NOVEL ANTI-BCMA CHIMERIC ANTIGEN RECEPTOR, ITS NUCLEIC ACID SEQUENCE, AND ITS PREPARATION

FIELD OF INVENTION

The present invention relates to novel chimeric antigen receptor polypeptide (CAR), wherein the CAR comprises an extracellular antigen-binding domain, comprising an antibody or antibody fragment thereof that binds to a B-cell Maturation Antigen (BCMA) polypeptide. The invention also relates to a nucleic acid molecule encoding the CAR of the invention, a genetically modified immune cell, expressing the CAR of the invention and the use of said cell in the treatment of various medical disorders.

BACKGROUND OF INVENTION

CAR-T cell therapy is a type of adoptive immunotherapy that has revolutionized the treatment landscape for hematological malignancies. It involves the modification and reprogramming of a patient's own T cells to enhance their cancer-fighting capabilities. The therapy begins with isolation of T cells from patient, which are then genetically engineered to express chimeric antigen receptor (CAR) on their surface. A CAR is a recombinant receptor that possess both, antigen- binding as well as T cell-activating ability. It typically consists of a single-chain variable fragment (scFv) derived from an antibody linked to co-stimulatory and intracellular signaling domain via a hinge and transmembrane domain. These CARs are designed to recognize and bind to specific antigens present on cancer cells. Modified T cells are further expanded to achieve a desired dose and finally infused back into the patient's body, where the CAR-T cells recognize and bind to cancer cells, leading to their destruction. The reprogrammed T cells can proliferate and remain in the patient for several years, as a living drug. Because of this, the technology has several advantages over chemotherapy and antibody-based therapies.

CAR-T cell therapy has shown remarkable success in the treatment of hematological malignancies, particularly in patients with relapsed or refractory diseases. In clinical trials, CAR-T cell therapy has demonstrated high response rates and durable remissions in patients with B-cell lymphoma and leukemia. Recently, this scope of research has been expanded to the field of multiple myeloma. The choice of a suitable target antigen plays a central role in determining the success of CAR-T cell therapy. As plasma B-cells in multiple myeloma have poor CD19 expression, anti-CD19 CAR-T cells developed for B-cell leukemia and lymphoma did not generate encouraging response for myeloma. Therefore, identifying an antigen that is highly expressed on myeloma cells, and minimally expressed on normal tissues is essential to ensure CAR-T cells selectively attack cancer cells while sparing healthy cells.

B-cell Maturation Antigen (BCMA), a member of tumor necrosis factor receptor superfamily 17 (TNFRSF17), is now being investigated as a potential antigen for multiple myeloma. BCMA is expressed selectively on plasma cells, with higher levels of expression on malignant plasma cells as compared to normal plasma cells. As BCMA is exclusively expressed on the surface of plasmablasts and differentiated plasma cells, but not on other hematopoietic stem cells and normal tissue cells, it is regarded as an ideal target antigen.

US20120082661A1 and US20190359726A1 relates to antibodies that recognize the B-cell Maturation Antigen (BCMA) and that bind naïve B cells, plasma cells, and/or memory B cells.

Several on-going trials are being conducted to evaluate the efficacy of BCMA-directed CAR-T cells against multiple myeloma. So far, an overall response rates (ORR) ranging from 63% to 95% have been reported [*Mailankody, S., et al., JCARH125, anti-BCMA CAR-T cell therapy for relapsed/refractory multiple myeloma: initial proof of concept results from a phase 1/2 multicenter study (EVOLVE). 2018. 132: p. 957; Roex, G., et al., Safety and clinical efficacy of BCMA CAR-T cell therapy in multiple myeloma. 2020. 13(1): p. 1-14; Wang, B.-Y., et al., Long-term follow-up of a phase 1, first-in-human open-label study of LCAR- B38M,*

a structurally differentiated chimeric antigen receptor T (CAR-T) cell therapy targeting B- cell maturation antigen (BCMA), in patients (pts) with relapsed/refractory multiple myeloma (RRMM). 2019, American Society of Hematology Washington, DC].

These findings are noteworthy considering that most patients eligible for early trials had previously failed multiple chemotherapy regimens, including stem cell transplant in a few cases, and had lower chances of survival. The remarkable performance of two anti-BCMA CAR-T products i.e. Idecabtagene vicleucel and Ciltacabtagene autoleucel in clinical study resulted in their USFDA approval for the treatment of adults with r/r multiple myeloma. Considering the impressive success of CAR-T cell responses, several groups around the world have shifted their focus to CARs as the preferred approach for improving outcomes in previously known fatal situations, as well as exploring their utility in earlier stages of the treatment timeline. It represents a significant advancement in the fight against this disease.

Limitations

Despite showing promise in the treatment of hematological malignancies, CAR-T cell therapy does have several limitations. Severe life-threatening toxicities such as CRS and neurotoxicity can be considered as some of the common barriers to effective CAR-T cell therapy. This further requires special intensive care units, additional diagnostics, and drugs for management. The majority of CARs developed clinically today utilize scFv-derived from mouse monoclonal antibody (mAb). A thorough literature survey indicates that the presence of non-human components in CAR design, such as domain derived from mouse monoclonal antibodies, have been known to be immunogenic due to which the patients might end up rejecting the therapeutic cells [*Khan, A., et al., Immunogenicity of CAR-T Cell Therapeutics: Evidence, Mechanism and Mitigation. 2022*].

For an effective treatment response, it is essential that the generated CAR-T cells persist for a longer duration without inducing toxicity. Furthermore, such

incidences limit the overall clinical outcome of the therapy and render repeat dosing difficult. Developing strategies to prevent relapse and enhance long-term remission rates remains an ongoing challenge. Additionally, a single dose of CAR-T cell therapy costs as high as $1 million or more, depending upon the severity of side effects. This also serves as a significant impediment to effective implementation, limiting patient access in low- and middle-income countries (LMICs). Addressing these limitations and further optimizing CAR-T cell therapy will be crucial to maximizing its potential and making it a more widely accessible and effective treatment option for hematological malignancies.

B cell malignancies such as multiple myeloma, is an incurable hematologic disease. Current therapies often cause remissions, but nearly all patients eventually relapse and die. CAR-T cell therapy has emerged as a promising treatment modality for hematological malignancies, including multiple myeloma. However, several limitations have been identified, which hinder its widespread adoption and effectiveness. Such limitations therefore necessitate the development of affordable CAR-T treatments that offer improved efficacy and reduced toxicities. The present inventors have surprisingly developed novel chimeric antigen receptor polypeptide (CAR), wherein the CAR comprises an extracellular antigen-binding domain, comprising an antibody or antibody fragment thereof that binds to a B-cell Maturation Antigen (BCMA) polypeptide, and which ameliorates the aforesaid shortcomings of the prior arts.

OBJECTS OF THE INVENTION

It is an object of the present invention to overcome the drawbacks of the aforesaid prior arts.

It is an object of the present invention to provide anti-BCMA chimeric antigen receptor polypeptide (CAR).

It is an object of the present invention to provide murine anti-BCMA chimeric antigen receptor polypeptide (mCAR).

It is another object of the present invention to provide nucleic acid molecule encoding the murine anti-BCMA CAR of the invention, a genetically modified immune cell, expressing the CAR of the invention and the use of said cell in the treatment of various medical disorders.

It is yet another object of the present invention to provide humanized anti-BCMA chimeric antigen receptor polypeptide (hCAR).

It is yet another object of the present invention to provide nucleic acid molecule encoding the humanized anti-BCMA CAR of the invention, a genetically modified immune cell, expressing the CAR of the invention and the use of said cell in the treatment of various medical disorders.

It is yet another object of the present invention to provide a kit comprising the anti-BCMA CAR of the invention.

It is yet another object of the present invention to provide a pharmaceutical composition comprising the anti-BCMA CAR of the invention.

It is yet another object of the present invention to provide use of anti-BCMA CAR according to present invention in the manufacture of a medicament for use in preventing and/or treating various medical disorders in a subject (eg, a human).

SUMMARY OF THE INVENTION

According to an aspect of the present invention there is provided a recombinant nucleic acid molecule encoding an anti-BCMA chimeric antigen receptor (CAR).

According to another aspect of the present invention there is provided an anti-BCMA CAR polypeptide.

According to yet another aspect of the present invention there is provided a vector comprising the nucleic acid molecule encoding an anti-BCMA chimeric antigen receptor (CAR).

According to yet another aspect of the present invention there is provided a fusion recombinant protein comprising an anti-BCMA binding domain (scFv) fused to the constant region of an antibody (Fc).

According to yet another aspect of the present invention there is provided a linker of the anti-BCMA binding domain (scFv).

According to yet another aspect of the present invention there is provided an immune cell comprising the vector or expressing the anti-BCMA CAR polypeptide.

According to yet another aspect of the present invention there is provided a kit comprising the anti-BCMA CAR of the present invention.

According to yet another aspect of the present invention there is provided a pharmaceutical composition comprising the recombinant nucleic acid molecule or the CAR polypeptide or the vector or the immune cell, with a pharmaceutically acceptable carrier, diluent, or excipient, and optionally one or more further pharmaceutically active polypeptides and/or compounds.

According to yet another aspect of the present invention there is provided a method of preparing an anti-BCMA CAR.

According to yet another aspect of the present invention there is provided a method of treating a subject having a disease associated with expression of BCMA.

BRIEF DESCRIPTION OF THE DRAWINGS

The above and other aspects, features, and advantages of certain exemplary embodiments of the present invention will be more apparent from the following description taken in conjunction with the accompanying drawings wherein:

Figure 1 shows schematic representation of anti-BCMA CAR constructs: murine anti-BCMA CARs and humanized anti-BCMA CARs

Figure 2 shows that anti-BCMA CAR expression on surface of transduced-T cells were detected by flow cytometric analysis with Protein L staining.

Figure 3 shows the expansion kinetics of anti-BCMA CAR expressing T cells generated from healthy donor.

Figure 4 shows the antigen-dependent Cytotoxicity assay of anti-BCMA CAR-T cells against multiple myeloma cell line (RPMI8226), transgenic BCMA-positive cell line (Nalm6-BCMA) and non-myeloma cell lines (Nalm6 and K562). Effector (CAR-T) and Target (Tumor) cell were co-cultured at effector-to-target (E: T) ratio of 1:1 for 24 hours and the percentage killing was evaluated using FACS analysis.

Figure 5 shows the cytokine profile of anti-BCMA CAR-T cells after 24hours of co-culture with BCMA-positive target cells and BCMA-negative control cells.

(A) Bar graph depicting the concentration of Interferon-gamma (IFN-γ) secreted into the supernatant of co-cultures containing anti-BCMA CAR-expressing T cells and target cells after 24 hours of incubation

(B) Bar graph illustrating the concentration of Tumor necrosis factor-alpha (TNF-α) in the supernatant from the same co-cultures

(C) Bar graph showing the concentration of Interleukin-2 (IL-2) secreted into the supernatant after 24 hours

Figure 6 shows the *in vitro* persistence and functional capacity of anti-BCMA CAR-T cells subjected to multiple rounds of antigen challenge. CAR-T cells were co-cultured with BCMA-positive tumor cells at 1:1 ratio without any exogenous IL-2 support. After an interval of 3 days, CAR-T cells were re-stimulated with BCMA-positive tumor cells. Percentage killing was evaluated using FACS analysis, and the cells were counted using the trypan blue dye exclusion method.

(A) Graph depicting the proliferation dynamics of anti-BCMA CAR-T cells after each round of antigen stimulation

(B) Bar graph illustrating the percentage of tumor cell killing achieved after each interval

Figure 7 details the *in vivo* efficacy of anti-BCMA CAR-T cells.

(A) Schematic of experimental design to study the *in vivo* functional efficacy of anti-BCMA CAR-T cells in NOD/SCID mice

(B) Cumulative analysis of tumor burden (total flux in photons/sec unit) in untreated, mock controls and mice treated with anti-BCMA CAR-T cells until day 30. The disease progression was monitored by bioluminescence weekly

(C) Overall survival of untreated, mock controls, and mice treated with anti-BCMA CAR-T cells.

DETAILED DESCRIPTION OF THE INVENTION

The following description with reference to the accompanying drawings is provided to assist in a comprehensive understanding of exemplary embodiments of the invention. It includes various specific details to assist in that understanding but these are to be regarded as merely exemplary.

Accordingly, those of ordinary skill in the art will recognize that various changes and modifications of the embodiments described herein can be made without departing from the scope of the invention. In addition, descriptions of well-known functions and constructions are omitted for clarity and conciseness.

The terms and words used in the following description and claims are not limited to the bibliographical meanings, but, are merely used by the inventor to enable a clear and consistent understanding of the invention. Accordingly, it should be apparent to those skilled in the art that the following description of exemplary embodiments of the present invention are provided for illustration purpose only and not for the purpose of limiting the scope of the invention as defined by the appended claims and their equivalents.

It is to be understood that the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise.

Features that are described and/or illustrated with respect to one embodiment may be used in the same way or in a similar way in one or more other embodiments and/or in combination with or instead of the features of the other embodiments.

It should be emphasized that the term “comprises/comprising” when used in this specification is taken to specify the presence of stated features, steps or components but does not preclude the presence or addition of one or more other features, steps, components, or groups thereof.

The term “Chimeric Antigen receptor” (CAR) as used herein means a recombinant receptor that possess both, antigen- binding as well as T cell-activating ability. It typically consists of a single-chain variable fragment (scFv) derived from an antibody linked to co-stimulatory and intracellular signaling domain via a hinge and transmembrane domain.

An "intracellular signaling domain," as the term is used herein, refers to an intracellular portion of a molecule. In embodiments, the intracellular signal domain transduces the effector function signal and directs the cell to perform a specialized function. The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CAR-T cell.

The term "costimulatory molecule" refers to the cognate binding partner on a T cell that specifically binds with a costimulatory ligand, thereby mediating a costimulatory response by the T cell, such as, but not limited to, proliferation. Costimulatory molecules are cell surface molecules other than antigen receptors or their ligands that are required for an efficient immune response. A costimulatory intracellular signaling domain refers to the intracellular portion of a costimulatory molecule.

"Immune effector cell," as that term is used herein, refers to a cell that is involved in an immune response, e.g., in the promotion of an immune effector response. Examples of immune effector cells include T cells, e.g., alpha/beta T cells and gamma/delta T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells, and myeloid-derived phagocytes.

The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines.

The term "antibody," as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin molecule, which specifically binds with an antigen.

The term "scFv" refers to a fusion protein comprising at least one antibody fragment comprising a variable region of a light chain and at least one antibody fragment comprising a variable region of a heavy chain, wherein the light and heavy chain variable regions are contiguously linked via a short flexible polypeptide linker, and capable of being expressed as a single chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein an scFv may have the VL and VH variable regions in either order, e.g., with respect to the N-terminal and C-terminal ends of the polypeptide, the scFv may comprise VL-linker-VH or may comprise VH-linker-VL.

The terms "complementarity determining region" or "CDR," as used herein, refer to the sequences of amino acids within antibody variable regions which confer antigen specificity and binding affinity. For example, in general, there are three CDRs in each heavy chain variable region (e.g., HCDR1, HCDR2, and HCDR3) and three CDRs in each light chain variable region (LCDR1, LCDR2, and LCDR3).

The term "antigen" or "Ag" refers to a molecule that provokes an immune response. This immune response may involve either antibody production, or the activation of specific immunologically-competent cells, or both. The skilled artisan will understand that any macromolecule, including virtually all proteins or peptides, can serve as an antigen.

The term "isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated," but the same nucleic acid or peptide partially or completely separated

from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

A “murine” antibody is an immunoglobulin including a murine scFv. The VL and VH regions are of mouse origin. The framework (FR) region and CDRs are both of mouse origin.

A "humanized" antibody is an immunoglobulin including a human framework region (FR) and one or more CDRs from a non-human (for example a mouse, rat, or synthetic) immunoglobulin. The non-human immunoglobulin providing the CDRs is termed a "donor," and the human immunoglobulin providing the framework is termed an "acceptor."

The term "sequence identity" as used herein, refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity" may be calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base or the identical amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters. Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul et al., (1977) Nuc. Acids Res. 25:3389-3402; and Altschul et al., (1990) J. Mol. Biol. 215:403-410, respectively. Software for performing

BLAST analyses is publicly available through the National Centre for Biotechnology Information.

Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith and Waterman, (1970) Adv. Appl. Math. 2:482c, by the homology alignment algorithm of Needleman and Wunsch, (1970) J. Mol. Biol. 48:443, by the search for similarity method of Pearson and Lipman, (1988) Proc. Nat'l. Acad. Sci. USA 85:2444, by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection (see, e.g., Brent et al., (2003) Current Protocols in Molecular Biology).

The present invention relates to novel chimeric antigen receptor polypeptide (CAR), wherein the CAR comprises an extracellular antigen-binding domain, comprising an antibody or antibody fragment that binds to a B Cell Maturation Antigen (BCMA) polypeptide. The invention also relates to a nucleic acid molecule encoding the CAR of the invention, a genetically modified immune cell, expressing the CAR of the invention and the use of said cell in the treatment of various medical disorders.

In an embodiment of the present invention there is provided an anti-BCMA CAR polypeptide.

The anti-BCMA CAR comprises:

(a) a single chain antibody or single chain antibody fragment comprising a humanized anti-BCMA binding domain;
(b) a hinge region;
(c) a transmembrane domain; and
(d) a cytoplasmic domain comprising a costimulatory domain and a signaling domain.

Wherein said humanized anti-anti-BCMA binding domain is scFv comprising HCVR and LCVR attached by a linker.

The HCVR of the anti-BCMA binding domain comprises: a heavy chain framework region 1 (HFR1); a heavy chain framework region 2 (HFR2); a heavy chain framework region 3 (HFR3); and a heavy chain framework region 4 (HFR4), while the LCVR comprises: a light chain framework region 1 (LFR1), a light chain framework region 2 (LFR2), a light chain framework region 3 (LFR3) and a light chain framework region 4 (LFR4).

The HCVR of the anti-BCMA binding domain also comprises a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), a heavy chain complementary determining region 3 (HC CDR3), and similarly the LCVR comprises a light chain complementary determining region 1 (LC CDR1), a light chain complementary determining region 2 (LC CDR2), and a light chain complementary determining region 3 (LC CDR3).

The complementary determining regions of the HCVR and LCVR of the BCMA binding domain scFv of the present anti-BCMA CAR polypeptide is based on C11D5.3 antibody, while the framework regions of the HCVR and LCVR of the BCMA binding domain scFv is based on murine or human antibody.

In an embodiment, the said HCVR comprises a heavy chain complementary determining region 1 (HC CDR1) having nucleotide sequence of SEQ ID No:54, a heavy chain complementary determining region 2 (HC CDR2) having nucleotide sequence of SEQ ID No:55, a heavy chain complementary determining region 3 (HC CDR3) having nucleotide sequence of SEQ ID No:56.

In an embodiment, the said HCVR comprises a heavy chain complementary determining region 1 (HC CDR1) having amino acid sequence of SEQ ID No:51, a heavy chain complementary determining region 2 (HC CDR2) having amino acid

sequence of SEQ ID No:52, a heavy chain complementary determining region 3 (HC CDR3) having amino acid sequence of SEQ ID No: 53.

In an embodiment, the said LCVR comprises: a light chain complementary determining region 1 (LC CDR1) having nucleotide sequence of SEQ ID No: 38, a light chain complementary determining region 2 (LC CDR2) having nucleotide sequence of SEQ ID No:39, and a light chain complementary determining region 3 (LC CDR3) having nucleotide sequence of SEQ ID No: 40.

In an embodiment, the said LCVR comprises: a light chain complementary determining region 1 (LC CDR1) having amino acid sequence of SEQ ID No: 35, a light chain complementary determining region 2 (LC CDR2) having amino acid sequence of SEQ ID No: 36, and a light chain complementary determining region 3 (LC CDR3) having amino acid sequence of SEQ ID No: 37.

In a preferred embodiment the anti-BCMA CAR polypeptide comprises a BCMA binding domain scFv having HCVR and LCVR, where the HCVR and LCVR comprises framework regions from a murine antibody.

In a preferred embodiment the anti-BCMA CAR polypeptide comprises a BCMA binding domain scFv having HCVR and LCVR, where the HCVR and LCVR comprises framework regions from a human antibody.

Murine anti-BCMA Chimeric Antigen Receptor:

The BCMA binding domain scFv having murine antibody-based framework regions comprises a HCVR comprising a heavy chain framework region 1 (HFR1) having nucleotide sequence of SEQ ID No: 47; a heavy chain framework region 2 (HFR2) having nucleotide sequence of SEQ ID No: 48; a heavy chain framework region 3 (HFR3) having nucleotide sequence of SEQ ID No: 49; a heavy chain framework region 4 (HFR4) having nucleotide sequence of SEQ ID No: 50.

The HCVR of the BCMA binding domain scFv having murine antibody-based framework regions, comprises a heavy chain framework region 1 (HFR1) having amino acid sequence of SEQ ID No: 43, a heavy chain framework region 2 (HFR2) having amino acid sequence of SEQ ID No: 44, a heavy chain framework

region 3 (HFR3) having amino acid sequence of SEQ ID No: 45; a heavy chain framework region 4 (HFR4) having amino acid sequence of SEQ ID No: 46.

The LCVR of the BCMA binding domain scFv having murine antibody-based framework regions, comprises a light chain framework region 1 (LFR1) having nucleotide sequence of SEQ ID No: 31, a light chain framework region 2 (LFR2) having nucleotide sequence of SEQ ID No: 32, a light chain framework region 3 (LFR3) having nucleotide sequence of SEQ ID No: 33 and a light chain framework region 4 (LFR4) having nucleotide sequence of SEQ ID No: 34.

The LCVR of the BCMA binding domain scFv having murine antibody-based framework regions, comprises a light chain framework region 1 (LFR1) having amino acid sequence of SEQ ID No: 27, a light chain framework region 2 (LFR2) having amino acid sequence of SEQ ID No: 28, a light chain framework region 3 (LFR3) having amino acid sequence of SEQ ID No: 29 and a light chain framework region 4 (LFR4) having amino acid sequence of SEQ ID No: 30.

The heavy chain variable region (HCVR) of the BCMA binding domain scFv having murine antibody-based framework regions has a nucleotide sequence selected from SEQ ID No:42 and an amino acid sequence selected from SEQ ID No. 41.

The light chain variable region (LCVR) of the BCMA binding domain scFv having murine antibody-based framework regions has a nucleotide sequence selected from SEQ ID No: 26 and an amino acid sequence of SEQ ID No: 25.

In an embodiment, the BCMA binding domain scFv having murine antibody-based framework regions has a nucleotide sequence selected from SEQ ID No. 58 or nucleotide sequence with at least 95% identity thereof, and an amino acid sequence selected from SEQ ID No.57.

Humanized anti-BCMA Chimeric Antigen Receptor:

In another embodiment, the present invention provides a solution to the problem of rejection of therapeutic cells containing non-human based antibodies by providing a BCMA binding domain scFv with a human antibody-based

framework regions, where the BCMA binding domain scFv comprises complementary determining regions of murine based C11D5.3 CAR antibody.

Thus, there is provided a humanized anti-BCMA CAR polypeptide.

The BCMA binding domain scFv having human antibody-based framework regions comprises a HCVR comprising a heavy chain framework region 1 (HFR1) having nucleotide sequence of SEQ ID No: 81; a heavy chain framework region 2 (HFR2) having nucleotide sequence of SEQ ID No: 82; a heavy chain framework region 3 (HFR3) having nucleotide sequence of SEQ ID No: 83; a heavy chain framework region 4 (HFR4) having nucleotide sequence of SEQ ID No: 84.

The HCVR of the BCMA binding domain scFv having human antibody-based framework regions, comprises a heavy chain framework region 1 (HFR1) having amino acid sequence of SEQ ID No: 77, a heavy chain framework region 2 (HFR2) having amino acid sequence of SEQ ID No: 78, a heavy chain framework region 3 (HFR3) having amino acid sequence of SEQ ID No: 79; a heavy chain framework region 4 (HFR4) having amino acid sequence of SEQ ID No: 80.

The LCVR of the BCMA binding domain scFv having human antibody-based framework regions, comprises a light chain framework region 1 (LFR1) having nucleotide sequence of SEQ ID No:71, a light chain framework region 2 (LFR2) having nucleotide sequence of SEQ ID No: 72, a light chain framework region 3 (LFR3) having nucleotide sequence of SEQ ID No: 73 and a light chain framework region 4 (LFR4) having nucleotide sequence of SEQ ID No:74.

The LCVR of the BCMA binding domain scFv having human antibody-based framework regions, comprises a light chain framework region 1 (LFR1) having amino acid sequence of SEQ ID No: 67, a light chain framework region 2 (LFR2) having amino acid sequence of SEQ ID No: 68, a light chain framework region 3 (LFR3) having amino acid sequence of SEQ ID No: 69 and a light chain framework region 4 (LFR4) having amino acid sequence of SEQ ID No: 70.

In an embodiment the BCMA binding domain scFv having human antibody-based framework regions comprises a HCVR comprising a heavy chain complementary determining region (HCDR1) having nucleotide sequence of SEQ

ID No: 54; a heavy chain complementary determining region 2 (HCDR2) having nucleotide sequence of SEQ ID No: 55; and a heavy chain complementary determining region 3 (HCDR3) having nucleotide sequence selected from SEQ ID No: 56, and SEQ ID No: 108.

The BCMA binding domain scFv having human antibody-based framework regions comprises a HCVR comprising a heavy chain complementary determining region 1 (HCDR1) having amino acid sequence of SEQ ID No: 51; a heavy chain complementary determining region 2 (HCDR2) having amino acid sequence of SEQ ID No: 52; and a heavy chain complementary determining region 3 (HCDR3) having amino acid sequence selected from SEQ ID No: 53, and SEQ ID No: 107.

The BCMA binding domain scFv having human antibody-based framework regions comprises a LCVR comprising a light chain complementary determining region (LCDR1) having nucleotide sequence of SEQ ID No: 38; a light chain complementary determining region 2 (LCDR2) having nucleotide sequence of SEQ ID No: 39; and a light chain complementary determining region 3 (LCDR3) having nucleotide sequence selected from SEQ ID No: 40, and SEQ ID No: 101.

The BCMA binding domain scFv having human antibody-based framework regions comprises a LCVR comprising a light chain complementary determining region 1 (LCDR1) having amino acid sequence selected from SEQ ID No: 35 and SEQ ID No.: 98; a chain complementary determining region 2 (LCDR2) having amino acid sequence of SEQ ID No: 36; and a light chain complementary determining region 3 (LCDR3) having amino acid sequence selected from SEQ ID No: 37, and SEQ ID No: 99.

The heavy chain variable region (HCVR) of the BCMA binding domain scFv having human antibody-based framework regions has a nucleotide sequence selected from SEQ ID No:76 and SEQ ID No.: 106; while the amino acid sequences are selected from SEQ ID No. 75 and SEQ ID No.: 105.

The light chain variable region (LCVR) of the BCMA binding domain scFv having human antibody-based framework regions has a nucleotide sequence selected from SEQ ID No: 66 and SEQ ID No:97, while the amino acid sequences are selected from SEQ ID No: 65 and SEQ ID No:96.

In an embodiment, the BCMA binding domain scFv having human antibody-based framework regions has a nucleotide sequence selected from SEQ ID No. 64, SEQ ID No:92, SEQ ID No: 95 and SEQ ID No:104, while the amino acid sequence is selected from SEQ ID No.63, SEQ ID No:91, SEQ ID No:94 and SEQ ID No:103.

In an embodiment, there is provided an anti-BCMA CAR polypeptide comprising a single chain antibody or single chain antibody fragment having scFv as an anti-BCMA binding domain with a nucleotide sequence selected from SEQ ID No.: 58, SEQ ID No. 64, SEQ ID No:92, SEQ ID No: 95 and SEQ ID No:104.

In an embodiment, there is provided an anti-BCMA CAR polypeptide comprising a single chain antibody or single chain antibody fragment having scFv as an anti-BCMA binding domain with an amino acid sequence selected from SEQ ID No: 57, SEQ ID No: 63, SEQ ID No: 91, SEQ ID No: 94 and SEQ ID No: 103.

In an embodiment the anti-BCMA CAR polypeptide comprising a single chain antibody or single chain antibody fragment having scFv as an anti-BCMA binding domain comprises a linker for connecting Heavy chain variable region (HCVR) and light chain variable region (LCVR).

Linker

In an embodiment, the linker of the anti-BCMA binding domain (scFv) is a Whitlow linker comprising a nucleotide sequence selected from SEQ ID No. 4 and an amino acid sequence of SEQ ID No.3

In another aspect, the present invention provides a novel linker comprising a nucleotide sequence selected from SEQ ID No. 86, SEQ ID No. 87, SEQ ID No. 88, SEQ ID No. 89 or nucleotide sequence with at least 95% identity thereof. The novel linker comprises an amino acid sequence selected from SEQ ID No. 85.

Hinge Domain

In an embodiment the anti-BCMA CAR polypeptide further comprises a hinge region, which is further connected to a transmembrane domain. The hinge

region provides flexibility and length to the CAR polypeptide and affects its functionality.

The hinge domain of the present anti-BCMA CAR polypeptide is CD8α comprising a nucleotide sequence selected from SEQ ID No. 6, SEQ ID 7 or nucleotide sequence with at least 95% identity thereof.

The hinge domain CD8α in the present anti-BCMA CAR polypeptide comprises an amino acid sequence of SEQ ID No. 5.

Transmembrane Domain

The transmembrane domain in the present anti-BCMA CAR polypeptide helps for membrane anchorage of the antigen recognizing extracellular domain. The transmembrane domain is the connecting link between the extracellular domain and the intracellular domain of the CAR.

In an embodiment, the transmembrane domain is CD8α comprising a nucleotide sequence selected from SEQ ID No. 9, SEQ ID 10, or nucleotide sequence with at least 95% identity thereof.

The transmembrane domain is CD8α comprising an amino acid sequence of SEQ ID No. 8.

Intracellular domain:

The intracellular domain in the present anti-BCMA CAR polypeptide provides the anti-tumor activity to the cells preferably in T cells. The intracellular domain is derived from cytoplasmic domains of T cell receptor complex and other related receptor which play a role in the biological functions of the T cells. Therefore, intracellular domain usually plays a role in providing the effector function to the CAR-T cells. After antigen recognition, receptors cluster and a signal is transmitted to the cell. The T cell activation though occurs through T cell receptor signaling, an additional co-stimulatory activation is required for an enhanced T cell response. The intracellular domain in CAR polypeptide usually comprises a T cell receptor signaling domain and a cytoplasmic fragment of the costimulatory receptors. The choice of intracellular domain has an impact on the

activation of signaling cascades in the T cells which confer the effector functions like the anti-tumor response, cytokine secretion and the T cell phenotype.

In an embodiment, the anti-BCMA CAR polypeptide comprises a co-stimulatory domain.

The co-stimulatory domain in the present CAR polypeptide is selected from 4-1BB or CD28.

The 4-1BB co-stimulatory domain has a nucleotide sequence selected from SEQ ID No.12, 13, 14 or nucleotide sequence with at least 95% identity thereof, whereas the amino acid sequence is SEQ ID No.11.

The CD28 has a nucleotide sequence selected from SEQ ID No. 24 or nucleotide sequence with at least 95% identity thereof, whereas the amino acid sequence selected from SEQ ID No. 23.

In an embodiment, the anti-BCMA CAR polypeptide also comprises a signaling domain.

The signaling domain in the present anti-BCMA CAR polypeptide is CD3ζ having a nucleotide sequence selected from SEQ ID No. 16, 17, 18, 19, 20, 21, 22 or nucleotide sequence with at least 95% identity thereof; whereas the amino acid sequence is SEQ ID No.15.

Leader sequence:

The anti-BCMA chimeric antigen receptor polypeptide described herein comprises a leader sequence at the amino terminal (N-ter).

In an embodiment, the leader sequence has a nucleotide sequence of SEQ ID No. 2 or nucleotide sequence with at least 95% identity thereof.

In an embodiment, the leader sequence has an amino acid sequence of SEQ ID No. 1.

Thus, there is provided an anti-BCMA CAR polypeptide which comprises: (a) a single chain antibody or single chain antibody fragment comprising an anti-BCMA binding domain (scFv);

(b) a hinge region;

(c) a transmembrane domain; and

(d) a cytoplasmic domain comprising a costimulatory domain and a signaling domain;

wherein said anti-BCMA binding domain (scFv) comprises a Heavy chain variable region (HCVR) and a light chain variable region (LCVR); attached by a linker.

In an embodiment, there is provided a recombinant nucleic acid molecule for encoding an anti-BCMA chimeric antigen receptor (CAR) having an anti-BCMA binding domain.

The present invention provides a recombinant nucleic acid molecule encoding an anti-BCMA chimeric antigen receptor (CAR) having an anti-BCMA binding domain, where the anti-BCMA binding domain comprises, a murine antibody-based framework regions.

In an embodiment the recombinant nucleic acid molecule encoding anti-BCMA binding domain having murine antibody framework regions has a nucleotide sequence selected from SEQ ID No. 60 and SEQ ID No. 62.

In an embodiment there is provided an anti-BCMA CAR polypeptide comprising anti-BCMA binding domain having murine antibody framework regions with an amino acid sequence selected from SEQ ID No. 59 and SEQ ID No. 61.

The present invention provides anti-BCMA CAR constructs encoding an anti-BCMA binding domain having human antibody-based framework region **(Figure 1)**, with a focus on achieving a favorable efficacy-to-safety balance. This enhances the overall safety profile of the treatment, enabling more effective therapeutic outcomes while minimizing potential risks.

The anti-BCMA CAR constructs encoding anti-BCMA binding domain having human antibody-based framework region contribute significantly to the advancement of CAR-T cell therapy.

The anti-BCMA CAR polypeptide having human antibody-based framework region is developed by grafting complementary determining regions of monoclonal antibody C11D5.3 over the framework regions of a human antibody.

In an embodiment an anti-BCMA CAR construct encoding anti-BCMA CAR polypeptide having human antibody-based framework region is developed by providing a recombinant nucleic acid encoding the anti-BCMA CAR polypeptides having at least a single residue mutation.

The mutation in anti-BCMA CAR polypeptide may be provided to generate anti-BCMA CAR construct encoding anti-BCMA CAR polypeptide having human antibody-based framework region, where such mutations are provided in linker region of the anti-BCMA binding domain, CDR, co-stimulatory domain regions of the CAR.

The anti-BCMA CAR construct encoding anti-BCMA CAR polypeptide having human antibody-based framework region, are developed by providing a recombinant nucleic acid molecule.

The recombinant nucleic acid molecule encoding anti-BCMA CAR construct having an anti-BCMA CAR polypeptide, comprises a nucleic acid sequence optimized for human codon usage, and has a nucleotide sequence selected from SEQ ID No. 111, SEQ ID No. 112, SEQ ID No. 113, SEQ ID No. 114 and SEQ ID No. 115.

The anti-BCMA CAR polypeptide so obtained comprises human antibody-based framework regions, has an amino acid sequence selected from SEQ ID No. 90, SEQ ID No. 93, SEQ ID No. 102, SEQ ID No. 109 and SEQ ID No. 110.

The anti-BCMA CAR contains all human genes to improve the efficacy and reduce toxicity and immunogenicity. The antigen binding domain described herein, recognizing BCMA, is an anti-BCMA binding domain comprising human antibody-based framework regions.

In an embodiment there is provided a method for generation of anti-BCMA CAR polypeptide comprising human antibody-based framework regions.

The present invention provides humanization of murine antibody based anti-BCMA CAR antibody.

Humanization is a process by which xenogeneic antibody sequences are modified to reduce this immunogenicity. In order to humanize an antibody/binding domain, it includes various critical choices including the extents of the CDRs, the human frameworks to use and the substitution of residues from the murine antibody into the human framework regions. There is no single parameter to decide the right choice of the heavy and light chain. However, these choices can severely impact the safety, efficacy, toxicity, and immunogenicity of the CAR-T cells.

The present invention provides method for humanizing anti-BCMA scFv constructs having murine monoclonal antibody CDRs for enhanced expression and stability in human T cells.

The method for development of humanized anti-BCMA binding domains comprises steps of:

i. sequence analysis of monoclonal antibody to identify FRs and CDRs using Kabat numbering,

ii. identification of acceptor frameworks sequences by multiple sequence alignment of CDR of the monoclonal antibody over a human germline framework sequences and selection of a suitable acceptor FR,

iii. sequence analysis of acceptor framework to identify FRs and CDRs of human antibody using Kabat numbering, and

iv. CDR grafting and replacement of CDRs from the murine monoclonal anti-BCMA onto the selected human acceptor framework.

This replacement was performed while preserving critical residues within the FRs necessary for maintaining the structural integrity and antigen-binding affinity of the scFv.

Vector

In an embodiment there is provided a vector molecule containing nucleotide sequence required for encoding anti-BCMA CAR.

The nucleotide sequence of the CAR construct is cloned into an expression vector suitable for integration and stable expression in mammalian cells with techniques known in art. The CAR construct can also be synthesized chemically and cloned into the expression vector.

The present invention provides a vector encoding an anti-BCMA CAR polypeptide having murine antibody-based framework regions, comprising nucleotide sequence selected from SEQ ID No. 117 or SEQ ID No. 118.

The present invention provides a vector encoding an anti-BCMA CAR polypeptide having human antibody-based framework regions, comprising nucleotide sequence selected from SEQ ID No. 119, SEQ ID No. 120, SEQ ID No. 121, SEQ ID No. 122 and SEQ ID No. 123, or nucleotide sequence with at least 95% identity thereof.

The expression of CAR is under the control of a constitutive promoter usually derived from a mammalian cell. A promoter is a DNA sequence usually present upstream of the gene of interest which drives the expression of the target gene in the cells.

The choice of the promoter has an impact on the expression in a particular cell type and pertaining to this invention can have a role in determining the efficacy of the CAR. The promoter is chosen from a group of widely studied constitutive promoters from mammalian origin.

In an embodiment, there is provided a promoter used for development of CAR constructs.

The promoter hEF-1α enhances expression of the anti-BCMA CAR gene. This is crucial in a clinical setting, as the presence of CAR on the surface of T cells significantly affects the efficacy of CAR-T cell therapy. The hEF-1α promoter offers several advantages over the MND promoter, for example: its inherent human origin reduces the risk of immunogenicity and unwanted immune responses in clinical applications.

In an embodiment, the promoter is hEF-1α promoter comprising the nucleotide sequence of SEQ ID No. 116 or nucleotide sequence with at least 95% identity thereof.

Immune cell

The invention also provides immune cell which comprises a nucleic acid according to the invention. The immune cell may be capable of expressing a CAR polypeptide according to the invention.

In an embodiment, the immune cell is selected from a human T lymphocyte, B lymphocyte, Natural Killer cells or a combination thereof.

Fusion recombinant scFv-Fc protein

In another aspect, the invention is directed to a fusion recombinant protein comprising an scFv-Fc construct comprising the scFv as described above. The scFv-Fc may be dimerized. Or, the Fc component may be mutated so that scFv-Fc is monomeric. It is a small, targeted antibody fragment that can still trigger immune responses by attaching to the Fc receptor on immune cells.

The fusion recombinant protein comprising an anti-BCMA binding domain (scFv) fused to the constant region of an antibody (Fc); wherein the said scFv-Fc construct wherein the said scFv-Fc construct has a nucleotide sequence selected from SEQ ID No. 125, SEQ ID No.127, SEQ ID No.129, SEQ ID No.131 and SEQ ID No. 133 encoding an amino acid sequence selected from SEQ ID No. 124 , SEQ ID No.126, SEQ ID No.128, SEQ ID No.130 and SEQ ID No. 132.

Kits:

The present invention also provides a kit comprising vector encoding the nucleic acid molecule for the anti-BCMA CAR of the present invention and reagents for immune cell activation, expansion, and engineering.

Pharmaceutical composition

The present invention also provides a pharmaceutical composition encoding the murine anti-BCMA CAR or the murine anti-BCMA polypeptide or humanized anti-BCMA CAR or the humanized CAR polypeptide or the vector or the cell, with a pharmaceutically acceptable carrier, diluent, or excipient, and optionally one or more further pharmaceutically active polypeptides and/or compounds.

The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

As used herein "pharmaceutically acceptable carrier, diluent or excipient" includes without limitation any adjuvant, carrier, excipient, glidant, sweetening agent, diluent, preservative, dye/colorant, flavour enhancer, surfactant, wetting agent, dispersing agent, suspending agent, stabilizer, isotonic agent, solvent, surfactant, or emulsifier which has been approved by the Food and Drug Administration as being acceptable for use in humans or domestic animals. Exemplary pharmaceutically acceptable carriers include, but are not limited to, to sugars, such as lactose, glucose and sucrose, starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate, tragacanth, malt, gelatine, talc, cocoa butter, waxes, animal and vegetable fats, paraffins, silicones, bentonites, silicic acid, zinc oxide, oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil, glycols, such as propylene glycol, polyols, such as glycerine, sorbitol, mannitol and polyethylene glycol, esters, such as ethyl oleate and ethyl laurate, agar; buffering agents, such as magnesium hydroxide and aluminium hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, phosphate buffer solutions, and any other compatible substances employed in pharmaceutical formulations.

Pharmaceutical compositions of the present invention comprising a CAR-expressing immune effector cell population, such as T cells, may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextran, mannitol, proteins, polypeptides, or amino acids such as glycine, antioxidants, chelating agents such as EDTA or glutathione, adjuvants (e.g., aluminium hydroxide), and preservatives. Compositions of the present invention are preferably formulated for parenteral administration, e.g., intravascular (intravenous or intraarterial), intraperitoneal or intramuscular administration.

The liquid pharmaceutical compositions, whether they be solutions, suspensions or other like form, may include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which may serve as the solvent or suspending medium, polyethylene glycols, glycerine, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulphate; chelating agents such as ethylenediamine tetra acetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic. An injectable pharmaceutical composition is preferably sterile.

Method of preparing

In another aspect, the present invention provides a method of preparing an anti-BCMA CAR encoded by the nucleic acid of present invention comprising, transducing a T cell with a vector.

In another aspect, the present invention provides a method for preparing immune cells expressing an anti-BCMA CAR encoded by a nucleic acid of present invention, comprising:

(i) providing a population of immune cells;

(ii) introducing into the immune cells a nucleic acid encoding the anti-BCMA CAR;

(iii) culturing the immune cells under conditions allowing for expression of the chimeric receptor.

In an embodiment, the population of immune cells is derived from peripheral blood mononuclear cells (PBMC).

In another embodiment, the immune cells is selected from human T lymphocyte, B lymphocyte or Natural Killer cells.

In another embodiment, there is provided a method for manufacturing T cells expressing an anti-BCMA chimeric antigen receptor (CAR), the method comprising the steps of:

(a) isolation and selection of immune cells from a human donor;

(b) enriching CD3+ T cells from the immune cells using magnetic bead separation;

(c) activating the enriched CD3+ T cells by anti-CD3/anti-CD28 antibodies in a culture medium comprising recombinant interleukin-2 (IL-2) at 37°C with 5% CO_2 for 36-40 hours;

(d) transducing the activated T cells with a lentiviral vector encoding an anti-BCMA CAR nucleic acid, at a multiplicity of infection (MOI) of up to 10, and incubating the transduced cells at 37°C with 5% CO_2 for 24-30 hours; and

(e) expanding the transduced T cells to obtain a population of T cells expressing the anti-BCMA CAR on their cell surface at a transduction efficiency of at least 10%.

Method of treatment

In another aspect of the present invention, there is provided a method of treating a subject having a disease associated with expression of BCMA, comprising

administering to the subject an effective amount of an immune effector cell expressing the anti-BCMA CAR polypeptide.

The genetically modified immune effector cells contemplated herein provide improved methods of adoptive immunotherapy for use in the treatment of B cell related conditions that include, but are not limited to immunoregulatory conditions and hematological malignancies.

In particular embodiments, compositions comprising CAR-modified T cells contemplated herein are used in the treatment of hematologic malignancies, including but not limited to B cell malignancies such as, for example, multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL). Chronic lymphocytic leukemia (CLL). Illustrative examples of conditions that can be treated, prevented or ameliorated using the immune effector cells comprising the CARs contemplated herein include, but are not limited to: systemic lupus erythematosus, rheumatoid arthritis, myasthenia gravis, autoimmune hemolytic anemia, idiopathic thrombocytopenia purpura, anti-phospholipid syndrome, Chagas' disease, Grave's disease, Wegener's granulomatosis, poly-arteritis nodosa, Sjogren's syndrome, pemphigus vulgaris, scleroderma, multiple sclerosis, anti-phospholipid syndrome, ANCA associated vasculitis, Goodpasture's disease, Kawasaki disease, and rapidly progressive glomerulonephritis.

EXAMPLES:

The following examples are meant to illustrate the present invention. The examples are presented to exemplify the invention and are not to be considered as limiting the scope of the invention.

Example 1: Design and development of anti-BCMA CAR constructs

The present invention pertains to the development of novel BCMA-specific CAR constructs tailored for therapeutic applications, particularly for the treatment of multiple myeloma.

Anti-BCMA scFv

The BCMA-specific scFv is based on the C11D5.3 clone (US20120082661A1). The heavy (VH) and light (VL) chain variable regions of mouse-derived C11D5.3 clone were identified using Kabat numbering and subsequently utilized to construct a murine scFv.

Humanization of Murine C11D5.3-derived scFv

The present invention relates to the humanization of murine C11D5.3-derived scFv, to develop a anti-BCMA binding domain. Humanization is a crucial process in reducing immunogenicity for clinical applications. It involves designing the framework regions (FRs) and complementarity-determining regions (CDRs) based on the Kabat numbering system. This approach ensures precise identification and alignment of amino acids critical for maintaining binding affinity and specificity of the antibody.

The following methodology outlines the steps involved in the development of novel humanized anti-BCMA binding domains:

Sequence analysis of murine monoclonal antibody: The murine C11D5.3-derived anti-BCMA scFv sequence was analysed to identify FRs and CDRs using Kabat numbering.

Identification of acceptor frameworks sequences: Human germline framework sequences were sourced from the *VBASE2* database according to the CDR canonical structure of the donor.

Sequence analysis of acceptor framework: The selected human acceptor frameworks underwent sequence analysis to identify their FRs and CDRs using Kabat numbering.

CDR grafting and replacement: The CDRs from the murine anti-BCMA scFv were then grafted onto the selected human acceptor framework.

Table 1 shows the similarity of light chain variable regions of human germline sequences available in *VBASE2* database with murine C11D5.3 light chain variable regions.

Light chain human germline sequence of VBASE database	% Identity with C11D5.3 light chain
VK3-A27	61.62%
VK3-A11	59.6%
VK3-L25	60.61%

Table 2 shows the similarity of heavy chain variable regions of human germline sequences available in VBASE database with murine C11D5.3 heavy chain variable regions.

Heavy chain human germline sequence of VBASE database	% Identity with C11D5.3 heavy chain
VH1-18	56.7%
VH1-e	54.64%
VH1-69	54.64%
VH1-f	58.76%
VH5-51	55.67%
VH5-a	54.64%
VH7-4.1	72.16%
VH7-81	67.01%

Tables 3 shows the similarity of framework 4 region (FR4) of human light chain variable regions with murine C11D5.3 respectively.

Light chain FR4 sequences of human germline VBASE database	% Identity with C11D5.3 FR4 region
JK1	80%
JK2	90%
JK3	70%
JK4	90%
JK5	80%

Tables 4 shows the similarity of framework 4 region (FR4) of human heavy chain variable regions with murine C11D5.3 respectively.

Heavy chain FR4 sequences of human germline VBASE database	% Identity with C11D5.3 FR4 region
JH1	90.91%
JH2	81.82%
JH3	90.91%
JH6	90.91%

Anti-BCMA CAR

In accordance with the present invention, two murine and five humanized 2nd generation anti-BCMA CAR constructs were constructed **(Figure 1).** The scFv domains of these constructs were adapted from the murine clone C11D5.3 using the methodology described in Example 1.

The anti-BCMA CARs in addition incorporated human CD8α hinge and transmembrane domain, the 4-1BB (CD137) co-stimulatory domain, and the CD3ζ

signaling domain, all of which were sourced from a lentiviral vector (PCT/IN2019/050111).

To ensure the efficacy of the anti-BCMA CAR-T cells, particularly in their expression on the T cell surface, the following steps were involved:

Codon optimization and gene synthesis: The efficacy of humanized anti-BCMA CAR-T cells is critically dependent on the expression of the CAR on the T cell surface. The nucleotide sequence encoding the CAR was optimized to ensure high expression in human T cells. Gene optimization was performed using a multi-parametric approach via the Gene-Optimizer tool, which considered factors such as codon usage, GC content, and the avoidance of sequence motifs that could negatively impact gene expression. Codon usage was tailored to match the tRNA abundance in human cells, and the GC content was adjusted to enhance mRNA stability. The optimized gene was then synthesized and cloned into a third-generation lentiviral vector for efficient gene delivery.

Promoter Selection and Expression: All the anti-BCMA CAR constructs were expressed under the control of the hEF-1α promoter, known for its robust expression in human T cells. The constructs were cloned into a third-generation lentiviral vector system, to offer enhanced safety and efficiency in clinical applications.

Tables 5 and 6 sets forth the nucleotide and amino acid sequence IDs respectively of the scFv and full-length sequences of the prepared murine anti-BCMA CARs and humanized anti-BCMA CARs.

Table 5: Anti-BCMA CAR nucleotide SEQ ID

Anti-BCMA CAR designation	LCVR	LFR1	LCCDR1	LFR2	LCCDR2	LFR3	LCCDR3	LF4	Linker	HCVR	HFR1	HCCDR1	HFR2	HCCDR2	HFR3	HCCDR3	HFR4	scFv	Full length

m1 CAR	26	31	38	32	39	33	40	34	4	42	47	54	48	55	49	56	50	58	60
m 2 CAR	26	31	38	32	39	33	40	34	4	42	47	54	48	55	49	56	50	58	62
h 1 CAR	66	71	38	72	39	73	40	74	4	76	81	54	82	55	83	56	84	64	111
h2 CAR	66	71	38	72	39	73	40	74	86 87 88 89	76	81	54	82	55	83	56	84	92	112
h3 CAR	97	71	100	72	39	73	101	74	4	76	81	54	82	55	83	56	84	95	113
h4 CAR	97	71	100	72	39	73	101	74	86 87 88 89	106	81	54	82	55	83	108	84	104	114
h5 CAR	97	71	100	72	39	73	101	74	86 87 88 89	106	81	54	82	55	83	108	84	104	115

Table 6: Anti-BCMA CAR amino acid SEQ ID

Anti-BCMA CAR design ation	LCVR	LFR1	LCCDR1	LFR2	LCCDR2	LFR3	LCCDR3	LF4	Linker	HCVR	HFR1	HCCDR1	HFR2	HCCDR2	HFR3	HCCDR3	HFR4	scFv	Full length
m1 CAR	25	27	35	28	36	29	37	30	3	41	43	51	44	52	45	53	46	57	59
m2 CAR	25	27	35	28	36	29	37	30	3	41	43	51	44	52	45	53	46	57	61
h1 CAR	65	67	35	68	36	69	37	70	3	75	77	51	78	52	79	53	80	63	90
h2 CAR	65	67	35	68	36	69	37	70	85	75	77	51	78	52	79	53	80	91	93
h3 CAR	96	67	98	68	36	69	99	70	3	75	77	51	78	52	79	53	80	94	102
h4 CAR	96	67	98	68	36	69	99	70	85	105	77	51	78	52	79	107	80	103	109
h5 CAR	96	67	98	68	36	69	99	70	85	105	77	51	78	52	79	107	80	103	110

Example 2: Lentiviral vector production

The lentiviral vector was produced using a third-generation lentiviral packaging system. HEK293T cells were co-transfected with the transfer plasmid, packaging plasmids, and envelope plasmid using the calcium phosphate precipitation method. After 24 hours, the culture medium containing the lentiviral particles was collected and filtered through a 0.45μm syringe filter to remove cellular debris. The filtered supernatant was then concentrated by ultracentrifugation (Beckman, USA), and the resulting virus pellet was resuspended in PBS and stored overnight at 4°C to allow the pellet to dissolve. The following day, the virus was further purified by passing it through a 100 kDa cutoff filter, then resuspended in PBS, and stored at -80°C until use.

The titer of the produced lentiviral particles was determined by transducing HEK293T cells with serially diluted lentivirus in the presence of Polybrene. After 24 hours, the cells were supplemented with fresh media and maintained at 37°C with 5% CO2 for an additional 48 hours. The viral titer was then calculated based on flow cytometric analysis of CAR-expressing transduced cells.

The anti-BCMA CARs successfully yielded optimal viral titers using this transfection protocol. Among the constructs, m1CAR, h1CAR, and h2CAR exhibited significantly higher viral titers compared to the other humanized CARs.

Example 3: Generation of novel anti-BCMA CAR-T cells

This example outlines a comprehensive methodology for generating T cells expressing novel anti-BCMA CARs and assessing their proliferation kinetics.

Isolation and activation of peripheral blood mononuclear cells (PBMCs)

PBMCs were isolated from healthy donors using a standard density gradient centrifugation method. CD3+ T cells were then enriched using the Dynabeads™ Untouched Human T Cells Isolation Kit (Invitrogen: Cat# 11344D). The collected T cells were activated using anti-CD3/anti-CD28 magnetic beads at a bead-to-T cell ratio of 3:1. The cells were cultured in T-cell media consisting of AIM-V supplemented with 5% heat-inactivated human serum (HI-HS) along with 50 ng/ml

recombinant interleukin-2 (IL-2) and incubated at 37ºC with 5% CO2 for 36-40 hours.

Transduction of T cells with anti-BCMA CAR encoding lentiviral vector

The activated T cells were washed and resuspended in fresh T-cell media supplemented with protamine sulphate (20µg/ml) and IL-2 (100ng/ml). The T cells were then transduced with virus supernatants containing the novel anti-BCMA CAR construct, with a multiplicity of infection (MOI) ranging from 5 to 10 followed by a 24-30 hours of incubation at 37°C with 5% CO2. The following day, the virus and beads were removed, and the cells were resuspended in fresh T-cell media containing 20 ng/ml IL-2 for further expansion.

Characterization of CAR expression and proliferation kinetics

On day 5/6 post-transduction, the T cells were analyzed for surface anti-BCMA CAR expression using Recombinant Protein L staining. PE-labeled Streptavidin antibody was added after subsequent washes to remove any nonspecifically bound Protein L, followed by analysis via flow cytometry. Untransduced T cells served as a control to account for any non-specific binding. Lentiviral-mediated T cell transduction resulted in distinct surface CAR expression, with a transduction efficiency of > 15% **(Figure 2).**

Following confirmation of transduction efficiency, the CAR-expressing T cells were maintained in T-cell media supplemented with 20 ng/ml IL-2 at a cell density of 0.3 - 0.5 x 10^6 cells/ml. The proliferation kinetics and viability of these cells were monitored over 7-10 days using the trypan blue dye exclusion method.

All anti-BCMA CAR-T cells exhibited optimal proliferation kinetics within 7 days of *ex vivo* culture. Notably, T cells transduced with m1CAR, h1CAR, and h2CAR constructs demonstrated a nearly two-fold higher proliferation rate compared to those transduced with other CARs **(Figure 3).**

Example 4: Functional characterization of novel anti-BCMA CAR-T cells

The novel anti-BCMA CAR-T cells were further evaluated for their ability to target and kill BCMA-positive tumor cells through a series of *in vitro* and *in vivo* experiments.

***In vitro* Cytotoxicity assay**

To assess the anti-tumor potential of anti-BCMA CAR-T cells, the expanded CAR-T cells (effector cells) were co-cultured with BCMA-positive tumor cell lines (RPMI8226 and transgenic Nalm6-BCMA) as well as BCMA-negative tumor cells (Nalm6 and K562) at an effector-to-target (E: T) ratio of 1:1 for 24 hours. The cell pellet was used determine the antigen-specific killing of BCMA-positive tumor cells by flow cytometry, in comparison to untransduced T cells.

Anti-BCMA CAR-T cells demonstrated robust elimination of BCMA-positive tumor cells at a 1:1 ratio, with negligible cytotoxicity against BCMA-negative cell lines, thereby confirming the antigen-specific action of the novel anti-BCMA CAR-T cells **(Figure 4).**

Cytokine release assay

To evaluate the cytokine release of T cells upon antigen recognition, the levels of key immune effector cytokines, including IFN-γ, IL-2, and TNF-α, were measured. Cell-free supernatants from the co-culture assay were collected and analyzed for cytokine levels using ELISA (Thermo Fisher Scientific). The novel anti-BCMA CAR-T cells secreted significantly higher levels of cytokines when co-cultured with BCMA-positive tumor cells. Importantly, negligible cytokine secretion was observed when exposed to BCMA-negative tumor cells, confirming their robust antigen-specific activation and reduced likelihood of off-target effects.

However, the humanized CAR-T cells secreted several-fold lower cytokine levels compared to murine CAR-T cells under the same conditions. This reduced cytokine release is a pivotal factor contributing to their lower risk of cytokine release syndrome (CRS), a common and severe adverse event associated with CAR-T therapies **(Figure 5A-C)**.

***In vitro* persistence of anti-BCMA CAR-T cells**

To examine the robustness of the novel anti-BCMA CAR-T cells *in vitro*, we performed a repeat antigen stimulus stress test. CAR-T cells were co-cultured with BCMA-positive tumor cells at a 1:1 ratio without the addition of exogenous IL-2. Every three days, CAR-T cells were re-stimulated with fresh BCMA-positive tumor cells, mimicking repeated antigen exposure. At each interval, the absence of tumor cells was verified using flow cytometry, and the cell count was assessed using the trypan blue dye exclusion method. h2CAR-T cells exhibited robust and sustained proliferation in response to repeated antigen exposure, surpassing both m1CAR and h1CAR-T cells **(Figure 6A).** In addition, flow cytometric analysis confirmed efficient elimination of BCMA-positive target cells at each time point **(Figure 6B).** These findings provide strong evidence supporting the clinical potential of h2CAR-T cells to deliver improved and durable treatment outcomes for patients with BCMA-expressing malignancies.

***In vivo* anti-tumor activity of novel anti-BCMA CAR-T cells**

To assess the efficacy of the novel anti-BCMA CAR-T cells *in vivo*, 5×10^5 luciferase-positive human Nalm6-BCMA cells were implanted in 6–8-week-old immunocompromised NOD/SCID mice post 2.5 Gy irradiation. The treated group received an infusion of 5×10^6 anti-BCMA CAR-T cells, while the untreated control group did not receive any CAR-T infusion. Tumor burden was monitored weekly using bioluminescence imaging (BLI) with the Perkin Elmer IVIS 100 Imaging System **(Figure 7A)**. Mice were imaged under 2% isoflurane anesthesia with 2 L/min O_2 and were followed up until day 30 for tumor burden.

The treated mice exhibited negligible tumor burden at both 5×10^6 and 10×10^6 doses of CAR-T cells throughout the 30-day observation period, in contrast to the untreated and Mock T cell-treated group **(Figure 7B).** Additionally, the treated group showed a substantial survival benefit compared to the untreated and Mock group **(Figure 7C)**.

It is to be understood that the present invention is susceptible to modifications, changes, and adaptations by those skilled in the art. Such modifications, changes, and adaptations are intended to be within the scope of the present invention.

CLAIMS:

1. A recombinant nucleic acid molecule encoding a chimeric antigen receptor (CAR), wherein the CAR comprises:

(a) a single chain antibody or single chain antibody fragment comprising an anti-BCMA binding domain, wherein the said anti-BCMA binding domain is a murine anti-BCMA binding domain or a humanized anti-BCMA binding domain;

(b) a hinge region;

(c) a transmembrane domain; and

(d) a cytoplasmic domain comprising a costimulatory domain and a signaling domain;

Wherein said anti-BCMA binding domain is an scFv comprising a Heavy chain variable region (HCVR) and a light chain variable region (LCVR) attached by a linker;

Wherein the said HCVR comprises:

a heavy chain complementary determining region 1 (HC CDR1) having nucleotide sequence of SEQ ID No: 54;

a heavy chain complementary determining region 2 (HC CDR2) having nucleotide sequence of SEQ ID No:55;

a heavy chain complementary determining region 3 (HC CDR3) having nucleotide sequence of SEQ ID No: 56 or 108; and

Wherein the said LCVR comprises:

a light chain complementary determining region 1 (LC CDR1) having the nucleotide sequence of SEQ ID No: 38 or 100;

a light chain complementary determining region 2 (LC CDR2) having the nucleotide sequence of SEQ ID No: 39;

and a light chain complementary determining region 3 (LC CDR3) having the nucleotide sequence of SEQ ID No: 40 or 101.

2. The nucleic acid molecule as claimed in claim 1, wherein the said HCVR comprises a humanized framework region:

a heavy chain framework region 1 (HFR1) having the nucleotide sequence of SEQ ID No: 81;

a heavy chain framework region 2 (HFR2) having the nucleotide sequence of SEQ ID No: 82;

a heavy chain framework region 3 (HFR3) having the nucleotide sequence of SEQ ID No: 83;

a heavy chain framework region 4 (HFR4) having the nucleotide sequence of SEQ ID No: 84 and;

Wherein said LCVR comprises a humanized framework region:

a light chain framework region 1 (LFR1) having the nucleotide sequence of SEQ ID No: 71;

a light chain framework region 2 (LFR2) having the nucleotide sequence of SEQ ID No: 72;

a light chain framework region 3 (LFR3) having the nucleotide sequence of SEQ ID No: 73; and

a light chain framework region 4 (LFR4) having the nucleotide sequence of SEQ ID No: 74.

3. The nucleic acid molecule as claimed in claim 1, wherein said HCVR comprises the murine framework region:

a heavy chain framework region 1 (HFR1) having the nucleotide sequence of SEQ ID No: 47;

a heavy chain framework region 2 (HFR2) having the nucleotide sequence of SEQ ID No: 48;

a heavy chain framework region 3 (HFR3) having the nucleotide sequence of SEQ ID No: 49

a heavy chain framework region 4 (HFR4) having the nucleotide sequence of SEQ ID No: 50; and

Wherein said LCVR comprises the murine framework region:

a light chain framework region 1 (LFR1) having the nucleotide sequence of SEQ ID No:31;

a light chain framework region 2 (LFR2) having the nucleotide sequence of SEQ ID No: 32;

a light chain framework region 3 (LFR3) having the nucleotide sequence of SEQ ID No: 33; and

a light chain framework region 4 (LFR4) having the nucleotide sequence of SEQ ID No:34.

4. The nucleic acid molecule as claimed in claim 1, wherein the said nucleic acid encodes a humanized heavy chain variable region (HCVR) having nucleotide sequence selected from SEQ ID No: 76 or SEQ ID No: 106 and a humanized light chain variable region (LCVR) having nucleotide sequence of SEQ ID No: 66 or SEQ ID No. 97.

5. The nucleic acid molecule as claimed in claim 1, wherein the said nucleic acid encodes a murine heavy chain variable region (HCVR) selected from SEQ ID No: 42 and a murine light chain variable region (LCVR) of SEQ ID No: 26.

6. The nucleic acid molecule as claimed claim 1, wherein the said linker comprises a nucleotide sequence selected from SEQ ID No. 4, 86, 87, 88 or 89 or nucleotide sequence with at least 95% identity thereof.

7. The nucleic acid molecule as claimed in claim 1, wherein the humanized scFv has a nucleotide sequence selected from SEQ ID No. 64, 92, 95, 104 or nucleotide sequence with at least 95% identity thereof.

8. The nucleic acid molecule as claimed in claim 1, wherein the murine scFv has a nucleotide sequence selected from SEQ ID No. 58 or nucleotide sequence with at least 95% identity thereof.

9. The nucleic acid molecule as claimed in claim 1, wherein the hinge domain is CD8α comprising a nucleotide sequence selected from SEQ ID No. 6, SEQ ID 7 or nucleotide sequence with at least 95% identity thereof.

10. The nucleic acid molecule as claimed in claim 1, wherein the transmembrane domain is CD8α comprising a nucleotide sequence selected from SEQ ID No. 9 or SEQ ID 10.

11. The nucleic acid molecule as claimed in claim 1, wherein the co-stimulatory domain is selected from 4-1BB or CD28.

12. The nucleic acid molecule as claimed in claim 11, wherein the 4-1BB has a nucleotide sequence selected from SEQ ID No.12, 13, 14 or nucleotide sequence with at least 95% identity thereof.

13. The nucleic acid molecule as claimed in claim 11, wherein CD28 has a nucleotide sequence selected from SEQ ID No. 24 or nucleotide sequence with at least 95% identity thereof.

14. The nucleic acid molecule as claimed in claim 1, wherein the signaling domain is CD3ζ having a nucleotide sequence selected from SEQ ID No. 16, 17,18, 19,20, 21, 22 or nucleotide sequence with at least 95% identity thereof.

15. The nucleic acid molecule as claimed in claim 1, wherein the encoded humanized CAR further comprises a leader sequence.

16. The nucleic acid molecule as claimed in claim 15, wherein the leader sequence has a nucleotide sequence of SEQ ID No. 2 or nucleotide sequence with at least 95% identity thereof.

17. A recombinant nucleic acid molecule encoding a humanized chimeric antigen receptor (hCAR) having an anti-BCMA binding domain, wherein it comprises a nucleic acid sequence optimized for human codon usage and

selected from SEQ ID No. 111, SEQ ID No. 112, SEQ ID No. 113 and SEQ ID No. 114, SEQ ID No. 115.

18. A recombinant nucleic acid molecule encoding a murine chimeric antigen receptor having an anti-BCMA binding domain, wherein it comprises a nucleic acid sequence selected from SEQ ID No. 60 and SEQ ID No. 62.

19. A chimeric antigen receptor (CAR) polypeptide, wherein the CAR comprises:

(a) a single chain antibody or single chain antibody fragment comprising an anti-BCMA binding domain, wherein the said anti-BCMA binding domain is a murine anti-BCMA binding domain or a humanized anti-BCMA binding domain;

(b) a hinge region;

(c) a transmembrane domain; and

(d) a cytoplasmic domain comprising a costimulatory domain and a signaling domain;

Wherein said anti-BCMA binding domain is an scFv comprising a HCVR and LCVR attached by a linker;

Wherein said HCVR comprises:

a heavy chain complementary determining region 1 (HC CDR1) having amino acid sequence of SEQ ID No: 51;

a heavy chain complementary determining region 2 (HC CDR2) having amino acid sequence of SEQ ID No:52;

a heavy chain complementary determining region 3 (HC CDR3) having amino acid sequence of SEQ ID No: 53 or 107; and

Wherein said LCVR comprises:

a light chain complementary determining region 1 (LC CDR1) having amino acid sequence of SEQ ID No: 35 or 98;

a light chain complementary determining region 2 (LC CDR2) having amino acid sequence of SEQ ID No: 36;

and a light chain complementary determining region 3 (LC CDR3) having amino acid sequence of SEQ ID No: 37 or 99.

20. The CAR polypeptide as claimed in claim 19, wherein said HCVR comprises a humanized framework region:

a heavy chain framework region 1 (HFR1) having amino acid sequence of SEQ ID No: 77;

a heavy chain framework region 2 (HFR2) having amino acid sequence of SEQ ID No: 78;

a heavy chain framework region 3 (HFR3) having amino acid sequence of SEQ ID No: 79;

a heavy chain framework region 4 (HFR4) having amino acid sequence of SEQ ID No: 80; and

Wherein said LCVR comprises a humanized framework region:

a light chain framework region 1 (LFR1) having amino acid sequence of SEQ ID No: 67;

a light chain framework region 2 (LFR2) having amino acid sequence of SEQ ID No: 68;

a light chain framework region 3 (LFR3) having amino acid sequence of SEQ ID No: 69; and

a light chain framework region 4 (LFR4) having amino acid sequence of SEQ ID No: 70.

21. The CAR polypeptide as claimed in claim 19, wherein said HCVR comprises a murine framework region:

a heavy chain framework region 1 (HFR1) having amino acid sequence of SEQ ID No: 43

a heavy chain framework region 2 (HFR2) having amino acid sequence of SEQ ID No: 44

a heavy chain framework region 3 (HFR3) having amino acid sequence of SEQ ID No: 45

a heavy chain framework region 4 (HFR4) having amino acid sequence of SEQ ID No: 46 and;

Wherein said LCVR comprises a murine framework region:

a light chain framework region 1 (LFR1) having amino acid sequence of SEQ ID No: 27,

a light chain framework region 2 (LFR2) having amino acid sequence of SEQ ID No: 28,

a light chain framework region 3 (LFR3) having amino acid sequence of SEQ ID No: 29 and

a light chain framework region 4 (LFR4) having amino acid sequence of SEQ ID No: 30.

22. The CAR polypeptide as claimed claim 19, which comprises a humanized heavy chain variable region (HCVR) having an amino acid sequence selected from SEQ ID No. 75 or SEQ ID No. 105, and a humanized light chain variable region (LCVR) having an amino acid sequence of SEQ ID No: 65 or SEQ ID No. 96.

23. The CAR polypeptide as claimed claim 19, which comprises a murine heavy chain variable region (HCVR) having an amino acid sequence selected from SEQ ID No. 41, and a light chain variable region (LCVR) having an amino acid sequence of SEQ ID No: 25.

24. The CAR polypeptide as claimed in claim 19, wherein the linker comprises an amino acid sequence selected from SEQ ID No.3 or SEQ ID No.85.

25. The CAR polypeptide as claimed in claim 19, wherein the humanized scFv has an amino acid sequence selected from SEQ ID No. 63, 91, 94 or 103.

26. The CAR polypeptide as claimed in claim 19, wherein the murine scFv has an amino acid sequence selected from SEQ ID No.57.

27. The CAR polypeptide as claimed in claim 19, wherein the hinge domain is CD8α comprising an amino acid sequence of SEQ ID No. 5.

28. The CAR polypeptide as claimed in claim 19, wherein the transmembrane domain is CD8α comprising an amino acid sequence of SEQ ID No. 8.

29. The CAR polypeptide as claimed in claim 19, wherein the co-stimulatory domain is selected from 4-1BB or CD-28.

30. The CAR polypeptide as claimed in claim 29, wherein the 4-1BB has an amino acid sequence of SEQ ID No.11.

31. The CAR polypeptide as claimed in claim 29, wherein CD-28 has an amino acid sequence selected from SEQ ID No. 23.

32. The CAR polypeptide as claimed in claim 19, wherein the signaling domain is CD3ζ having an amino acid sequence of SEQ ID No.15.

33. The CAR polypeptide as claimed in claim 19, wherein the encoded humanized CAR further comprises a leader sequence.

34. The CAR polypeptide as claimed in claim 33, wherein the leader sequence has an amino acid sequence of SEQ ID No. 1.

35. A humanized chimeric antigen receptor (CAR) polypeptide, wherein it comprises an amino acid sequence selected from SEQ ID No. 90, SEQ ID No. 93, SEQ ID No. 102 and SEQ ID No. 109, 110 or an amino acid sequence with at least 95% identity thereof.

36. A murine chimeric antigen receptor (CAR) polypeptide, wherein it comprises an amino acid sequence selected from SEQ ID No. 59 or SEQ ID No.61.

37. A vector comprising the nucleic acid molecule as claimed in any of claims 1 to 18.

38. The vector as claimed in claim 37, further comprising a promoter, wherein the promoter is hEF-lα promoter comprising the nucleotide sequence of SEQ ID No. 116 or nucleotide sequence with at least 95% identity thereof.

39. The vector as claimed in claim 37, wherein the said vector comprises the nucleotide sequence selected from SEQ ID No. 117, SEQ ID No. 118, SEQ

ID No. 119, SEQ ID No. 120, SEQ ID No. 121, SEQ ID No. 122 and SEQ ID No. 123, or nucleotide sequence with at least 95% identity thereof.

40. A kit comprising a vector encoding the nucleic acid molecule as claimed in any one of claims 1 to 18 and reagents for immune cell activation, expansion, and engineering.

41. A fusion recombinant protein comprising an anti-BCMA binding domain (scFv) fused to a constant region of an antibody (Fc); wherein the said scFv-Fc construct has a nucleotide sequence selected from SEQ ID No. 125, SEQ ID No.127, SEQ ID No.129, SEQ ID No.131 and SEQ ID No. 133 and encoding an amino acid sequence selected from SEQ ID No. 124, SEQ ID No.126, SEQ ID No.128, SEQ ID No.130 and SEQ ID No. 132.

42. A linker of the anti-BCMA binding domain (scFv) comprising a nucleotide sequence selected from SEQ ID No. 86, SEQ ID No. 87, SEQ ID No. 88, SEQ ID No. 89 or nucleotide sequence with at least 95% identity thereof and encoding an amino acid sequence of SEQ ID No. 85.

43. An immune cell comprising the vector as claimed in any one of claims 37 to 39 or expressing the CAR polypeptide as claimed in any one of claims 19 to 36.

44. The immune cell as claimed in claim 43, wherein the immune cell is selected from a T lymphocyte, B lymphocyte, Natural Killer (NK) cells, or a combination thereof.

45. A pharmaceutical composition comprising the recombinant nucleic acid molecule as claimed in any one of claims 1 to 18 or the CAR polypeptide as claimed in any one of claims 19 to 36 or the vector as claimed in any one of claims 37 to 39 or the immune cell as claimed in any one of claims 43 to 44, with a pharmaceutically acceptable carrier, diluent, or excipient, and optionally one or more further pharmaceutically active polypeptides and/or compounds.

46. A method of preparing an anti-BCMA CAR encoded by the nucleic acid as claimed in any one of claims 1 to 18, comprising, transducing a T cell with a vector as claimed in claims 37 to 39.

47. A method for preparing immune cells expressing an anti-BCMA CAR encoded by a nucleic acid according to any one of claims 1 to 18, comprising:

(i) providing a population of immune cells;

(ii) introducing into the immune cells a nucleic acid according to claims 1 to 18; and

(iii) culturing the immune cells under conditions allowing for expression of the chimeric receptor.

48. The method as claimed in claim 47, wherein the population of immune cells is derived from peripheral blood mononuclear cells (PBMC).

49. The method as claimed in claim 47, wherein the immune cells is selected from human T lymphocyte, B lymphocyte or Natural Killer cells.

50. A method for manufacturing T cells expressing an anti-BCMA chimeric antigen receptor (CAR), the method comprising the steps of:

(a) isolation and selection of immune cells from a human donor;

(b) enriching CD3+ T cells from the immune cells using magnetic bead separation;

(c) activating the enriched CD3+ T cells by anti-CD3/anti-CD28 antibodies in a culture medium comprising recombinant interleukin-2 (IL-2) at 37°C with 5% CO_2 for 36-40 hours;

(d) transducing the activated T cells with a lentiviral vector encoding an anti-BCMA CAR according to any one of claims 1 to 18, at a multiplicity of infection (MOI) of up to 10, and incubating the transduced cells at 37°C with 5% CO_2 for 24-30 hours; and

(e) expanding the transduced T cells to obtain a population of T cells expressing the anti-BCMA CAR on their cell surface at a transduction efficiency of at least 10%.

51. A method of treating a subject having a disease associated with expression of BCMA, comprising administering to the subject an effective amount of an immune effector cell according to claims 43 to 44.

52. The method as claimed in claim 51, wherein the disease associated with BCMA expression is selected from multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL). Chronic lymphocytic leukemia (CLL), systemic lupus erythematosus, rheumatoid arthritis, myasthenia gravis, autoimmune hemolytic anemia, idiopathic thrombocytopenia purpura, anti-phospholipid syndrome, Chagas' disease, Grave's disease, Wegener's granulomatosis, poly-arteritis nodosa, Sjogren's syndrome, pemphigus vulgaris, scleroderma, multiple sclerosis, anti-phospholipid syndrome, ANCA associated vasculitis, Goodpasture's disease, Kawasaki disease, and rapidly progressive glomerulonephritis.