extracellular domain comprises the antibody of the first or second aspect of the invention, and the antibody preferably is a single-chain antibody or domain antibody.

In a specific embodiment, the intracellular signal domain comprises one or more co-stimulatory signal domains and/or primary signal domains.

15 In a specific embodiment, the chimeric antigen receptor further comprises a hinge domain.

In a specific embodiment, the transmembrane domain is selected from the group consisting of alpha, beta, zeta chain of TCR, transmembrane regions of CD3 ϵ , CD3 ζ , CD4, CD5, CD8 α , CD9, CD16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80,
20 CD86, CD134, CD137, CD152, CD154 and PD1; and/or

the costimulatory signal domain is selected from the group consisting of intracellular signal regions of CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54, CD83, OX40, CD137, CD134, CD150, CD152, CD223, CD270, PD-L2, PD-L1, CD278, DAP10, LAT, NKD2C SLP76, TRIM, Fc ϵ RI γ , MyD88 and 41BBL; and/or

25 the primary signal domain is selected from the group consisting of TCR ξ , FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, CD278 (also named as "ICOS"), CD66d and CD3 ζ ,

preferably,

30 the transmembrane domain is selected from the group consisting of transmembrane domains of CD8 α , CD4, CD45, PD1, CD154 and CD28; and/or

the co-stimulatory signal domain is selected from the group consisting of CD137, CD134, CD28 and OX40; and/or

the primary signal domain is selected from CD3 ζ ,

35 most preferably, the transmembrane domain is selected from CD8 α or CD28, the co-stimulatory signal domain is selected from the intracellular signal domain of CD137 or

CD28, and the primary signal domain is selected from CD3ζ.

In a specific embodiment, the chimeric antigen receptor comprises the following sequentially linked an antibody, a transmembrane region and an intracellular signal region:

5 the antibody of the first or second aspect of the invention, the transmembrane region of CD8 and CD3ζ;

the antibody of the first or second aspect of the invention, the transmembrane region of CD8, the intracellular signal region of CD137 and CD3ζ;

10 the antibody of the first or second aspect of the invention, the transmembrane region of CD28, the intracellular signal region of CD28, and CD3ζ; or

the antibody of the first or second aspect of the invention, the transmembrane region of CD28, the intracellular signal region of CD28, CD137 and CD3ζ.

15 In an eleventh aspect, a nucleic acid is provided in the present invention, encoding the chimeric antigen receptor of the tenth aspect of the invention.

In a twelfth aspect, an expression vector is provided in the present invention, comprising the nucleic acid of the eleventh aspect of the invention.

20 In a thirteenth aspect, a virus is provided in the present invention, comprising the vector of the twelfth aspect of the invention.

In a preferred embodiment, the virus is a lentivirus.

25 In a fourteenth aspect, the use of the chimeric antigen receptor of the tenth aspect of the present invention, or the nucleic acid of the eleventh aspect of the present invention, or the expression vector of the twelfth aspect of the present invention, or the virus of the thirteenth aspect of the present invention is provided in the present invention, for the preparation of genetically modified immune cells targeting a tumor cell expressing BCMA.

30 In a preferred embodiment, the tumor expressing BCMA is multiple myeloma.

In a fifteenth aspect, a genetically modified immune cell is provided in the present invention, which is transduced with the nucleic acid of the eleventh aspect of the invention, or the expression vector of the twelfth aspect of the invention or the thirteenth aspect of the invention or the virus of the thirteenth aspect of the present invention; or

expresses the chimeric antigen receptor of the tenth aspect of the invention.

The immune cells are preferably selected from T lymphocytes, NK cells or NKT cells.

In a specific embodiment, the genetically modified immune cell further expresses a
5 sequence other than the chimeric antigen receptor of the tenth aspect of the invention, and the other sequence comprises a cytokine, or another chimeric antigen receptor, or a chemokine receptor, or an siRNA that reduces PD-1 expression, or a protein that blocks PD-L1, or a TCR, or a safety switch;

Preferably, the cytokine comprises IL-12, IL-15, IL-21, or type I interferon;

10 Preferably, the chemokine receptor comprises CCR2, CCR5, CXCR2, or CXCR4;

Preferably, the safety switch comprises iCaspase-9, Truncated EGFR or RQR8.

In a sixteenth aspect, the use of the genetically modified immune cell of the fifteenth aspect of the present invention is provided in the present invention, for preparing a
15 tumor-suppressing drug, and the tumor is a tumor expressing BCMA.

In a preferred embodiment, the tumor expressing BCMA is multiple myeloma.

In a seventeenth aspect, a pharmaceutical composition is provided in the present invention, comprising:

20 an antibody of the first or second aspect of the invention or a nucleic acid encoding the antibody; or

an immunoconjugate of the seventh aspect of the invention or a nucleic acid encoding the immunoconjugate; or

25 a chimeric antigen receptor of the tenth aspect of the invention, or a nucleic acid encoding the chimeric antigen receptor; or

a genetically modified immune cell of the fifteenth aspect of the invention.

It is to be understood that the various technical features of the present invention mentioned above and the various technical features specifically described hereinafter (as
30 in the Examples) may be combined with each other within the scope of the present invention to constitute a new or preferred technical solution, which will not be repeated one by one herein.

Description of figures

35 Fig. 1 shows SDS electropherograms of BCMA_huFc and BCMA_muFc (reduction

conditions).

Figure 2 shows the expression of BCMA in a stable cell line K562-BCMA detected by FACs.

Figure 3 shows the results of ELISA assays for antibodies 7A12, 7G2 and 23F10.

5 Figure 4 shows the binding of antibodies 7A12, 7G2 and 23F10 to K562-BCMA and K562 detected by FACs.

Figure 5 shows analysis of purified anti-BCMA scFv_Fc antibody by SDS PAGE (reduction conditions).

10 Figure 6 shows the binding of gradient-diluted purified scFv_Fc to K562-BCMA determined by FACs assay.

Figure 7 shows the affinity of antibodies 7A12, 7G2 and 23F10 to BCMA determined by Biacore.

Figure 8 shows the binding of antibodies 7A12, 7G2 and 23F10 to RPMI8226 cell line determined by FACs.

15 Figure 9 shows the competitive binding of antibodies and APRIL to BCMA determined by ELISA.

Figure 10 shows the positive rate of BCMA-CAR T virus-infected T lymphocytes detected by FACS.

20 Figure 11A shows the results of *in vitro* toxicity tests of BCMA-CAR T on BCMA-expression positive and negative cells, and Figure 11B shows the results of *in vivo* experiments of BCMA-CAR T in mice.

Figure 12 compares the heavy chain amino acid sequences of clones 25C2, 25D2 and 23F10.

25 Fig. 13A shows the aggregation of antibody 25C2, Figure 13B shows the aggregation of antibody 25D2, and Figure 13C shows the aggregation of antibodies 23F10 and 7A12.

Figure 14A shows the binding of 25C2 and 25D2 to K562-BCMA and K562 cells, and Figure 14B shows the specificity of antibodies 23F10, 25C2 and 25D2 determined by ELISA assay.

30 Figure 15 shows the experiment results of cell killing of 25C2-BBZ, 25D2-BBZ, 7A12-BBZ, C11D5.3-BBZ and sPD-1-7A12-BBZ.

Figure 16 shows the results of subcutaneous xenografts of 25C2-BBZ, 25D2-BBZ, C11D5.3-BBZ, 7A12-BBZ.

35 **Modes for carrying out the invention**

Through extensive and intensive research, the inventors have unexpectedly discovered antibodies that specifically bind to BCMA, and these antibodies can be applied to prepare various targeted antitumor drugs and drugs for diagnosing tumors. The present invention has been completed based on the above findings.

5 The technical terms used herein have the same or similar meanings as conventionally understood by a skilled person. Some terms are defined as follows for understanding the invention,.

10 The term "BCMA" as used herein refers to a B cell maturation antigen, which is a type III transmembrane protein consisting of 184 amino acid residues (NCBI Reference Sequence: NP_001183.2), and the amino acid sequence is shown in SEQ ID No: 37. In a specific embodiment, BCMA refers to human BCMA.

 The term "APRIL" as used herein refers to A proliferation-inducing ligand, which is a proliferation-inducing ligand consisting of 184 amino acid residues (NCBI Reference Sequence: NP_003799.1), and belongs to TNF superfamily

15 The term "antibody" as used herein refers to an antigen-binding protein of the immune system. The term "antibody" as used herein includes an intact full length antibody having an antigen binding region and any fragments thereof retaining an "antigen-binding portion" or "antigen-binding region", or a single strand thereof, such as a single chain variable fragment (scFv). A native antibody refers to a glycoprotein
20 comprising at least two heavy (H) chains and two light (L) chains or antigen-binding fragments thereof interconnected by a disulfide bond. The term "antibody" also includes all recombinant forms of antibodies, particularly the antibodies described herein, such as antibodies expressed in prokaryotic cells, unglycosylated antibodies, and antibody fragments that bind to antigens and derivatives hereinafter. Each heavy chain consists of a
25 heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. Each light chain consists of a light chain variable region (abbreviated herein as VL) and a light chain constant region. VH and VL can be further subdivided into hypervariable regions named complementarity determining regions (CDRs), which are interspersed in more conserved regions named framework regions (FR). Each VH and VL consists of
30 three CDRs and four FRs, from the amino terminus to the carboxy terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain binding domains that interact with an antigen. The constant region of the antibody can mediate binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (such as effector cells)
35 and the first component (C1q) of the classical complement system.

Antibody fragments include, but are not limited to, (i) Fab fragments consisting of VL, VH, CL and CH1 domains, including Fab' and Fab'-SH, (ii) Fd fragments consisting of VH and CH1 domains, (iii) Fv fragment consisting of VL and VH domains of a single antibody; (iv) a dAb fragment consisting of a single variable region (Ward et al, 1989, Nature 341: 544-546); (v) F(ab')₂ fragment, a bivalent fragment comprising two linked Fab fragments; (vi) a single-chain Fv molecule antigen binding site (Bird et al, 1988, Science 242: 423-426; Huston et al, 1988, Proc. Natl. Acad. Sci. USA 85:5879-5883); (vii) bispecific single-chain Fv dimer (PCT/US92/09965); (viii) "dibody" or "tribody", multi-valent or multi-specific fragments constructed by gene fusion (Tomlinson et al, 2000, Methods Enzymol. 326: 461-479; WO94/13804; Holliger et al, 1993, Proc. Natl. Acad. Sci. USA 90:6444-6448); and (ix) scFv genetically fused to identical or different antibodies (Coloma & Morrison, 1997, Nature Biotechnology 15, 159-163).

The term "Fc" or "Fc region" as used herein includes a polypeptide comprising an antibody constant region other than the first constant region immunoglobulin domain. Therefore, Fc refers to the last two constant region immunoglobulin domains of IgA, IgD and IgG, and the last three constant region immunoglobulin domains of IgE and IgM, and flexible hinges at N-terminus of these domains. For IgA and IgM, Fc can include J chain. For IgG, Fc includes hinges between immunoglobulin domains Cγ2 and Cγ3 as well as Cγ1 and Cγ2. Boundaries of Fc region may vary, however, the human IgG heavy chain Fc region is generally defined as comprising residues C226 or P230 at its carboxy terminus, where numbering is based on EU index of Kabat. For human IgG1, Fc is defined herein to include residue P232 to its carboxy terminus, where numbering is based on EU index of Kabat. Fc may refer to the isolated region, or the region in the environment of Fc polypeptide, such as an antibody. The "hinge" as said above includes a flexible polypeptide comprising amino acids between the first and second constant domains of an antibody. Structurally, IgG CH1 domain ends at position EU220 and IgG CH2 domain begins at residue EU237. Therefore, for IgG, the antibody hinge herein is defined to include 221 (D221 of IgG1) to 231 (A231 of IgG1), where the numbering is based on EU index of Kabat.

The term "parent antibody" or "parent immunoglobulin" as used herein includes an unmodified antibody which is to be modified to produce variants. The parent antibody can be a naturally occurring antibody, or a variant or modified version of a naturally occurring antibody. A parent antibody can refer to the antibody itself, a composition comprising the parent antibody, or a nucleic acid sequence encoding the same. The term "parent antibody" or "parent immunoglobulin" as used herein includes a murine or chimeric

antibody that is to be modified to produce a humanized antibody.

The term "variant antibody" or "antibody variant" as used herein includes an antibody sequence that differs from the parent antibody sequence by at least one amino acid modification compared with the parent antibody. A variant antibody sequence
5 herein has at least about 80%, preferably at least about 90%, more preferably at least about 95% amino acid sequence identity to the parent antibody sequence. An antibody variant can refer to the antibody itself, a composition comprising the parent antibody, or a nucleotide sequence encoding the same.

The term "variant" as used herein includes an antibody sequence that differs from
10 the parent antibody sequence by at least one amino acid modification compared with the parent antibody. In a specific embodiment, a variant antibody sequence herein has at least about 80%, preferably at least about 90%, more preferably at least about 95%, more preferably at least about 97%, more preferably at least about 98%, most preferably at least about 99% amino acid sequence identity to the parent antibody sequence. An
15 antibody variant can refer to the antibody itself, a composition comprising the parent antibody, or a nucleotide sequence encoding the same. The term "amino acid modification" includes amino acid substitution, addition and/or deletion, and "amino acid substitution" refers to the replacement of an amino acid at a particular position in a parent polypeptide sequence with another amino acid. For example, substitution R94K
20 means that the arginine at position 94 is replaced by lysine, and "amino acid insertion" as used herein refers to the addition of an amino acid at a particular position in a parent polypeptide sequence. As used herein, "amino acid deletion" or "deletion" refers to removal of an amino acid at a particular position in a parent polypeptide sequence.

The term "conservative modification" or "conservative sequence modification" as
25 used herein refers to an amino acid modification that does not significantly affect or alter the binding characteristics of an antibody comprising the amino acid sequence. Such conservative modifications include amino acid substitutions, insertions, and deletions. Modifications can be introduced into the antibodies of the invention by standard techniques known in the art, such as site-directed mutagenesis and
30 PCR-mediated mutagenesis. Conservative amino acid substitutions are substitutions in which amino acid residues are replaced with amino acid residues having similar side chains. A family of amino acid residues having similar side chains has been defined in the art. These families include amino acids containing basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged
35 acute side chains (e.g., glycine, asparagine, serine, threonine, tyrosine, cysteine,

tryptophan), non-polar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), β -branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Therefore, one or more amino acid residues in the CDR regions or the framework regions of the antibody of the present invention can be replaced with amino acid residues of other families with identical side chain, and the function retained by the altered antibody (variant antibody) can be tested.

All positions of immunoglobulin heavy chain constant region discussed in the present invention are numbered based on EU index of Kabat (Kabat et al., 1991, sequences of proteins of immunological interest, 5th edition, United States Public Health Service, National Institutes of Health, Bethesda, incorporated herein by reference in its entirety). "EU index of Kabat" refers to the residue numbering of human IgG1 EU antibody as described by Edelman et al., 1969, Biochemistry 63: 78-85.

The term "antigenic determinant" as used herein, also named as antigenic epitope, may consist of a contiguous sequence of BCMA protein sequence or a discontinuous three-dimensional structure of BCMA protein sequence.

The term "chimeric antigen receptor" or "CAR" as used herein, refers to a polypeptide comprising an extracellular domain capable of binding an antigen, a transmembrane domain, and a cytoplasmic signaling domain (i.e., an intracellular signal domain), and the intracellular signal domain refers to a protein that transmits signals into a cell by producing a second messenger through a defined signaling pathway, thereby regulating cellular activities, or a protein that correspondes to such a messenger and acts as an effector, including a primary signal domain and a functional signaling domain (i.e., a co-stimulatory signal domain) derived from a stimulatory molecule as defined below. The intracellular signal domain produces a signal that promotes the immune effector function of cells of the CAR (e.g., CAR T cells), and examples of immune effector functions, such as in CART cells, includes cell lytic activity and helper activity, including secretion of cytokine.

The term "primary signal domain" refers to modulating the initial activation of a TCR complex in an irritating manner. In one aspect, the primary signal domain is elicited by, for example, binding of a TCR/CD3 complex to a peptide-loaded MHC molecule, thereby mediating a T cell response (including, but not limited to, proliferation, activation, differentiation, etc.). The primary signal domain that functions in a stimulatory manner may comprise an immunoreceptor tyrosine activation motif or a signaling motif of ITAM. Examples of primary signal domains comprising ITAM that

are particularly useful in the present invention include, but are not limited to, the sequence derived from TCR ξ , FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, CD278 (also referred to as "ICOS") and CD66d. In an exemplary CAR of the invention, in any one or more of the CARs of the invention, the intracellular signaling domain comprises an intracellular signaling sequence, such as the primary signal domain of CD3 ξ .

The term "co-stimulatory signal domain" refers to a "co-stimulatory molecule" which is a related binding partner on a T cell that specifically binds to a co-stimulatory ligand, thereby mediating a co-stimulatory response of a T cell, such as, but not limited to, proliferation. Co-stimulatory molecules are cell surface molecules or ligands thereof which are required for an effective immune response and non-antigen receptors. Co-stimulatory molecules include, but are not limited to, MHC class I molecules, BTLA and Toll ligand receptors, as well as OX40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18) and 4-1BB (CD137).

In the present invention, in one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain, and the intracellular signaling domain comprises a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain, and the intracellular signaling domain comprises a functional signaling domain derived from a co-stimulatory molecule and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen recognition domain, a transmembrane domain and an intracellular signaling domain, and the intracellular signaling domain comprises at least two functional signaling domains derived from one or more co-stimulatory molecules and a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises an optional leader sequence at the amino acid (ND end) of the CAR fusion protein. In one aspect, the CAR further comprises a leader sequence at N-terminus of the extracellular antigen recognition domain, wherein the leader sequence is optionally cleaved from the antigen recognition domain (e.g., scFv) during processing and localization of the CAR to the cell membrane.

The term "CD3 ξ " as used herein is defined as a protein provided by GenBan Accession No. BAG36664.1, or equivalent residues from a non-human species such as a mouse, rodent, monkey, ape, and the like. "CD3 ξ domain" as used herein is defined as

amino acid residues from the cytoplasmic domain of ξ chain sufficient to functionally deliver the initial signal required for T cell activation. In one aspect, the cytoplasmic domain of ξ comprises residues 52 to 164 of GenBank Accession No. BAG36664.1, a functional ortholog thereof - equivalent residues from non-human species such as a mouse, rodents, monkey, ape, etc.

The term "4-1BB" as used herein refers to a member of TNFR superfamily having the amino acid sequence of GenBank Acc. No. AAA62478.2, or equivalent residues from a non-human species such as a mouse, rodent, monkey, ape and the like. "4-1BB co-stimulatory domain" is defined as amino acid sequence 214-255 of GenBank ACC. No. AAA62478.2, or equivalent residues from non-classified species such as mouse, rodent, monkey, ape, etc. In one aspect, the "4-1BB co-stimulatory domain" is the sequence provided in SEQ ID NO: 35, or equivalent residues from a non-human species such as a mouse, rodent, monkey, ape, and the like.

The term "interferon" as used herein refers to a full-length interferon, or an interferon fragment (truncated interferon) or interferon mutant substantially retaining the biological activities of a full-length wild-type interferon (e.g., retaining at least 80%, preferably at least 90%, more preferably at least 95%, 98% or 99% of those of a full length interferon). Interferons include type I interferons (e.g., interferon α and interferon β) and type II interferons (e.g., interferon γ).

The antibody of the present invention or a variant thereof can be applied to prepare various targeted antitumor drugs as well as drugs for diagnosing tumors, in particular, for preparing immune effector cells targeting BCMA.

Anti-BCMA antibody

In the present disclosure, antigen binding proteins having an antigen-binding region based on scFv, including antibodies, are described. A recombinant BCMA was used to select scFv from a human scFv phage display library. These molecules display fine specificity. For example, the antibody only recognizes K562 cells stably expressing BCMA and does not recognize K562 cells.

In some embodiments, the invention encompasses an antibody having scFv sequence, which is fused to one or more heavy chain constant regions to form an antibody having a human immunoglobulin Fc region to produce a bivalent protein, thereby increasing overall affinity and stability of an antibody. In addition, the Fc portion allows for direct conjugation of other molecules (including but not limited to fluorescent dyes, cytotoxins, radioisotopes, etc.) to, for example, antibodies used in antigen quantification

studies in order to immobilize antibodies for affinity measurement, targeted delivery of therapeutic drugs, use of immune effector cells to test Fc-mediated cytotoxicity and many other applications.

The results presented herein highlight the specificity, sensitivity and utility of the antibodies of the invention in targeting BCMA.

The molecules of the invention are based on single-chain variable fragments (scFv) identified and selected by phage display, the amino acid sequence of which confers specificity to BCMA and forms the basis of all antigen binding proteins of the present disclosure. Therefore, the scFv can be used to design various different "antibody" molecules, including, for example, full length antibodies, fragments thereof such as Fab and F(ab')₂, fusion proteins (including scFv_Fc), multivalent antibodies, i.e., an antibody having more than one specificity to the same or different antigens, for example, bispecific T cell-binding antibody (BiTE), tri-antibody, etc. (Cuesta et al, Multivalent antibodies: when design surpasses evolution, Trends in Biotechnology 28: 355-362, 2010).

In one embodiment where the antigen binding protein is a full length antibody, the heavy and light chains of the antibodies of the invention may be of full length (for example, the antibody may comprise at least one, preferably two intact heavy chains, and at least one, preferably two intact light chains), and alternatively may comprise an antigen binding moiety (Fab, F(ab')₂, Fv or scFv). In other embodiments, the antibody heavy chain constant region is selected, for example, from IgG1, IgG2, IgG3, IgG4, IgM, IgA1, IgA2, IgD, and IgE. The selection of antibody type will depend on the immune effector function that the designed antibody is intended to elicit. Suitable amino acid sequences for the constant regions of various immunoglobulin isotypes and methods for producing a wide variety of antibodies are known to a skilled person in the construction of recombinant immunoglobulins.

In a first aspect, an antibody or fragment thereof binding to BCMA is provided in the present invention, comprising heavy chain CDR1 comprising an amino acid sequence of any one of SEQ ID NO: 1, 60, 62, and/or heavy chain CDR2 comprising an amino acid sequence of any of SEQ ID NO: 2, 61, 63, and/or heavy chain CDR3 comprising an amino acid sequence of any one of SEQ ID NOs: 3, 4, 5. In another aspect, an antibody or fragment thereof binding to BCMA is provided in the present invention, comprising light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 6, and/or light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 7, and/or light chain CDR3 comprising an amino acid sequence of any of SEQ ID NO: 8, 9, 10. In another aspect, an antibody or fragment thereof binding to BCMA is provided in the present invention,

comprising heavy chain CDR1 comprising an amino acid sequence of any one of SEQ ID NO: 1, 60, 62, and/or heavy chain CDR2 comprising an amino acid sequences of any one of SEQ ID NO: 2, 61, 63, and/or heavy chain CDR3 comprising an amino acid sequence of any one of SEQ ID NOs: 3, 4, 5, and/or light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 6, and/or light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 7, and/or light chain CDR3 comprising an amino acid sequence of any of SEQ ID NO: 8, 9, 10. Preferably, the BCMA-binding antibody or fragment thereof comprises heavy chain CDR1 comprising an amino acid sequence of any one of SEQ ID NO: 1, 60, 62, and heavy chain CDR2 comprising an amino acid sequence of any one of SEQ ID NO: 2, 61, 63, and heavy chain CDR3 comprising an amino acid sequence of any one of SEQ ID NO: 3, 4, 5, and/or light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 6, and light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 7, and light chain CDR3 comprising an amino acid sequence of any one of SEQ ID NOs: 8, 9, 10. More preferably, the BCMA-binding antibody or fragment thereof comprises heavy chain CDR1 comprising an amino acid sequence of any one of SEQ ID NO: 1, 60, 62, and heavy chain CDR2 comprising an amino acid sequence of any one of SEQ ID NO: 2, 61, 63, and heavy chain CDR3 comprising an amino acid sequence of any one of SEQ ID NO: 3, 4, 5, and light chain CDR1 comprising an amino acid sequence of SEQ ID NO: 6, and light chain CDR2 comprising an amino acid sequence of SEQ ID NO: 7, and light chain CDR3 comprising an amino acid sequence of any one of SEQ ID NOs: 8, 9, 10.

In another aspect, an antibody or fragment thereof binding to BCMA is provided in the present invention, comprising a heavy chain variable region sequence selected from the group consisting of SEQ ID NOs: 13, 17, 21, 56 and 58.

In another aspect, an antibody or fragment thereof binding to BCMA is provided in the present invention, comprising a light chain variable region sequence selected from the group consisting of SEQ ID NOs: 11, 15 and 19.

Each of the heavy and light chain variable region sequences can bind to BCMA, therefore, the heavy and light chain variable region sequences can be "mixed and matched" to produce anti-BCMA binding molecules of the invention.

In another aspect, variants of an antibody or fragment thereof binding to BCMA is provided in the present invention. Accordingly, an antibody or fragment thereof is provided in the present invention, having a heavy chain and/or light chain variable region that is at least 80% identical to the variable region sequence of the heavy or light chain.

Preferably, the amino acid sequence identity of the heavy and/or light chain variable

regions is at least 85%, preferably at least 90%, more preferably at least 95%, more preferably 96%, more preferably 97%, even more preferably 98%, the most preferably 99%, including, for example, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% and 100%. The variant can be
5 obtained from the antibody described in the present application as a parent antibody by yeast library screening, phage library screening, point mutation or the like. As in the method used in Example 10 of the present application, the antibody 23F10 was used as the parent antibody, and the phage library screening method was used for mutation modification.

10 In another aspect, an antibody that recognizes the same antigenic determinant as the anti-BCMA antibody described above is provided in the present invention.

Properties of Anti-BCMA antibody

Standard assays for assessing the binding ability of an antibody, such as an
15 anti-BCMA antibody, are known in the art and include, for example, ELISA, Western blot and flow cytometry analysis. Suitable assays are described in detail in the examples.

Nucleic acids, vectors and host cells

An isolated nucleic acid encoding an antibody binding to BCMA and fragment
20 thereof, a vector and a host cell comprising the nucleic acid or vector, are also provided in the present invention. The nucleic acid can be present in an intact cell, cell lysate, or can be in a partially purified or substantially purified form.

The nucleic acid of the invention can be obtained using standard molecular biology techniques, for example, standard PCR amplification or cDNA cloning techniques,
25 thereby obtaining cDNA encoding the light and heavy chains of an antibody or encoding VH and VL segments. For antibodies obtained from immunoglobulin gene libraries (e.g., using phage display technology), one or more nucleic acids encoding the antibodies can be recovered from the library. Methods for introducing foreign nucleic acids into host cells are generally known in the art and can vary with the used host cell.

30 Preferred nucleic acid molecules of the invention are those selected from the group consisting of SEQ ID NOs: 12, 16 and 20 which encode a light chain variable region, and/or those selected from the group consisting of SEQ ID NO: 14, 18, 22, 57 and 59 which encode a heavy chain variable region. A more preferred nucleic acid molecule comprises a sequence of SEQ ID NO: 14 encoding a heavy chain and a sequence of SEQ
35 ID NO: 12 encoding a light chain, or comprises a sequence of SEQ ID NO: 18 encoding a

heavy chain and a sequence of SEQ ID NO: 16 encoding a light chain, or comprises a sequence of SEQ ID NO: 22 encoding a heavy chain and a sequence of SEQ ID NO: 20 encoding the light chain, or comprises a sequence of SEQ ID NO: 57 encoding a heavy chain and a sequence of SEQ ID NO: 20 encoding the light chain, or comprises a sequence of SEQ ID NO: 59 encoding a heavy chain and a sequence of SEQ ID NO: 20 encoding the light chain.

For expressing a protein, a nucleic acid encoding an antibody of the invention can be integrated into an expression vector. A variety of expression vectors are available for protein expression. Expression vectors can include self-replicating extra-chromosomal vectors, or vectors integrated into the host genome. Expression vectors used in the present invention include, but are not limited to, those which enable expression of proteins in mammalian cells, bacteria, insect cells, yeast, and *in vitro* systems. As is known in the art, a variety of expression vectors which are commercially available or otherwise available, can be used in the present invention to express antibodies.

Immunoconjugate

In the present invention, a multifunctional immunoconjugate is also provided, comprising the antibodies described herein and further comprising at least one functional molecule of other type. The functional molecule is selected from, but not limited to, a molecule that targets a tumor surface marker, a tumor-suppressing molecule, a molecule that targets a surface marker of an immune cell, or a detectable label. The antibody and the functional molecule may form a conjugate by covalent attachment, coupling, attachment, cross-linking, or the like.

As a preferred mode, the immunoconjugate may comprise an antibody of the invention and at least one molecule that targets a tumor surface marker or a tumor-suppressing molecule. The tumor-suppressing molecule may be anti-tumor cytokines or anti-tumor toxins. Preferably, the cytokines include but are not limited to IL-2, IL-7, IL-12, IL-15, type I IFN, TNF-alpha. In a specific embodiment, the molecule that targets a tumor surface marker is a molecule that targets the same tumor surface marker as the antibody of the invention. For example, the molecule that targets a tumor surface marker can be an antibody or ligand that binds to a tumor surface marker, for example, can act synergistically with the antibodies of the invention to more precisely target tumor cells.

As a preferred mode, the immunoconjugate may comprise an antibody of the present invention and a detectable label. Such detectable labels include, but are not limited to,

fluorescent labels, chromogenic labels such as enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron-emitting metals and non-radioactive paramagnetic metal ion. More than one marker can also be included. The label used to label the antibody for the purpose of
5 detection and / or analysis and / or diagnosis depends on the used particular detection / analysis / diagnosis technique and / or method, eg, immunohistochemical staining (tissue) samples, flow cytometry, and the like. Suitable labels for detection / analysis / diagnosis techniques and / or methods known in the art are well known to those skilled in the art.

As a preferred mode, the immunoconjugate may comprise: an antibody of the
10 invention and a molecule that targets a surface marker of an immune cell. The molecule targeting a surface marker of a immune cell may be an antibody or a ligand binding to a surface marker of a immune cell, capable of recognizing the immune cell, and carry the antibody of the present invention to the immune cell. The antibody of the present invention can target the immune cell to tumor cells, thereby inducing the immune cell to
15 specifically kill tumors. The immune cell surface marker may be selected from the group consisting of CD3, CD16, CD28, and preferably, the antibody binding to the immune cell surface marker is an anti-CD3 antibody. The immune cells can be selected from the group consisting of T cells, NK cells, and NKT cells.

As a means of chemically generating an immunoconjugate by conjugation,
20 either directly or indirectly (eg, by a linker), the immunoconjugate can be produced as a fusion protein comprising an antibody of the invention and other suitable proteins. The fusion protein can be produced by a method known in the art, for example recombinantly produced by constructing and subsequently expressing the nucleic acid molecule which comprises the nucleotide sequence encoding the
25 antibody in frame with a nucleotide sequence encoding a suitable label.

In another aspect of the invention, a nucleic acid molecule encoding at least one antibody of the invention, a functional variant, or an immunoconjugate thereof is provided. Once obtaining the relevant sequence, the recombination method can be used to obtain the relevant sequence in large quantities. This is usually done by
30 cloning it into a vector, transferring it to a cell, and then isolating the relevant sequence from the proliferating host cells by conventional methods.

The present invention also relates to vectors comprising the appropriate DNA sequences described above as well as appropriate promoters or control sequences. These vectors can be used to transform an appropriate host cell to enable expression of the
35 protein. The host cell may be a prokaryotic cell, such as a bacterial cell; or a lower

eukaryotic cell, such as a yeast cell; or a higher eukaryotic cell, such as a mammalian cell.

Chimeric antigen receptor containing anti-BCMA antibody

A plurality of chimeric antigen receptors (CAR) are provided in the present invention, comprising an antibody or antibody fragment of the present invention. The CAR T cell exhibits anti-tumor properties. In some embodiments, cells (e.g., T cells) are transduced with a viral vector encoding CAR. In some embodiments, the viral vector is a lentiviral vector. In some embodiments, the cells can stably express CAR.

In a preferred embodiment, the BCMA binding portion of a CAR is a scFv antibody fragment that retains an equivalent binding affinity, for example it binds to the same antigen with comparable efficacy, as compared with the IgG antibody from which it is derived. The antibody fragment is functional, thereby providing a biochemical reaction, which can include, but is not limited to, activating an immune response, inhibiting the initiation of signaling from its target antigen, inhibiting kinase activity, and the like. Accordingly, a BCMA-CAR which comprises a WT1 binding domain and engineered into a T cell, and a method for using it in adoptive immunotherapy are provided in the present invention.

In one aspect, the anti-BCMA antigen binding domain of CAR is a scFv antibody fragment that is humanized relative to the murine sequence scFv from which it is derived.

In one aspect, the CAR of the invention combines the antigen binding domain of a particular antibody with an intracellular signaling molecule. For example, in some aspects, intracellular signaling molecules include, but are not limited to, CD3 ξ chain, 4-1BB and CD28 signaling modules, and combinations thereof.

In one aspect, the BCMA-CAR comprises at least one intracellular signaling domain that is selected from a CD137 (4-1BB) signaling domain, a CD28 signaling domain, a CD3 ξ signaling domain, or any combination thereof. In one aspect, the BCMA-CAR comprises at least one intracellular signaling domain derived from one or more co-stimulatory molecules that are not CD137 (4-1BB) or CD28.

Exemplarily, the sequence of BCMA-CAR can be 7A12-BBZ (SEQ ID NO: 75), 25C2-BBZ (SEQ ID NO: 76), 25D2-BBZ (SEQ ID NO: 77), 7G2-BBZ (SEQ ID NO: 78), 7A12-28Z (SEQ ID NO: 79), 7A12-28BBZ (SEQ ID NO: 80), 7G2-28Z (SEQ ID NO: 81), 7G2-28BBZ (SEQ ID NO: 82), 25C2-28Z (SEQ ID NO: 83), 25C2-28BBZ (SEQ ID NO: 84), 25D2-28Z (SEQ ID NO: 85), 25D2-28BBZ (SEQ ID NO: 86). Conventional transmembrane domain and intracellular domain can be selected by a skilled person to replace the transmembrane domain and intracellular domain of the above SEQ ID NO:

75-86, which will fall within the scope of this application.

Chimeric antigen receptor modified T cell

An immune cell comprising a chimeric antigen receptor of the invention is also
5 provided in the present invention.

In another aspect, the chimeric antigen receptor-modified T cell provided in the present invention further carries an encoding sequence for a foreign cytokine; preferably, the cytokine comprises: IL-12, IL-15 or IL-21. The immune cells are preferably selected from T lymphocytes, NK cells or NKT cells.

10 In another aspect, the chimeric antigen receptor-modified T cell provided in the present invention further comprise a PD-L1 blocker or a protein that blocks PD-L1, such as native PD-1, or a mutant PD-1 capable of binding to PD-L1, or a fragment of native or mutant PD-1 capable of binding to PD-L1, or an antibody against PD-L1. Exemplarily, the PD-L1 blocker may comprise an amino acid sequence encoded by SEQ ID NO:70.

Pharmaceutical composition

The antibodies, immunoconjugates comprising the antibodies, and genetically modified immune cells of the present invention can be used in the preparation of a pharmaceutical composition or diagnostic reagent. In addition to an effective amount of
20 the antibody, immunological conjugate, or immune cell, the composition may further comprise a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" means that when the molecular entities and compositions are properly administered to animals or humans, they do not cause adverse, allergic or other untoward reactions.

Specific examples of some of the substances which may be used as
25 pharmaceutically acceptable carriers or components thereof are sugars, such as lactose, dextrose and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as carboxymethylcellulose sodium, ethylcellulose and methylcellulose; gum tragacanth; malt; gelatin; talc; solid lubricants such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil,
30 olive oil, corn oil and cocoa butter; polyhydric alcohols such as propylene glycol, glycerin, sorbitol, mannitol and polyethylene glycol; alginic acid; emulsifiers such as Tween®; wetting agents such as sodium lauryl sulfate; coloring agents; flavoring agents; tablets, stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline solutions; and phosphate buffers and the like.

35 The composition of the present invention can be prepared into various dosage forms

as needed, and the dosage to be administered to a patient can be determined by a physician according to factors, such as type, age, body weight, and general disease condition of a patient, mode of administration, and the like. For example, injection or other treatment may be used.

5

Advantages of the invention:

1. Specific antibodies against BCMA are provided in the invention;
 2. Immune effector cells that target BCMA are provided in the invention; and
 3. The antibody of the present invention is capable of efficiently binding to tumor
- 10 cells expressing BCMA, and the immune effector cells of the present invention exhibit significant killing ability against tumor cells expressing BCMA, and therefore, the antibody and immune effector cells of the present invention can be efficiently and safely applied to the treatment of multiple myeloma, thereby constituting a material foundation for the treatment of multiple myeloma.

15

The invention will be further illustrated hereinafter in conjunction with specific examples. It is to be understood that the examples are not intended to limit the scope of the invention. The experimental methods in the following examples which do not specify the specific conditions are usually prepared according to conventional conditions such as J. Sambrook et al., Molecular Cloning Experimental Guide, Third Edition, Science Press,

20 Sambrook et al., Molecular Cloning Experimental Guide, Third Edition, Science Press, 2002, or according to the conditions recommended by the manufacturer.

Example 1. Preparation of BCMA Recombinant Protein

a. Construction of BCMA_huFc, BCMA_muFc expression plasmid

25 The gene (SEQ ID NO: 39) of extracellular segment of human BCMA, Met1-Ala54 (SEQ ID NO: 38), was *in vitro* synthesized, inserted into the eukaryotic expression plasmid containing the Fc fragment Asp104-Lys330 of human IgG1 heavy chain constant region, and linked with “GS” to form a fusion expression protein BCMA_huFc (SEQ ID NO: 40), and the corresponding gene sequence is shown in SEQ ID NO: 41.

30 The gene (SEQ ID NO: 39) of extracellular segment of human BCMA was inserted into the eukaryotic expression plasmid containing Fc fragment Arg100-Lys324 of murine IgG1 heavy chain constant region, and linked with “GS” to form a fusion expression protein BCMA_muFc (SEQ ID NO: 42), and the corresponding gene sequence is shown in SEQ ID NO: 43.

35 b. Expression of BCMA_huFc, BCMA_muFc by transient transfection

1) One day before transfection, $6-7 \times 10^5$ /ml 293F cells were inoculated in 125 ml culture flasks;

2) On the day of transfection, 3×10^7 cells were adjusted in 28 ml FreeStyle™ 293 expression medium;

5 3) Lipid-DNA complex was prepared by the following steps:

30 ug of DNA was diluted with Opti-MEM I at final volume of 1 ml, and mixed thoroughly;

60 ul of 293fectin™ was diluted with Opti-MEM I to a final volume of 1 ml and mixed thoroughly;

10 the mixture was incubated for 5 minutes at room temperature;

4) the diluted DNA was diluted with 293fectin™ and incubated for 20 minutes at room temperature;

5) 2 ml of DNA-293fectin complex was added to 28 ml of cells, cultured at 37°C, under 8% CO₂, 125 rpm for 3-4 days, and the supernatant was collected.

15 c. Purification of BCMA_huFc, BCMA_muFc

1) The supernatant was centrifuged at 13000 rpm for 15 min;

2) Protein A filler was used in affinity purification with the steps being listed as follows:

Balance: 10 column volumes of balance buffer was used for protein A filler.

20 Loading: the sample processed with 0.45 µm filter was loaded.

Washing: 20 column volume balance buffer were used for removing impurities until there was no flow-through.

Elution: 10 column volumes of elution buffer were added to elute the protein of interest (6% of neutralization buffer was pre-added to the collection tube).

25 Solution formulation:

Balance buffer: PBS pH 7.4

Elution buffer: 0.1 M glycine pH 2.6

Neutralize buffer: 1 M Tris

30 3) The elution was filtered through a 0.22 µm membrane, concentrated in a millipore ultrafiltration tube with a cut-off of 10 KD to a volume of 1 ml, and desalted using a PD-Midi desalting column. 1.5 ml of the sample was collected. Protein concentration was measured by OD280/1.47.

2 µg was taken for SDS-PAGE and the results are shown in Figure 1.

35 **Example 2. Construction of K562-BCMA stable cell line**

1. Construction of pWPT-BCMA packaging plasmid

The full length gene (SEQ ID NO: 37) of human BCMA was synthesized *in vitro*, and cleavage sites MluI, Sall (SEQ ID NO: 44) were introduced, which were inserted into the lentiviral packaging plasmid pWPT by double digestion.

2. Packaging of lentiviruses

a) Lenti-x 293T was digested and plated to a 10 cm dish at 8×10^6 cells, and cultured at 37°C.

b) The next morning: plasmid/PEI mixture was prepared

pWPT-BCMA 5ug

psPAX.2 7.5ug

pMD2.G 2.5ug

added into 800 uL of DMEM and incubated. The corresponding PEI volume was 45 uL, and the incubation was carried out for 5 min in 800 uL of OMEM.

c) the plasmid mixture was added dropwise to PEI incubation solution, mixed gently and incubated for 20 min at room temperature.

d) the prepared plasmid/PEI mixture was added dropwise into cells and mixed. The solution was changed after 5 hours.

e) virus supernatant was collected after 72 h, filtered through a 0.45 um filter and temporarily stored at 4°C.

3. BCMA virus-infected K562 cells

a) The afternoon of Day 1: well-grown K562 cells were plated at 1×10^5 cells to a 6 cm dish.

b) The afternoon of Day 2: supernatant of K562 cells was discarded, 3 mL of fresh complete medium was added, and 1 mL of virus stock solution was added to a final concentration of 6 ug/mL of polybrene.

c) The morning of Day 3: the supernatant was discarded and 5 mL of fresh complete medium was added.

d) The morning of Day 6: some cells were taken for flow detection.

4. Identification of K562-BCMA mixed clone

a) K562-BCMA mixed clones and K562 negative cells were washed with 1% NCS (PBS containing 1% calf serum) for 2 times and then incubated with primary antibody: huBCMA antibody (abcam, #17323) diluted with 1% NCS at 1:1000 (each 50 uL) and incubated for 50 min at 4°C.

b) Cells were washed twice with 1% NCS and then incubated with secondary antibody: DyLight488-labeled goat anti-rat IgG (abcam, #ab98420), diluted with 1% NCS

at 1: 200 (each 50 uL), and incubated for 45 min at 4°C.

c) Cells were washed 3 times with 1% NCS and resuspended in 1% NCS and detected using a Guava easyCyte™ HT System instrument. The results are shown in Figure 2A.

5 5. K562-BCMA monoclonal plating

a) Cells in K562-BCMA mixed clone were counted and monoclonal clones were plated by limiting dilution.

b) The growth of clones was observed one week later and the medium was supplemented.

10 c) Two weeks later, cells in the wells of the monoclonal growth were taken and expanded for culture.

6. Identification of K562-BCMA monoclonal

The detection method was the same as identification of the mixed clone, and the experimental results are shown in Fig. 2B. 4 of the monoclonal clones were BCMA
15 positive clones.

Example 3. Screening for BCMA-specific scFv using a whole human phage display library

The phage display library used in the present invention is a whole human natural
20 scFv phage library constructed by the present company, and has a storage capacity of 1E+11. The scFv fragment highly specific for BCMA was obtained using screening methods known to a skilled person. Briefly, 10 ug/ml antigen BCMA_huFc and human Fc fragment were coated in immunotubes, respectively. To reduce the effect from Fc fragment, the phage library was added to the immunotube coated with human Fc fragment
25 for 1 hr. The supernatant was taken and added to the immunotube coated with BCMA_huFc for 1.5 hours, then the non-specific phage was washed away, and the bound phage was eluted and used to infect *E. coli* TG1 in logarithmic growth phase. The phage eluted was expanded and the expanded phage library was purified using PEG/NaCl precipitation for the next round of screening. Panning was performed for 3-4 cycles to
30 enrich scFv phage clones that specifically bind to BCMA. Positive clones were determined by standard ELISA methods for BCMA_huFc. Human Fc fragment was used as an unrelated antigen in ELISA to verify the specificity of the antibody. A total of 2470 clones were screened, in which 160 clones specifically bound to BCMA_huFc, while did not bind to human Fc fragment in ELISA assays. 76 clones with high signal values were
35 picked for sequencing, and 23 single sequences were obtained. These 23 clones were

purified and expressed to obtain three clones specifically binding to K562-BCMA cells (Fig. 4), and the clone were named as 7G2, 7A12 and 23F10. By sequencing analysis, the heavy chain variable region of 7A12 is the amino acid sequence shown in SEQ ID NO: 13, and the light chain variable region is the amino acid sequence shown in SEQ ID NO: 11; the heavy chain variable region of 7G2 is the amino acid sequence shown in SEQ ID NO: 17, and the light chain variable region is the amino acid sequence shown in SEQ ID NO: 15; and the heavy chain variable region of 23F10 is the amino acid sequence shown in SEQ ID NO: 21, and the light chain variable region is the amino acid sequence shown in SEQ ID NO: 19.

Amino acid sequence of the heavy chain variable region of 7A12 (SEQ ID NO: 13):
EVQLLES GGGLVQPGGSLRLSCAASGFTFSS**SYAMS**WVRQAPGKGLEWVSA**ISGSGGSTYYADSVKGR**FTISRDN SKNTLYLQMNSLRAEDTAVYYCARY**PYLA****FDY**WGQGT LVT VSS (CDR sequences are shown in bold and underlined)

Nucleotide sequence of the heavy chain variable region of 7A12 (SEQ ID NO: 14):
GAGGTGCAATTGCTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG
TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTACCTTTAGCAGTTATGCCAT
GAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATT
AGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCA
CCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAGATGAACAGCCT
GAGAGCCGAGGACACGGCCGTATATTACTGTGCGCGTTACCCATACCTGGCA
TTCGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT

Amino acid sequence of the light chain variable region of 7A12 (SEQ ID NO: 11):
EIVLTQSPGTLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPRLLIYGA**SSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQYGPPSY**TFGQG TKVEI

K (CDR sequences are shown in bold and underlined)

Nucleotide sequence of the light chain variable region of 7A12 (SEQ ID NO: 12):
GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG
AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTT
AGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGA
GCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCA GTGGCAGTGGATCCG
GGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGT
GTATTACTGTCAGCAGTACGGTTACCCACCATCTTACACGTTTCGGCCAGGGG
ACCAAAGTGGAATCAAA

Amino acid sequence of the heavy chain variable region of 7G2 (SEQ ID NO: 17):
EVQLLES GGGLVQPGGSLRLSCAASGFTFSS**SYAMS**WVRQAPGKGLEWVSA

ISGSGGSTYYADSVKGR**FTISR****DNSKNTLYLQMNSLRAEDTAVYYCAK****LSGDA**
AMDYWGQGT**LVTVSS** (CDR sequences are shown in bold and underlined)

Nucleotide sequence of the heavy chain variable region of 7G2 (SEQ ID NO: 17):

GAGGTGCAATTGCTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG
5 TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTACCTTTAGCAGTTATGCCAT
GAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATT
AGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCA
CCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAGATGAACAGCCT
GAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAACTGTCTGGTGATGCA
10 GCAATGGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT

Amino acid sequence of the light chain variable region of 7G2 (SEQ ID NO: 15):

EIVLTQSPGTLSPGERATLSC**RASQSVSSSYLA**WYQQKPGQAPRLLIY**GA**
SSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQYGYPPRY**TFGQGTKVEI
K (CDR sequences are shown in bold and underlined)

15 Nucleotide sequence of the light chain variable region of 7G2 (SEQ ID NO: 16):

GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG
AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTT
AGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGA
GCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTCAAGTGGCAGTGGATCCG
20 GGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGT
GTATTACTGTCAGCAGTACGGTTACCCACCAAGATACACGTTCCGGCCAGGGG
ACCAAAGTGGAATCAAA

Amino acid sequence of the heavy chain variable region of 23F10 (SEQ ID NO: 21):

25 EVQLLESGGGLVQPGGSLRLSCAASGFTF**SSYAMS**WVRQAPGKGLEWV**SA**
ISGSGGSTYYADSVKGR**FTISR****DNSKNTLYLQMNSLRAEDTAVYYCAK****VRPFW**
GTFDY**WGQGT****LVTVSS** (CDR sequences are shown in bold and underlined)

Amino acid sequence of the heavy chain variable region of 23F10 (SEQ ID NO: 22):

30 GAGGTGCAATTGCTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGG
TCCCTGAGACTCTCCTGTGCAGCCTCCGGATTACCTTTAGCAGTTATGCCAT
GAGCTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATT
AGTGGTAGTGGTGGTAGCACATACTACGCAGACTCCGTGAAGGGCCGGTTCA
CCATCTCCAGAGACAATTCCAAGAACACGCTGTATCTGCAGATGAACAGCCT
35 GAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAGTTCGTCCATTCTGG

GGTACTTTCGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT

Amino acid sequence of the heavy chain variable region of 23F10 (SEQ ID NO: 19):

EIVLTQSPGTLSPGERATLSC**RASOSVSSSYLA**WYQQKPGQAPRLLIYGA
5 **SSRAT**GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYC**QQYFNPPEY**TFGQGTKVEI
K (CDR sequences are shown in bold and underlined)

Amino acid sequence of the heavy chain variable region of 23F10 (SEQ ID NO: 20):

GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGG
10 AAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTT
AGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGA
GCATCCAGCAGGGCCACTGGCATCCCAGACAGGTTTCAGTGGCAGTGGATCCG
GGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGT
GTATTACTGTCAGCAGTACTTCAACCCACCAGAATACACGTTCGGCCAGGGG
15 ACCAAAGTGGAAATCAAA

Example 4. Construction of anti-BCMA scFv_Fc fusion antibody and transient expression, purification and activity identification thereof in eukaryotic cells

Primers were designed for VH and VL fragments of 7G2, 7A12, 23F10, respectively,
20 and a linker consisting of 15 flexible amino acids (GGGGS GGGGS GGGGS) was
introduced to form a scFv; a NheI cleavage site and protective bases were introduced
upstream to VH, and a BamHI cleavage site and protective bases were introduced
downstream to VL. The PCR product was analyzed by 1% agarose gel electrophoresis,
purified and recovered. After digestion, it was ligated into V152 eukaryotic expression
25 vector (purchased from Shanghai Ruijin Biotechnology Co., Ltd.). 293F cells in
logarithmic growth phase were transiently transfected with 293fectin™ Transfection
reagent (Invitrogen, 12347-019) or polyethyleneimine (PEI) (Sigma-Aldrich, 408727). At
5-7 days after transfection, the supernatant was collected and subjected to affinity
purification of Protein A. The obtained antibodies were quantitatively and qualitatively
30 analyzed by SDS PAGE (Fig. 5).

The binding of the antibody to K562 stably expressing BCMA was tested by flow
cytometry. The method for FACs detection is as follows: cells were harvested, washed
once with growth medium, and resuspended in PBS. The cell concentration was adjusted
to 4E+5 cells/ml. The gradient-diluted scFv_Fc fusion antibody was incubated with the
35 cells for 30 minutes on ice, the initial concentration of the antibody was 500 nM, which

was 5-fold diluted for 7 gradients in total. Thereafter, the antibody was incubated with FITC-labeled anti-mouse IgG secondary antibody, and, after washed twice, detected using Guava easyCyte™ HT System. Figure 6 shows the binding of scFv_Fc fusion forms of antibody 7A12, 7G2 and 23F10 to K562-BCMA. All the three antibodies exhibited a concentration-dependent binding with an EC50 of 3.13 nM, 3.42 nM and 5.61 nM, respectively.

Example 5. Determination of antibody affinity using surface plasmon resonance (SPR)

The affinities of different antibodies to BCMA were determined using biacore T200. The used method was as follows:

BCMA_huFc was coated on a CM5 chip by amino coupling to about 500 RU, and the gradient-diluted antibody as a mobile phase was passed through the antigen-coated channel at a flow rate of 30 ul/min. The running buffer was HBS-N and the temperature was 25°C. The experimental data was analyzed by BIAevaluation 3.2 and the kinetic curves were fitted using 1:1 langmuir model. KD of 7A12 (scFv_Fc) was 663 pM, KD of 7G2 (scFv_Fc) was 499 pM, and KD of 23F10 (scFv_Fc) was 667 pM (see Figure 7). The parameters are shown in the following table:

Clone	ka (1/Ms)	kd (1/s)	KD (M)
7G2	7.52E+04	3.75E-05	4.99E-10
7A12	9.84E+04	6.53E-05	6.63E-10
23F10	6.64E+04	4.43E-05	6.67E-10

Example 6. Determination of binding of antibodies to tumor cell lines by FACs

RPMI8226 is a peripheral blood B lymphocyte of human multiple myeloma. The method for FACs detection is as follows: cells were harvested, washed once with growth medium, and resuspended in PBS. The cell concentration was adjusted to 4E+5 cells/ml. The gradient-diluted scFv_Fc fusion antibody was incubated with the cells for 30 minutes on ice, and the initial concentration of the antibody was 500 nM, and 5-fold diluted for 7 gradients in total. Thereafter, the antibody was incubated with a FITC-labeled anti-mouse IgG secondary antibody, and, after washed twice, detected by Guava easyCyte™ HT System. Figure 8 shows the concentration-dependent binding of scFv_Fc fusion forms of antibody 7A12, 7G2 and 23F10 on cell line RPMI8226.

Example 7. Competitive binding assay of anti-BCMA antibody to BCMA ligand APRIL

1. Expression of purified recombinant APRIL fusion protein

The fusion protein of human APRIL His115-Leu250 and Fc fragment Asp104-Lys330 of human IgG1 heavy chain constant region linked by “GS” was recombinantly expressed. The fusion protein APRIL_huFc (SEQ ID NO: 45), the corresponding gene sequence was SEQ ID NO: 46. Transient transfection, expression and purification were performed as described in Example 1.

2. Competitive ELISA

A ELISA plate was coated with 50 ng/ml 100 ul/empty BCMA_muFc at 4°C overnight. On the next day, the plate was washed with PBS for 3 times, and PBS containing 2% skim milk powder was added and blocked at room temperature for 1 hour. 40 ng/ml APRIL_huFc and gradient-diluted antibody 7A12, 7G2 or 23F10 (starting concentration 200 nM, 3-fold dilution, 7 gradients) were simultaneously added. The resulted mixture was incubated for 1 hour at room temperature, washed for three times with PBST, and three times with PBS. A 1: 1000 dilution of HRP-labeled mouse anti-human Fc antibody was added, incubated for 1 hour at room temperature, and washed three times with PBST, and three times with PBS. TMB was added for development and read with a microplate reader.

The experimental results are shown in Fig. 9. All of 7A12, 7G2 and 23F10 can significantly inhibit the binding of APRIL to BCMA, which demonstrates that the antibodies of the invention can inhibit the binding of BCMA to its natural ligand.

Example 8. Construction of anti-BCMA chimeric antigen receptor plasmid (CAR)

a. Construction of anti-BCMA antibody 7A12 chimeric antigen receptor plasmid

Lentiviral plasmids expressing the second and third generation chimeric antigen receptors of antibody 7A12 were constructed using PRRLSIN-cPPT.EF-1 α as a vector, including PRRLSIN-cPPT.EF-1 α -7A12-28Z, PRRLSIN-cPPT.EF-1 α -7A12-BBZ and PRRLSIN-cPPT.EF-1 α -7A12-28BBZ. 7A12-28Z sequence consists of CD8 α signal peptide (SEQ ID NO: 23), 7A12 scFv (SEQ ID NO: 47), CD8 hinge (SEQ ID NO: 25), CD28 transmembrane region (SEQ ID NO: 27), intracellular signaling domain (SEQ ID NO: 29) and intracellular segment CD3 ξ (SEQ ID NO: 31) of CD3; 7A12-BBZ sequence consists of CD8 α signal peptide (SEQ ID NO: 23), 7A12 scFv (SEQ ID NO: 47), CD8 hinge (SEQ ID NO: 25), transmembrane region (SEQ ID NO: 33), CD137 intracellular signaling domain (SEQ ID NO: 35) and CD3 ξ (SEQ ID NO: 31); 7A12-28BBZ sequence consists of CD8 α signal peptide (SEQ ID NO: 23), 7A12-scFv (SEQ ID NO: 47), CD8

hinge (SEQ ID NO: 25), CD28 transmembrane region (SEQ ID NO: 27), intracellular segment (SEQ ID NO: 29), CD137 intracellular signaling domain (SEQ ID NO: 35) and CD3 ξ (SEQ ID NO: 31).

b. Construction of plasmid for chimeric antigen receptor of anti-BCMA antibody

5 7G2

Lentiviral plasmids expressing the second and third generation chimeric antigen receptors of antibody 7G2 were constructed using PRRLSIN-cPPT.EF-1 α as a vector, including PRRLSIN-cPPT.EF-1 α -7G2-28Z, PRRLSIN-cPPT.EF-1 α -7G2-BBZ and PRRLSIN-cPPT.EF-1 α -7G2-28BBZ. 7G2-28Z sequence consists of CD8 α signal peptide (SEQ ID NO: 23), 7G2 scFv (SEQ ID NO: 48), CD8 hinge (SEQ ID NO: 25), CD28 transmembrane region (SEQ ID NO: 27), intracellular signaling domain (SEQ ID NO: 29) and intracellular segment CD3 ξ (CD ID NO: 31) of CD3; 7G2-BBZ sequence consists of CD8 α signal peptide (SEQ ID NO: 23), 7G2 scFv (SEQ ID NO: 48), CD8 hinge (SEQ ID NO: 25), transmembrane region (SEQ ID NO: 33), CD137 intracellular signaling domain (SEQ ID NO: 35) and CD3 ξ (SEQ ID NO: 31); 7G2-28BBZ sequence consists of CD8 α signal peptide (SEQ ID NO: 23), 7G2-scFv (SEQ ID NO: 48), CD8 hinge (SEQ ID NO: 25), CD28 transmembrane region (SEQ ID NO: 27), intracellular segment (SEQ ID NO: 29), CD137 intracellular signaling domain (SEQ ID NO: 35) and CD3 ξ (SEQ ID NO: 31).

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Example 9. Preparation of CAR-T cells

1. Lentiviral packaging, virus concentration and titer determination of lentiviral vector of CAR targeting BCMA

a. Lentiviral packaging

25 1) 293T cells were inoculated in a 10 cm cell culture dish, and cultured overnight at 37°C, 5% CO₂ for transfection, and the medium was DMEM containing 10% fetal bovine serum (Gibico);

2) 5.4 μ g of target gene plasmid PRRLSIN-cPPT.EF-1 α -EGFP (Mock) or related CAR plasmid and 6.2 μ g of packaging plasmid pRsv-REV, 6.2 μ g of RRE-PMDLg, 2.4 μ g of Vsvg were dissolved in 800 μ L blank DMEM medium and mixed;

30 3) 60 μ g of PEI was dissolved in 800 μ L of serum-free DMEM medium, mixed gently (or vortexed at 1000 rpm for 5 seconds), and incubated for 5 min at room temperature;

4) Formation of transfection complex: the plasmid mixture was added to PEI mixture, and immediately after the addition, the mixture was vortexed or gently mixed,

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and incubated at room temperature for 20 min;

5) 1.6 ml of transfection complex was added to a 10 cm culture dish containing 11 ml of DMEM medium (unnecessary to change the medium); after 4-5 hours, the transfected 293T cells were exchanged with DMEM medium containing 10% FBS and incubated for 72 h at 37°C and the viral supernatant was collected.

b. Lentivirus concentration

1) Preparation of 5X PEG8000 NaCl: 8.766 g of NaCl and 50g of PEG8000 were weighed and dissolved in 200 ml Milli-Q pure water; sterilized at 121°C for 30 min; and stored at 4°C;

2) Lentiviral supernatant was filtered with a 0.45 µm filter; 7.5 ml of 5X PEG-8000 NaCl stock solution was added per 30 ml of the filtered virus initial solution; mixed once every 20 to 30 minutes for 3-5 times; placed at 4°C overnight; and centrifuged at 4°C, 4000 g for 20 min;

3) The supernatant was aspirated and discarded, the tube was placed for 1 to 2 minutes, and the residual liquid was aspirated and discarded; an appropriate amount of lentivirus solution was added to dissolve the lentiviral precipitate; and dispensed and stored at -80°C.

c. Titer determination of lentiviral

1) 293T cells were inoculated in a 6-well culture plate at 2×10^5 cells, 1 ml/well; 10 µg/µl (initial concentration) polybrene solution was added at 0.6 µl/ml to a final concentration of 6 µg/ml; cultured at 37°C, 5% CO₂ for 1 hours, and the medium was DMEM containing 10% fetal bovine serum;

2) virus concentrate was added at 10 µL/well, 5-fold dilution, 3 gradients, and cultured at 37°C, 5% CO₂;

3) After 72 hours of infection, trypsin was used to digest (30s) 293T cells, 1 ml DMEM (10% FBS) was added to quench digestion, the cell suspension was transferred into a 2 ml centrifuge tube (two aliquots), centrifuged at 5000 rpm for 5 min, and the supernatant was discarded; the cells were washed twice with PBS (2% NBS);

4) 50 µl of PE-SA (1:200 dilution) antibody was added into cells in Control group, incubated for 45 min on ice, washed twice with PBS (2% NBS), and resuspended as a control;

5) 50 µl of 1: 50 diluted biotin-Goat anti human IgG, F(ab')₂ antibody was added into cells in Test group cells, incubated on ice for 45 min; and washed twice with PBS (2% NBS); 50 µl of PE-SA (1: 200 dilution) antibody was added and incubated on ice for 45 min;

6) 2 ml of PBS (2% NBS) was added to resuspend the cells, and centrifuged at 4°C, 5000 rpm/min for 5 minutes; the supernatant was discarded; which was repeated twice;

7) 500 µl of PBS (2% NBS) was added and transferred to a flow tube. PE channel was detected by a flow cytometry, and the number of cells with a positive rate of 5-20% was appropriate. Titer (PFUs/mL) = cell number × positive rate / virus volume was calculated.

2. Lentiviral-transduced T lymphocyte ----- Preparation of CAR-positive T lymphocytes

1) Activation of T lymphocyte: lymphocytes were added into a lymphocyte culture medium at a density of about 1×10^6 /mL, and magnetic beads (Invitrogen) coated with anti-CD3 and CD28 antibodies at a magnetic bead:cell ratio of 2:1 and recombinant human IL-2 (Shanghai Huaxin Biotech Co., Ltd.) at a final concentration of 500 U/mL were simultaneously added and incubated for 48 h;

2) One day before infection, a 24-well plate was coated by Retronectin at a final concentration of 5 µg/ml, and incubated overnight at 4°C;

3) the retronectin solution (PBS) in the 24-well plate was discarded and the plate was washed twice with 1 ml of PBS;

4) the concentrated lentivirus was added to PBMCs cells at MOI = 10, centrifuged at 1000g for 40min, and transferred to a cell incubator;

5) Amplification: The infected cells were passaged every other day at a density of 5×10^5 /mL, and recombinant human IL-2 at a final concentration of 500 U/mL was supplemented in the lymphocyte culture solution.

3. Expression of chimeric antigen receptor of T lymphocyte

1) On the day 7 of culture, 1×10^6 of lentivirus-infected T lymphocytes were taken in a centrifuge tube;

2) the T cells were centrifuged at 4°C, 5000 rpm for 5 min, the supernatant was discarded, and the residue was washed twice with PBS;

3) 50 µl of biotin-Goat anti human IgG, F(ab')₂ antibody (1:50 dilution) were added into the cells to be tested, incubated for 45 min on ice; washed twice with PBS (2% NBS); and 50 µl of PE-SA (1:200 dilution) antibody was added and incubated on ice for 45 min;

4) 2 ml of PBS (2% NBS) was added to resuspend the cells and centrifuged at 4°C, 5000 rpm/min for 5 minutes, the supernatant was discarded; which was repeated twice;

5) 500 µl of PBS (2% NBS) was added and transferred to a flow tube. PE channel was detected by a flow cytometry to determine the proportion of CAR-positive T cells.

The positive infection rates of Mock, 7A12-28Z, 7A12-BBZ, 7A12-28BBZ,

7G2-28Z, 7G2-BBZ and 7G2-28BBZ T cell in the *in vitro* toxicity killing experiment are shown in Figure 10, which are 72.8%, 60.8%, 48.7%, 57.4%, 67.5%, 68.8%, 63.6%, respectively.

4. Cytotoxicity assay of CAR T cells targeting BCMA

CytoTox 96 non-radioactive cytotoxicity assay kit (Promega) was used with reference to the instructions of CytoTox 96 non-radioactive cytotoxicity assay kit.

Target cells: 75 μ l of 2×10^5 /mL K562, K562-BCMA and RPMI-8226 cells were inoculated into 96 well plates, respectively. Effector cells: T-Mock and CAR T cells expressing different chimeric antigen receptors were added at an effector target ratio of 3:1, 1:1 or 1:3. Quadruplicate wells were set for each group, and the average of 4 replicate wells was taken. The detection time was hour 18 of incubation of the cells. Each experimental group and each control group are as follows:

Each experimental group: each target cell + CAR T expressing different chimeric antigen receptors;

Control group 1: maximum release of LDH from target cells;

Control group 2: spontaneous release of LDH from target cells;

Control group 3: spontaneous release of LDH from effector cells;

The cytotoxicity calculation formula is: cytotoxicity % = [(experimental group - effector cell control - target cell control) / (target cell maximum - target cell control)] $\times$ 100%.

The results showed that each of the CAR T cells expressing different chimeric antigen receptors had significant *in vitro* killing activities against BCMA-positive K562-BCMA and RPMI-8226 cells, especially for RPMI-8226 cells endogenously expressing BCMA, while almost no killing effect on BCMA-negative K562 cells (Fig. 11A).

5. Treatment of NOD/SCID mice loaded with peripheral blood B lymphocytes RPMI-8226 of multiple myeloma

RPMI-8226 cells were inoculated into 40 NOD/SCID mice at 8×10^6 /mice, respectively. On Day 12 after subcutaneous inoculation of tumor cells, the average tumor volume was 75 mm³, the mice were randomly divided into 4 groups, and 1×10^7 CAR T were injected into the tail vein. And cyclophosphamide was intraperitoneally injected before the injection at a dosage of 100 mg/kg for clearing residual T cells in mice in advance. On Day 17 of CAR T injection, the mice were sacrificed by cervical dislocation.

The tumor size of the mice was analyzed. The results are shown in Fig. 11B. Compared with UTD group, the antitumor effects in 7A12-28Z, 7A12-BBZ and

7A12-28BBZ treatment groups were significant, and on Day 17 of CAR T injection, there was 1 case of tumor regression in 7 mice of 7A12-28Z treatment group, 2 cases of tumor regression in 7 mice of 7A12-BBZ treatment group, and 7 cases of tumor regression in 7 mice of 7A12-28BBZ treatment group. The tumor inhibition rates were 7A12-28Z (84.6%), 7A12-BBZ (65.4%) and 7A12-28BBZ (100%), respectively.

Example 10. Modification of Antibody 23F10

In this example, 23F10 was used as a parent antibody, and 23F10 was modified by phage display method. A phage library was constructed based on 23F10 with CDR3 regions of the light and heavy chain being retained, and two phage libraries were constructed by randomizing CDR1 and CDR2 of the light chain or CDR1 and CDR2 of the heavy chain with degenerate primers, respectively. Primer information is as follows:

SEQ ID NO	name	Sequence
49	Primer LMF	CAGGAAACAGCTATGACCATGATTAC
50	Primer BH1R	TGAGACCCACTCCAGCCCCTTCCCTGGAGCCTGGCGGACCCAMNNM NNMNNMNNMNNMNNMNNAAAGGTGAATCCGGAGGCTG
51	Primer BH2F	GGCTGGAGTGGGTCTCANNKATTNNKNNKNNKNNKGGTNNKACAN NKTACGCAGACTCCGTGAAGGG
52	Primer FdR	GACGTTAGTAAATGAATTTTCTGTATGAGG
53	Primer BL1R	GATGAGGAGCCTGGGAGCCTGGCCAGGTTTCTGCTGGTACCAMNNT AAMNNMNNMNNMNNMNNMNNMNNCTGACTGGCCCTGCAAGAG
54	Primer BL2F	CCAGGCTCCCAGGCTCCTCATCNNKNNKNNKNNKNNKAGGGCCACT GGCATCCCAGAC

2.1 Construction of 23F10 mutant

A template plasmid was firstly constructed based on antibody 23F10 (scFv) (SEQ ID NO: 55). For phage libraries of randomized light chain CDR1 and CDR2, primers LMF and BL1R were used to PCR-amplify fragment 1; primers BL2F and FdR were used to PCR-amplify fragment 2; then fragment 1 and fragment 2 were ligated by bridge-PCR to obtain a full length scFv containing the randomized sequence, and afterwards the full-length fragment was digested with NcoI and NotI and ligated into an identically digested template plasmid by T4 ligase. The plasmid was transduced into TG1 competent cells by electroporation, the storage capacity of which was 1.50E+9. For phage libraries of randomized heavy chain CDR1 and CDR2, primers LMF and BH1R were used to PCR-amplify fragment 3; primers BH2F and FdR were used to PCR-amplify fragment 4; then fragment 3 and fragment 4 were ligated by bridge-PCR to obtain a full length scFv containing the randomized sequence, and afterwards the full-length fragment was digested with NcoI and NotI and ligated into an identically digested template plasmid by T4 ligase.

The plasmid was transduced into TG1 competent cells by electroporation, the storage capacity of which was 2.2E+9.

Screening of phage libraries. Referring to the method in Example 3, the initial concentration of antigen BCMA_huFc was 20 nM, and a 5-fold gradient dilution was performed for the next round of screening. Panning was performed for 2-3 cycles to enrich scFv phage clones specifically binding to BCMA_huFc. Positive clones were determined by standard ELISA methods for BCMA_huFc. In ELISA, human Fc fragment was used as an unrelated antigen to verify the specificity of the antibody. A total of 80 ELISA-positive clones were picked and the dissociation constant K_d of the supernatant was determined by biacore after reinduction. Among them, there are two clones, 25C2 and 25D2, the K_d of which is 10 times lower than the parental clone 23F10, as shown in the following table:

Clone	Dissociation constant (K_d, S^{-1})
23F10	3.43E-03
25C2	3.64E-04
25D2	3.74E-04

The light chains of clones 25C2 and 25D2 were sequenced as being identical to 23F10. In Figure 12, the heavy chain amino acid sequences of clones 25C2, 25D2 and 23F10 were compared, wherein, compared with the parent antibody 23F10, there are 5 point mutations on the heavy chain in clone 25C2 (SEQ ID NOs: 56, 57 are the amino acid sequence and the nucleotide sequence of 25C2 heavy chain variable region, respectively), and there are 2 point mutations on CDR1, serine to glycine at 31st position and tyrosine to asparagine at 32nd position; there are 2 point mutations on CDR2, serine to asparaginy at the 54th position and tyrosine to phenylalanine at the 59th position, and there is 1 point mutation in the framework region, serine to glycine at the 30th position.

Compared with the parent antibody 23F10, there are 4 point mutations on the heavy chain of Clone 25D2 (SEQ ID NO: 58, 59 are the amino acid sequence and nucleotide sequence of the heavy chain variable region of 25D2, respectively), wherein there are 3 point mutations in CDR2 region, serine to glycine at the 54th position, serine to asparagine at the 57th position and tyrosine to phenylalanine at the 59th position, and there is 1 point mutation in the framework region, serine to arginine at the 30th position.

The sequence of HCDR1 of 25C2 is set forth in SEQ ID NO: 60, and the sequence of HCDR2 of 25C2 is set forth in SEQ ID NO: 61. The sequence of HCDR1 of 25D2 is set forth in SEQ ID NO: 62, and the sequence of HCDR2 of 25D2 is set forth in SEQ ID NO: 63. The nucleotide sequence and amino acid sequence of 25C2 scFv are shown in SEQ ID

NO: 64, 65, respectively, and the nucleotide sequence and amino acid sequence of the 25D2 scFv are shown in SEQ ID NO: 66, 67, respectively.

2.2 Expression and purification of clone 25C2, 25D2 (scFv_Fc)

According to Example 4, appropriate cleavage sites and protecting bases were introduced upstream to VH, and appropriate cleavage sites and protecting bases were introduced downstream to VL. The PCR product was analyzed by 1% agarose gel electrophoresis, purified and recovered. After digestion, it was ligated into eukaryotic expression vector V152 containing human Fc fragment (purchased from Shanghai Ruijin Biotechnology Co., Ltd.), and transiently transfected into 293F cells by 293Fectin and expressed.

The aggregation of 25C2 and 25D2 was analyzed by SEC. As shown in Figs. 13A and 13B, the antibody in a monomer form accounted for 91% and 97%, respectively. Compared with the parent antibody 23F10 (30% monomer rate), the monomer rate was increased by 61% and 67%, respectively, and the aggregation was significantly reduced. After concentration by ultrafiltration, the obtained antibodies were quantitatively and qualitatively analyzed by SDS PAGE. The yields were 80 ug/ml and 60 ug/ml, respectively (yield = weight of final product/transfection volume).

2.3 Binding characteristics of Clones 25C2, 25D2

K562 and K562 cells (K562-BCMA) stably expressing human BCMA were used and harvested, washed with complete growth medium, and plated into U-bottom microtiter plates at about 1 to 5×10^5 cells/well. The gradient-diluted scFv_Fc fusion antibody was incubated with K562-BCMA/K562 for 30 minutes on ice, and then incubated with FITC-labeled anti-human Fc as a secondary antibody. After two washing steps, the analysis was performed using a Guava easyCyte™ HT System, and the experimental data was processed using GraphPad Prism to obtain an EC50. Figure 14 shows the binding of 25C2, 25D2 to K562-BCMA and K562 cells. The results showed that EC50s of two clones, 25C2, 25D2 with improved stability and reduced aggregation binding to K562-BCMA were 2.594 nM and 1.891 nM, respectively, which, compared with 23F10, were increased by 3 to 4 times.

2.5 Determination of specificity of Clones 25C2, 25D2

The specificity of the antibodies 23F10, 25C2, 25D2 was determined by ELISA.

2 ug/ml recombinant human BCMA_Fc, mouse BCMA_Fc, TACI_huFc (R&D, #174TC), BAFF R (R&D, #1162-BR) were coated on immunoplates at 4°C overnight. The next day, 300 µl/well of 2% MPBS was added for 2 hours, then 200 nM purified antibody (scFv format) was added and incubated at 37°C for 1 hour, washed three times

with PBST (PBS containing 0.05% Tween-20), and washed three times with PBS. And then 1: 4000 diluted HRP-labeled anti-Flag tag antibody (sigma, #A8592-1MG) was added, incubated for 1 hour at 37°C, washed three times with PBST (PBS containing 0.05% Tween-20) and washed three times with PBS. 100 ul/well of TMBS substrate was added and developed for 10-15 minutes. The reaction was quenched by adding 50 ul of 2 M sulfuric acid.

Results are shown in Fig. 14B, wherein antibodies 7A12, 23F10, 25C2, 25D2 specifically bind to human BCMA, and do not bind human TACI and human BAFF R. Among them, the binding of antibodies 25C2, 25D2 to mouse BCMA is weaker.

Example 11. Preparation of 25C2, 25D2 CAR-T cells

According to the procedure of Example 8, plasmids of chimeric antigen receptor of 25C2, 25D2 were constructed, respectively.

a. Construction of plasmid of chimeric antigen receptor of 25C2

Lentiviral plasmid PRRLSIN-cPPT.EF-1 α -25C2-BBZ expressing the second-generation chimeric antigen receptor of antibody 25C2 was constructed using PRRLSIN-cPPT.EF-1 α as a vector. Lentiviral plasmid PRRLSIN-cPPT.EF-1 α -25D2-BBZ expressing the second-generation chimeric antigen receptor of antibody 25D2 was constructed using PRRLSIN-cPPT.EF-1 α as a vector.

25C2-BBZ sequence consists of CD8 α signal peptide (SEQ ID NO: 23), 25C2 scFv (SEQ ID NO: 64), CD8 hinge (SEQ ID NO: 25), transmembrane region (SEQ ID NO: 33), CD137 intracellular signaling domain (SEQ ID NO: 35) and CD3 ξ (SEQ ID NO: 31).

25D2-BBZ sequence consists of CD8 α signal peptide (SEQ ID NO: 23), 25D2 scFv (SEQ ID NO: 66), CD8 hinge (SEQ ID NO: 25), transmembrane region (SEQ ID NO: 33), CD137 intracellular signaling domain (SEQ ID NO: 35) and CD3 ξ (SEQ ID NO: 31).

According to the procedure of Example 9, the plasmids PRRLSIN-cPPT.EF-1 α -25C2-BBZ, PRRLSIN-cPPT.EF-1 α -25D2-BBZ were subjected to lentiviral packaging, T cell infection and amplification, respectively, to obtain chimeric antigen receptor-modified T cells 25C2-BBZ and 25D2-BBZ.

Example 12. Preparation of CAR-T cells expressing soluble PD1

In this example, CAR-T cells expressing soluble PD1 were prepared using scFv of antibody 7A12. The preparation method is listed as follows:

1. The signal peptide sequence of PD-1 (SEQ ID NO: 68), PD-1 extracellular segment sequence (SEQ ID NO: 69) and the sequence of CH3 (SEQ ID NO: 70) were

synthesized and cloned into T Vector to obtain plasmid T-sPD1-Fc.

Using the T-sPD1-Fc plasmid as a template, the upstream primer 5'-acgcgtcctagcgtaccggctgccaccatgcagatcccacaggcgccc-3' (SEQ ID NO: 71) and the downstream primer 5'-ctctcggggctgccaccatacaccagggttggactggc-3' (SEQ ID NO: 72) were used in PCR amplification to obtain sPD1 sequence; and the upstream primer 5'-tatggtgggcagccccgagagccacag-3' (SEQ ID NO: 73), downstream primer 5'-aaaattcaaagtctgtttcactttaccgggagacagggag-3' (SEQ ID NO: 74) were used in amplification to obtain sPD1-CH3 fragment.

The sPD1-CH3 fragment and the fragment of 7A12-BBZ (SEQ ID NO: 75) were PCR-spliced and amplified to obtain sPD1-CH3-7A12-BBZ, and the sequence of 7A12-BBZ consists of CD8 α signal peptide (SEQ ID NO: 23), 7A12 scFv (SEQ ID NO: 47), CD8 hinge (SEQ ID NO: 25), transmembrane region (SEQ ID NO: 33), CD137 intracellular signaling domain (SEQ ID NO: 35) and CD3 ξ (SEQ ID NO: 31).

The above constructed fragment sPD1-CH3-7A12-BBZ has a MluI cleavage site at 5' end and a SalI cleavage site at 3' end, which was double-digested with MluI and SalI and ligated into indentically double-digested PRRLSIN-cPPT.EF-1 α vector to obtain a plasmid expressing sPD-1-CH3 protein and a chimeric antigen receptor targeting BCMA.

According to the procedure of Example 9, T cells sPD-1-7A12-BBZ expressing sPD1 and 7A12-BBZ were obtained.

Example 13. *In vitro* cell killing experiment

In vitro killing experiments were performed using 25C2-BBZ T cells, 25D2-BBZ T cells, 7A12-BBZ T cells, C11D5.3-BBZ T cells, and sPD-1-7A12-BBZ T cells as effector cells, among which C11D5.3-BBZ (SEQ ID NO: 87) is a second generation CAR prepared by using anti-BCMA mouse anti-C11D5.3 (see CN201580073309.6). Target cells were human myeloma cells NCI-H929 and multiple myeloma peripheral blood B lymphocytes RPMI-8226.

CytoTox 96 non-radioactive cytotoxicity assay kit (Promega) was used according to the instruction of CytoTox 96 non-radioactive cytotoxicity test kit.

Effector cells were inoculated in 96-well plates at a effector target ratio of 3:1, 1:1 or 1:3, and 50 μ L of 2×10^5 /mL NCI-H929 and RPMI-8226 cells were inoculated into the corresponding 96-well plates.

Pentaplicate wells were set for each group, and the plates were incubated in an incubator for 18 h.

The experimental groups and the control groups were set as follows: experimental

group: each target cell + T lymphocytes expressing different chimeric antigen receptors; control group 1: maximal release of LDH from target cells; control group 2: spontaneous release of LDH from target cells; Control group 3: spontaneous release of LDH from Effector cells. The calculation formula is: % cytotoxicity = [(experimental group - effector cell spontaneous group - target cell spontaneous group) / (target cell maximum - target cell spontaneous)] * 100.

The experimental results of cell killing are shown in Figure 15.

Example 14. *In vivo* Cell killing experiment in mice

8×10⁶ RPMI-8226 cells were subcutaneously inoculated into the right iliac crest of B-NDG mice, and on Day 18, the average tumor volume was about 243 mm³, thereby obtaining a subcutaneous xenograft model of B-NDG mice loaded with peripheral blood B lymphocytes RPMI-8226 of multiple myeloma.

The mouse subcutaneous xenograft model was divided into 3 groups (4 in each group), and injected with 25C2-BBZ, 25D2-BBZ and untransfected T cells (UTD) at a dose of 5×10⁶, respectively. The results are shown in the following table. On Day 32 and Day 36 of inoculation of tumor cells, in all 4 mice of the 25C2-BBZ and 25D2-BBZ treatment groups, tumors regressed.

CAR T Dose: 5×10 ⁶	Cancer Free Days after tumor cell inoculation					
	Day25	Day29	Day32	Day36	Day39	Day42
UTD	0/4	0/4	0/4	0/4	0/4	0/4
25C2-BBZ	1/4	2/4	4/4	4/4	4/4	4/4
25D2-BBZ	0/4	1/4	3/4	4/4	4/4	4/4

The mouse subcutaneous xenograft model was divided into 3 groups (4 in each group), and injected with 25C2-BBZ, 25D2-BBZ, C11D5.3-BBZ, 7A12-BBZ and untransfected T cells (UTD) at an injection dose of 1 x 10⁶ CAR T. The tumor regression was shown in the following table and Figure 16.

CAR T Dose: 1×10 ⁶	Cancer Free Days after tumor cell inoculation						
	Day29	Day32	Day36	Day39	Day42	Day45	Day49
UTD	0/4	0/4	0/4	0/4	0/4	0/4	0/4
25C2-BBZ	1/4	2/4	3/4	4/4	4/4	4/4	4/4
25D2-BBZ	0/4	0/4	1/4	1/4	1/4	1/4	2/4
C11D5.3-BBZ	0/4	1/4	1/4	2/4	3/4	3/4	3/4
7A12-BBZ	0/4	0/4	0/4	1/4	2/4	2/4	2/4

All documents mentioned in the present application are hereby incorporated by

reference in their entirety as if each document is separately cited as a reference. In addition, it is to be understood that various modifications and changes may be made by a skilled person in the art, after reading the above teachings of the present invention, and the equivalent forms also fall within the scope defined by the claims appended hereto.

Sequence listing

SEQ ID NO	name	Sequence
1	7A12, 7G2, 23F10 HCDR1	SYAMS
2	7A12, 7G2, 23F10 HCDR2	AISGSGGSTYYADSVKG
3	7A12 HCDR3	YPYLAFDY
4	7G2 HCDR3	LSGDAAMDY
5	23F10 HCDR3	VRPFWGTFDY
6	7A12, 7G2, 23F10 LCDR1	RASQSVSSSYLA
7	7A12, 7G2, 23F10 LCDR2	GASSRAT
8	7A12 LCDR3	QQYGYPPSY
9	7G2 LCDR3	QQYGYPPRY
10	23F10 LCDR3	QQYFNPPEY
11	Amino acid sequence of 7A12 light chain variable region	EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFS GSGSGTDFLTISRLEPEDFAVYYCQQYGYPPSYTFGQGTKVEIK
12	Nucleotide sequence of 7A12 light chain variable region	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTGTCTCCAGGGGAAAGAGCCACC CTCTCTTGCAAGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAA CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATCCAGCAGGGCCACTGGCATCCCA GACAGGTTCACTGGCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGA GCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTACGGTTACCCACCATCTTACACGTTC GGCCAGGGGACCAAAAGTGGAAATCAAA
13	Amino acid sequence of 7A12 heavy chain variable region	EVQLLESGGGLVQPGGSLRLSAAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYA DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARYPYLAFDYWGQGT LVTVSS
14	Nucleotide sequence of 7A12 heavy chain variable region	GAGGTGCAATTGCTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACT CTCTGTGCAGCCTCCGGATTACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACT ACGCAGACTCCGTGAAGGGCCGGTTACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGCGTTA CCCATACCTGGCATTTCGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT
15	Amino acid sequence of 7G2 light chain variable region	EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFS GSGSGTDFLTISRLEPEDFAVYYCQQYGYPPRYTFGQGTKVEIK
16	Nucleotide sequence of 7G2 light variable region	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTGTCTCCAGGGGAAAGAGCCACC CTCTCTTGCAAGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAA CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATCCAGCAGGGCCACTGGCATCCCA GACAGGTTCACTGGCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGA GCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTACGGTTACCCACCAAGATACACGTT CGGCCAGGGGACCAAAAGTGGAAATCAAA
17	Amino acid sequence of 7G2 heavy chain variable region	EVQLLESGGGLVQPGGSLRLSAAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYA DSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKLSGDAAMDYWGQGT LVTVSS
18	Nucleotide sequence of 7G2 heavy chain variable region	GAGGTGCAATTGCTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACT CTCTGTGCAGCCTCCGGATTACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACT ACGCAGACTCCGTGAAGGGCCGGTTACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAACT GTCTGGTGATGCAGCAATGGACTACTGGGGCCAAGGAACCCTGGTCACCGTCTCGAGT
19	Amino acid sequence of 23F10 light chain variable region	EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFS GSGSGTDFLTISRLEPEDFAVYYCQQYFNPPEYTFGQGTKVEIK

20	Nucleotide sequence of 23F10 light chain variable region	GAAATCGTGTTAACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCTTGCAGGGCCAGTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAA CCTGGCCAGGCTCCCAGGCTCCTCATCTATGGAGCATCCAGCAGGGCCACTGGCATCCCA GACAGGTTCAGTGGCAGTGGATCCGGGACAGACTTCACTCTCACCATCAGCAGACTGGA GCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTACTTCAACCCACCAGAATACACGTT CGGCCAGGGGACCAAAGTGGAAATCAAA
21	Amino acid sequence of 23F10 heavy chain variable region	EVQLLESGGGLVQPGGSLRLSCAASGFTFSYAMSWVRQAPGKGLEWVSAISGSGGSTYYA DSVKGRFTISRDN SKNTLYLQMNSLR AEDTAVYYCAKVRPFWGTFDYWGQGLTVTVSS
22	Nucleotide sequence of 23F10 heavy chain variable region	GAGGTGCAATTGCTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGACT CTCTGTGCAGCCTCCGGATTACCTTTAGCAGTTATGCCATGAGCTGGGTCCGCCAGGC TCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACT ACGCAGACTCCGTGAAGGGCCGGTTACCATCTCCAGAGACAATTCCAAGAACACGCTG TATCTGCAGATGAACAGCCTGAGAGCCGAGGACACGGCCGTATATTACTGTGCGAAAGT TCGTCCATTCTGGGGTACTTTCGACTACTGGGGCC AAGGAACCTGGTACCGTCTCGAG T
23	Amino acid sequence of CD8α signal peptide	Malpvtallplallhaarp
24	Nucleotide sequence of CD8α signal peptide	atggccttaccagtaccgccttgcctcctgccgtggccttgcctccacgccgcaggccg
25	Amino acid sequence of CD8 hinge	Ttpprptptaptiasqplsrpeacrpaaggavhtrgldfacd
26	Nucleotide sequence of CD8 hinge	Accacgacgccagcgcgcgaccaccaacaccggcgccaccatcgcgtcgcagccctgtccctgcgccagaggcgtgccggccagcgg cggggggcgagtcacacgagggggctggactcgcctgtgat
27	Amino acid sequence of CD28 transmembrane region	Fwvlvvgvvlacysllvtvafiifwv
28	Nucleotide sequence of CD28 transmembrane region	ttttgggtgctgggtggtggtggtggagtcctggctgctatagcttgctagtaacagtgccctttattttctgggtg
29	Amino acid sequence of CD28 intracellular region	Rsksrllhsdymnmtprrpgptrkhyqpyapprdfaays
30	Nucleotide sequence of CD28 intracellular region	Aggagtaagaggagcaggtcctgcacagtactacatgaacatgactccccgccgccccgggccaacccgcaagcattaccagccctatgcc caccacgcgacttcgagcctatcgtcc
31	Amino acid sequence of CD3Z domain	Rvkfsrsadapayqqgnqlynelnlgrrreydvldkrrrdpempgkqrrknpqeglynelqkdmaeyseigmkgerrrgkghdgl yqglstatkdydalhmqlppr
32	Nucleotide sequence of CD3Z domain	Agagtgaaagtcagcaggagcgcagacgccccgcgtaccagcaggccagaaccagctctataacgagctcaatctaggacgaagagagga gtacgatgtttGgacaagagacgtgcccgggacccctgagatgggggaaagccgagagaaggaagaccctcaggaagccctgtacaatga actgcagaaagataagatggcGgagggcctacagtgagattgggatgaaagcgagcggcgagggggaaggggcacgatggcctttaccagg gtctcagtacagccaccaaggacacctacgacgccccttcacatgcaggccctgccccctcgc
33	Amino acid sequence of CD8 transmembrane region	Iyiwaplagtcgvllslvit
34	Nucleotide sequence of CD8 transmembrane region	Atctacatctggggcgccttggccgggacttgtgggtcctctctctgctactggtatcacc
35	Amino acid sequence of CD137 intracellular region	Krgkrklllyfkqpfrpvqqtqeedgcsrfpeeeeggcel

36	Nucleotide sequence of CD137 intracellular region	Aaacggggcagaaagaaactctgtatatattcaaacaccatttatgagaccagtacaaactactcaagaggagatggctgtagctgccgatttccagaagaagaagaggagatgtgaactg
37	BCMA amino acid sequence	MLQMAGQCSQNEYFDSLHLHACIPQLRCSSNTPPLTCQRYCNASVNSVKGTNAILWTCLGLSLIISLAVFVLMFLLRKINSEPLKDEFKNTGSGLLGMANIDLEKSRGTGEIILPRGLEYTVEECTCEDCIKSKPKVDSHDFPLPAMEEGATILVTTKTNDYCKSLPAALSATEIEKSISAR
38	Human BCMA extracellular segment Met1-Ala54	MLqmagqcsqneyfdslhacipqlrcssntppltcqrycnasvtnsvkgtna
39	Nucleotide sequence of human BCMA extracellular segment Met1-Ala54	Atgctgcagatggccggccagtgacagcagaacgagtactcgacagcctgctgcacgcctgcatccctgccagctcgggtgcagcagcaaca cccccctgacctgccagcggtagctgcaacgccagcgtgaccaacagcgtgaagggcaccaagcgc
40	BCMA_huFc	MLqmagqcsqneyfdslhacipqlrcssntppltcqrycnasvtnsvkgtnagsdkthtppcpapellggpsvflfpkpdkdlmirsrtpevtcvvvdvshEdpevkfnwyvdgvevhnaktkpreeqynstyrsvslvllhqdwlngkeykckvsnkalpapietkiskakgpprepqvytlppsrldeltnkqvslwclvkgfypsdiavewesngqpennykttppvldsdgsfflysklvtvdkrsrwqqgnvfscsvmhealhnhytkslspsgk
41	Nucleotide sequence of BCMA_huFc	Atgctgcagatggccggccagtgacagcagaacgagtactcgacagcctgctgcacgcctgcatccctgccagctcgggtgcagcagcaaca cccccctGacctgccagcggtagctgcaacgccagcgtgaccaacagcgtgaagggcaccaacgccggatccgacaaaactcacacatgcc caccgtgccagcactgaaCtctggggggaccgtgacttctctctcccccaaaaccaaaggacacctcatgatccccggaccctcgagg tcatatgcgtggtgtggacgtgagccacgaAgacctgaggtcaagttcaactggtagctggagcggcgtggaggtgcataatgcgaagacaag ccggggaggagcagtagacaacagcagctaccgtgtgtgcagCgtctcaccgtcctgcaccaggactggctgaatggcaaggagtacaagtgc aaggtctccaaagccctccagccccatcgagaaaacatctccaaagccaAagggcagccccgagaaccacaggtgtacacctgcccc atccccgggatgagctgaccaagaaccaggtcagcctgtgtgtcgtgtgtaaaaggtcttatccagCgacatcgccgtggagtgggagagaatg ggagccgggagaaactacaagaccacgcctccctgctggtgactccgacggctcctctctctatagcaagctcaCcgtaggaacagagcagg tggcagcaggggaacgtctctcatgctccgtgatgcagtaggctctgcacaaccactacacgcagaagacgtctcctgctccgggtaaa
42	BCMA_muFc	MLqmagqcsqneyfdslhacipqlrcssntppltcqrycnasvtnsvkgtnagsrdcgckpcictvpevssvflfpkpdkdlmirsrtpevtcvvvdskddpevQfswfvddvevhtaqtqpreeqfnstfrsvslpimhqdwlngkefkcrvnsaafpapietkisktgrpkapqvytipppk eqmakdkvsltcmidtfpeditvewqwnngpaenykntqpmidtdgsyfvysklvqksnweagntfsvlhelghhhtekslshspgk
43	Nucleotide sequence of BCMA_muFc	Atgctgcagatggccggccagtgacagcagaacgagtactcgacagcctgctgcacgcctgcatccctgccagctcgggtgcagcagcaaca cccccCccctgacctgccagcggtagctgcaacgccagcgtgaccaacagcgtgaagggcaccaacgccggatccaggaggtgtggtgtaagcc ttgcataatgacAgtcaccagaagtatcatctgtcttcatcttcccccaaaagccaaggtgtgctcaccattactctaaaggtcagctgtgtgtt ggtagacatcagaagGatgatccccgaggtccagttcagctgggtttgatgatgtggaggtgcacacagctcagacgcaacccccggaggagc agttcaacagcactttccgtcagTcagtgaacttccatcatgcaccaggactggctcaatggcgaagagttcaaatcagggtcacaacgtgcagc ttccctgcccccatcgagaaaacatctccAaaaccaaaggcagaccgaaggctccacaggtgtacaccattccacctcccaaggagcagatgg ccaaggataaagtgcagctgacatgataacagacTtctccctgaagacattactgtggagtggcagtggaatgggcagcagcggagaact acaagaacactcagcccatcatggacacagatggctcttacttctcgtcAcagcaagctcaatgtgcagaagagcaactgggagggcaggaatactt caccgtcctgtgttcatatgaggcctgcacaaccaccatactgagaagagccttccccacttctcgtgtaaa
44	Nucleotide sequence of human BCMA with introduced restriction sites MluI, SalI	Acgctgctacgctacccgtcgccaccatgttgcagatggctgggcagtgctcccaaatgaataattttgacagtttgtgcatgcttcatactgt caacttcgAtgttcttataactcctccttaacatgcagcgttattgtaatgcaagtgtagcaaatcagtgaaaggaacgaatgcattcttgacc tgtttgggactgagcttAataatttcttggcagttttcgtgctaattgttttctaaggagaataaactctgaaccattaaaggacaggtttaaacaacag gatcaggtctcctggcagtggttaaCattgacctggaagaagcagcagcagtgatgataaattattctccagagccctcagtagcacgttggaaga atgcacactgtgaagactgcataagagcaaacggAaggtcgaactctgaccattgcttccactccagctatggaggaagggcgaacaccttctgtc accacgaaaacgaatgactattgcaagagcctgccagctgcttgaagtgtctacggagatagagaatacaattctgctagtagtaagtcgac
45	APRIL_huFc	Hsvlhlvpinatksddsdvtevmwqpalrrgrlgaqgygvriqdagvyllysqvlfqdvftmgqvvsreggrqretlfrcirsmphspdray nscysagVfhlhgqdlsviipraraklnlshphtflgfvklsgsdktthtppcpapellggpsvflfpkpdkdlmirsrtpevtcvvvdvshedpe vkfnwyvdgvevhnAktkpreeqynstyrsvslvllhqdwlngkeykckvsnkalpapietkiskakgpprepqvytlppsrldeltnkqvslwclvkgfypsdiavewesngqpennykttppvldsdgsfflysklvtvdkrsrwqqgnvfscsvmhealhnhytkslspsgk
46	Nucleotide sequence of APRIL_huFc	Cacagcgtgctgcacctggtgccatcaacgccaccagcaaggacgacagcagcgtgaccaggtgatgtggcagcccgccctgcggcgggg ccggggcctgcaggccagggctacggcgtcggtatccaggacggcggcgtgtacctgctgtacagccaggtgctgttccaggacgtgacctca ccatgggccagtggtgagccgggagggccagggcggcagagaccctgttccggtgcacccgagcatgccagccaccccgaccggggcc tacaacagctgctacagcggcggcgtgttccactgcaccagggcgacatcctgagcgtgatcatccccgggccggggcgaagctgaacctgag cccccacggcaccttctgggtctgtgaagctgggattccgacaaaactcacatgccaccgtgccagcagcctgaactcctggggggaacctg agtcttctcttcccccaaaaccaaaggacacctcatgatctccggaccctgaggtcacatgcgtgggtgtggagcgtgagccacgaagacct gaggtcaagttcaactgtacgtggcggcgtggaggtgcataatccaaagacaaagccggggagagcagtagtaaacagcagcagctaccgtgtgg tcagctcctcacctcctgcaccaggactggctgaatggcgaaggagtacaagtgcagggtctccaaagaagccctccagcccccatcgagaaaa accatctccaaagccaaaggcagcccccagaaaccaggtgtacacacctgcccccatccgggatgagctgaccaagaaccaggtcagcctgt ggtgctgtgtcaaaagcttctatccagcagatccgctggaggtggagagcaatgggcagccggagagaacactacaagaccacgctccctgctgagactccgacggctccttctctatgcaagctaccgtggagaagagcaggtggcagcaggggaacgtcttctatgctccgtgatcatg aggcctcgcacaaccatacagcagaagagccttccctgctcctccgggtaaa
47	7A12 scFv	Evqllesggglvqpqgsrlrscasgftfssyamswwrqapkglewwsaisgsggstyyadsvkgrfuisrdnsntlylqmnsiraedtavyy carypylafDywgqgtlvtvssgggsgggsgggseivltqspgtlslspgeratlsrscasqsvssylawyqqkpgqaprllygassratgi pdrfsgsgsgtdfllisrlpepfavyyccqygypprytfggqtkveikr
48	7G2 scFv	Evqllesggglvqpqgsrlrscasgftfssyamswwrqapkglewwsaisgsggstyyadsvkgrfuisrdnsntlylqmnsiraedtavyy caklsgdaAmdywgqgtlvtvssgggsgggsgggseivltqspgtlslspgeratlsrscasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdfllisrlpepfavyyccqygypprytfggqtkveikr

49	Primer LMF	CAGGAAACAGCTATGACCATGATTAC
50	Primer BH1R	TGAGACCCACTCCAGCCCTTCCTGGAGCCTGGCGGACCCAMNNMNNMNNMNNMNN MNNAAGGTGAATCCGGAGGCTG
51	Primer BH2F	GGCTGGAGTGGGTCTCANNKATTNNKNNKNNKNNKGGTNNKACANNKTACGCAGACTC CGTGAAGGG
52	Primer FdR	GACGTTAGTAAATGAATTTTCTGTATGAGG
53	Primer BL1R	GATGAGGAGCCTGGGAGCCTGGCCAGGTTTCTGCTGGTACCAMNNTAAMNNMNNMNN MNNMNNMNNCTGACTGGCCCTGCAAGAG
54	Primer BL2F	CCAGGCTCCCAGGCTCCTCATCNNKNNKNNKNNKAGGGCCACTGGCATCCCAGAC
55	23F10 scFv	Evqllesggglvqpggsrlrscasgftfssyamswvrqapgkglewvsaisgsgstyadsvkgrftisrdsnkntlylqmnsraedtavyy cakvrpfwgtfdywgqgtltvssggsgsgsgsgsgseivltqspgtlslspgeratlsrasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdflltislrepedfavyycqqyfnppeytfqggtkveikr
56	25C2 VH(AA)	evqllesggglvqpggsrlrscasgftfsgnamswvrqapgkglewvsaisgsgstyadsvkgrftisrdsnkntlylqmnsraedtavyy cakvrpfwgtfdywgqgtltvss
57	25C2 VH	gaggtgcaattgctggagctctggggaggcttggtacagcctgggggctcctgagactctctgtgcagcctccggattcacctttggcggtaag ccatgctcctgggtccgccagcctccagggaagggtgctgagtggtctcagcaattagtgtaagtggttagtacattctacgcagactccgtgaa gggccgggttcaccatctccagagacaattccaagaacacgctgtatctgcagatgaacagcctgagagccgaggacacggccgtatattactgtgc gaaagtctgcattctggggtactttgactactggggccaaggaacctgtgacacgtctcgaagt
58	25D2 VH(AA)	evqllesggglvqpggsrlrscasgftfssyamswvrqapgkglewvsaisgsgstyadsvkgrftisrdsnkntlylqmnsraedtavyy cakvrpfwgtfdywgqgtltvss
59	25D2 VH	gaggtgcaattgctggagctctggggaggcttggtacagcctgggggctcctgagactctctgtgcagcctccggattcacctttaggagctatgc catgagctgggtccgccagcctccagggaagggtgctgagtggtctcagctattagtgccgggtgtggttaacacattctacgcagactccgtgaa gggccgggttcaccatctccagagacaattccaagaacacgctgtatctgcagatgaacagcctgagagccgaggacacggccgtatattactgtgc gaaagtctgcattctggggtactttgactactggggccaaggaacctgtgacacgtctcgaagt
60	25C2 HCDR1	gnams
61	25C2 HCDR2	aisnggstfyadsvkg
62	25D2 HCDR1	syams
63	25D2 HCDR2	aisgggntfyadsvkg
64	25C2 scFv	gaggtgcaattgctggagctctggggaggcttggtacagcctgggggctcctgagactctctgtgcagcctccggattcacctttggcggtaag ccatgctcctgggtccgccagcctccagggaagggtgctgagtggtctcagcaattagtgtaagtggttagtacattctacgcagactccgtgaa gggccgggttcaccatctccagagacaattccaagaacacgctgtatctgcagatgaacagcctgagagccgaggacacggccgtatattactgtgc gaaagtctgcattctggggtactttgactactggggccaaggaacctgtgacacgtctcgaagtgtggaaggcgggttcaggcggaggtggttct ggcgggtggcggatcgaaatcgtgttaacgagctctcaggcacctgtctttgtctccaggggaagaccacctctctgcaggcggagctcaga gtgttagcagcagctacttagcctggtaccagcagaacctggccaggtcctccaggtcctcatctatgagagatccagcaggccactggcatccc agacaggttcagtgagcagtgatccgggacagacttactctaccatcagcagactggagcctgaagattttgagtgattactgtcagcagtaactt caaccaccagaatacacgttcggccaggggaccgaagtggaaatcaaacgt
65	25C2 scFv(AA)	evqllesggglvqpggsrlrscasgftfsgnamswvrqapgkglewvsaisgsgstyadsvkgrftisrdsnkntlylqmnsraedtavyy cakvrpfwgtfdywgqgtltvssggsgsgsgsgsgseivltqspgtlslspgeratlsrasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdflltislrepedfavyycqqyfnppeytfqggtkveikr
66	25D2 scFv	gaggtgcaattgctggagctctggggaggcttggtacagcctgggggctcctgagactctctgtgcagcctccggattcacctttaggagctatgc catgagctgggtccgccagcctccagggaagggtgctgagtggtctcagctattagtgccgggtgtggttaacacattctacgcagactccgtgaa gggccgggttcaccatctccagagacaattccaagaacacgctgtatctgcagatgaacagcctgagagccgaggacacggccgtatattactgtgc gaaagtctgcattctggggtactttgactactggggccaaggaacctgtgacacgtctcgaagtgtggaaggcgggttcaggcggaggtggttct ggcgggtggcggatcgaaatcgtgttaacgagctctcaggcacctgtctttgtctccaggggaagaccacctctctgcaggcggagctcaga gtgttagcagcagctacttagcctggtaccagcagaacctggccaggtcctccaggtcctcatctatgagagatccagcaggccactggcatccc agacaggttcagtgagcagtgatccgggacagacttactctaccatcagcagactggagcctgaagattttgagtgattactgtcagcagtaactt caaccaccagaatacacgttcggccaggggaccgaagtggaaatcaaacgt
67	25D2 scFv(AA)	evqllesggglvqpggsrlrscasgftfssyamswvrqapgkglewvsaisgsgstyadsvkgrftisrdsnkntlylqmnsraedtavyy cakvrpfwgtfdywgqgtltvssggsgsgsgsgsgseivltqspgtlslspgeratlsrasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdflltislrepedfavyycqqyfnppeytfqggtkveikr
68	PD-1 signal peptide sequence	ATGCAGATCCACAGGCGCCCTGGCCAGTCTGCTTGGGCGGTGCTACAACTGGGCTGGCG G
69	PD-1 extracellular segment sequence	CCAGGATGGTCTTTAGACTCCCCAGACAGGCCCCTGGAACCCCCACCTTCTCCCCAGCC CTGCTCGTGGTGACCGAAGGGGACAAACGCCACCTTCACCTGCAGCTTCTCCAACACATCG GAGAGCTTCGTGCTAAACTGGTACCGCATGAGCCCCAGCAACCAGACGGACAAGCTGGC CGCCTTCCCGGAGGACCGCAGCCAGCCCGGCCAGGACTGCCGCTTCCGTGTACACAACCT GCCCCAACGGGCGTGACTTCCACATGAGCGTGGTCAGGGCCCCGGCGCAATGACAGCGGCA CCTACCTCTGTGGGGCCATCTCCCTGGGCCCCAAGGCGCAGATCAAGAGAGCCTTGGCG GCAGAGCTCAGGGTGACAGAGAGAAGGGCAGAAAGTGCCACAGCCCCACCCAGCCCCCTC ACCCAGGCCAGCCGGCCAGTTCCAAACCCTGGTG
70	DNA sequence of CH3 domain	CCCCCATGCCCCACCATGCCCCAGCACCTGAGTTCCTGGGGGGACCATCAGTCTTCTCTGTTC CCCCAAAACCCAAGGACATCTCATGATCTCCCGGACCCTGAGGTACAGTGGTGGTG GTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGA GGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTACCGTGTGG TCAGCGTCTTACCGTCTGTGCACAGGACTGGCTGAACGGCAAGGAGTACAAGTGCAAG GTCTCCAACAAAGGCCTCCCGTCC
71	primer	acgcgtctagcgtaccgggtcggaccatgcagatccacagcgccc
72	Primer	ctctcgggctgccaccatacaccagggttggaaactggc
73	Primer	tatggtggcgagccccgagaccacag

74	primer	aaaattcaaatgctgttctacttaccggagacaggag
75	7A12-BBZ	Evqllesggglvqppgslrlscaasgftfssyamswvrqapgkglewvsaisggsgstyadsvkgrftisrdsnkntlylqmnsraedtavvy carypylafDywgqgtltvtssggsgsgsgsgsgsgseivltqspgtlslsperatlscrasqsvssylawyqqkpgqaprllygassratgi pdrfsgsgsgtdfilitrllepedfavyyccqygyppsytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEED GCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEM GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALH MQALPPR
76	25C2-BBZ	evqllesggglvqppgslrlscaasgftfsgnamswvrqapgkglewvsaisngsgstyadsvkgrftisrdsnkntlylqmnsraedtavvy cakvrpfwgtfdywgqgtltvtssggsgsgsgsgsgsgseivltqspgtlslsperatlscrasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdfilitrllepedfavyyccqygnppeytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDAL HMQALPPR
77	25D2-BBZ	evqllesggglvqppgslrlscaasgftfssyamswvrqapgkglewvsaisggsgntfyadsvkgrftisrdsnkntlylqmnsraedtavvy cakvrpfwgtfdywgqgtltvtssggsgsgsgsgsgsgseivltqspgtlslsperatlscrasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdfilitrllepedfavyyccqygnppeytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDAL HMQALPPR
78	7G2-BBZ	Evqllesggglvqppgslrlscaasgftfssyamswvrqapgkglewvsaisggsgstyadsvkgrftisrdsnkntlylqmnsraedtavvy caklsgdaAmdywgqgtltvtssggsgsgsgsgsgsgseivltqspgtlslsperatlscrasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdfilitrllepedfavyyccqygypprytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEE DGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDAL HMQALPPR
79	7A12-28Z	Evqllesggglvqppgslrlscaasgftfssyamswvrqapgkglewvsaisggsgstyadsvkgrftisrdsnkntlylqmnsraedtavvy carypylafDywgqgtltvtssggsgsgsgsgsgsgseivltqspgtlslsperatlscrasqsvssylawyqqkpgqaprllygassratgi pdrfsgsgsgtdfilitrllepedfavyyccqygyppsytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDFWVLLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPG PTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDT YDALHMQALPPR
80	7A12-28BBZ	Evqllesggglvqppgslrlscaasgftfssyamswvrqapgkglewvsaisggsgstyadsvkgrftisrdsnkntlylqmnsraedtavvy carypylafDywgqgtltvtssggsgsgsgsgsgsgseivltqspgtlslsperatlscrasqsvssylawyqqkpgqaprllygassratgi pdrfsgsgsgtdfilitrllepedfavyyccqygyppsytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDFWVLLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPG PTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQALPPR
81	7G2-28Z	Evqllesggglvqppgslrlscaasgftfssyamswvrqapgkglewvsaisggsgstyadsvkgrftisrdsnkntlylqmnsraedtavvy caklsgdaAmdywgqgtltvtssggsgsgsgsgsgsgseivltqspgtlslsperatlscrasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdfilitrllepedfavyyccqygypprytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDFWVLLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPG PTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKD TYDALHMQALPPR
82	7G2-28BBZ	Evqllesggglvqppgslrlscaasgftfssyamswvrqapgkglewvsaisggsgstyadsvkgrftisrdsnkntlylqmnsraedtavvy caklsgdaamdywgqgtltvtssggsgsgsgsgsgsgseivltqspgtlslsperatlscrasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdfilitrllepedfavyyccqygypprytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDFWVLLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPG PTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQALPPR
83	25C2-28Z	Evqllesggglvqppgslrlscaasgftfssyamswvrqapgkglewvsaisggsgstyadsvkgrftisrdsnkntlylqmnsraedtavvy cakvrpfwgtfdywgqgtltvtssggsgsgsgsgsgsgseivltqspgtlslsperatlscrasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdfilitrllepedfavyyccqygnppeytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDFWVLLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPG PTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKD TYDALHMQALPPR
84	25C2-28BBZ	Evqllesggglvqppgslrlscaasgftfssyamswvrqapgkglewvsaisggsgstyadsvkgrftisrdsnkntlylqmnsraedtavvy cakvrpfwgtfdywgqgtltvtssggsgsgsgsgsgsgseivltqspgtlslsperatlscrasqsvssylawyqqkpgqaprllygassrat gipdrfsgsgsgtdfilitrllepedfavyyccqygnppeytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDFWVLLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPG PTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQGGQNQLYNELNLGRREEYDVLDKRRGRD PEMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGDGLYQGLSTATKDTYDALHMQALPPR

85	25D2-28Z	evqllesggglvqppgslrlscaasgftfrsyamswvrqapgkglewvsaisgggntfyadsvkgrftisrdnsntlylqmnsraedtavyy cakvrpfwgtfdywgqgtltvtvssgggsgggsgggsgggseivltqspgtlslspgeratlscrasqsvssylawyqqkpgqaprrliygassrat gipdrfsgsgsgtdfilitisrlepedfavyyccqyfnppeytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRP GPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRG RDPPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKD TYDALHMQALPPR
86	25D2-28BBZ	evqllesggglvqppgslrlscaasgftfrsyamswvrqapgkglewvsaisgggntfyadsvkgrftisrdnsntlylqmnsraedtavyy cakvrpfwgtfdywgqgtltvtvssgggsgggsgggsgggseivltqspgtlslspgeratlscrasqsvssylawyqqkpgqaprrliygassrat gipdrfsgsgsgtdfilitisrlepedfavyyccqyfnppeytfqggtkveikrTTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRP GPTRKHYQPYAPPRDFAAYRSRGRKRLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCE LRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPPRRKNPQEGL YNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR
87	C11D5.3-BBZ	Qiqlvqsgpelkpggetvkiackasgytftdysinwvkrapgkglkwmgwintetrepayaydfgrgrfafsletsastaylqinnlkyedtatyf caldysyamdywgqgtsvtvssgggsgggsgggsgggdivltqspplamslgkratiscrasesvtlilgshlihyqqkpgqppptliqlasn TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVI TLYCKRGRKRLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEEGGCELRVKFSRSADAPAYKQG QNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPPRRKNPQEGLYNELQKDKMAEAYSEIGM KGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR

Claims

1. An antibody that targets BCMA, wherein the antibody is selected from the group consisting of:

5 (1) an antibody, comprising a heavy chain variable region comprising HCDR1 as shown in SEQ ID NO: 1, 60 or 62, and/or comprising HCDR2 as shown in SEQ ID NO: 2, 61 or 63, and/or HCDR3 shown in any one of SEQ ID NO: 3, SEQ ID NO: 4 or SEQ ID NO: 5;

10 (2) an antibody, comprising a light chain variable region comprising LCDR1 as shown in SEQ ID NO: 6, and/or comprising LCDR2 as shown in SEQ ID NO: 7, and/or comprising LCDR3 as shown in any one of SEQ ID NO: 8, SEQ ID NO: 9 or SEQ ID NO: 10;

 (3) an antibody, comprising a heavy chain variable region of the antibody of (1) and a light chain variable region of the antibody of (2);

15 (4) an antibody, which is a variant of the antibody of any one of (1) to (3) and has the same or similar activity as the antibody of any one of (1) to (3).

2. The antibody of claim 1, wherein the antibody is selected from the group consisting of:

20 (1) an antibody, comprising HCDR1 as shown in SEQ ID NO: 1, HCDR 2 as shown in SEQ ID NO: 2, HCDR3 as shown in SEQ ID NO: 3, and LCDR1 as shown in SEQ ID NO: 6, LCDR2 as shown in SEQ ID NO: 7 and LCDR3 as shown in SEQ ID NO: 8;

 (2) an antibody, comprising HCDR1 as shown in SEQ ID NO: 1, HCDR 2 as shown in SEQ ID NO: 2, HCDR3 as shown in SEQ ID NO: 4, LCDR1 as shown in SEQ ID NO: 6, LCDR2 as shown in SEQ ID NO: 7 and LCDR3 as shown in SEQ ID NO: 9;

25 (3) an antibody, comprising HCDR1 as shown in SEQ ID NO: 1, HCDR2 as shown in SEQ ID NO: 2, HCDR3 as shown in SEQ ID NO: 5, LCDR1 as shown in SEQ ID NO: 6, LCDR2 as shown in SEQ ID NO: 7 and LCDR3 as shown in SEQ ID NO: 10;

 (4) an antibody, comprising HCDR1 as shown in SEQ ID NO: 60, HCDR2 as shown in SEQ ID NO: 61, HCDR3 as shown in SEQ ID NO: 5, LCDR1 as shown in SEQ ID NO: 6, LCDR2 as shown in SEQ ID NO: 7 and LCDR3 as shown in SEQ ID NO: 10;

30 (5) an antibody, comprising HCDR1 as shown in SEQ ID NO: 62, HCDR2 as shown in SEQ ID NO: 63, HCDR3 as shown in SEQ ID NO: 5, LCDR1 as shown in SEQ ID NO: 6, LCDR2 as shown in SEQ ID NO: 7 and LCDR3 as shown in SEQ ID NO: 10;

 (6) an antibody, which is a variant of any one of (1) to (5) and has the same or

similar activity as the antibody of any one of (1) to (5).

3. The antibody of claim 1, wherein the antibody is selected from the group consisting of:

(1) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 13, the amino acid sequence of SEQ ID NO: 17, the amino acid sequence of SEQ ID NO: 21, or the amino acid sequence of SEQ ID NO: 56 or the amino acid sequence of SEQ ID NO: 58;

(2) an antibody, wherein the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 11, the amino acid sequence of SEQ ID NO: 15, or the amino acid sequence of SEQ ID NO: 19;

(3) an antibody, comprising a heavy chain variable region of the antibody of (1) and a light chain variable region of the antibody of (2);

(4) an antibody, which is a variant of any one of (1) to (3) and has the same or similar activity as the antibody of any one of (1) to (3).

4. The antibody of claim 3, wherein the antibody is selected from the group consisting of:

(1) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 13 and the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 11;

(2) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 17 and the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 15;

(3) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 21 and the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 19;

(4) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 56 and the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 19;

(5) an antibody, wherein the heavy chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 58 and the light chain variable region of the antibody has the amino acid sequence of SEQ ID NO: 19;

(6) an antibody, which is a variant of any one of (1) to (5) and has the same or similar activity as the antibody of any one of (1) to (5).

5. An antibody recognizing the same antigenic determinant as the antibody of any one of claims 1-4.

6. A nucleic acid encoding the antibody of any one of claims 1-5.

7. An expression vector comprising the nucleic acid of claim 6.

8. A host cell comprising the expression vector of claim 7 or has the nucleic acid of claim 6 integrated in the genome.

5 9. Use of the antibody of any one of claims 1-5 for the preparation of a targeting drug, an antibody drug conjugate or a multifunctional antibody which specifically targets tumor cells expressing BCMA; or

for the preparation of an agent for diagnosis of a tumor expressing BCMA; or

for the preparation of a chimeric antigen receptor-modified immune cell; and

10 preferably, the immune cell includes T lymphocyte, NK cell or NKT lymphocyte.

10. A multifunctional immunoconjugate, comprising

an antibody of any one of claims 1-5; and

a functional molecule linked thereto; and said functional molecule is selected from the group consisting of a molecule that targets a tumor surface marker, a molecule that
15 inhibits tumors, a molecule that targets a surface marker of an immune cell, and a detectable label.

11. The multifunctional immunoconjugate of claim 10, wherein the molecule that inhibits tumors is an antitumor cytokine or an antitumor toxin; preferably, the cytokine comprises: IL-12, IL-15, type I interferon, TNF-alpha.

20 12. The multifunctional immunoconjugate of claim 10, wherein the molecule that targets a surface marker of an immune cell is an antibody or a ligand that binds to a surface marker of an immune cell; and preferably, the surface marker of an immune cell comprises: CD3, CD16, CD28, and more preferably, the antibody that binds to a surface marker of an immune cell is an anti-CD3 antibody.

25 13. The multifunctional immunoconjugate of claim 12, wherein the molecule that targets a surface marker of an immune cell is an antibody that binds to a surface marker of a T cell.

14. A nucleic acid encoding the multifunctional immunoconjugate of any one of claims 10-13.

30 15. Use of the multifunctional immunoconjugate of any one of claims 10-13, for the preparation of an antitumor drug, or

for the preparation of an agent for diagnosis of a tumor expressing BCMA; or

for the preparation of a chimeric antigen receptor-modified immune cell; and

preferably, the immune cell comprises: T lymphocytes, NK cells or NKT lymphocytes.

35 16. A chimeric antigen receptor comprising an extracellular domain, transmembrane

domain and intracellular signal domain, wherein the extracellular domain comprises the antibody of any one of claims 1-5, and the antibody preferably is a single-chain antibody or domain antibody.

17. The chimeric antigen receptor of claim 16, wherein the intracellular signal domain comprises one or more co-stimulatory signal domains and/or primary signal domains.

18. The chimeric antigen receptor of claim 16, wherein the chimeric antigen receptor further comprises a hinge domain.

19. The chimeric antigen receptor of claim 17, wherein the transmembrane domain is selected from the group consisting of alpha, beta, zeta chain of TCR, transmembrane regions of CD3 ϵ , CD3 ζ , CD4, CD5, CD8 α , CD9, CD16, CD22, CD27, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, CD152, CD154 and PD1; and/or

the costimulatory signal domain is selected from the group consisting of intracellular signal regions of CARD11, CD2, CD7, CD27, CD28, CD30, CD40, CD54, CD83, OX40, CD137, CD134, CD150, CD152, CD223, CD270, PD-L2, PD-L1, CD278, DAP10, LAT, NKD2C SLP76, TRIM, Fc ϵ RI γ , MyD88 and 41BBL; and/or

the primary signal domain is selected from the group consisting of TCR ξ , FcR γ , FcR β , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, CD278 (also named as "ICOS"), CD66d and CD3 ζ ,

preferably,

the transmembrane domain is selected from the group consisting of transmembrane domains of CD8 α , CD4, CD45, PD1, CD154 and CD28; and/or

the co-stimulatory signal domain is selected from the group consisting of CD137, CD134, CD28 and OX40; and/or

the primary signal domain is selected from CD3 ζ ,

most preferably, the transmembrane domain is selected from CD8 α or CD28, the co-stimulatory signal domain is selected from the intracellular signal domain of CD137 or CD28, and the primary signal domain is selected from CD3 ζ .

20. The chimeric antigen receptor of claim 16, wherein the chimeric antigen receptor comprises the following sequentially linked an antibody, a transmembrane region and an intracellular signal region:

the antibody of any one of claims 1-5, the transmembrane region of CD8 and CD3 ζ ;

the antibody of any one of claims 1-5, the transmembrane region of CD8, the intracellular signal region of CD137 and CD3 ζ ;

the antibody of any one of claims 1-5, the transmembrane region of CD28, the

intracellular signal region of CD28, and CD3 ζ ; or

the antibody of any one of claims 1-5, the transmembrane region of CD28, the intracellular signal region of CD28, CD137 and CD3 ζ .

21. A nucleic acid encoding the chimeric antigen receptor of any one of claims
5 16-20.

22. An expression vector comprising the nucleic acid of claim 21.

23. A virus comprising the vector of claim 22.

24. Use of the chimeric antigen receptor of any one of claimss 16-20, or the nucleic acid of claim 21, or the expression vector of claim 22, or the virus of claim 23, for the
10 preparation of genetically modified immune cells targeting a tumor cell expressing BCMA.

25. A genetically modified immune cell, which is transduced with the nucleic acid of claim 21, or the expression vector of claim 22 or the virus of claim 23; or expresses the chimeric antigen receptor of any one of claims 16-20,

15 the immune cells are preferably selected from T lymphocytes, NK cells or NKT cells.

26. The genetically modified immune cell of claim 25, wherein it further expresses a sequence other than the chimeric antigen receptor of the tenth aspect of the invention, and the other sequence comprises a cytokine, or another chimeric antigen receptor, or a
20 chemokine receptor, or an siRNA that reduces PD-1 expression, or a protein that blocks PD-L1, or a TCR, or a safety switch;

Preferably, the cytokine comprises IL-12, IL-15, IL-21, or type I interferon;

Preferably, the chemokine receptor comprises CCR2, CCR5, CXCR2, or CXCR4;

Preferably, the safety switch comprises iCaspase-9, Truncated EGFR or RQR8.

25 27. Use of the genetically modified immune cell of any one of claims 25-27, for preparing a tumor-suppressing drug, and the tumor is a tumor expressing BCMA.

28. A pharmaceutical composition, comprising:

an antibody of any one of claims 1-5 or a nucleic acid encoding the antibody; or

30 an immunoconjugate of any one of claims 10-15 or a nucleic acid encoding the immunoconjugate; or

a chimeric antigen receptor of any one of claims 16-20 or a nucleic acid encoding the chimeric antigen receptor; or

a genetically modified immune cell of any one of claims 25-26.