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(54) Titre : RECEPTEUR ANTIGENIQUE CHIMERIQUE CIBLANT BCMA, SON PROCEDE DE PREPARATION ET SON UTILISATION  
(54) Title: BCMA-TARGETED CHIMERIC ANTIGEN RECEPTOR AS WELL AS PREPARATION METHOD THEREFOR AND APPLICATION THEREOF

(57) **Abrégé/Abstract:**

The present invention provides a BCMA-targeted chimeric antigen receptor (CAR) as well as a preparation method therefor and an application thereof. Specifically, the present invention provides the BCMA-targeted CAR, which comprises a BCMA-targeted scFv, a hinge region, a transmembrane region, and an intracellular signal structure domain. The present invention provides a nucleic acid molecule for coding the CAR and a corresponding expression vector as well as CAR-T cells and application thereof. The CAR of the present invention targets BCMA-positive cells, and can be used for treating diseases such as BCMA-positive B cell lymphoma, multiple myeloma, and plasma cell leukemia.

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**Abstract**

The present invention provides a BCMA-targeted chimeric antigen receptor (CAR) as well as a preparation method therefor and an application thereof. Specifically, the present invention provides the BCMA-targeted CAR, which comprises a BCMA-targeted scFv, a hinge region, a transmembrane region, and an intracellular signal structure domain. The present invention provides a nucleic acid molecule for coding the CAR and a corresponding expression vector as well as CAR-T cells and application thereof. The CAR of the present invention targets BCMA-positive cells, and can be used for treating BCMA-positive B-cell lymphoma, multiple myeloma and plasma cell leukemia.

## **BCMA-targeted chimeric antigen receptor as well as preparation method therefor and application thereof**

### **Technical field**

5       The invention relates to the field of bio-medicine, and more particularly to a BCMA targeting chimeric antigen receptor as well as preparation method and application thereof.

### **Background technique**

10       BCMA is a B cell maturation antigen, also known as CD269 or TNFRSF17, and is a member of the tumor necrosis factor receptor superfamily. Its ligands are B cell activating factor (BAFF) and a proliferation-induced ligand (APRIL).

15       Binding of BCMA to BAFF and APRIL activates NF- $\kappa$ B and induces up-regulation of anti-apoptotic Bcl-2 members such as Bcl-xL or Bcl-2 and Mcl-1. The interaction between BCMA and its ligands regulates humoral immunity as well as the growth and differentiation of B cells from different aspects to maintain a stable and balanced environment in the human body.

20       The expression of BCMA is restricted to B cell lines. It is expressed on plasmablasts, plasma cells and a portion of mature B cells, and increased at the differentiation of terminal B cells. While in most B cells, such as naive B cells, memory B cells and B cell germinal centers and other organs, BCMA is not expressed. It has been reported that the expression of BCMA is important for long-lived, fixed plasma cells in the bone marrow. Therefore, plasma cells in the bone marrow are reduced in BCMA-deficient mice, but plasma cell level in the spleen is not affected. Mature B cells can normally differentiate into plasma cells in BCMA knockout mice. The BCMA knockout mice looked normal and seemed healthy, and the number of B cells was normal, but the plasma cells could not survive for a long time.

25       BCMA is also highly expressed in malignant plasma cells, such as multiple myeloma and plasma cell leukemia. BCMA is also detected in HRS cells of patients with Hodgkin's lymphoma. In America, malignant tumors of blood system account for about 10% of all malignant tumors, and myeloma accounts for 15% of all malignant hematological tumors.

30       According to the literature, the expression of BCMA is associated with progression of multiple myeloma disease. The BCMA gene is highly expressed in myeloma samples, but is low expressed in chronic lymphocytic leukemia, acute lymphocytic leukemia, and acute T-cell lymphocytic leukemia. B cell lymphomas were significantly increased in a mouse model overexpressing BCMA ligands BAFF and APRIL. Ligands that bind to BCMA have been

35       shown to regulate the growth and survival of multiple myeloma cells expressing BCMA. The combination of BCMA with BAFF and APRIL can make malignant plasma cells survive. Therefore, loss of tumor cells expressing BCMA and distribution of the interaction between BCMA ligand and receptor can improve outcome in the treatment of multiple myeloma or other BCMA positive B cell lines malignant lymphoma.

Multiple myeloma, also known as plasmacytoma or Keller's disease, is a malignant tumor of the refractory B cell line, characterized by abnormal proliferation of plasma cells. Plasma cells are a type of leukocyte that is responsible for production of antibodies. According to data released by the National Cancer Institute in 2017, myeloma accounts for 1.8% of all tumor cases, with a mortality rate of 2.1%. The statistical results of 2010-2014 show that the incidence rate is about 6.6 in 100,000 per year and the mortality rate is about 50%. Multiple myeloma is a middle-aged disease. The median age of onset in Europe and the United States is 68 years old. There are more males than females. The peak age of onset in China is 55-65 years old, and the ratio of male to female is 2.35:1. There is no confirmed epidemiological data on multiple myeloma in China. It is generally estimated that the incidence rate is similar to that in surrounding southeast Asia and Japan, about one in 100,000. Traditional treatments for multiple myeloma include chemotherapy and hematopoietic stem cell transplantation, but these methods have a high recurrence rate. Bortezomib (PS -341) is first proteasome inhibitor, which is approved by the FDA in 2003 for the treatment of relapsed refractory multiple myeloma, either alone or in combination with existing medications. The results were gratifying. The drug was also marketed in China in 2005 and has become one of the options for the treatment of multiple myeloma with thalidomide and dexamethasone. The treatment of multiple myeloma is usually combined. However, if multiple drugs are used at the same time, there are also negative effects of costly and cumulative side effects. There is still a clinical need to develop new methods for the treatment of multiple myeloma.

Recently, immunotherapy, especially adoptive T-cell therapy, has shown strong efficacy and bright prospects in clinical trials for the treatment of malignant tumors of the blood system. T cells can be genetically modified to express a chimeric antigen receptor (CAR), which includes an antigen recognition portion and a T cell activation region. Using the antigen binding properties of monoclonal antibodies, CAR can redirect the specificity and reactivity of T cell and target in a non-MHC restricted manner. This non-MHC restricted antigen recognition allows CAR-expressing T cells to recognize antigen without antigen processing, thus avoiding a major mechanism of tumor escape. In addition, CAR does not produce dimers with alpha chain and beta chain of the endogenous TCR.

At present, two chimeric antigen receptor T cell therapy (CAR-T) products targeting CD19 have been approved for the treatment of acute lymphoblastic leukemia in children and young adult patients and adult second-line or multi-line system therapy of recurrent or refractory large B-cell lymphoma. However, CD19 is rarely expressed in malignant plasma cells of multiple myeloma. There is an urgent need in the art to develop a CAR-T product that targets BCMA for the treatment of multiple myeloma.

### **Summary of the invention**

It is an object of the present invention to provide a BCMA targeted chimeric antigen receptor as well as preparation method and application thereof.

Specifically, it is an object of the present invention to provide a sequence of BCMA targeted chimeric antigen receptor as well as preparation method and activity identification of a modified T cell (CART-BCMA) thereof.

The present invention provides a chimeric antigen receptor structure for use in the treatment of BCMA positive B cell lymphoma.

In a first aspect of the invention, it provides a chimeric antigen receptor (CAR) (sequence), and its antigen binding domain is an antibody single chain variable region sequence that targets extracellular region of BCMA.

In another preferred embodiment, the antigen binding domain is an antibody single chain variable region sequence that targets amino acid residues at positions 24 to 41 of the BCMA sequence.

In another preferred embodiment, the NCBI accession number of the BCMA sequence is AY684975.1.

In another preferred embodiment, the structure of the antigen binding domain is shown in formula I as below:



wherein  $V_H$  is an antibody heavy chain variable region;  $V_L$  is an antibody light chain variable region; and "-" is a linker peptide or a peptide bond;

and, the amino acid sequence of  $V_L$  is as shown in SEQ ID NO: 1, and the amino acid sequence of  $V_H$  is as shown in SEQ ID NO: 2;

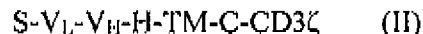
or, the amino acid sequence of  $V_L$  is as shown in SEQ ID NO: 3, and the amino acid sequence of  $V_H$  is as shown in SEQ ID NO: 4;

or, the amino acid sequence of  $V_L$  is as shown in SEQ ID NO: 5, and the amino acid sequence of  $V_H$  is as shown in SEQ ID NO: 6.

In another preferred embodiment, the amino acid sequence of the linker peptide is as shown in SEQ ID NO: 10 or SEQ ID NO: 11.

In another preferred embodiment, the antibody single chain variable region comprises a human, mouse, human-mouse chimeric antibody single chain variable region.

In another preferred embodiment, the structure of the chimeric antigen receptor is shown in formula II as below:



wherein,

S is an optional signal peptide;

H is a hinge region;

TM is a transmembrane domain;

C is a co-stimulatory signaling molecule;

CD3 $\zeta$  is a cytoplasmic signaling sequence derived from CD3 $\zeta$ ;

$V_H$  and  $V_L$  are as described above.

In another preferred embodiment, the S is a signal peptide of a protein selected from the group consisting of CD8, CD28, GM-CSF, CD4, CD137, or a combination thereof.

In another preferred embodiment, the S is a signal peptide derived from CD8.

In another preferred embodiment, the amino acid sequence of S is as shown in SEQ ID  
5 NO: 9.

In another preferred embodiment, the H is a hinge region of a protein selected from the group consisting of CD8, CD28, CD137, or a combination thereof.

In another preferred embodiment, the H is a hinge region derived from CD8.

In another preferred embodiment, the amino acid sequence of H is as shown in SEQ ID  
10 NO: 12.

In another preferred embodiment, the TM is a transmembrane region of a protein selected from the group consisting of CD28, CD3ε, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, or a combination thereof.

In another preferred embodiment, the TM is a transmembrane region derived from CD8.

In another preferred embodiment, the sequence of TM is as shown in SEQ ID NO: 13.

In another preferred embodiment, the C is a co-stimulatory signaling molecule of a protein selected from the group consisting of OX40, CD2, CD7, CD27, CD28, CD30, CD40, CD70, CD134, 4-1BB (CD137), PD1, Dap10, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), NKG2D, GITR, TLR2, or a combination thereof.

In another preferred embodiment, C is a co-stimulatory signaling molecule derived from  
20 4-1BB.

In another preferred embodiment, the amino acid sequence of C is as shown in SEQ ID  
NO: 14.

In another preferred embodiment, the amino acid sequence of CD3ζ is as shown in SEQ  
25 ID NO: 15.

In a second aspect of the invention, it provides a nucleic acid molecule, encoding the chimeric antigen receptor (CAR) of the first aspect of the invention.

In another preferred embodiment, the nucleic acid molecule is isolated.  
30

In a third aspect of the invention, it provides a vector, comprising the nucleic acid molecule of the second aspect of the invention.

In another preferred embodiment, the vector is selected from the group consisting of DNA, RNA, plasmid, lentiviral vector, adenoviral vector, retroviral vector, transposon, or a  
35 combination thereof.

In another preferred embodiment, the vector is a lentiviral vector.

In a fourth aspect of the invention, it provides a host cell, comprising the vector of the third aspect of the invention or having the exogenous nucleic acid molecule of the second

aspect of the invention integrated into the chromosome or expressing the CAR of the first aspect of the invention.

In another preferred embodiment, the cell is an isolated cell, and/or the cell is a genetically engineered cell.

5 In another preferred embodiment, the cell is a mammalian cell.

In another preferred embodiment, the cell is a T cell.

In a fifth aspect of the invention, it provides a method for preparing a CAR-T cell expressing the CAR of the first aspect of the invention, and the method comprises the steps of:

10 transducing the nucleic acid molecule of the second aspect of the invention or the vector of the third aspect of the invention into a T cell, thereby obtaining the CAR-T cell.

In a sixth aspect of the invention, it provides a preparation, comprising the chimeric antigen receptor of the first aspect of the invention, the nucleic acid molecule of the second aspect of the invention, the vector of the third aspect of the invention, or the cell of the fourth aspect of the invention, and a pharmaceutically acceptable carrier, diluent or excipient.

In another preferred embodiment, the preparation is a liquid preparation.

In another preferred embodiment, the dosage form of the preparation is injection.

20 In another preferred embodiment, the concentration of the CAR-T cells in the preparation is  $1 \times 10^3$ - $1 \times 10^8$  cells / ml, preferably  $1 \times 10^4$ - $1 \times 10^7$  cells / ml.

In a seventh aspect of the invention, it provides the use of the chimeric antigen receptor of the first aspect of the invention, the nucleic acid molecule of the second aspect of the invention, the vector of the third aspect of the invention, or the cell of the fourth aspect of the invention, for the preparation of a medicine or a preparation for preventing and/or treating tumor or cancer.

In another preferred embodiment, the tumor is selected from the group consisting of a hematological tumor, a solid tumor, or a combination thereof.

30 In another preferred embodiment, the blood tumor is selected from the group consisting of acute myeloid leukemia (AML), multiple myeloma (MM), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), diffuse large B cell lymphoma (DLBCL), or a combination thereof.

35 In another preferred embodiment, the solid tumor is selected from the group consisting of gastric cancer, peritoneal metastasis of gastric cancer, liver cancer, leukemia, renal cancer, lung cancer, small intestine cancer, bone cancer, prostate cancer, colorectal cancer, breast cancer, large intestine cancer, cervical cancer, ovarian cancer, lymphoma, nasopharyngeal carcinoma, adrenal tumor, bladder tumor, non-small cell lung cancer (NSCLC), glioma, endometrial cancer, or a combination thereof.

In another preferred embodiment, the tumor is a BCMA positive tumor, preferably a



BCMA positive B cell lymphoma, multiple myeloma, or plasma cell leukemia.

In an eighth aspect of the invention, it provides a kit for the preparation of the cell of the fourth aspect of the invention, the kit comprises a container, and the nucleic acid molecule of the second aspect of the invention or the vector of the third aspect of the invention is located in the container.

In a ninth aspect of the invention, it provides a use of the cell of the fourth aspect of the invention, or the preparation of the sixth aspect of the invention for the prevention and/or treatment of cancer or tumor.

In a tenth aspect of the invention, it provides a method of treating a disease comprising administering an appropriate amount of the cell of the fourth aspect of the invention, or the preparation of the sixth aspect of the invention, to a subject in need of treatment.

In another preferred embodiment, the disease is cancer or tumor.

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 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 needs not be described one by one, due to space limitations.

### Description of Drawings

Figure 1 shows screening result of CART-BCMA comparative examples. Figure 1A shows detection of transfection efficiency of engineered T cell with chimeric antigen receptors targeting BCMA. The expression level of the CAR gene-encoded protein on the surface of the T cell membrane in CAR-BCMA cells cultured on day 6 was identified by Fc fragment staining method of recombinant human BCMA protein.  $1 \times 10^5$  of CART-BCMA cells cultured on day 10 were cultured respectively with BCMA-positive K562-BCMA-B9 tumor cell line, MM.1S and RPMI8226 tumor cell lines naturally expressing BCMA, and BCMA-negative K562 tumor cell line, or without tumor cells, in 200  $\mu$ l GT-551 medium for 18h at a ratio of 1:1. Then the expression level of CD137 on the surface of T cell membrane (Fig. 1B) and the secretion level of IFN $\gamma$  in the culture supernatant was detected respectively (Fig. 1C).

Figure 2 shows structure of chimeric antigen receptor targeting BCMA. The structure of CAR includes a leader sequence, an antigen recognition sequence, a linker region, a transmembrane region, a co-stimulatory factor signal region, and a CD3 $\zeta$  signaling region.

Figure 3 shows detection of transfection efficiency of engineered T cell with chimeric antigen receptors targeting BCMA. The expression levels of the CAR gene-encoded protein on the surface of the T cell membrane in CAR-BCMA cells cultured on day 7 (Fig. 3A) day 21

(Fig. 3B) and day 29 (Fig. 3C) were identified by Fc fragment staining method of recombinant human BCMA protein.

Figure 4 shows the expression level of CD137 on the surface of T cell membrane (Fig. 4A) and the secretion level of IFN $\gamma$  in the culture supernatant (Fig. 4B). Specifically,  $1 \times 10^5$  of  
 5 CART-BCMA cells cultured on day 7 were cultured respectively with BCMA-positive K562-BCMA-E7 tumor cell line and BCMA-negative K562 tumor cell line, or without tumor cells, in 200  $\mu$ l GT-551 medium for 18h at a ratio of 1:1. Then the expression level of CD137 on the surface of T cell membrane and the secretion level of IFN $\gamma$  in the culture supernatant was detected respectively.

Figure 5 shows detection of advanced apoptosis level of tumor cells induced by CART-BCMAs. Specifically,  $1 \times 10^4$  CFSE-labeled BCMA-negative (NH929) or BCMA-positive (NH929-BCMA) tumor cell lines were co-cultured respectively with  
 10 corresponding T cells in 100  $\mu$ l of GT-551 medium for 4 h at a ratio as shown in the figure. The proportion of PI-positive cells in CFSE-positive cells was analyzed by flow cytometry after staining with 100  $\mu$ l of 25% PI dye for 15 min. The figure shows the statistical analysis of PI  
 15 positive cells in the corresponding co-culture samples.

Figure 6 shows detection of advanced apoptosis level of tumor cells induced by CART-BCMAs. Figure 6A shows ratio of CART positive cells in the analyzed samples, wherein NT and BCMA-MO6 were calculated at 60%.  $1 \times 10^4$  CFSE-labeled BCMA-negative  
 20 (NH929) or BCMA-positive (NH929-BCMA, MM.1S) tumor cell lines were co-cultured respectively with corresponding T cells in 100  $\mu$ l of GT-551 medium for 4 h at a ratio as shown in the figure. The proportion of PI-positive cells in CFSE-positive cells was analyzed by flow cytometry after staining with 100  $\mu$ l of 25% PI dye for 15 min. Figure 6B shows the statistical analysis of PI positive cells in the corresponding co-culture samples.

Figure 7 shows the inhibitory effect of CART-BCMAs on the proliferation *in vivo* of myeloma cell line RPMI-8226 in B-NDG mice. RPMI-8226 cells in logarithmic growth phase were collected, and  $4.0 \times 10^6$  tumor cells were inoculated subcutaneously in the right back of mice. When the tumor volume reached about 120 mm<sup>3</sup>, the animals were randomly divided into  
 25 4 groups according to tumor volume. Then solvent control,  $7.5 \times 10^6$  NT (T cells only) and  $7.5 \times 10^6$  CART-BCMAs cells were injected through tail vein respectively. Figure 7A shows that single injection of CART-BCMA-1 and CART-BCMA-20 through tail vein can effectively inhibit the growth of human myeloma RPMI-8226 cells. Figure 7B shows that CART-BCMA-1 and CART-BCMA-20 can significantly prolong the survival of tumor-bearing mice with human myeloma RPMI-8226 cells.

### Modes for Carrying Out the Present Invention

After extensive and intensive studies and screening, the inventors have obtained a chimeric antigen receptor targeting BCMA antigen for the first time. Specifically, the present invention obtains a chimeric antigen receptor structure targeting BCMA based on four

monoclonal antibody sequences of BCMA-1, BCMA-20, BCMA-CA8, and BCMA-MO6, and completes the analysis and identification of the expression level in primary T cells, *in vitro* activation ability and tumor cell killing efficacy of these chimeric antigen receptors. Studies have shown that the chimeric antigen receptors of the present invention target BCMA positive cells and can be used to treat BCMA positive B cell lymphoma, multiple myeloma, plasma cell leukemia or other diseases.

Specifically, the present invention identifies the correlation between the expression time and the expression intensity of different CAR structures on the surface of the cell membrane after virus infection, and further identifies the difference in expression of different CAR structural proteins. This finding suggests that different CAR structures exist a difference in the expression level of CAR protein on the membrane surface and the persistence of CART *in vivo* activity under same infection condition. After extensive screening, the CAR structure of the present invention was obtained. The results show that the protein encoded by the CAR structure of the present invention can be fully expressed and membrane-localized.

In the present invention, the preparation process of CAR-modified T cell targeting BCMA antigen is improved. Primarily, GT-551 serum-free medium supplemented with 1% human albumin was selected to culture lymphocytes *in vitro*.

#### **Term**

To make the disclosure easier to understand, some terms are firstly defined. As used in this application, unless expressly stated otherwise herein, each of the following terms shall have the meanings given below. Other definitions are set forth throughout the application.

The term "about" may refer to a value or composition within an acceptable error range for a particular value or composition as determined by those skilled in the art, which will depend in part on how the value or composition is measured or determined.

The term "administering" refers to the physical introduction of a product of the invention into a subject using any one of various methods and delivery systems known to those skilled in the art, including intravenous, intramuscular, subcutaneous, intraperitoneal, spinal or other parenteral administration, such as by injection or infusion.

The term "antibody" (Ab) may include, but is not limited to, an immunoglobulin that specifically binds an antigen and contains at least two heavy (H) chains and two light (L) chains linked by disulfide bonds, or an antigen binding parts thereof. Each H chain contains a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region contains three constant domains, CH1, CH2, and CH3. Each light chain contains a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region contains a constant domain CL. The VH and VL regions can be further subdivided into hypervariable regions called complementarity determining regions (CDR), which are interspersed within more conservative regions called framework regions (FR). Each VH and VL contains three CDRs and four FRs, which are arranged from

amino terminal to carboxy terminal in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen.

## 5           **Chimeric antigen receptor (CAR)**

The chimeric antigen receptor (CAR) of the invention comprises an extracellular domain, a transmembrane domain, and an intracellular domain. The extracellular domain comprises a target-specific binding element (also known as an antigen binding domain). The intracellular domain includes a co-stimulatory signaling region and a  $\zeta$  chain. The co-stimulatory signaling region refers to a part of the intracellular domain that includes a co-stimulatory molecule. The co-stimulatory molecule is a cell surface molecule required for efficient response of lymphocytes to antigens, rather than a antigen receptor or its ligand.

A linker can be incorporated between the extracellular domain and the transmembrane domain of the CAR, or between the cytoplasmic domain and the transmembrane domain of the CAR. As used herein, the term "linker" generally refers to any oligopeptide or polypeptide that plays a role of linking the transmembrane domain to the extracellular domain or the cytoplasmic domain in a polypeptide chain. The linker may comprise 0-300 amino acids, preferably 2-100 amino acids and most preferably 3-50 amino acids.

In a preferred embodiment of the invention, the extracellular domain of the CAR provided by the invention comprises an antigen binding domain targeting BCMA. When the CAR of the present invention is expressed in T cell, antigen recognition can be performed based on antigen binding specificity. When the CAR binds to its associated antigen, it affects tumor cell, causing tumor cell to fail to grow, to death or to be affected otherwise, causing the patient's tumor burden to shrink or eliminate. The antigen binding domain is preferably fused to the intracellular domain from one or more of the co-stimulatory molecule and the  $\zeta$  chain. Preferably, the antigen binding domain is fused with an intracellular domain of a combination of a 4-1BB signaling domain and a CD3 $\zeta$  signaling domain.

As used herein, the "antigen binding domain" and "single-chain antibody fragment" refer to a Fab fragment, a Fab' fragment, a F(ab')<sub>2</sub> fragment, or a single Fv fragment that has antigen-binding activity. The Fv antibody contains the heavy chain variable region and the light chain variable region of the antibody, but has no constant region. The Fv antibody has the smallest antibody fragment with all antigen-binding sites. Generally, Fv antibodies also include a polypeptide linker between the VH and VL domains, and can form the structure required for antigen binding. The antigen binding domain is usually a scFv (single-chain variable fragment). The size of scFv is typically 1/6 of a complete antibody. The single-chain antibody is preferably an amino acid chain sequence encoded by a nucleotide chain. As a preferred mode of the present invention, the scFv comprises an antibody which specifically recognizes the extracellular region of BCMA, particularly an antibody which specifically recognizes amino acid residues at positions 24 to 41 of the BCMA sequence, preferably a single chain antibody.

As for the hinge region and the transmembrane region (transmembrane domain), the CAR can be designed to comprise a transmembrane domain fused to the extracellular domain of the CAR. In one embodiment, a transmembrane domain that is naturally associated with one of the domains in the CAR is used. In some embodiments, transmembrane domains may be selected or modified by amino acid substitutions to avoid binding such domains to the transmembrane domain of the same or different surface membrane proteins, thereby minimizing the interaction with other members of the receptor complexes.

The intracellular domain in the CAR of the invention comprises the signaling domain of 4-1BB and the signaling domain of CD3 $\zeta$ .

Preferably, the CAR structure of the present invention comprises a signal peptide, an antigen recognition sequence (antigen-binding domain), a linker region, a transmembrane region, a co-stimulatory factor signal region, and a CD3zeta signaling region ( $\zeta$  chain portion). The order of connection is as follows:

[CD8 S]-[VL-Linker-VH]-[hinge-CD8TM]-[4-1BB]-[CD3zeta]

Specifically, the sequence selected in the present invention is as follows:

(1) The signal peptide is a signal peptide sequence derived from CD8:

MALPVTALLPLALLHAARP (SEQ ID NO: 9)

(2) light chain (VL) sequence of single-chain variable region derived from BCMA-1 antibody:

DIVLTQSPPSLAMS LGKRATISCRASESVTILGSHLIHWYQQKPGQPPTLLIQLASN  
VQTGVPARFSGS GSRTDFTLTIDPVEEDDVAVYYCLQSRTIPRTFGGGTKLEIK (SEQ ID  
NO: 7)

(3) heavy chain (VH) sequence of single-chain variable region derived from BCMA-1 antibody:

QIQLVQSGPELKKPGETVKISCKASGYTFTDYSINWVKRAPGKGLKWMGWINTETREP  
AYAYDFRGRFAFSLETSASTAYLQINNLYEDTATYFCALDYSYAMDYWGQGTSTVTV  
SS (z: 8)

Among them, BCMA-1 is an antibody sequence contained in a published Car-T sequence, and is used as a control in the present application.

(4) light chain (VL) sequence of single-chain variable region derived from BCMA-20 antibody:

DIQMTQSPSSLSASVGDRVITICRASQGISNYLNWYQQKPGKAPKPLIYYTSNLQS  
GVPSRFSGSGSGT DYTLTISSLQPEDFATYYCMGQTISSYTFGQGTKLEIK (SEQ ID NO:  
1)

(5) heavy chain (VH) sequence of single-chain variable region derived from BCMA-20 antibody:

EVQLVESGGGLVQPGGSLRLSCAASGFTFSNFDMAWVRQAPGKGLVWVSSITG  
ADHAIYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCVRHGYDGYHFLFDY  
WGQGTLVTVSS (SEQ ID NO: 2)

(6) light chain (VL) sequence of single-chain variable region derived from BCMA-CA8 antibody:

DIQLTQTTSSLSASLGDRVTISCSASTTTSNYLNWYQQKPDGTVELVIYYTSNLHG  
GGPSRFSGSGSGTDYSLTIGYLEPEDVATYYCQQYRKLPWTFGGGSKLEIKR (SEQ ID  
5 NO: 3)

(7) heavy chain (VH) sequence of single-chain variable region derived from BCMA-CA8 antibody:

EVQLQQSGAVLARPGASVKMSCKGSGYTFTNYWMHWVKQRPGQGLEWIGATY  
RGHSPTYYNQKFKGKAKLTAVTSTSTAYMELSSLTNEDSAVYYCTRGAIYNGYDVL  
10 NWGQGTSLTVSS (SEQ ID NO: 4)

(8) light chain (VL) sequence of single-chain variable region derived from BCMA-MO6 antibody:

DIQMTQSPSSLSASVGDRTITCSASQDISNYLNWYQQKPGKAPKLLIYYTSNLHS  
GVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYRKLPWTFGQGTKLEIKR (SEQ ID  
15 NO: 5)

(9) heavy chain (VH) sequence of single-chain variable region derived from BCMA-MO6 antibody:

QVQLVQSGAEVKKPGSSVKVSCASGGTFSNYWMHWVRQAPGQGLEWMGAT  
YRGHSPTYYNQKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARGAIYDGYDVL  
20 DNWGQGTSLTVSS (SEQ ID NO: 6)

(10) The linker sequence between heavy chain and light chain of BCMA-1 single-chain variable region is:

GSTSGSGKPGSGEGSTKG (SEQ ID NO: 10)

(11) The linker sequence between heavy chain and light chain of BCMA-20,  
25 BCMA-CA8, and BCMA-MO6 single-chain variable region is:

GGGGSGGGGSGGGGS (SEQ ID NO: 11)

(12) Sequence of hinge region and linker region:

FVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD  
(SEQ ID NO: 12)

30 (13) The transmembrane region is a transmembrane region sequence of CD8 (CD8TM) antigen:

IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 13)

(14) The co-stimulatory factor signal region is derived from the sequence of 4-1BB cytoplasmic signaling motif:

35 KRGRKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 14)

(15) The signaling region of CD3 zeta is derived from the sequence of immunoreceptor tyrosine-based activation motif (ITAM) of CD3zeta in the TCR complex:

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPGEMGGKPQRRK  
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA

LPPR (SEQ ID NO: 15)

### **Chimeric antigen receptor T cells (CAR-T cells)**

As used herein, the terms "CAR-T cell", "CAR-T", "CART", "CAR-T cell of the  
5 invention" all refer to the CAR-T cell of the second aspect of the invention.

The present invention relates to the construction of a chimeric antigen receptor structure targeting BCMA, a preparation method of a chimeric antigen receptor engineered T cell targeting BCMA, and activity identification thereof.

### **10 Vector**

The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques.  
15 Alternatively, the gene of interest can be produced synthetically.

The present invention also provides vectors in which the expression cassette of the present invention is inserted. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the advantage over  
20 vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non-proliferating cells, such as hepatocytes. They also have the advantage of low immunogenicity.

In brief summary, the expression cassette or nucleic acid sequence of the invention is typically and operably linked to a promoter, and incorporated into an expression vector. The  
25 vectors can be suitable for replication and integration in eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

The expression constructs of the present invention may also be used for nucleic acid immune and gene therapy, using standard gene delivery protocols. Methods for gene delivery  
30 are known in the art. See, e.g., U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466, incorporated by reference herein in their entireties. In another embodiment, the invention provides a gene therapy vector.

The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a  
35 phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al, (2001 , Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New

York), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326, 193).

A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant virus can then be isolated and delivered to cells of the subject either *in vivo* or *ex vivo*. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivirus vectors are used.

Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-1 10 bp upstream of the start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto. Another example of a suitable promoter is Elongation Growth Factor-1 $\alpha$  (EF- 1 $\alpha$ ). However, other constitutive promoter sequences may also be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters, inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionein promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.



In order to assess the expression of a CAR polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects, the selectable  
 5 marker may be carried on a separate piece of DNA and used in a co- transfection procedure. Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like.

Reporter genes are used for identifying potentially transfected cells and for evaluating the  
 10 functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase,  
 15 beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be  
 20 linked to a reporter gene and used to evaluate agents for the ability to modulate promoter-driven transcription.

Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the  
 25 expression vector can be transferred into a host cell by physical, chemical, or biological means.

Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2001, Molecular Cloning: A  
 30 Laboratory Manual, Cold Spring Harbor Laboratory, New York). A preferred method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection.

Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human cells. Other viral  
 35 vectors can be derived from lentivirus, poxviruses, herpes simplex virus 1, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat, Nos. 5,350,674 and 5,585,362.

Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and

lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. An exemplary colloidal system for use as a delivery vehicle *in vitro* and *in vivo* is a liposome (e.g., an artificial membrane vesicle).

In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a liposome. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (*in vitro*, *ex vivo* or *in vivo*). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

In a preferred embodiment of the invention, the vector is a lentiviral vector.

### **Preparation**

The invention provides a preparation comprising the CAR-T cell according to the first aspect of the invention, and a pharmaceutically acceptable carrier, diluent or excipient. In one embodiment, the preparation is a liquid preparation. Preferably, the preparation is an injection. Preferably, the concentration of the CAR-T cells in the preparation is  $1 \times 10^3$ - $1 \times 10^8$  cells / ml, more preferably  $1 \times 10^4$ - $1 \times 10^7$  cells / ml.

In one embodiment, the preparation may comprises buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. The preparation of the invention is preferably formulated for intravenous administration.

### **Therapeutic application**

The invention comprises therapeutic applications using cells (e.g., T cells) transduced with a lentiviral vector (LV) encoding the expression cassette of the invention. The transduced T cells can target the tumor cell marker BCMA, synergistically activate T cells, and cause T

cell immune responses, thereby significantly increasing the killing efficiency against tumor cells.

Thus, the present invention also provides a method for stimulating a T cell-mediated immune response to a target cell population or tissue in a mammal comprising the step of  
5 administering to the mammal a CAR-T cell of the invention.

In one embodiment, the present invention comprises a class of cell therapies, wherein T cells from autologous patient (or heterologous donor) are isolated, activated and genetically modified to generate CAR-T cells, and then injected into the same patient. The probability of graft versus host disease in the way is extremely low, and antigens are recognized by T cells in  
10 a non-MHC-restricted manner. In addition, one CAR-T can treat all cancers that express the antigen. Unlike antibody therapies, CAR-T cells are able to replicate *in vivo* resulting in long-term persistence that can lead to sustained tumor control

In one embodiment, the CAR-T cells of the invention can undergo robust *in vivo* T cell expansion and can persist for an extended amount of time. In addition, the CAR mediated  
15 immune response may be part of an adoptive immunotherapy approach in which CAR-modified T cells induce an immune response specific to the antigen binding moiety in the CAR. For example, an anti-BCMA CAR-T cell elicits an immune response specific against cells expressing BCMA.

Although the data disclosed herein specifically disclose lentiviral vector comprising  
20 BCMA scFv, hinge and transmembrane domain, and 4-1BB and CD3 $\zeta$  signaling domains, the invention should be construed to include any number of variations for each of the components of the construct as described elsewhere herein.

Cancers that may be treated include tumors that are unvascularized or largely unvascularized, and tumors that are vascularized. Cancers may include non-solid tumors (such  
25 as hematological tumors, for example, leukemias and lymphomas) or solid tumors. Types of cancers to be treated with the CARs of the invention include, but are not limited to, carcinoma, blastoma, and sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies e.g., sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

Hematologic cancers are cancers of the blood or bone marrow. Examples of hematological (or hematogenous) cancers include leukemias, including acute leukemias (such  
30 as acute lymphocytic leukemia, acute myelocytic leukemia, acute myelogenous leukemia and myeloblasts, promyelocytic, myelomonocytic, monocytic and erythroleukemia), chronic leukemias (such as chronic myelocytic (granulocytic) leukemia, chronic myelogenous  
35 leukemia, and chronic lymphocytic leukemia), polycythemia vera, lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma (indolent and high grade forms), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, myelodysplastic syndrome, hairy cell leukemia and myelodysplasia.

Solid tumors are abnormal masses of tissue that usually do not contain cysts or liquid

areas. Solid tumors can be benign or malignant. Different types of solid tumors are named for the type of cells that form them (such as sarcomas, carcinomas, and lymphomas). Examples of solid tumors, such as sarcomas and carcinomas, include fibrosarcoma, myxosarcoma, liposarcoma, mesothelioma, malignant lymphoma, pancreatic cancer and ovarian cancer.

5       The CAR-modified T cells of the invention may also serve as a type of vaccine for *ex vivo* immunization and/or *in vivo* therapy in a mammal. Preferably, the mammal is a human.

With respect to *ex vivo* immunization, at least one of the following occurs *in vitro* prior to administering the cell into a mammal: i) expanding the cells, ii) introducing a nucleic acid encoding a CAR to the cells, and/or iii) cryopreservation of the cells.

10       *Ex vivo* procedures are well known in the art and are discussed more fully below. Briefly, cells are isolated from a mammal (preferably a human) and genetically modified (i.e., transduced or transfected *in vitro*) with a vector expressing a CAR disclosed herein. The CAR-modified cell can be administered to a mammalian recipient to provide a therapeutic benefit. The mammalian recipient may be a human and the CAR-modified cell can be  
15 autologous with respect to the recipient. Alternatively, the cells can be allogeneic, syngeneic or xenogeneic with respect to the recipient.

In addition to using a cell-based vaccine in terms of *ex vivo* immunization, the present invention also provides compositions and methods for *in vivo* immunization to elicit an immune response directed against an antigen in a patient.

20       The present invention provides methods for treating tumors comprising administering to a subject in need thereof, a therapeutically effective amount of the CAR-modified T cells of the invention.

The CAR-modified T cells of the present invention may be administered either alone, or as a pharmaceutical composition in combination with diluents and/or with other components  
25 such as IL-2, IL-17 or other cytokines or cell populations. Briefly, pharmaceutical compositions of the present invention may comprise a target cell population as described herein, in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or  
30 dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present invention are preferably formulated for intravenous administration.

35       Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

When "an immunologically effective amount", "an anti-tumor effective amount", "an

tumor-inhibiting effective amount", or "therapeutic amount" is indicated, the precise amount of the compositions of the present invention to be administered can be determined by a physician with consideration of individual differences in age, weight, tumor size, extent of infection or metastasis, and condition of the patient (subject). It can generally be stated that a pharmaceutical composition comprising the T cells described herein may be administered at a dosage of  $10^4$  to  $10^9$  cells/kg body weight, preferably  $10^5$  to  $10^6$  cells/kg body weight, including all integer values within those ranges. T cell compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al., New Eng. J. of Med. 319: 1676, 1988). The optimal dosage and treatment regime for a particular patient can readily be determined by one skilled in the art of medicine by monitoring the patient for signs of disease and adjusting the treatment accordingly.

The administration of the subject compositions may be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (*i.v.*) injection, or intraperitoneally. In one embodiment, the T cell compositions of the present invention are administered to a patient by intradermal or subcutaneous injection. In another embodiment, the T cell compositions of the present invention are preferably administered by *i.v.* injection. The compositions of T cells may be injected directly into a tumor, lymph node, or site of infection.

In certain embodiments of the present invention, cells activated and expanded using the methods described herein, or other methods known in the art where T cells are expanded to therapeutic levels, are administered to a patient in conjunction with (e.g., before, simultaneously or following) any number of relevant treatment modalities, including but not limited to treatment with agents such as antiviral therapy, cidofovir and interleukin-2, Cytarabine (also known as ARA-C) or natalizumab treatment for MS patients or efalizumab treatment for psoriasis patients or other treatments for PML patients. In further embodiments, the T cells of the invention may be used in combination with chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunotherapeutic agents. In a further embodiment, the cell compositions of the present invention are administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, or the use of chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the present invention. In an additional embodiment, expanded cells are administered before or following surgery.

The dosage of the above treatments to be administered to a patient will vary with the precise nature of the condition being treated and the recipient of the treatment. The scaling of dosages for patient administration can be performed according to art-accepted practices. In general,  $1 \times 10^6$  to  $1 \times 10^{10}$  of the modified T cells of the invention (e.g., CAR-T-BCMA cells) can be applied to patients by means of, for example, intravenous infusion each treatment or each course of treatment.

**The main advantages of the present invention are:**

(a) As for the chimeric antigen receptor of the present invention, the extracellular antigen binding domain is a specific anti-BCMA scFv; the CAR formed by binding the specific anti-BCMA scFv to a specific hinge region and an intracellular domain shows a great ability of killing tumor cells with low cytotoxicity and low side effects.

(b) The chimeric antigen receptor provided by the invention can achieve stable expression and membrane localization of CAR protein after T cells infected by lentivirus carrying CAR gene.

(c) The CAR-modified T cells of the present invention have a longer survival time in vivo and strong anti-tumor effect. The scFv used in the present invention is a humanized or human-derived antibody, and is less likely to produce specific immunological rejection.

The present invention will be further illustrated below with reference to the specific examples. It is to be understood that these examples are for illustrative purposes only and are not intended to limit the scope of the invention. For the experimental methods in the following examples the specific conditions of which are not specifically indicated, they are performed under routine conditions, e.g., those described by Sambrook, et al., in *Molecule Clone: A Laboratory Manual*, New York: Cold Spring Harbor Laboratory Press, 1989, or as instructed by the manufacturers, unless otherwise specified. Percentages and parts are by weight unless otherwise stated.

**Example 1**

**Construction of lentiviral expression vector**

The full-length DNA synthesis and cloning were commissioned by Shanghai Boyi Biotechnology Co., Ltd to achieve the construction of coding plasmids. The pWPT lentiviral vector was selected as a cloning vector, and the cloning sites were BamH I and Sal I sites. The specific sequence is as described above.

**Example 2**

**Preparation of CAR-T cell**

(1) Mononuclear cells (PBMCs) were isolated from venous blood of healthy people by density gradient centrifugation.

(2) On day 0, PBMCs were seeded in a cell culture flask previously coated with CD3 monoclonal antibody (OKT3) at a final concentration of 5  $\mu\text{g/mL}$  and Retronectin (purchased from TAKARA) at a final concentration of 10  $\mu\text{g/mL}$ . The medium was GT-551 cell culture medium containing 1% human albumin. Recombinant human interleukin 2 (IL-2) was added to the medium at a final concentration of 1000 U/mL. The cells were cultured in a CO<sub>2</sub> incubator with a saturated humidity of 5% at 37 °C.

(3) On day 1, the supernatant of the cultured PBMCs was slowly aspirated and discarded. New GT-551 cell culture medium containing 1% human albumin was added, and recombinant human interleukin 2 (IL-2) was added to the medium at a final concentration of 1000 U/mL. The cells were continuously cultured in a CO<sub>2</sub> incubator with a saturated humidity of 5% at 37 °C.

(4) On day 3, fresh medium, concentrated and purified CAR-BCMAs lentivirus solution, protamine sulfate (12  $\mu\text{g/mL}$ ), and IL-2 (at a final concentration of 1000 U/mL) were added. After 12 hours of infection in a 5% CO<sub>2</sub> incubator at 37 °C, the culture medium was discarded, fresh medium was added, and cultivation was continued in a 5% CO<sub>2</sub> incubator at 37 °C.

(5) Starting from day 6, CAR-BCMAs cells can be taken for the corresponding activity assay.

### Example 3

**Detection of the integration rate of the CAR gene in the T cell genome and the expression level of the encoded protein thereof on the membrane surface**

0.5 $\times 10^6$  of CART-BCMAs cell samples cultured on day 7 (Fig. 3A), day 21 (Fig. 3B) and day 29 (Fig. 3C) in Example 2 were taken, respectively. The expression level of CAR-BCMA protein on the surface of T cell membrane was analyzed by flow cytometry after Fc fragment staining of recombinant human BCMA protein.

The result is shown in Fig. 3, and four CAR structures designed in the present invention can be expressed in their corresponding modified T cells and complete the cell membrane surface localization.

### Example 4

**Detection of the in vitro activation ability of CAR-BCMAs**

Cell activation level indicator proteins CD137 and IFN $\gamma$  was detected using CART-BCMAs cells cultured on day 7 in Example 2. 1 $\times 10^5$  of CART-BCMA cells cultured on day 7 were cultured respectively with BCMA-positive K562-BCMA+E7 tumor cell line and BCMA-negative K562 tumor cell line, or without tumor cells, in 200  $\mu\text{L}$  GT-551 medium for 18h at a ratio of 1:1. Then the expression level of CD137 on the surface of T cell membrane was detected by flow cytometry and the secretion level of IFN $\gamma$  in the culture supernatant was detected by ELISA.

The results are shown in Fig. 4A and Fig. 4B, the expression of CD137 was detected on

the surface of four CART cells, and the expression of IFN $\gamma$  was detected in the culture supernatant. Among them, CAR-BCMA-20 shows best CD137 activation level and IFN $\gamma$  release level. CART-BCMA-MO6 which is constructed based on humanized MO6 antibody sequence shows a weaker level of CD137 activation but a higher level of IFN $\gamma$  release than  
 5 CART-BCMA-CA8 which is constructed based on mouse antibody sequence.

### Example 5

#### Detection of advanced apoptosis activity of tumor cells induced by CART-BCMAs cells

10 (1) The CART-BCMAs cells cultured on day 17 in Example 2 were mixed respectively with  $1 \times 10^4$  CFSE-labeled BCMA-negative cells (NH929) or BCMA-positive self-constructed cells (NH929-BCMA overexpressing tumor cell line) at a ratio of 1:1, 2.5:1, 5:1, 10:1, 20:1 (as shown in Fig. 5). The mixed cells were co-cultured in 100  $\mu$ l GT-551 medium for 4 h, and then stained with 100  $\mu$ l 25% PI dye for 15 min. The proportion of PI positive cells in CFSE positive  
 15 cells was analyzed by flow cytometry.

(2) The CART-BCMAs cells cultured on day 22 in Example 2 were mixed respectively with  $1 \times 10^4$  CFSE-labeled BCMA-negative cells (NH929), BCMA-positive self-constructed cells (NH929-BCMA overexpressing tumor cell line) or MM.1S cell line naturally expressed BCMA at a ratio of 1:1, 5:1, 10:1, 10:1, 40:1 (as shown in Fig. 6B). The mixed cells were  
 20 co-cultured in 100  $\mu$ l GT-551 medium for 4 h, and then stained with 100  $\mu$ l 25% PI dye for 15 min. The proportion of PI positive cells in CFSE positive cells was analyzed by flow cytometry.

The results are shown in Fig. 5 and Fig. 6, all four CART cells can induce apoptosis of BCMA-positive tumor cells. Among them, CART-BCMA-20 can induce advanced apoptosis of  
 25 BCMA-positive tumor cells better than CART-BCMA-1. The ability of CART-BCMA-MO6 and CART-BCMA-CA8 to induce advanced apoptosis of BCMA-positive tumor cells is similar.

### Example 6

#### Inhibition of CART-BCMAs on RPMI-8226 myeloma xenograft model

30 RPMI-8226 cells in logarithmic growth phase were collected, and  $4.0 \times 10^6$  tumor cells were inoculated subcutaneously in the right back of 6-8 week old B-NDG mice. When the tumor volume reached about 120 mm<sup>3</sup>, the animals were randomly divided into 4 groups according to tumor volume, so that the tumor volume difference of each group was less than  
 35 10% of the mean value. Then the solvent control,  $7.5 \times 10^6$  NT and  $7.5 \times 10^6$  CART-BCMAs cells were injected through tail vein respectively.

The results are shown in Fig. 7, compared with the control group, single injection of CART-BCMA-1 and CART-BCMA-20 through tail vein can effectively inhibit the growth of human myeloma RPMI-8226 cells (relative tumor proliferation rate %T/CRTV $\leq$ 40%, P<0.05),



and can significantly prolong the survival time of human myeloma-bearing mice (median survival of the control group is 23 days, median survival of the CART-BCMAs treatment group > 33 days). There was no significant difference in tumor proliferation rate and median survival between the mice treated with CART-BCMA-1 and CART-BCMA-20.

5

### **Comparative example**

In the screening process of the chimeric antigen receptor of the present application, the inventors tested a large number of candidate sequences, which are illustrated below with examples.

10 The antibodies to be screened include: BCMA-1, BCMA-2, BCMA-69, BCMA-72, BCMA-2A1, BCMA-1E1, BCMA-J22.9, BCMA-20, BCMA-CA8, and BCMA-MO6. Structures of chimeric antigen receptor targeting BCMA were constructed on the basis of the above antibodies. Among them, BCMA-1 and BCMA-2 are published Car-T sequences and used as a positive control for screening. CAR-T cells were prepared in the same way as in  
15 Example 2, and detected in the same way as in Examples 3 and 4.

The results are shown in Figure 1, which are the experimental results of two batches of CAR-T cells. The expression of Car-T was detected with BCMA-Fc fusion protein. It can be seen that there is a high expression in primary T cells, as seen in Fig. 1A. In Fig. 1B, it can be seen that BCMA-1, BCMA-20, BCMA-1E1, BCMA-CA8, BCMA-MO6, and BCMA-J22.9  
20 can be activated by the BCMA antigen. Fig. 1C shows that the activated BCMA-1, BCMA-20, BCMA-1E1, BCMA-CA8, BCMA-MO6, and BCMA-J22.9 CAR-Ts can produce higher levels of IFN- $\gamma$ . The results show that the CAR-T functions obtained by BCMA-1, BCMA-20, CA8 and MO6 are similar, so BCMA-20, CA8 and MO6CAR-T were further analyzed and studied.

25 All literatures mentioned in the present application are incorporated herein by reference, as though each one is individually incorporated by reference. In addition, it should also be understood that, after reading the above teachings of the present invention, those skilled in the art can make various changes or modifications, equivalents of which falls in the scope of claims as defined in the appended claims.

30

## Claims

1. A chimeric antigen receptor, wherein the antigen binding domain of which is an antibody single chain variable region sequence that targets extracellular region of BCMA.

2. The chimeric antigen receptor of claim 1, wherein the antigen binding domain is an antibody single chain variable region sequence that targets amino acid residues at positions 24 to 41 of the BCMA sequence.

3. The chimeric antigen receptor of claim 1, wherein structure of the antigen binding domain is shown in formula I as below:

$$10 \qquad V_L - V_H \qquad (I)$$

wherein V<sub>H</sub> is an antibody heavy chain variable region; V<sub>L</sub> is an antibody light chain variable region; and “-” is a linker peptide or a peptide bond;

and, the amino acid sequence of V<sub>L</sub> is as shown in SEQ ID NO: 1, and the amino acid sequence of V<sub>H</sub> is as shown in SEQ ID NO: 2;

15 or, the amino acid sequence of V<sub>I</sub> is as shown in SEQ ID NO: 3, and the amino acid sequence of V<sub>II</sub> is as shown in SEQ ID NO: 4;

or, the amino acid sequence of V<sub>L</sub> is as shown in SEQ ID NO: 5, and the amino acid sequence of V<sub>H</sub> is as shown in SEQ ID NO: 6.

4. The chimeric antigen receptor of claim 1, wherein structure of the chimeric antigen  
20 receptor is shown in formula II as below:

$$S-V_L-V_H-H-TM-C-CD3\zeta \quad (II)$$

wherein,

S is an optional signal peptide;

H is a hinge region:

25 TM is a transmembrane domain:

C is a co-stimulatory signaling molecule;

CD3ζ is a cytoplasmic signaling sequence derived from CD3ζ;

$V_H$  and  $V_L$  as described above.

5. A nucleic acid molecule encoding the chimeric antigen receptor of claim 1.

30 6. A vector comprising the nucleic acid molecule of claim 5.

7. A host cell comprising the vector of claim 6 or having an exogenous nucleic acid molecule of claim 5 integrated into a chromosome or expressing the chimeric antigen receptor of claim 1.

8. A method for preparing a CAR-T cell expressing the chimeric antigen receptor of claim 35 1, which comprises a step of:

transducing the nucleic acid molecule of claim 5 or the vector of claim 6 into a T cell, thereby obtaining the CAR-T cell.

9. A preparation comprising the chimeric antigen receptor of claim 1, the nucleic acid molecule of claim 5, the vector of claim 6, or the host cell of claim 7, and a pharmaceutically

acceptable carrier, diluent or excipient.

10. A use of the chimeric antigen receptor of claim 1, the nucleic acid molecule of claim 5, the vector of claim 6, or the host cell of claim 7, for preparing a medicament or a preparation for preventing and/or treating tumor or cancer.

5 11. The use of claim 10, wherein the tumor is BCMA positive tumor.

12. The use of claim 10, wherein the tumor is BCMA positive B cell lymphoma, multiple myeloma, or plasma cell leukemia.

10

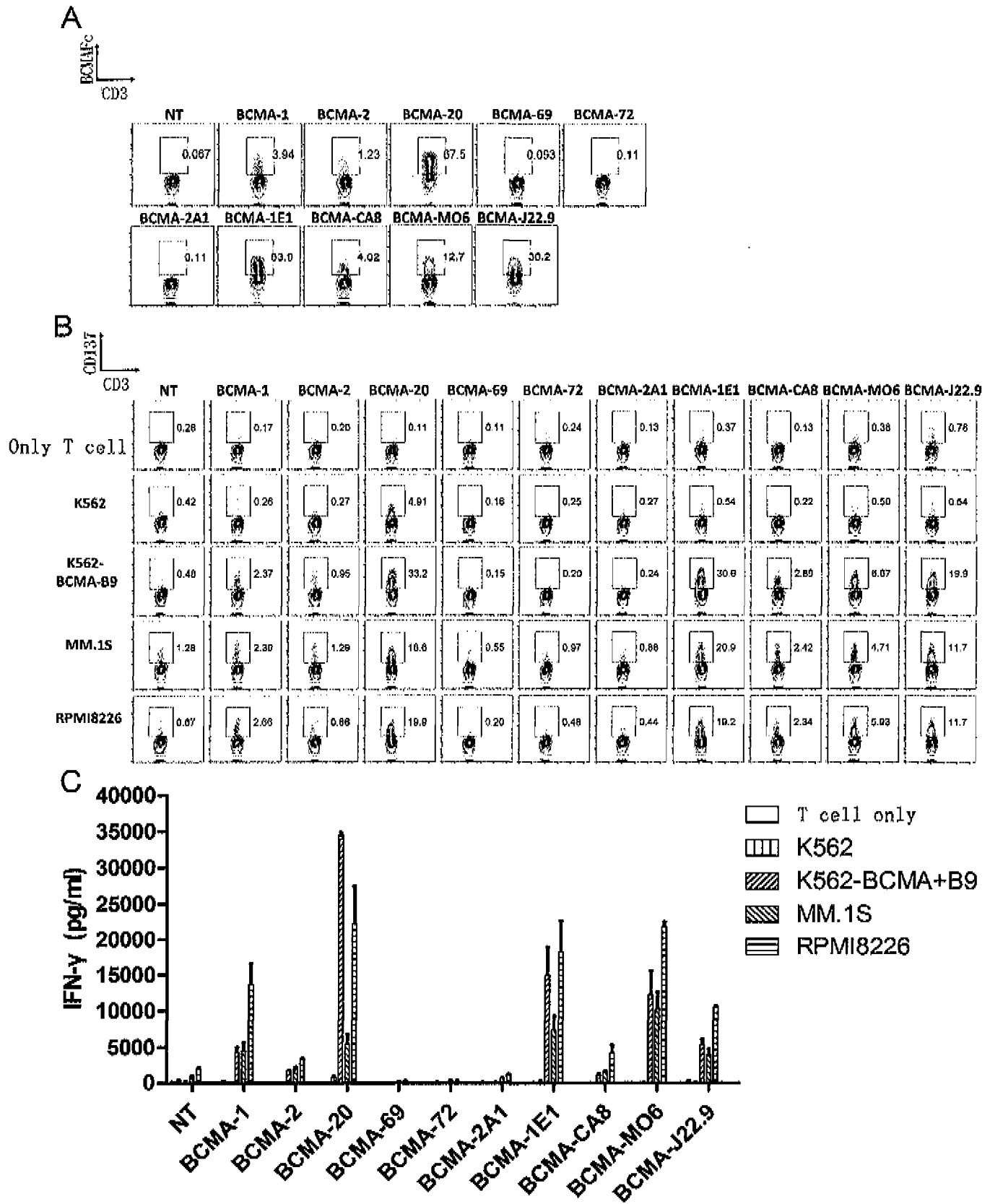
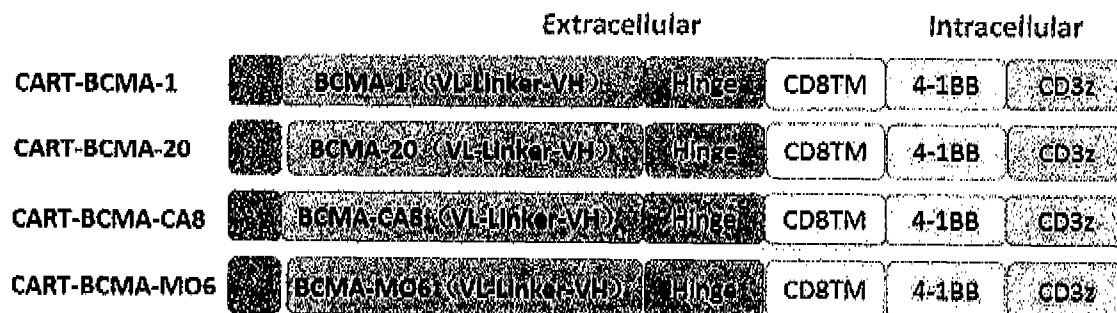


FIG. 1



 CD8 leader sequence

FIG. 2

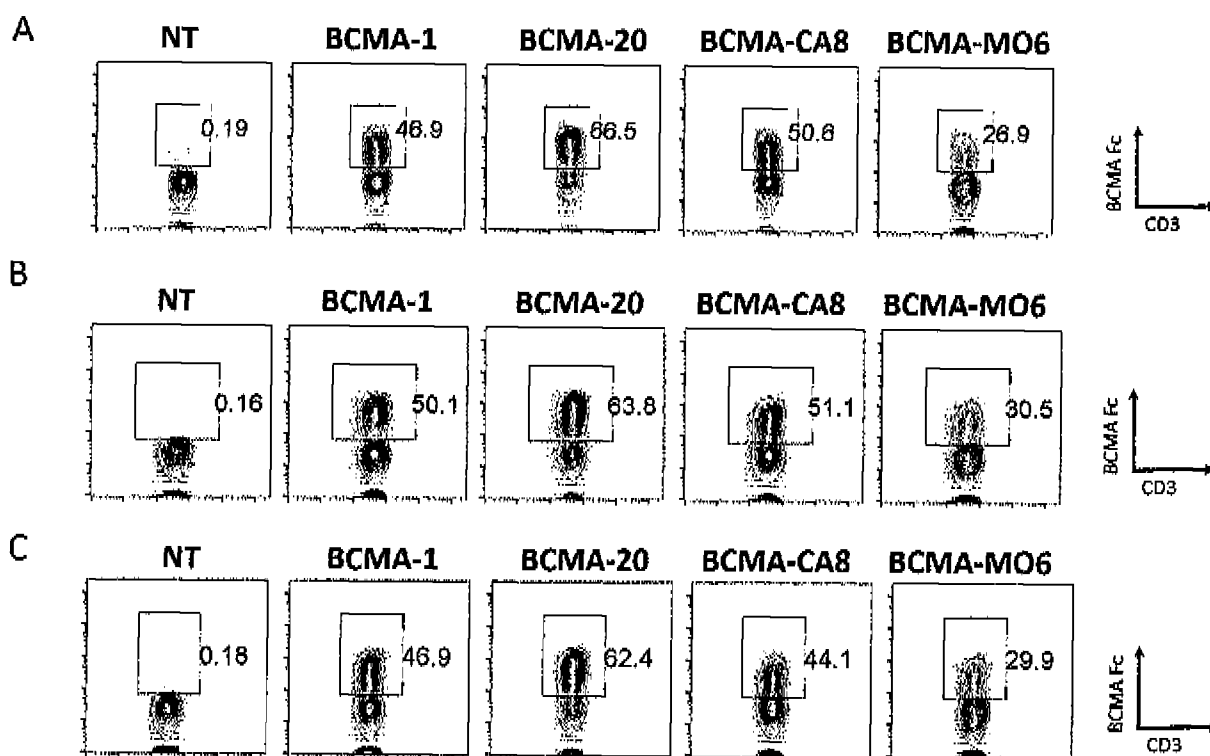


FIG. 3

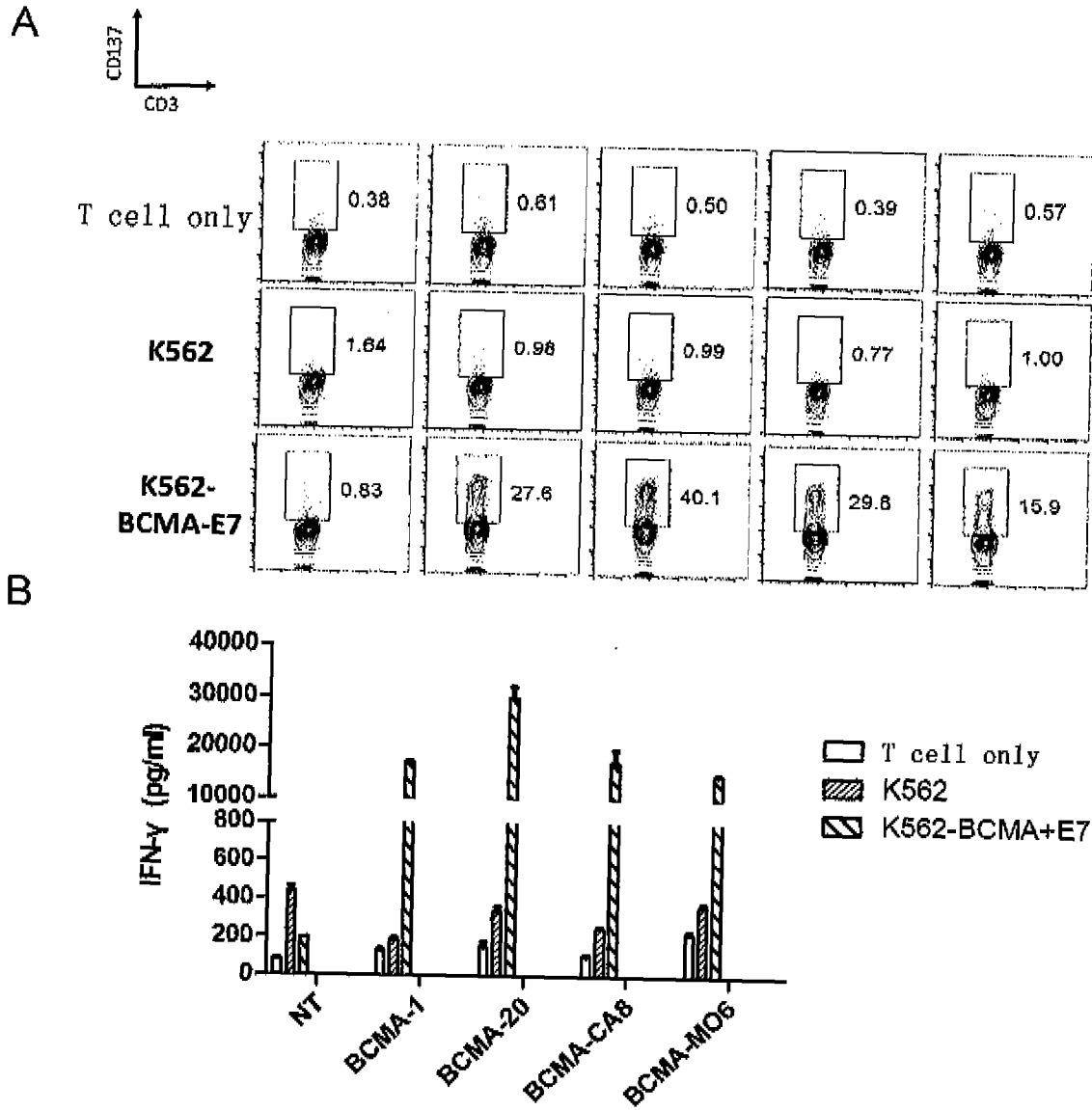


FIG. 4

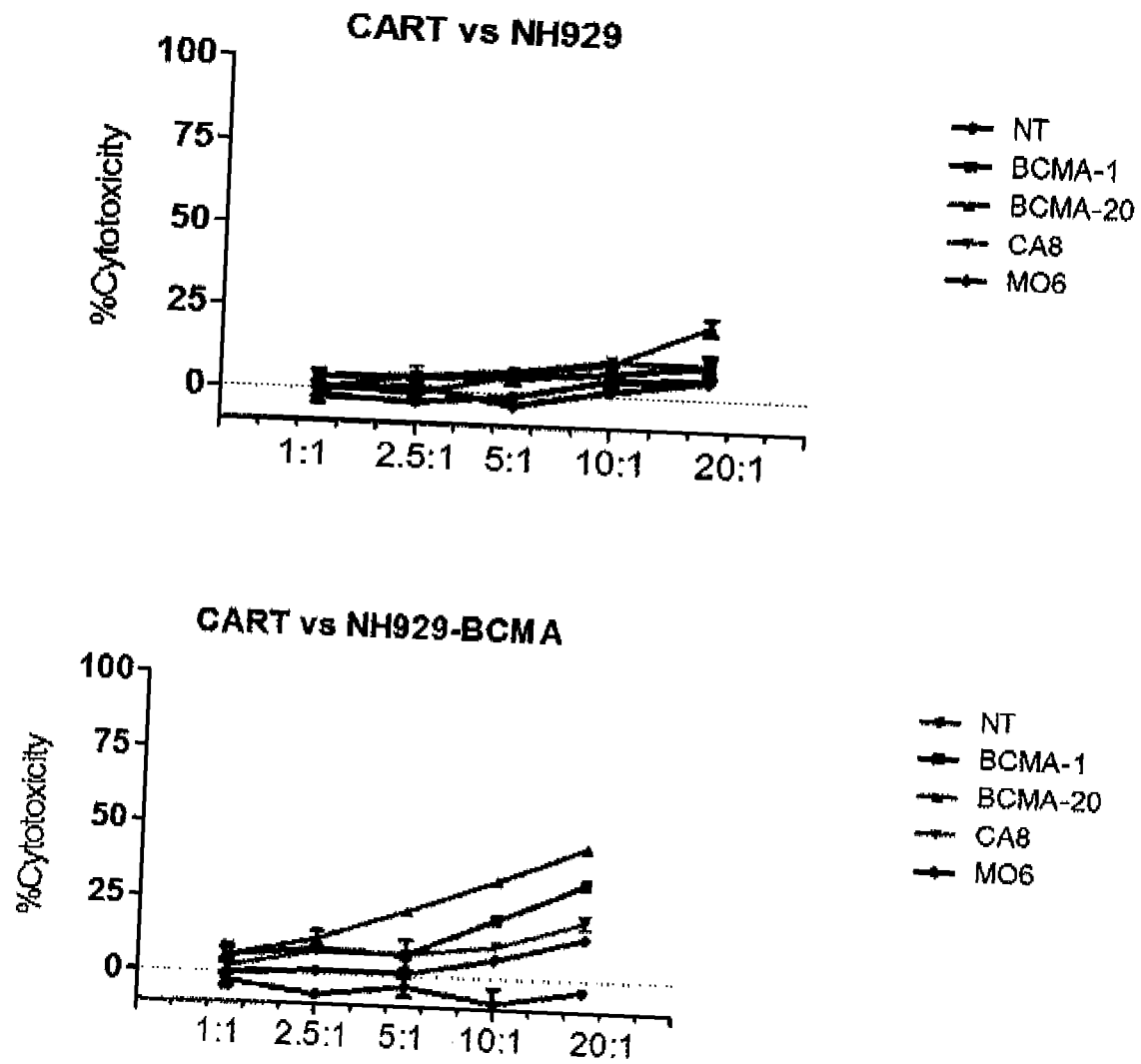


FIG. 5

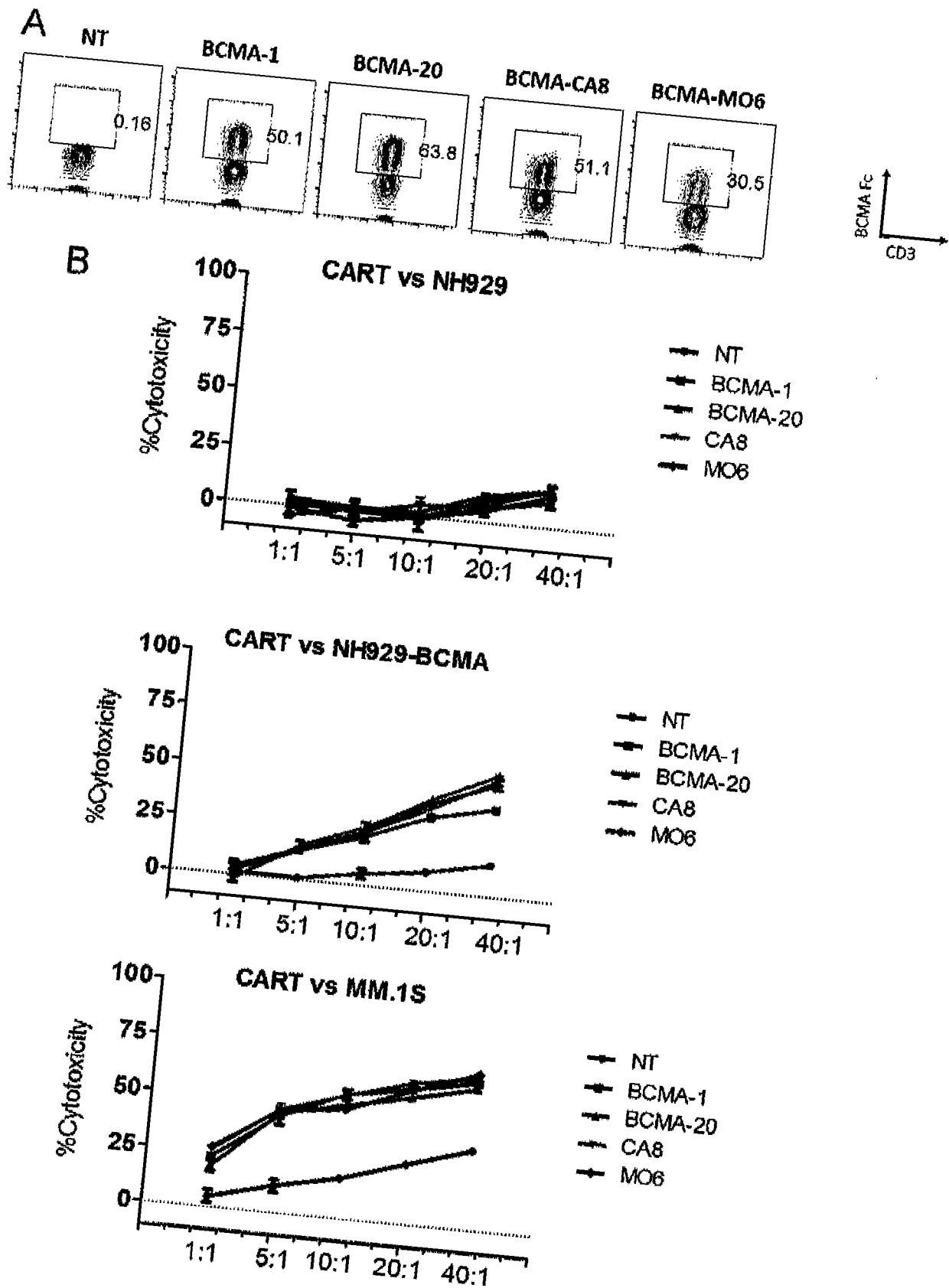


FIG. 6



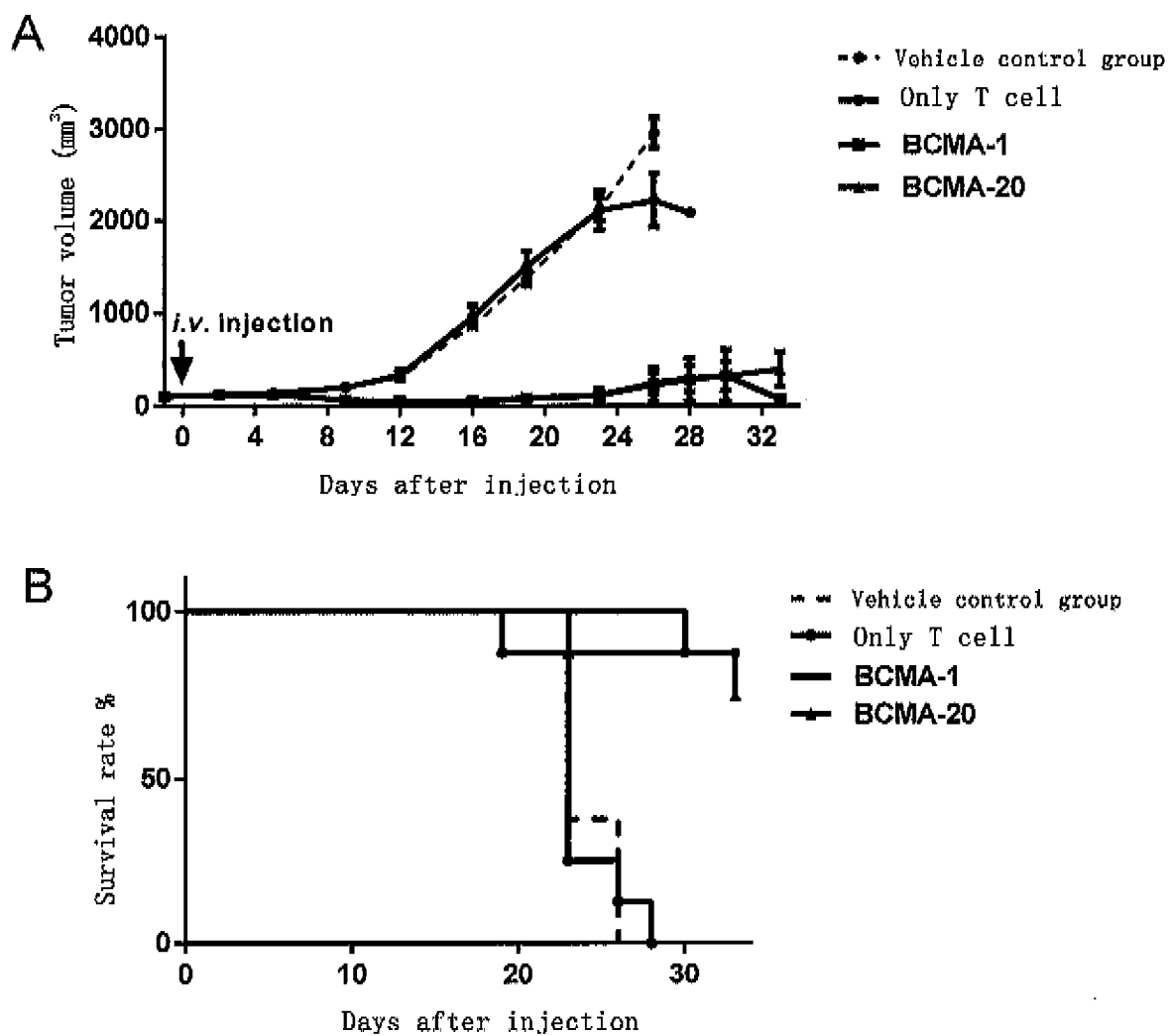


FIG. 7