

CD7-TARGETING HUMANIZED ANTIBODY, CHIMERIC ANTIGEN RECEPTOR, AND USE THEREOF

TECHNICAL FIELD

The present disclosure relates to the field of biomedicine. More specifically, the present disclosure relates to CD7-targeting humanized antibody, chimeric antigen receptor, and use thereof.

BACKGROUND

T-cell malignancy is a kind of lymphoid system malignancy originating from T cells. Due to the lack of understanding of their biology, the classification thereof is difficult to accomplish, and many classifications remain belonging to “temporary classification” in the World Health Organization (WHO) classification of diseases. Currently, the WHO classifies T-cell malignancy as comprising immature T-cell neoplasm and mature T-cell neoplasm; wherein immature T-cell neoplasm comprises T acute lymphoblastic leukemia (T-ALL), T-lymphoblastic lymphoma (T-LBL); and mature T-cell neoplasm comprises various types of T-cell leukemia and T-cell lymphoma. T-cell malignancy is characterized by high aggressiveness, diverse clinical symptoms, generally poor prognosis, high recurrence rates (30%-40%), unclear pathogenic factors, and lack of biological research. Although current treatment strategies have improved effects for pediatric T-ALL, with event-free survival (EFS) rates reaching 85%, the EFS rates for relapsed T-ALL remains <15%, the 5-year overall survival (OS) rates of adult T-ALL patients are only 40%–60%, with poor prognosis. Therefore, there is also a need for novel and targeted therapeutic schemes to improve the prognosis of T-cell malignancies, particularly for the patients with relapsed and refractory T-cell malignancies.

Chimeric Antigen Receptor (CAR) is the core component of CAR cell therapy drugs, which can include an antigen recognition domain, a hinge region, a transmembrane region, and an intracellular domain. So far, the antigen recognition domain has been derived from the single-chain variable fragment (abbreviated as scFv) of an antibody, or from receptor-ligand interactions, TCR mimics, or variable lymphocyte receptors (VLRs), and most commonly from scFv antibodies. CAR-T cell immunotherapy is considered to be one of the most promising methods to conquer tumors. CAR-T cells are generated by genetically modifying T cells to express CAR proteins, and such CAR protein has the ability to recognize intact proteins on the membrane surface independently of antigen presentation, thereby causing T cell activation and functional effects. At present, CAR-T cell immunotherapy has achieved significant effects in the treatment of various hematological tumors, for example, CD19 CAR-T for the treatment of B-cell lymphoma and BCMA CAR-T for the treatment of multiple myeloma have been commercially available as drugs.

CD7 is a transmembrane glycoprotein and a member of the immunoglobulin supergene

family. CD7 is generally overexpressed in immature T-cell tumors, whereas in mature T-cell tumors, the expression pattern and intensity of CD7 vary. In addition to T-cell malignancies, leukemia blasts and malignant progenitor cells in approximately 30% of patients with acute myeloid leukemia (AML) also express CD7. Therefore, CAR-T cells targeting CD7 are expected to treat the corresponding diseases as described above.

SUMMARY OF THE INVENTION

The present application provides a CD7-targeting humanized antibody, chimeric antigen receptor and use thereof. Based on the existing murine-derived antibody sequences, the inventors designed five humanized Th69 scFv antibodies to reduce their heterology. However, during the humanization process, the binding affinity, specificity, and other functionality of the antibody for specific antigen may be compromised. Therefore, obtaining humanized antibodies with reduced immunogenicity and uncompromised or even enhanced functionality has practical significance. The inventors used the five humanized Th69 scFv antibodies as the extracellular antigen recognition domain of CD7 CARs, and evaluated the proliferation, specificity, and functionality of CAR-T cells of humanized Th69 scFv antibodies through *in vitro* experiments and *in vivo* animal experiments. Three humanized Th69 scFv antibodies with best comprehensive effects were screened.

The present application provides a CD7-targeting humanized antibody or antigen-binding fragment thereof, comprising: the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2;

or, the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30;

or, the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30.

In certain embodiments of the above-mentioned humanized antibody or antigen-binding fragment thereof, the humanized antibody or antigen-binding fragment thereof is a scFv antibody, a sc(Fv)₂ antibody, or a [sc(Fv)₂]₂ antibody.

In certain embodiments the above-mentioned humanized antibody or antigen-binding fragment thereof, the humanized antibody or antigen-binding fragment thereof is the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 3;

or, the humanized antibody or antigen-binding fragment thereof is the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 35;

or, the humanized antibody or antigen-binding fragment thereof is the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 36.

In another aspect, the present application also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the above-mentioned humanized antibody or antigen-binding fragment thereof.

In certain embodiments of the above-mentioned isolated nucleic acid molecule, the nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1, is shown in SEQ ID NO: 4;

and/or, the nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2, is shown in SEQ ID NO: 5;

and/or, the nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28, is shown in SEQ ID NO: 37;

and/or, the nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30, is shown in SEQ ID NO: 38.

In yet another aspect, the present application also provides a vector comprising the above-mentioned isolated nucleic acid molecule.

In yet another aspect, the present application also provides a cell comprising any one of the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule or the vector.

In yet another aspect, the present application also provides a pharmaceutical composition comprising any one of the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule, the vector or the cell, and a pharmaceutically acceptable adjuvant.

In yet another aspect, the present application also provides use of the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule, the vector or the cell in the preparation of a detection reagent for diagnosing diseases or conditions related to the expression of CD7.

In certain embodiments of the above-mentioned use, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain embodiments of the above-mentioned use, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia (T-ALL), CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia (AML).

In certain embodiments of the above-mentioned use, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia (ETP-ALL) and other T acute lymphoblastic leukemia.

In certain embodiments of the above-mentioned use, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma (T-LBL), CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In yet another aspect, the present application also provides use of the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule, the vector or the cell in the preparation of a medicament for treating diseases or conditions related to the expression of CD7.

In certain embodiments of the above-mentioned use, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain embodiments of the above-mentioned use, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain embodiments of the above-mentioned use, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain embodiments of the above-mentioned use, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In yet another aspect, the present application also provides a method for treating diseases or conditions, including the following steps: administering an effective amount of a medicament comprising any one of the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule, the vector or the cell to a subject having a need for treatment of the diseases or conditions related to the expression of CD7.

In certain embodiments of the above-mentioned method, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain embodiments of the above-mentioned method, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain embodiments of the above-mentioned method, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain embodiments of the above-mentioned method, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In yet another aspect, the present application also provides a medicament comprising the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule, the vector or the cell, for use in treating diseases or conditions related to the expression of CD7.

In certain embodiments of the above-mentioned medicament, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain embodiments of the above-mentioned medicament, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain embodiments of the above-mentioned medicament, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain embodiments of the above-mentioned medicament, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-Lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In yet another aspect, the present application also provides an antibody drug comprising any one of the above-mentioned humanized antibody or antigen-binding fragment thereof.

In yet another aspect, the present application also provides an antibody-drug conjugate comprising any one of the above-mentioned humanized antibody or antigen-binding fragment thereof.

In yet another aspect, the present application also provides a CD7-targeting chimeric antigen receptor, comprising a CD7-targeting extracellular antigen recognition domain, a hinge region, a transmembrane region, and an intracellular domain, wherein the CD7-targeting extracellular antigen recognition domain comprises:

the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2;

or, the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30;

or, the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30.

In certain embodiments of any one of the above-mentioned chimeric antigen receptor, the extracellular antigen recognition domain comprises any one structure selected from the group consisting of: (the amino acid sequence as shown in SEQ ID NO: 1)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 2), (the amino acid sequence as shown in SEQ ID NO: 2)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 1)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 30), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 28)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 30), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 28);

optionally, the extracellular antigen recognition domain comprises any one structure selected from the group consisting of: (the amino acid sequence as shown in SEQ ID NO: 2)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 28);

further optionally, the extracellular antigen recognition domain is any one structure selected from the group consisting of: (the amino acid sequence as shown in SEQ ID NO: 2)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 28).

In certain embodiments of any one of the above-mentioned chimeric antigen receptor, the linker sequence is selected from one or more of the following sequences: SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

In certain embodiments of any one of the above-mentioned chimeric antigen receptor,

the extracellular antigen recognition domain comprises: the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 3, or the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 35, or the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 36;

optionally, the amino acid sequence of the extracellular antigen recognition domain is shown in SEQ ID NO: 3, or is shown in SEQ ID NO: 35, or is shown in SEQ ID NO: 36.

In certain embodiments of any one of the above-mentioned chimeric antigen receptor, the hinge region is derived from one or more of IgG1, IgG4, CD4, CD7, CD28, CD84, CD8 α ; optionally, the amino acid sequence of the hinge region is derived from CD8 α ; further optionally, the amino acid sequence of the hinge region comprises the amino acid sequence as shown in SEQ ID NO: 9; even further optionally, the amino acid sequence of the hinge region is shown in SEQ ID NO: 9.

In certain embodiments of any one of the above-mentioned chimeric antigen receptor, the transmembrane region is derived from one or more of CD3, CD4, CD7, CD8 α , CD28, CD80, CD86, CD88, 4-1BB, CD152, OX40, Fc70; optionally, the amino acid sequence of the transmembrane region is derived from CD8 α ; further optionally, the amino acid sequence of the transmembrane region comprises the amino acid sequence as shown in SEQ ID NO: 10; even further optionally, the amino acid sequence of the transmembrane region is shown in SEQ ID NO: 10.

In certain embodiments of any one of the above-mentioned chimeric antigen receptor, the intracellular domain comprises an intracellular signal transduction region; optionally, the intracellular domain further comprises a costimulatory signal transduction region.

In certain embodiments of any one of the above-mentioned chimeric antigen receptor, the intracellular signal transduction region is derived from one or more of CD3 ζ , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, FcR γ , FcR β , CD66d, DAP10, DAP12, Syk; optionally, the intracellular signal transduction region is derived from CD3 ζ ; further optionally, the amino acid sequence of the intracellular signal transduction region comprises the amino acid sequence as shown in SEQ ID NO: 11; even further optionally, the amino acid sequence of the intracellular signal transduction region is shown in SEQ ID NO: 11.

In certain embodiments of any one of the above-mentioned chimeric antigen receptor, the costimulatory signal transduction region is derived from one, two, or more of CD2, CD3, CD7, CD27, CD28, CD30, CD40, CD83, CD244, 4-1BB, OX40, LFA-1, ICOS, LIGHT, NKG2C, NKG2D, DAP10, B7-H3, MyD88; optionally, the costimulatory signal transduction region is derived from CD28 or 4-1BB; further optionally, the amino acid sequence of the costimulatory signal transduction region comprises the amino acid

sequence as shown in SEQ ID NO: 12; even further optionally, the amino acid sequence of the costimulatory signal transduction region is shown in SEQ ID NO: 12.

In certain embodiments of any one of the above-mentioned chimeric antigen receptor, the chimeric antigen receptor further comprises a guiding peptide located at the N-terminus of the amino acid sequence of the chimeric antigen receptor; optionally, the guiding peptide is derived from CD8 α ; further optionally, the amino acid sequence of the guiding peptide comprises the amino acid sequence as shown in SEQ ID NO: 13; even further optionally, the amino acid sequence of the guiding peptide is shown in SEQ ID NO: 13.

In certain embodiments of any one of the above-mentioned chimeric antigen receptor, the chimeric antigen receptor comprises any one of the following sequences: the amino acid sequence as shown in SEQ ID NO: 14, or the amino acid sequence as shown in SEQ ID NO: 18; or the amino acid sequence as shown in SEQ ID NO: 19.

optionally, the amino acid sequence of the chimeric antigen receptor is shown in SEQ ID NO: 14, or is shown in SEQ ID NO: 18, or is shown in SEQ ID NO: 19.

In yet another aspect, the present application also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding any one of the above-mentioned chimeric antigen receptor.

In certain embodiments of the above-mentioned isolated nucleic acid molecule, the nucleotide sequence encoding the chimeric antigen receptor comprises:

a nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1, which is shown in SEQ ID NO: 4; and a nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2, which is shown in SEQ ID NO: 5;

or, a nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1, which is shown in SEQ ID NO: 4; and a nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30, which is shown in SEQ ID NO: 38;

or, a nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28, which is shown in SEQ ID NO: 37; and a nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30, which is shown in SEQ ID NO: 38.

In certain embodiments of the above-mentioned isolated nucleic acid molecule, the nucleotide sequence encoding the chimeric antigen receptor comprises:

a nucleotide sequence encoding the amino acid sequence as shown in SEQ ID NO: 14, which is shown in SEQ ID NO: 15;

or, a nucleotide sequence encoding the amino acid sequence as shown in SEQ ID NO: 18, which is shown in SEQ ID NO: 39;

or, a nucleotide sequence encoding the amino acid sequence as shown in SEQ ID NO: 19, which is shown in SEQ ID NO: 40.

In yet another aspect, the present application also provides a vector comprising the above-mentioned isolated nucleic acid molecule.

In certain embodiments of the above-mentioned vector, the vector is an expression vector; in certain embodiments, the vector is a viral vector; in certain embodiments, the vector is a lentiviral vector.

In yet another aspect, the present application also provides an engineered immune effector cell comprising the above-mentioned chimeric antigen receptor, the above-mentioned isolated nucleic acid molecule, or the above-mentioned vector.

In certain embodiments of the above-mentioned engineered immune effector cell, the engineered immune effector cell is selected from one or more of a T lymphocyte, natural killer cell (NK cell), peripheral blood mononuclear cell (PBMC cell), induced pluripotent stem cell (iPSC), T cell differentiated from an induced pluripotent stem cell (iPSC-T), NK cell differentiated from an induced pluripotent stem cell (iPSC-NK) and embryonic stem cell.

In certain embodiments of the above-mentioned engineered immune effector cell, the engineered immune effector cell is a T lymphocyte; optionally, the source of the T lymphocyte is an autologous T lymphocyte or allogeneic T lymphocyte.

In certain embodiments of the above-mentioned engineered immune effector cell, the allogeneic T lymphocyte comprises gene-edited *CD7/TRAC* double-negative cell; further, the allogeneic T lymphocyte comprises more than 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 95%, 95%, 96%, 97%, 98%, 99% gene-edited *CD7/TRAC* double-negative cell.

In certain embodiments of the above-mentioned engineered immune effector cell, the T lymphocyte is an $\alpha\beta$ T lymphocyte or a $\gamma\delta$ T lymphocyte.

In yet another aspect, the present application also provides a pharmaceutical composition comprising the above-mentioned engineered immune effector cell, and a pharmaceutically acceptable adjuvant.

In certain embodiments of the above-mentioned pharmaceutical composition, the pharmaceutically acceptable adjuvant includes a protective agent.

In certain embodiments of the above-mentioned pharmaceutical composition, the pharmaceutically acceptable adjuvant includes a cell cryopreservation solution.

In certain embodiments of the above-mentioned pharmaceutical composition, the pharmaceutical composition is a cell suspension or cryopreserved cells thereof.

In certain embodiments of the above-mentioned pharmaceutical composition, the pharmaceutical composition is an intravenous injection.

In yet another aspect, the present application also provides a method for preparing the engineered immune effector cell, including the following steps: introducing a nucleotide sequence encoding any one of the above-mentioned chimeric antigen receptor into an immune effector cell.

In certain embodiments of the above-mentioned method, the immune effector cell is selected from one or more of a T lymphocyte, natural killer cell (NK cell), peripheral blood mononuclear cell (PBMC cell), induced pluripotent stem cell (iPSC), T cell differentiated from an induced pluripotent stem cell (iPSC-T), NK cell differentiated from an induced pluripotent stem cell (iPSC-NK) and embryonic stem cell.

In certain embodiments of the above-mentioned method, the immune effector cell is a T lymphocyte; optionally, the source of the T lymphocyte is an autologous T lymphocyte or allogeneic T lymphocyte.

In certain embodiments of the above-mentioned method, the allogeneic T lymphocyte comprises more than 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 95%, 95%, 96%, 97%, 98%, 99% gene-edited *CD7/TRAC* double-negative cell.

In certain embodiments of the above-mentioned method, the T lymphocyte is an $\alpha\beta$ T lymphocyte or a $\gamma\delta$ T lymphocyte.

In certain embodiments of the above-mentioned method, *Trac* gene and *CD7* gene of T cell are gene-edited with gene editing tool to obtain *CD7/TRAC* double-negative cell; wherein the gene editing tool is selected from the group consisting of CRISPR/Cas system, zinc-finger nuclease system, transcription activator-like effector nuclease system; optionally, the gene editing tool is selected from CRISPR/Cas system; further optionally, the CRISPR/Cas system comprises Cas9 protein and sgRNA.

In certain embodiments of the above-mentioned method, the sgRNA of the CRISPR/Cas system targeting *CD7* gene is shown in SEQ ID NO: 33.

In certain embodiments of the above-mentioned method, the chRDNA of the CRISPR/Cas system targeting *Trac* gene is shown in SEQ ID NO: 34.

In certain embodiments of the above-mentioned method, the method of introducing the nucleotide sequence encoding any one of the above-mentioned chimeric antigen receptor into the immune effector cell is selected from one or more of: the method of using virus or the method of using non-virus; optionally, the method of using virus comprises using one or more of the following viral vectors: γ -retroviral vector, lentiviral vector, adenovirus-associated viral vector; the method of using non-virus comprises one or more of the following methods: gene transfer with transposon, gene transduction with mRNA-mediating, electrotransformation.

In yet another aspect, the present application also provides use of the above-mentioned chimeric antigen receptor, the above-mentioned isolated nucleic acid molecule, the above-mentioned vector, or the above-mentioned engineered immune effector cell in the preparation of a medicament, wherein the medicament is used for treating diseases or conditions related to the expression of CD7.

In certain embodiments of the above-mentioned use, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain embodiments of the above-mentioned use, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain embodiments of the above-mentioned use, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain embodiments of the above-mentioned use, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In yet another aspect, the present application also provides a method for treating diseases or conditions related to the expression of CD7, including the following steps: administering an effective amount of the above-mentioned engineered immune effector cell or pharmaceutical composition to a subject having a need for treatment of the diseases or conditions related to the expression of CD7.

In certain embodiments of the above-mentioned method, the administration can be performed in different ways, such as intravenous, intratumoral, intraperitoneal, subcutaneous, intramuscular, topical, or intradermal administration. For example, the administration can be performed by intravenous injection to a subject. In certain

embodiments, an effective dose of engineered immune effector cell or the pharmaceutical composition can be administered to a subject in a single time, or in divided doses within a certain period, such as once a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months, or once every 3-6 months.

In certain embodiments of the above-mentioned method, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain embodiments of the above-mentioned method, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain embodiments of the above-mentioned method, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain embodiments of the above-mentioned method, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In certain embodiments of the above-mentioned method, the administration method is an intravenous injection.

In certain embodiments of the above-mentioned method, the effective amount of the engineered immune effector cell or the pharmaceutical composition is at a dose of 1×10^5 to 1×10^7 cells/kg.

In yet another aspect, the present application also provides a medicament comprising the above-mentioned engineered immune effector cell or pharmaceutical composition, for use in treating diseases or conditions related to the expression of CD7.

In certain embodiments of the above-mentioned medicament, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain embodiments of the above-mentioned medicament, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain embodiments of the above-mentioned medicament, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain embodiments of the above-mentioned medicament, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the detection results of CAR⁺ expression rates in CAR-T cells after transduction of T cells and expansion culture for different times in Example 2, wherein: UTD-NoEP represents the untransduced and non-knockout T cell group, UTD-KO TRAC+CD7 represents the untransduced but double-knockout T cell group, the other six groups represent double-knockout T cell group transduced with different CARs, respectively.

FIG. 2 shows the detection results of total T cell number after transduction of T cells and expansion culture for different times in Example 2, wherein: UTD-NoEP represents the untransduced and non-knockout T cell group, UTD-KO TRAC+CD7 represents the untransduced but double-knockout T cell group, the other six groups represent double-knockout T cell group transduced with different CARs, respectively.

FIG. 3A shows the short-term killing activity of CAR-T cells using murine-derived or 5 types of humanized Th69 scFv against positive target cell MOLT4 under effector-target ratios of 1:3, 1:1, and 3:1 in Example 3;

FIG. 3B shows the short-term killing activity of CAR-T cells using murine-derived or 5 types of humanized Th69 scFv against negative target cell KO CD7-MOLT4 under effector-target ratios of 1:3, 1:1, and 3:1 in Example 3;

FIG. 3C shows the long-term killing activity of CAR-T cells using murine-derived or 5 types of humanized Th69 scFv against positive target cell MOLT4 under effector-target ratios of 1:32, 1:16, 1:4, and 1:1 in Example 3;

FIG. 3D shows the long-term killing activity of CAR-T cells using murine-derived or 5 types of humanized Th69 scFv against positive target cell jurkat under effector-target ratios of 1:32, 1:16, 1:4, and 1:1 in Example 3;

FIG. 3E shows the long-term killing activity of CAR-T cells using murine-derived or 5 types of humanized Th69 scFv against negative target cell KO CD7-jurkat under effector-target ratios of 1:32, 1:16, 1:4, and 1:1 in Example 3;

Wherein: in FIG. 3A-FIG. 3E, NoEP represents the untransduced and non-knockout T cells, UTD represents untransduced but CD7 and TRAC double-knockout T cells, the other six groups represent double-knockout T cell group transduced with different CARs, respectively.

FIG. 4 shows the cumulative expansion folds of T cells in each group during rounds 1–4 in the multiple rounds of stimulation experiments in Example 4, wherein: NoEP represents the untransduced and non-knockout T cells, UTD represents untransduced but CD7 and TRAC double-knockout T cells, the other six groups represent double-knockout T cell group transduced with different CARs, respectively.

FIG. 5 shows the survival rates of mice at different times after infusion with different test samples in Table 3 in *in vivo* experiment in Example 6.

FIG. 6 shows the *in vivo* tumor imaging signal values of mice at different times after infusion with different test samples in Table 3 in *in vivo* experiment in Example 6.

DETAILED DESCRIPTION OF THE INVENTION

The embodiments of the present invention will be illustrated with the following specific examples. Those familiar with this technology can easily understand other advantages and effects of the present invention from the disclosure of the present specification.

The present application is further described below: in the present invention, unless otherwise stated, the scientific and technical terms used herein have the meanings commonly understood by those skilled in the art. Furthermore, the terms and laboratory procedures related to protein and nucleic acid chemistry, molecular biology, cell and tissue culture, microbiology, and immunology used herein are terms and conventional procedures widely used in the corresponding fields. Meanwhile, in order to better understand the present invention, definitions and explanations of relevant terms are provided below.

In the present application, the term “antibody” has the conventional meaning in the art and used in its broadest meaning in the present application. The antibody generally comprises heavy (H) chain and/or light (L) chain, analysis of amino acid sequences of the heavy chain and light chain in different antibodies revealed that the amino acid sequence near the N-terminus of heavy chain and light chain varies considerably, while the amino acid sequence of the other portions is relatively constant. Therefore, the region near the N-terminus in antibody light chain and heavy chain, where the amino acid sequence varies greatly, is referred to as a variable region (V), and the region near the C-terminus, where the amino acid sequence is relatively constant, is referred to as a constant region (C). The V regions of a heavy chain and a light chain are respectively abbreviated as VH and VL, and the C regions of a heavy chain and a light chain are respectively abbreviated as CH and CL. Small portions of amino acid residues within an antibody variable region vary strongly, and these regions where the residue composition and order of amino acids vary more easily are referred to as hypervariable regions (HVRs); there are three hypervariable regions in each V region of L chain and H chain, which can form a precise complement with an antigenic determinant spatially,

and thus a hypervariable region is also referred to as a complementarity determining region (CDR). In antibodies, the common rules for dividing CDR include Kabat, AbM, Chothia, Contact, and IMGT, which are well-known to those skilled in the art. When applying a website that implements these rules, once the VH and VL sequences are entered and the corresponding rules are selected, CDR sequences according to different rules can be obtained.

In the present application, the term “antigen-binding fragment” has the conventional meaning in the art, referring to a key fragment on an antibody that can specifically recognize and bind to an antigen, comprising VH region and/or VL region.

In the present application, the term “humanized antibody” is also referred to as a humanized engineered antibody. The humanized engineered methods are known in the art (e.g., WO96/02576). The purpose of humanized modification is to reduce its heterology while substantially retain the affinity and specificity of the parent antibody. For example, when the CDRs are derived from a murine antibody, primers can be synthesized (the corresponding primers can be obtained by referring to the method described in WO98/13388) and used to connect the CDR of the murine antibody with the framework region (FR) of a human antibody.

In the present application, the terms “Th-69 antibody” and “Th69 antibody” refer to an existing murine-derived antibody. It is an IgG1 monoclonal antibody generated by immunizing mice with T acute lymphoblastic leukemia (T-ALL) cell lines CEM and HSB-2, by Gramatzki M et al. in 1985. Th-69 can specifically bind to the extracellular domain of human CD7 with high affinity. When the scFv derived from murine-derived antibody is directly used in CAR-T drugs, it may increase immunological risk. For reducing the immunogenicity of scFv derived from murine-derived antibody and improving the safety of therapy, humanized modification can be performed on the scFv derived from murine-derived antibody. However, during the humanized modification process, the binding affinity, specificity, and other functionality of the antibody for specific antigen may be compromised. Therefore, obtaining humanized antibodies with reduced immunogenicity and uncompromised or even enhanced functionality has practical significance.

In the present application, the term “scFv” has the conventional meaning in the art, referring to a Single Chain Variable Fragment (abbreviated as scFv), which is an antibody composed of an antibody heavy chain variable region and a light chain variable region linked together via a short peptide (linker, linker sequence).

In the present application, nouns not explained specifically such as terms “Sc(Fv)₂” and “[Sc(Fv)₂]₂” have the conventional meaning in the art.

In the present application, the term “antibody drug” has the conventional meaning in the art, i.e. a drug with an antibody as active ingredient, such as monoclonal antibody

drug and bispecific antibody drug.

In the present application, the term “antibody-drug conjugate” has the conventional meaning in the art, i.e. antibody-drug conjugate, abbreviated as ADC. It is formed by connecting a biologically active small-molecule drug to a monoclonal antibody via a chemical chain, wherein the monoclonal antibody serves as a vector to deliver the small-molecule drug to the target cell.

In the present application, the term “Chimeric Antigen Receptor (CAR)” represents the core component of CAR cell therapy drugs, which may include an extracellular antigen recognition domain (for example, the portion that binds to a Tumor-Associated Antigen (TAA)), a hinge region, a transmembrane region and an intracellular domain. CAR-T (Chimeric Antigen Receptor T) cell immunotherapy is considered to be one of the most promising methods to conquer tumors. CAR-T cells are generated by genetically modifying T cells to express CAR proteins, and such CAR protein has the ability to recognize intact proteins on the membrane surface independently of antigen presentation, thereby causing T cell activation and functional effects.

In the present application, the term “extracellular antigen recognition domain” refers to the Antigen Recognition Domain (ARD). The reason why CAR cell therapy products (such as CAR-T cells) can specifically recognize and/or bind to target antigens expressed by tumor cells depends on the extracellular antigen recognition domain. So far, the antigen recognition domain has been derived from Single Chain Variable Fragment (abbreviated as scFv) of an antibody, or from receptor-ligand interactions, TCR mimics, Variable Lymphocyte Receptors (VLR). So far, the most common source is scFv segment of the antibody.

In the present application, the term “specific recognition and/or binding” refers to the recognition and/or binding between CAR and a specific target, wherein CAR binding to this target is with greater affinity, greater avidity, more easily, and/or of greater duration than CAR binding to other targets.

In the present application, CD7 is a transmembrane glycoprotein comprising 240 amino acid residues, and is a member of immunoglobulin supergene family. CD7 is generally overexpressed in immature T-cell tumors, whereas in mature T-cell tumors, the expression pattern and intensity of CD7 vary. In addition to T-cell malignancies, leukemia blasts and malignant progenitor cells in approximately 30% of patients with AML also express CD7. Therefore, drugs targeting CD7 may be also used for the treatment of CD7⁺ mixed-phenotype AML, as well as most NK lymphomas and NKT lymphomas. However, CD7 CAR-T cells may also kill CD7 CAR-T “sibling” while killing tumor cells with high CD7 expression, since CD7 is also expressed on the surface of T cells, which results in a significant reduction in the number of CAR-T cells during *in vitro* culture phase on Day 5.

In the present application, the terms “*Trac* gene” and “*TRAC* gene” have the conventional meaning in the art, referring to the T cell receptor α chain constant region. The T cell receptor (TCR) primarily recognizes antigen signals through the TCR-CD3 complex (comprising α chain, β chain, CD3 γ , CD3 δ , etc. of TCR). TCR is consisted of α chain and β chain, the translated α chain and β chain assemble into a heterodimer, which then associates with multiple CD3 subtype molecules. In cells, redundant TCR will be degraded if it fails to form a complete complex with CD3 molecules. Knockout of the *Trac* gene may affects the formation of TCR-CD3 complex, meaning that T cell receptors on the T cell surface will be eliminated, thereby preventing the occurrence of graft-versus-host disease (GVHD).

In the present application, the term “GVHD (graft-versus-host disease)” has the conventional meaning in the art, generally referring to graft-versus-host disease, it is resulted from T lymphocytes derived from allogeneic donor graft are stimulated by a series of “cytokine storms” initiated by the recipient after transplantation, which significantly enhances their immune response against recipient antigens and triggers cytotoxic attacks targeting recipient target cells, with the skin, liver and intestines as the major target.

In the present application, the term “*CD7/TRAC* double-negative allogeneic T lymphocytes” refers to the allogeneic T lymphocytes in which *CD7* gene and *TRAC* gene are abnormally expressed, or in which CD7 protein and T cell receptor α chain constant region are not properly expressed.

In the present application, the term “gene is abnormally expressed” has the conventional meaning in the art. Gene expression refers to the process by which genetic information derived from a gene is synthesized into functional gene expression product, the gene expression product generally refers to protein.

In the present application, the terms “gene editing” and “gene editing technology” have the conventional meaning in the art, referring to a technology that enables site-specific modification of the genome. Using this technology, a specific site in the genome can be precisely targeted, followed by cleavage of the target DNA fragment, knock-in or knock-out of the target gene segment at this site. As a molecular biological technology, gene editing technology can achieve precise modification of chromosomes, thereby altering the existing functions of cells. Compared with cell lines generally used in basic research, T cell, as a primary cell, exhibits no particular specificity other than the inability to proliferate long-term, can likewise be edited by the gene editing technology. At present, there are three major gene editing tools, namely zinc finger nuclease (ZFN) technology, transcription activator-like effector nuclease (TALEN) technology, and RNA-guided CRISPR/Cas nuclease technology. Compared with traditional gene targeting technology, new gene editing technologies retains the feature of site-specific modification, and can be applied to more species and cell types, with higher efficiency, shorter construction cycles and lower cost.

In the present application, the terms “CRISPR/Cas technology” and “CRISPR/Cas system” have the conventional meaning in the art, with the full name of clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins, it is an acquired immune system discovered in most bacteria and all archaea, which can specifically cleave foreign gene fragments. At present, diverse types of CRISPR/Cas systems have been identified, among which the construction of Type II system is relatively simple, with Cas9 protein and guide RNA (Gna, also referred to as sgRNA) as the core components. Compared with the earlier ZFN and TALEN technologies, CRISPR/Cas has the following advantages: low off-target rate, high efficiency, cost-effectiveness and wide application range. In addition, studies have reported that compared with the full RNA guide technology, the CRISPR chimeric RNA-DNA (chRDNA) guide technology can significantly enhance the specificity of Cas9 protein, thereby achieving high-level expected genome editing in cells and minimizing off-target rate.

In the present application, the terms “zinc finger nuclease technology” and “zinc finger nuclease system” have the conventional meaning in the art. The core design principle of zinc finger nuclease technology is the ingenious chimerization of two domains with specific functions, i.e. a specific recognition module and a functional module. The most typical zinc finger nuclease is constructed by fusing the non-specific endonuclease Fok I with a zinc finger-containing domain, which is capable of recognizing specific DNA sequence.

In the present application, the terms “transcription activator-like effector nuclease technology” and “transcription activator-like effector nuclease system” have the conventional meaning in the art. TALE effector is initially discovered as an invasion strategy employed by bacteria to infect plants. By fusing the Fok I nuclease to an artificial TALE with sequence-specific binding capacity, the researchers constructed a powerful class of tools with specific gene editing function, i.e., the TALEN protein. The typical TALEN protein consists of: a N-terminal domain containing a nuclear localization signal (NLS), a central domain containing a typical tandem TALE repeats which is capable of recognizing specific DNA sequence, and a C-terminal domain with the Fok I endonuclease function. The core principle of TALEN technology is to orderly achieve three different functions within a single TALEN protein: guide into cell nucleus, specific recognition of target DNA sites, and cleavage of target DNA sites.

In the present application, the term “hinge region” refers to the linker segment between the extracellular antigen recognition domain and the transmembrane domain, and the hinge region allows the CAR to recognize the antigen by providing a certain range of movement for the antigen recognition domain. The hinge regions currently used are mainly derived from one or more of IgG1, IgG4, CD4, CD7, CD28, CD84, and CD8 α .

In the present application, a “transmembrane region” refers to the transmembrane

domain that connects the intracellular and extracellular components of the CAR structure. Different transmembrane domains can affect the expression and stability of CAR to some extent, but they are not directly involved in signal transduction, and they improve downstream signal transduction by interactions. The transmembrane region may be derived from one or more of CD3, CD4, CD7, CD8 α , CD28, CD80, CD86, CD88, 4-1BB, CD152, OX40, and Fc70.

In the present application, the term “intracellular domain” comprises an intracellular signal transduction region and may further comprise a costimulatory signal transduction region.

In the present application, the term “intracellular signal transduction region” refers to the activation of at least one normal effector function of immune effector cells responsible for expressing CAR. The intracellular signal transduction region may be derived from one or more of CD3 ζ , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, FcR γ , FcR β , CD66d, DAP10, DAP12, and Syk.

In the present application, the term “costimulatory signal transduction region” exists because in addition to stimulation by antigen-specific signal, many immune effector cells require costimulation to promote cell proliferation, differentiation, and survival, as well as effector function of activated cells. In certain examples, the CAR may further comprise one or more costimulatory signal transduction region, wherein the costimulatory signal transduction region may be derived from one, two or more of CD2, CD3, CD7, CD27, CD28, CD30, CD40, CD83, CD244, 4-1BB, OX40, LFA-1, ICOS, LIGHT, NKG2C, NKG2D, DAP10, B7-H3 and MyD88.

In the present application, the term “guiding peptide” refers to the short peptide before the extracellular antigen recognition domain (such as the scFv sequence), whose function is to guide the recombinant protein synthesized in the cell to be exported to the outside of the cell. Commonly used guiding peptides include human CD8 α signaling peptide or human GM-CSF receptor α signaling peptide.

In the present application, one of the key factors that determine the therapeutic effect of CAR-immune cells is the choice of tumor target antigens, but the choice of tumor target antigens does not have to be a single one, thus, the extracellular antigen recognition domain may also comprise the extracellular antigen recognition domain (e.g. scFv antibody) targeting any one of the following targets: CD5, CD19, CD20, CD22, CD33, CD123, CLL1, BCMA, CD138, and CS1. For instance, the extracellular antigen recognition domain in dual-target CAR-T products comprises the scFv sequences targeting two targets. The scFv antibody targeting a single target comprises antibody heavy chain variable region (VH) and light chain variable region (VL), which are linked by linker sequence. The scFv antibody targeting two or more targets comprises the VH and VL regions targeting different targets, different regions are also linked by linker sequence directly or indirectly, and the arrangement can be any of the

following forms: Target 1 VL-Target 1 VH-Target 2 VL-Target 2 VH, Target 2 VL-Target 2 VH-Target 1 VL-Target 1 VH, Target 1 VL-Target 2 VL-Target 2 VH-Target 1 VH, and Target 2 VL-Target 1 VL-Target 1 VH-Target 2 VH. The hyphen “-” mentioned above represents linkage via a linker sequence.

In the present application, the term “linker sequence” generally refers to an oligopeptide or polypeptide region of about 1 to 100 amino acids in length, which connects any structure/region of the antibody or chimeric antigen receptor of the present invention together. The linker sequence may consist of different amino acid residues (e.g., glycine and serine) so that adjacent protein domains can move freely relative to each other. A longer linker sequence may be used when it is desirable to ensure that two adjacent domains do not spatially interfere with each other.

In the present application, the term “isolated” generally means those obtained from the natural state by artificial means. If an “isolated” substance or component occurs in nature, it may be that the natural environment in which it is located has changed, or that the substance has been separated from its natural environment, or both. For example, a certain unisolated polynucleotide or polypeptide naturally exists in a living animal, and the high purity of the same polynucleotide or polypeptide isolated from this natural state is referred to as isolated. The term “isolated” does not exclude substances that have been artificially or synthetically after being obtained from their natural state by artificial means, nor does it exclude the presence of other impure substances that do not affect the activity of the substance.

In the present application, the term “isolated nucleic acid molecule” generally refers to an isolated form of a nucleotide, deoxyribonucleotide or ribonucleotide of any length, which may be isolated from its natural environment or may be an artificial synthetic analog.

In the present application, when performing CAR gene transduction/transfection and target gene expression, gene transduction/transfection methods mainly include viral and non-viral methods. For example: the methods through a γ -retroviral vector, lentiviral vector, adenovirus-associated viral vector, plasmid DNA-dependent vector, transposon-dependent gene transfer, and mRNA-mediated gene transduction.

The term “vector” generally refers to a nucleic acid delivery vehicle into which a polynucleotide encoding a protein can be inserted and the protein can be expressed. The vector may be transformed, transduced, or transfected into the host cell so that the genetic material elements it carries can be expressed in the host cell. For example, vectors include: plasmids; phagemids; cosmids; artificial chromosomes such as yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), or P1-derived artificial chromosomes (PACs); phages such as lambda phage or M13 phage; and animal viruses, etc. The types of animal viruses used as vectors include retroviruses (including lentiviruses), adenoviruses, adeno-associated viruses, herpesviruses (such as

herpes simplex virus), poxviruses, baculoviruses, papillomaviruses, and papovaviruses (such as SV40). A vector may contain a variety of elements that control expression, including promoter sequences, transcription initiation sequences, enhancer sequences, selection elements, and reporter genes. In addition, the vector may also contain a replication origin. A vector may also contain components that assist them in entering into cells, such as viral particles, liposomes, or protein coats, but not just these substances. However, the method of introducing a polynucleotide encoding a certain protein into a cell does not necessarily rely on a vector, and the methods such as transposon and electrotransformation can be used instead. The term “transposon” refers to a discontinuous DNA segment that has the ability to migrate and carry genetic information between chromosomal sites, such as the Sleeping Beauty (SB) system and the PB system derived from *lepidopteran* insects. In certain examples, mRNA may also be transduced into T cells by means of electrotransformation.

In the present application, the term “immune effector cell” generally refers to a cell that participates in immune response, for example a cell that promotes immune effector response. An immune effector cell may be selected from the group consisting of: one or more of a T lymphocyte, natural killer cell (NK cell), peripheral blood mononuclear cell (PBMC cell), induced pluripotent stem cell, T lymphocyte differentiated from an induced pluripotent stem cell, NK cell differentiated from an induced pluripotent stem cell, and embryonic stem cell.

In the present application, the term “pharmaceutical composition” generally refers to a pharmaceutical composition suitable for administration to a patient, which may comprise the immune effector cell as described in the present application, and may further comprise one or more pharmaceutically acceptable adjuvants, such as: one or more of a carrier, protective agent, stabilizer, excipient, diluent, solubilizer, surfactant, emulsifier, and preservative. In certain examples, the pharmaceutically acceptable adjuvants comprise a protective agent, such as a cell cryopreservation solution. In certain examples, the pharmaceutical composition of the present application is a cell suspension or cryopreserved cells thereof.

In the present application, the term “subject” generally refers to a human or non-human animal, including but not limited to a mouse, rat, cat, dog, rabbit, horse, pig, cow, sheep, or monkey.

In the present application, the term “comprise/comprising” generally refers to the inclusion of explicitly specified features, but not the exclusion of other elements.

In the present application, the term “about” generally refers to a range of fluctuation above or below the specified value that is acceptable to those skilled in the art, such as: variation within the range of $\pm$ (0.5%–10%), such as variation within the range of 0.5%, 1%, 1.5%, 2%, 2.5%, 3%, 3.5%, 4%, 4.5%, 5%, 5.5%, 6%, 6.5%, 7%, 7.5%, 8%, 8.5%, 9%, 9.5%, or 10% above or below the specified value.

A CD7-targeting humanized antibody or antigen-binding fragment thereof, corresponding nucleic acid molecule, corresponding vector, corresponding cell, corresponding pharmaceutical composition

In one aspect, the present application provides a CD7-targeting humanized antibody or antigen-binding fragment thereof, comprising:

the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2;

or the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30;

or the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30.

In the present application, the biocomputational evaluation results indicated that all the humanization designs for the murine-derived antibody Th69 improved the humanization degree of the antibody, and the immunogenicity prediction results showed that humanization reduced immunogenicity of the antibodies, and the immunogenicity risk of all five candidate antibodies was low. The inventors further verified the proliferation, specificity and functionality of the chimeric antigen receptors consisted of five humanized scFv antibodies through experiments: the non-specific killing of the five groups of humanized CAR-T cells against negative target cells was significantly weaker than that of murine-derived mTh69 CAR-T cells; the CD107a expression of humanized Th69 was higher than that of murine-derived mTh69 CAR-T cells after co-culture with antigen-positive target cells, while no significant difference in CD107a expression after co-culture with antigen-negative target cells, suggesting that humanized Th69 scFv enhanced the specific binding capacity to CD7 antigen; the results of expansion capacity assay by multi-round stimulation showed that the proliferation capacity of humanized Th69 CAR-T cells was significantly superior to that of murine-derived CAR-T cells, among which the hu11 CAR-T cells, hu12 CAR-T cells and hu21 CAR-T cells exhibited a significant advantage in proliferation ability; the *in vivo* efficacy results showed that the humanized sequence hu21 had a stronger tumor-killing effect and prolonged the survival time of mice. In conclusion, humanized hu21, hu11 and hu12 were selected as the candidate sequences.

In certain examples of the above-mentioned humanized antibody or antigen-binding fragment thereof, the humanized antibody or antigen-binding fragment thereof is a scFv antibody, a sc(Fv)₂ antibody, or a [sc(Fv)₂]₂ antibody.

In certain examples the above-mentioned humanized antibody or antigen-binding fragment thereof, the humanized antibody or antigen-binding fragment thereof is the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 3;

or the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 35;

or the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 36.

In yet another aspect, the present application also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding the above-mentioned humanized antibody or antigen-binding fragment thereof.

In certain examples of the above-mentioned isolated nucleic acid molecule, the nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1, is shown in SEQ ID NO: 4;

and/or, the nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2, is shown in SEQ ID NO: 5;

and/or, the nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28, is shown in SEQ ID NO: 37;

and/or, the nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30, is shown in SEQ ID NO: 38.

In yet another aspect, the present application also provides a vector comprising the above-mentioned isolated nucleic acid molecule. The vector can be optionally selected from one or more of the following vectors: plasmids; phagemids; cosmids; artificial chromosomes such as yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), or P1-derived artificial chromosomes (PACs); phages such as lambda phage or M13 phage; and animal viruses, etc. The types of animal viruses used as vectors include retroviruses (including lentiviruses), adenoviruses, adeno-associated viruses, herpesviruses (such as herpes simplex virus), poxviruses, baculoviruses, papillomaviruses, and papovaviruses (such as SV40).

In yet another aspect, the present application also provides a cell comprising any one of the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule or the vector.

In yet another aspect, the present application also provides a pharmaceutical composition comprising any one of the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule, the vector or the cell, and a pharmaceutically acceptable adjuvant. The pharmaceutically acceptable

adjuvant comprises but not limited to one or more of carrier, protective agent, stabilizer.

Use of CD7-targeting humanized antibody or antigen-binding fragment thereof

In yet another aspect, the present application also provides use of the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule, the vector or the cell in the preparation of a detection reagent for diagnosing diseases or conditions related to the expression of CD7.

In certain examples of the above-mentioned use, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain examples of the above-mentioned use, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain examples of the above-mentioned use, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain examples of the above-mentioned use, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In yet another aspect, the present application also provides use of the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule, the vector or the cell in the preparation of a medicament for treating diseases or conditions related to the expression of CD7.

In certain examples of the above-mentioned use, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain examples of the above-mentioned use, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain examples of the above-mentioned use, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain examples of the above-mentioned use, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous

T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In yet another aspect, the present application also provides a method for treating diseases or conditions, including the following steps: administering an effective amount of a medicament comprising any one of the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule, the vector or the cell to a subject having a need for treatment of the diseases or conditions related to the expression of CD7.

In certain examples of the above-mentioned method, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain examples of the above-mentioned method, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain examples of the above-mentioned method, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain examples of the above-mentioned method, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In certain examples of the above-mentioned method, the administration can be performed in different ways, such as oral, intravenous, intratumoral, intraperitoneal, subcutaneous, intramuscular, topical, or intradermal administration.

In yet another aspect, the present application also provides a medicament comprising the above-mentioned humanized antibody or antigen-binding fragment thereof, the isolated nucleic acid molecule, the vector or the cell, for use in treating diseases or conditions related to the expression of CD7.

In certain examples of the above-mentioned medicament, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain examples of the above-mentioned medicament, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain examples of the above-mentioned medicament, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain examples of the above-mentioned medicament, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

An antibody drug, an antibody-drug conjugate comprising CD7-targeting humanized antibody or antigen-binding fragment thereof

In yet another aspect, the present application also provides an antibody drug comprising any one of the above-mentioned humanized antibody or antigen-binding fragment thereof.

In some examples of the above-mentioned antibody drug, the antibody drug is a monospecific antibody drug, a bispecific antibody drug, a trispecific antibody drug, or a tetraspecific antibody drug.

In yet another aspect, the present application also provides an antibody-drug conjugate comprising any one of the above-mentioned humanized antibody or antigen-binding fragment thereof.

A CD7-targeting chimeric antigen receptor, corresponding nucleic acid molecule, corresponding vector, corresponding immune effector cell, pharmaceutical composition

In yet another aspect, the present application also provides a CD7-targeting chimeric antigen receptor, comprising a CD7-targeting extracellular antigen recognition domain, a hinge region, a transmembrane region, and an intracellular domain, wherein the CD7-targeting extracellular antigen recognition domain comprises:

the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2;

or the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30;

or the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30.

In certain examples of any one of the above-mentioned chimeric antigen receptor, the extracellular antigen recognition domain comprises any one structure selected from the

group consisting of: (the amino acid sequence as shown in SEQ ID NO: 1)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 2), (the amino acid sequence as shown in SEQ ID NO: 2)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 1)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 30), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 28)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 30), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 28);

optionally, the extracellular antigen recognition domain comprises any one structure selected from the group consisting of: (the amino acid sequence as shown in SEQ ID NO: 2)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 28);

further optionally, the extracellular antigen recognition domain is any one structure selected from the group consisting of: (the amino acid sequence as shown in SEQ ID NO: 2)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 28).

In the above description, the hyphen “-” represents linkage, and it has directionality in above-mentioned description, indicating the linkage from the N-terminal to the C-terminal of the amino acid; optionally, “-” represents direct linkage.

In certain examples of any one of the above-mentioned chimeric antigen receptor, the linker sequence is selected from one or more of the following sequences: SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

In certain examples of any one of the above-mentioned chimeric antigen receptor, the extracellular antigen recognition domain comprises: the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 3, or the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 35, or the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 36;

optionally, the amino acid sequence of the extracellular antigen recognition domain is shown in SEQ ID NO: 3, or is shown in SEQ ID NO: 35, or is shown in SEQ ID NO: 36.

In certain examples of any one of the above-mentioned chimeric antigen receptor, the

hinge region is derived from one or more of IgG1, IgG4, CD4, CD7, CD28, CD84, CD8 α ; optionally, the amino acid sequence of the hinge region is derived from CD8 α ; further optionally, the amino acid sequence of the hinge region comprises the amino acid sequence as shown in SEQ ID NO: 9; even further optionally, the amino acid sequence of the hinge region is shown in SEQ ID NO: 9.

In certain examples of any one of the above-mentioned chimeric antigen receptor, the transmembrane region is derived from one or more of CD3, CD4, CD7, CD8 α , CD28, CD80, CD86, CD88, 4-1BB, CD152, OX40, Fc70; optionally, the amino acid sequence of the transmembrane region is derived from CD8 α ; further optionally, the amino acid sequence of the transmembrane region comprises the amino acid sequence as shown in SEQ ID NO: 10; even further optionally, the amino acid sequence of the transmembrane region is shown in SEQ ID NO: 10.

In certain examples of any one of the above-mentioned chimeric antigen receptor, the intracellular domain comprises an intracellular signal transduction region; optionally, the intracellular domain further comprises a costimulatory signal transduction region.

In certain examples of any one of the above-mentioned chimeric antigen receptor, the intracellular signal transduction region is derived from one or more of CD3 ζ , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, FcR γ , FcR β , CD66d, DAP10, DAP12, Syk; optionally, the intracellular signal transduction region is derived from CD3 ζ ; further optionally, the amino acid sequence of the intracellular signal transduction region comprises the amino acid sequence as shown in SEQ ID NO: 11; even further optionally, the amino acid sequence of the intracellular signal transduction region is shown in SEQ ID NO: 11.

In certain examples of any one of the above-mentioned chimeric antigen receptor, the costimulatory signal transduction region is derived from one, two, or more of CD2, CD3, CD7, CD27, CD28, CD30, CD40, CD83, CD244, 4-1BB, OX40, LFA-1, ICOS, LIGHT, NKG2C, NKG2D, DAP10, B7-H3, MyD88; optionally, the costimulatory signal transduction region is derived from CD28 or 4-1BB; further optionally, the amino acid sequence of the costimulatory signal transduction region comprises the amino acid sequence as shown in SEQ ID NO: 12; even further optionally, the amino acid sequence of the costimulatory signal transduction region is shown in SEQ ID NO: 12.

In certain examples of any one of the above-mentioned chimeric antigen receptor, the chimeric antigen receptor further comprises a guiding peptide located at the N-terminus of the amino acid sequence of the chimeric antigen receptor; optionally, the guiding peptide is derived from CD8 α ; further optionally, the amino acid sequence of the guiding peptide comprises the amino acid sequence as shown in SEQ ID NO: 13; even further optionally, the amino acid sequence of the guiding peptide is shown in SEQ ID NO: 13.

In certain examples of any one of the above-mentioned chimeric antigen receptor, the chimeric antigen receptor comprises any one of the following sequences: the amino acid sequence as shown in SEQ ID NO: 14, or the amino acid sequence as shown in SEQ ID NO: 18; or the amino acid sequence as shown in SEQ ID NO: 19.

optionally, the amino acid sequence of the chimeric antigen receptor is shown in SEQ ID NO: 14, or is shown in SEQ ID NO: 18, or is shown in SEQ ID NO: 19.

In yet another aspect, the present application also provides an isolated nucleic acid molecule comprising a nucleotide sequence encoding any one of the above-mentioned chimeric antigen receptor.

In certain examples of the above-mentioned isolated nucleic acid molecule, the nucleotide sequence encoding the chimeric antigen receptor comprises: a nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1, which is shown in SEQ ID NO: 4; and a nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2, which is shown in SEQ ID NO: 5;

or, a nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1, which is shown in SEQ ID NO: 4; and a nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30, which is shown in SEQ ID NO: 38;

or, a nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28, which is shown in SEQ ID NO: 37; and a nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30, which is shown in SEQ ID NO: 38.

In certain examples of the above-mentioned isolated nucleic acid molecule, the nucleotide sequence encoding the chimeric antigen receptor comprises:

a nucleotide sequence encoding the amino acid sequence as shown in SEQ ID NO: 14, which is shown in SEQ ID NO: 15;

or, a nucleotide sequence encoding the amino acid sequence as shown in SEQ ID NO: 18, which is shown in SEQ ID NO: 39;

or, a nucleotide sequence encoding the amino acid sequence as shown in SEQ ID NO: 19, which is shown in SEQ ID NO: 40.

In yet another aspect, the present application also provides a vector comprising the above-mentioned isolated nucleic acid molecule. The vector is selected from one or more of the following vectors: plasmids; phagemids; cosmids; artificial chromosomes

such as yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), or P1-derived artificial chromosomes (PACs); phages such as lambda phage or M13 phage; and animal viruses, etc. The types of animal viruses used as vectors include retroviruses (including lentiviruses), adenoviruses, adeno-associated viruses, herpesviruses (such as herpes simplex virus), poxviruses, baculoviruses, papillomaviruses, and papovaviruses (such as SV40).

In certain examples of the above-mentioned vector, the vector is an expression vector; in another examples, the vector is a viral vector; in another examples, the vector is a lentiviral vector.

In yet another aspect, the present application also provides an engineered immune effector cell comprising the above-mentioned chimeric antigen receptor, the above-mentioned isolated nucleic acid molecule, or the above-mentioned vector.

In certain examples of the above-mentioned engineered immune effector cell, the engineered immune effector cell is selected from one or more of a T lymphocyte, natural killer cell (NK cell), peripheral blood mononuclear cell (PBMC cell), induced pluripotent stem cell, T cell differentiated from an induced pluripotent stem cell, NK cell differentiated from an induced pluripotent stem cell and embryonic stem cell.

In certain examples of the above-mentioned engineered immune effector cell, the engineered immune effector cell is a T lymphocyte; optionally, the source of the T lymphocyte is an autologous T lymphocyte or allogeneic T lymphocyte.

In certain embodiments of the above-mentioned engineered immune effector cell, the allogeneic T lymphocyte comprises gene-edited *CD7/TRAC* double-negative cell; further, the allogeneic T lymphocyte comprises more than 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 95%, 95%, 96%, 97%, 98%, 99% gene-edited *CD7/TRAC* double-negative cell.

In certain examples of the above-mentioned engineered immune effector cell, the T lymphocyte is an $\alpha\beta$ T lymphocyte or a $\gamma\delta$ T lymphocyte.

In yet another aspect, the present application also provides a pharmaceutical composition comprising the above-mentioned engineered immune effector cell, and a pharmaceutically acceptable adjuvant.

In certain examples of the above-mentioned pharmaceutical composition, the pharmaceutically acceptable adjuvant includes a protective agent.

In certain examples of the above-mentioned pharmaceutical composition, the pharmaceutically acceptable adjuvant includes a cell cryopreservation solution.

In certain examples of the above-mentioned pharmaceutical composition, the pharmaceutical composition is a cell suspension or cryopreserved cells thereof.

In certain examples of the above-mentioned pharmaceutical composition, the pharmaceutical composition is an intravenous injection.

A preparing method of the engineered immune effector cell

In yet another aspect, the present application also provides a method for preparing the engineered immune effector cell, including the following steps: introducing a nucleotide sequence encoding any one of the above-mentioned chimeric antigen receptor into an immune effector cell.

In certain examples of the above-mentioned method, the immune effector cell is selected from one or more of a T lymphocyte, natural killer cell (NK cell), peripheral blood mononuclear cell (PBMC cell), induced pluripotent stem cell, T cell differentiated from an induced pluripotent stem cell (iPSC-T), NK cell differentiated from an induced pluripotent stem cell (iPSC-NK) and embryonic stem cell.

In certain examples of the above-mentioned method, the engineered immune effector cell is a T lymphocyte; optionally, the source of the T lymphocyte is an autologous T lymphocyte or allogeneic T lymphocyte.

In certain examples of the above-mentioned method, the allogeneic T lymphocyte comprises more than 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 95%, 95%, 96%, 97%, 98%, 99% gene-edited *CD7/TRAC* double-negative allogeneic T lymphocyte.

In certain examples of the above-mentioned method, *Trac* gene and *CD7* gene of T cell are gene-edited with gene editing tool to obtain *CD7/TRAC* double-negative cell. The gene editing tool is selected from the group consisting of CRISPR/Cas system, zinc-finger nuclease system, transcription activator-like effector nuclease system; optionally, the gene editing tool is selected from CRISPR/Cas system; further optionally, the CRISPR/Cas system comprises Cas9 protein and sgRNA.

In certain examples of the above-mentioned method, the sgRNA of the CRISPR/Cas system targeting *CD7* gene is shown in SEQ ID NO: 33.

In certain examples of the above-mentioned method, the chRDNA of the CRISPR/Cas system targeting *Trac* gene is shown in SEQ ID NO: 34.

In certain examples of the above-mentioned method, the T lymphocyte is an $\alpha\beta$ T lymphocyte or a $\gamma\delta$ T lymphocyte.

In certain examples of the above-mentioned method, the method of introducing the

nucleotide sequence encoding any one of the above-mentioned chimeric antigen receptor into the immune effector cell is selected from one or more of: the method of using virus or the method of using non-virus; optionally, the method of using virus comprises using one or more of the following viral vectors: γ -retroviral vector, lentiviral vector, adenovirus-associated viral vector; the method of using non-virus comprises one or more of the following methods: gene transfer with transposon, gene transduction with mRNA-mediating, electrotransformation.

Use of CD7-targeting chimeric antigen receptor

In yet another aspect, the present application also provides use of the above-mentioned chimeric antigen receptor, the above-mentioned isolated nucleic acid molecule, the above-mentioned vector, or the above-mentioned engineered immune effector cell in the preparation of a medicament, wherein the medicament is used for treating diseases or conditions related to the expression of CD7.

In certain examples of the above-mentioned use, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain examples of the above-mentioned use, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain examples of the above-mentioned use, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain examples of the above-mentioned use, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In yet another aspect, the present application also provides a method for treating a diseases or conditions related to the expression of CD7, including the following steps: administering an effective amount of the above-mentioned engineered immune effector cell or pharmaceutical composition to a subject having a need for treatment of the diseases or conditions related to the expression of CD7.

In certain examples of the above-mentioned method, the administration can be performed in different ways, such as intravenous, intratumoral, intraperitoneal, subcutaneous, intramuscular, topical, or intradermal administration. For example, the administration can be performed by intravenous injection to a subject. In certain embodiments, an effective dose of engineered immune effector cell or the pharmaceutical composition can be administered to a subject in a single time, or in

divided doses within a certain period, such as once a week, once every two weeks, once every three weeks, once every four weeks, once a month, once every 3 months, or once every 3-6 months.

In certain examples of the above-mentioned method, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain examples of the above-mentioned method, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain examples of the above-mentioned method, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain examples of the above-mentioned method, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

In certain examples of the above-mentioned method, the administration method is an intravenous injection.

In certain examples of the above-mentioned method, the effective amount of the engineered immune effector cell or the pharmaceutical composition is at a dose of 1×10^5 to 1×10^7 cells/kg. In certain examples, the doses for administration may vary for different indications; and the doses for administration may also vary depending on the severity of the patient's condition. The dose may be administered in a range of 1×10^5 CAR positive T cells/kg to 1×10^7 CAR positive T cells/kg, for example, 1×10^5 CAR positive T cells/kg to 1×10^6 CAR positive T cells/kg, 1×10^6 CAR positive T cells/kg to 1×10^7 CAR positive T cells/kg, 0.5×10^6 CAR positive T cells/kg, 0.6×10^6 CAR positive T cells/kg, 0.7×10^6 CAR positive T cells/kg, 0.8×10^6 CAR positive T cells/kg, 0.9×10^6 CAR positive T cells/kg, 1.0×10^6 CAR positive T cells/kg, 1.1×10^6 CAR positive T cells/kg, 1.2×10^6 CAR positive T cells/kg, 1.3×10^6 CAR positive T cells/kg, 1.4×10^6 CAR positive T cells/kg, 1.5×10^6 CAR positive T cells/kg, 1.6×10^6 CAR positive T cells/kg, 1.7×10^6 CAR positive T cells/kg, 1.8×10^6 CAR positive T cells/kg, 1.9×10^6 CAR positive T cells/kg, 2.0×10^6 CAR positive T cells/kg.

In yet another aspect, the present application also provides a medicament comprising the above-mentioned engineered immune effector cell or pharmaceutical composition, for use in treating diseases or conditions related to the expression of CD7.

In certain examples of the above-mentioned medicament, the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor.

In certain examples of the above-mentioned medicament, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

In certain examples of the above-mentioned medicament, the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia.

In certain examples of the above-mentioned medicament, the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

Without wishing to be bound by any theory, the following examples are only described to illustrate the chimeric antigen receptor, the engineered immune effector cell, the preparation method and use of the present application, and are not used to limit the scope thereof. The examples do not include detailed descriptions of traditional methods, such as those used to construct vectors and plasmids, methods of inserting genes encoding proteins into such vectors and plasmids, or methods of introducing plasmids into host cells. Such methods have been well-known to those of ordinary skill in the art, and described in many publications, including Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edition, Cold spring Harbor Laboratory Press.

Example 1: humanized modification design of murine-derived antibody and preparation of CD7 CAR-T cell

In the present application, the VH amino acid sequence (as shown in SEQ ID NO: 31) and VL amino acid sequence (as shown in SEQ ID NO: 32) of the murine-derived antibody sequence Th69 were subjected to CDR division according to KABAT rule, to obtain the amino acid sequences of VH CDR1, CDR2, CDR3 as shown in SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, respectively; and the amino acid sequences of VL CDR1, CDR2, CDR3 as shown in SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, respectively. Humanized modification was performed based on above sequences.

The template selected for the humanized modification of Th69 VH region was IGHv3-21*07. In the humanized modification of VH, different combinations of multiple key amino acids were chosen for back mutation, resulting in three humanized Th69 VH designs, namely Th69 VH-1, Th69 VH-2 and Th69 VH-3 (the amino acid sequences are as shown in SEQ ID NO: 1, SEQ ID NO: 27 and SEQ ID NO: 28, respectively). The multiple key amino acids include the amino acids at PHI-ANGLE position, the amino acids that are completely buried in the interior and have a low occurrence frequency at the corresponding position in human, and the amino acids at PHI-ANGLE

position and are also close to the CDR regions.

The template selected for the humanized modification of Th69 VL region was IGKv1-27*01. In the humanized modification of VL, different combinations of multiple key amino acids were chosen for back mutation, resulting in three humanized Th69 VL designs, namely Th69 VL-1, Th69 VL-2 and Th69 VL-3 (the amino acid sequences are as shown in SEQ ID NO: 29, SEQ ID NO: 30 and SEQ ID NO: 2, respectively). The multiple key amino acids include the amino acids at PHI-ANGLE position, the amino acids at VH/VL interface, upper core amino acids.

The humanized heavy chains and light chains were linked via linker sequences to form hu01 scFv, hu10 scFv, hu11 scFv, hu12 scFv and hu21 scFv, respectively. Each candidate humanized scFv antibody was screened based on the second-generation CAR structure, i.e., the second-generation CAR structure obtained by directly linking (a signal peptide)-(a extracellular antigen recognition domain)-(a hinge region)-(a transmembrane region)-(a costimulatory signal transduction region)-(a intracellular signal transduction region) in the order of N-terminal to C-terminal. In each CAR structure, CD8 α guiding chain was all used as the signal peptide (as shown in SEQ ID NO: 13), hu01 scFv, hu10 scFv, hu11 scFv, hu12 scFv, hu21 scFv and murine-derived control Th69 scFv were used as the extracellular antigen recognition domain, respectively, the hinge region (as shown in SEQ ID NO: 9) and the transmembrane region (as shown in SEQ ID NO: 10) adopted the structure of CD8 α , 4-1BB was used as the costimulatory signal transduction region (as shown in SEQ ID NO: 12), CD3 ζ was used as the intracellular signal transduction region (as shown in SEQ ID NO: 11). The corresponding amino acid sequences of the VH and VL of each candidate humanized scFv and the amino acid sequences of the corresponding CAR are shown in Table 1 in detail.

Table 1. Various CD7 CAR sequences

CAR name	CAR amino acid sequence NO.	Adopted scFv	scFv VH amino acid sequence NO.	scFv VL amino acid sequence NO.
hu01 CAR	SEQ ID NO: 16	hu01 scFv	SEQ ID NO: 1	SEQ ID NO: 29
hu10 CAR	SEQ ID NO: 17	hu10 scFv	SEQ ID NO: 27	SEQ ID NO: 30
hu11 CAR	SEQ ID NO: 18	hu11 scFv	SEQ ID NO: 1	SEQ ID NO: 30
hu12 CAR	SEQ ID NO: 19	hu12 scFv	SEQ ID NO: 28	SEQ ID NO: 30
hu21 CAR	SEQ ID NO: 14	hu21 scFv	SEQ ID NO: 1	SEQ ID NO: 2
Th69 CAR	SEQ ID NO: 20	Th69 scFv	SEQ ID NO: 31	SEQ ID NO: 32

The specific method for obtaining CD7 CAR-T cells was as follows:

1. Construction of lentiviral vectors

Based on Table 1, the nucleotide sequences encoding the above-mentioned 6 types of CD7 CAR structures were respectively constructed into empty lentiviral vector

(manufacturer: SBI, Cat. No.: CD500-CD800, subjected to conventional resistance modification as described in Example 1 of WO2021/121227) to obtain CAR expression vectors. The CAR expression vectors were then co-transfected with three packaging plasmids into 293T cells, and functional lentiviral vectors were obtained after collection and purification. The three packaging plasmids were pMD2.G (purchased from Biovector Corporation, Cat. No.: Biovector012259), pMDLg/pRRE (purchased from Biovector Corporation, Cat. No.: Biovector012251) and pRSV-Rev (purchased from Biovector Corporation, Cat. No.: Biovector012253), respectively.

2. Preparation of T cells

1) Sorting of T cells

Peripheral blood mononuclear cells (PBMC) were isolated from the subject's apheresis cells, and then T cells were sorted from the PBMC cells.

2) Activation of the T cells

The isolated T cells were resuspended with complete lymphocyte culture medium (X-VIVO15 medium + 5% fetal bovine serum + 300IU/ml IL-2 or X-VIVO15 medium + 5% fetal bovine serum + 5ng/ml IL-15 + 10ng/ml IL-7) to give a final concentration of $(1\sim3) \times 10^6$ cells/ml, and CD3/CD28 magnetic beads were added based on 1ul beads/ 1×10^6 cells to stimulate the cells. The mixture was well mixed, and placed in an incubator for culture for at least 24 h under the culture condition of 37°C+5% CO₂, to obtain the activated T cells.

3) Gene editing was performed on the activated T cells

Since 90% of T cells express CD7 on their surface, the inherent expression of CD7 on the surface of CD7 CAR-T cells will result in self-killing of the CD7 CAR-T cells. Knockout of the *CD7* gene in T cells by gene-editing methods to abolish surface CD7 expression is an effective strategy to prevent self-killing. Concurrently, the *Trac* gene in T cells was knocked out by gene-editing methods to make it suitable as a universal CAR-T product, and the specific methods were as follows:

RNP complex was formulated, each 11.5 μ L of RNP complex consists of 10 μ L Opti-MEM culture medium+0.6 μ L Cas9 (10 mg/mL)+0.45 μ L CD7 gRNA (100 μ M) +0.45 μ L TRAC chRDNA (100 μ M). The mixture was incubated at room temperature for 15 min. Wherein the sequence of the CD7 sgRNA is shown in SEQ ID NO: 33, and the sequence of the TRAC chRDNA is shown in SEQ ID NO: 34. The activated T cells were taken, the electroporation buffer was added at 10 μ L/M (1×10^6) cells to resuspend the cells, then mixed with the incubated RNP complex, 11.5 μ L of RNP complex was added per 1×10^6 cells, transferred to an electroporation cuvette. EH100 pulse program on the Lonza 4D electroporator was selected for electroporation. After electroporation,

pre-warmed T cell complete medium was added immediately to the electroporation cuvette, and the electroporation cuvette was incubated in the incubator for 15 min. Then the cells were transferred to a 96-well plate, supplemented with 100 μ L pre-warmed T cell complete medium, under the culture condition of 37°C+5% CO₂.

3. The sorted, activated, and gene-edited T cells (with double knockout of *CD7* and *Trac* genes) were transduced with lentivirus to prepare the corresponding CD7 CAR-T cells

The gene-edited T cells were taken, resuspended with X-VIVO15 medium alone containing polybrene at a final concentration of 8 μ g/ml and mixed well to obtain the cell suspension. 200 μ L of the lentiviral vector was slowly added per 800 μ L of the cell suspension (containing 2×10^6 cells). After well mixed, the mixture was placed in a well plate, placed in an incubator for culture for at least 4-6 h under the culture condition of 37°C+5% CO₂.

4. Expansion culture of transduced T cells

The transduced cells were taken, cultured with complete lymphocyte culture solution and passaged every other day to keep cell density at $(0.8 \sim 2) \times 10^6$ cells/ml for use in subsequent examples.

After infecting T cells with lentiviruses containing the CAR structures listed in Table 1, the obtained T cells were named according to their CAR sequence numbers. For example, T cells obtained with hu21 CAR were named hu21 CAR-T cells, and T cells obtained with hu12 CAR were named hu12 CAR-T cells.

On Day 4 after transduction, the cells were stained with fluorescent-labeled CD7 antibody (BioLegend, 395606)/CD3 antibody (BioLegend, 317318), then the proportion of CD7⁺/CD3⁺ cell was detected by flow cytometry, and was calculated to obtain that the knockout efficiency of CD7 was $94.481 \pm 3.656\%$, and the knockout efficiency of TRAC was $96.201 \pm 1.437\%$.

Example 2: Detection of CAR positive proportion of CAR-T cells and detection of CAR-T cells expansion

1. Detection of CAR positive proportion of CAR-T cells

The 6 types of CAR-T cells obtained in Example 1, untransduced and non-knockout T cells (UTD-NoEP group), and untransduced but double-knockout T cells (UTD-KO TRAC+CD7 group) were stained with FITC fluorescent-labeled CD7 antigen (manufacturer: ACRO Biosystems, Cat. No.: CD7-HF258). Various cells were detected for the positive proportion of CAR molecules by flow cytometry on different days after transduction, the detection results were shown in FIG. 1: the CAR expression levels on

the surface of the 5 types of CAR-T cells using humanized scFv were not significantly different from that of the CAR-T cell using murine-derived scFv, and the CAR-positive rates detected on Days 4, 6, and 11 after transduction were on the same level; and no CAR expression was detected in the two negative control groups: untransduced and non-knockout T cells and untransduced but double-knockout T cells.

2. Detection of total T cells expansion on different days after transduction

Samples of the 6 types of CAR-T cells obtained in Example 1, untransduced and non-knockout T cells (UTD-NoEP group), and untransduced but double-knockout T cells (UTD-KO TRAC+CD7 group) were taken on different days after transduction. The cells were stained with AO/PI double fluorescence (AO/PI double-stained cell apoptosis detection kit is commercially available; it detects the state of apoptotic cells by using the method of double-staining cell nucleus with AO/PI probes, which can distinguish normal cells and apoptotic cells). Each group of cells was counted by a cell counter to calculate the total T cell expansion fold during the culture process. The results were shown in FIG. 2: during culture process, the expansion folds of the 5 types of CAR-T cells using humanized scFv were all higher than that of the CAR-T cells using murine-derived scFv, but there was no significant difference in the expansion ability among the 5 types of CAR-T cells using humanized-derived scFv. In addition, the expansion ability of the double-knockout T cells was significantly higher than that of the non-knockout T cells, regardless of whether they were transduced with the CAR structure. It is speculated that the failure to knockout CD7 gene leads to self-killing of T cells, thereby affecting their expansion ability.

Example 3: *In vitro* killing ability of CAR-T cells

The *in vitro* killing experiments is divided into short-term killing experiment and long-term killing experiment, wherein: the short-term killing experiment means that the CAR-T cells using murine-derived or humanized Th69 scFv were co-cultured with target cells in X-VIVO15 medium under different effector-target ratios (1:3, 1:1, and 3:1) (effector cells were counted based on CAR⁺ cells) for 4-6 hours. The killing ratio of CAR-T cells to target cells was detected by detecting the activity of luciferase stably expressed in the target cells. The method for establishing the target cell line stably expressing luciferase was as follows: the target cells were infected with the viruses carrying luc-GFP sequence (the conventional luciferase and fluorescent protein sequences in the prior art could be used), followed by monoclonal culture via the infinite dilution method, and clones with GFP positivity and better growth status were picked up; the long-term killing experiment means that the CAR-T cells using murine-derived or humanized Th69 scFv were co-cultured with target cells in X-VIVO15 medium under different effector-target ratios (1:32, 1:16, 1:4, and 1:1) (effector cells were counted based on CAR⁺ cells) for 24 hours. The killing ratio of CAR-T cells to target cells was detected by detecting the activity of luciferase stably expressed in the target cells.

In the above-mentioned experiments, untransduced and non-knockout T cells (NoEP group) and untransduced but double-knockout T cells (UTD group) were used as negative effector cells; the target cells comprised Molt-4 cells (human T acute lymphoblastic leukemia cells) endogenously expressing CD7, Jurkat cells (human T lymphoblastic leukemia cells) endogenously expressing CD7, and KO CD7 Molt-4 cells (which refer to the KO CD7 Molt-4 cell lines established by knocking out CD7 by using CRISPR/Cas9 gene-editing technique, followed by selecting monoclonal CD7-Molt-4 cells via the infinite dilution method, the CD7 sgRNA used was identical to that in Example 1) as negative controls, and KO CD7 Jurkat cells (which refer to the KO CD7 Jurkat cell lines established by knocking out CD7 by using CRISPR/Cas9 gene-editing technique, followed by selecting monoclonal CD7-Jurkat cells via the infinite dilution method, the CD7 sgRNA used was identical to that in Example 1) as negative controls. The experimental results were as follows:

Short-term killing results: as shown in FIG. 3A, under effector-target ratios of 1:3, 1:1, and 3:1, the killing abilities of CAR-T cells using murine-derived or humanized Th69 scFv against positive target cells MOLT4 were on the same level. However, as shown in FIG. 3B, murine-derived Th69 CAR-T cells and humanized hu01 CAR-T cells exhibited non-specific killing effects against negative target cells KO CD7 MOLT4 under high effector-target ratio of 3:1, while the 4 groups of humanized hu10 CAR-T cells, humanized hu11 CAR-T cells, humanized hu12 CAR-T cells and humanized hu21 CAR-T cells exhibited no non-specific killing against negative target cells, with significant differences (*** represents $p < 0.001$, **** represents $p < 0.0001$), indicating that the safety of the 4 groups of humanized CAR-T cells was better.

Long-term killing results: as shown in FIG. 3C and FIG. 3D, under effector-target ratios of 1:32, 1:16, 1:4, and 1:1, the overall killing abilities of CAR-T cells using murine-derived or 5 types of humanized Th69 scFv against positive target cells MOLT4 and Jurkat were on the same level. However, humanized hu21 CAR-T cells exhibited a significant advantage in killing effects against positive target cells Jurkat under low effector-target ratios of 1:32 compared with murine-derived Th69 CAR-T cells (* represents $p < 0.05$); In addition, as shown in FIG. 3E, under high effector-target ratios of 1:4 and 1:1, murine-derived Th69 CAR-T cells exhibited non-specific killing against negative target cells KO CD7 Jurkat, while the non-specific killing of the 5 types of humanized CAR-T cells against negative target cells was significantly weaker than that of murine-derived Th69 CAR-T cells (* represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$, **** represents $p < 0.0001$), indicating that the safety of the 5 groups of humanized CAR-T cells was better.

Example 4: Multiple rounds of stimulation experiments of CAR-T cells

Antigen stimulation can activate CAR-T cells and cause CAR-T cell proliferation, while sustained activation of T cells will lead to cell exhaustion. The proliferation

ability and effector function of exhausted T cells will decrease. We verified the sustained proliferation ability of CD7 CAR-T cells by detecting the proliferation of CD3⁺ cells (i.e., T cell proliferation) after multiple rounds of antigen stimulation experiments. Humanized scFv clones with long-term proliferation ability were used as the screening criterion for selecting preferred antibodies.

The CAR positive ratios of each humanized CAR-T cells were adjusted to the same level as the group of CAR-T cells with the lowest CAR positive ratio using UTD. In the antigen stimulation experiment, each group of CAR-T cells was co-cultured with CD7⁺ target cells Molt4 in a 24 well plate under effector-target of 1:1, with 2ml of X-VIVO15 medium per well. Each group of cells was repeated in 3 wells. Fluorescent-labeled CD3 antibody (Manufacturer: BioLegend, Cat. No.: 300312) was used for T cells marking (CD3 was a marker to distinguish whether they are T cells). Detection and analysis were performed using flow cytometry to measure the proliferation of T cells. The cell number of CD3 positive cells was calculated based on the conversion of volume multiples. Then, according to the calculation results, a certain amount of CAR-T cells was taken from each group and added to the corresponding positive target cells under effector-target ratio of 1:1 for a new round of stimulation. This was repeated every 48h for 4 rounds of stimulation. The proliferation results were shown in FIG. 4: the humanized CAR-T cell groups all exhibited a significant advantage in proliferation ability compared with the Th69 CAR-T cell groups (** represents $p < 0.01$, *** represents $p < 0.001$, **** represents $p < 0.0001$), wherein hu21 CAR-T cells, hu11 CAR-T cells, and hu12 CAR-T cells exhibited more significant advantage in proliferation.

Example 5: Specific analysis

For verifying whether humanized modification of the sequence affects the specificity of the CD7 CAR, we designed cell-based experiments to evaluate the specificity of the 5 types of humanized Th69 CAR obtained from screening. The expression of lysosome-associated membrane protein 1 (CD107a) on the membrane was considered as a biomarker for activation of cytotoxic lymphocytes (CD8⁺ T cells and NK cells). When cytotoxic lymphocytes were stimulated with specific antigens, immune activation occurred, and CD107a was expressed. Therefore, the expression of CD107a can be used as a means of detecting CAR-T cell specificity.

Cell co-culture was performed according to the following method, and the specificity of CD7 CAR was evaluated by detecting CD107a via flow cytometry. The specific steps were as follows: tumor cells, CAR-T cells and untransduced T cells were counted. Tumor cells were adjusted to $2 \times 10^6/\text{ml}$ with X-VIVO culture medium, and CAR-T cells and untransduced T cells were adjusted to $1 \times 10^6/\text{ml}$ with X-VIVO culture medium. 20 μl of fluorescent-labeled CD107a antibodies (purchased from Biolegend, Model: 328608) were added to each well of a 96-well plate, then 100 μl of T cells (T cells refer to CAR-T cells or untransduced T cells) and 100 μl of tumor cells were added

to each well. After addition, the plates were centrifuged at 400r/min for 3 min. The cells were incubated in a 37°C, 5% CO₂ incubator for 60 min. After 60 min, 20 µl of Golgi stop working solution (i.e. 3ml X-VIVO15 culture medium plus 2µl Golgi stop protein transport inhibitor, Golgi stop was purchased from Biolegend, Model: 344722) was added to each well, then incubated in an incubator for 2.5 hours. Equal volumes of CD3 (purchased from Biolegend, Model: 300326) and CD8 antibodies (purchased from Biolegend, Model: 344722) were mixed, 10 µl of which was added to each well after mixing, and incubated in the incubator for 30 min. After 30 min, the cell cultures were centrifuged at 1500r/min for 5 min, the supernatant was shaken off, 200 µl of FACS buffer (PBS+0.5%BSA) was added and mixed well. The cell cultures were centrifuged at 1500r/min for 5 min, the supernatant was shaken off. 200 µl of FACS buffer (PBS+0.5%BSA) was added to each well and mixed well, then transferred to a labeled flow tube and then detected on a machine. The fluorescence signal of CD107a in the CD3⁺CD8⁺ cell population was analyzed. In the above-mentioned experiments, the CAR-T cells refer to the murine-derived Th69 CAR-T cells, the 5 types of humanized CAR-T cells, or the CAR-T cells prepared by installing h189-4 anti-CD7 scFv (VL and VH derived from SEQ ID NO: 17 and SEQ ID NO: 18 in CN112300282A, respectively, with the same linker sequence as other groups), 3A1e-LH anti-CD7 scFv (VL and VH derived from SEQ ID NO: 90 and SEQ ID NO: 91 in CN113383071A, respectively, with the same linker sequence as other groups) into the empty lentiviral vector mentioned in the Examples according to the same CAR construct sequence in Example 1 and prepared according to the method described in Example 1. The tumor cells refer to the cell lines with high CD7 expression (also referred to as CD7⁺ target cells in the present application): CCRF-CEM (T acute lymphoblastic leukemia cell line, commercial cell line), MOLT4 (T acute lymphoblastic leukemia cell line, commercial cell line); the cell lines with low CD7 expression (also referred to as CD7⁻ target cells in the present application): CD7 KO MOLT4 (the cell lines established by knocking out CD7 by using CRISPR/Cas9, followed by selecting and culturing CD7⁻ monoclonal MOLT4 cells), Nalm6 (human acute lymphoblastic leukemia cell, commercial cell line), 786-O (human clear cell renal adenocarcinoma cell line, commercial cell line), U266 (human multiple myeloma cell line, commercial cell line).

The CD107a detection results were shown in Table 2. After co-culture of CD7⁺ target cells and CD7⁻ target cells with untransduced T cells (UTD group), no significant difference was observed in CD107a expression levels. After co-culture of each group of CD7 CAR-T cells with CD7⁺ target cells CCRF/MOLT4, a high proportion of cells expressed CD107a, while positive proportion of CD107a was very low after co-culture of CAR-T cells with CD7⁻ target cells CD7 KO MOLT4/Nalm6/786-O/U266, indicating that the 5 types of humanized Th69 CARs exhibited excellent antigen recognition specificity. In addition, hu01 CAR-T, hu10 CAR-T, hu11 CAR-T, hu12 CAR-T, and hu21 CAR-T cells exhibited higher CD107a expression than that of murine-derived Th69 CAR-T cells after co-culture with antigen-positive target cells, suggesting that humanized Th69 scFv enhanced the specific binding capacity to CD7 antigen.

Table 2. CD107a expression of UTD or different CD7 CAR-T cells stimulated by various target cells

	CD7 expression	UTD	h189-4	3A1e	mTh69	hu01	hu10	hu11	hu12	hu21
NC (Background expression)	---	1.86%	3.00%	3.90%	2.01%	2.69%	2.50%	3.35%	2.59%	3.16%
CCRF-CEM	99.99%	4.06%	12.04%	33.70%	15.35%	26.33%	26.01%	27.08%	26.33%	25.70%
MOLT4	98.89%	4.79%	14.32%	31.21%	11.63%	22.78%	24.72%	28.07%	24.68%	25.38%
KO CD7-MOLT4	0.24%	4.35%	5.16%	8.39%	4.76%	6.28%	4.79%	6.68%	6.11%	5.08%
Nalm6	0.30%	4.10%	4.83%	7.22%	4.32%	6.34%	5.08%	5.33%	4.51%	5.96%
786-O	0.80%	3.46%	3.82%	3.21%	5.98%	4.85%	3.32%	4.35%	3.19%	3.29%
U266	1.28%	4.90%	4.54%	6.50%	5.05%	6.67%	5.27%	6.03%	6.39%	6.18%

Example 6: *In vivo* functional experiment

For further screening and identifying the humanized antibodies with optimal function, we performed *in vivo* pharmacological experiment of CD7 CAR-T. When selecting candidate sequences for *in vivo* functional experiments, after humanization scoring software (e.g., the classic humanization scoring system T20 score) and computational immunogenicity prediction (using AlphaMHC algorithm), hu01 scFv and hu10 scFv sequences exhibited high degree of humanization and low immunogenicity, and were therefore selected as candidate sequences for *in vivo* functional experiments. The results of *in vitro* functional experiments showed that hu21 CAR-T cells exhibited strong *in vitro* cell killing ability, weak non-specific killing, and strong proliferation ability after multiple rounds of stimulation, and were therefore also included as candidate sequence for *in vivo* functional experiments.

CCRF-CEM-GFP-Luc cells were inoculated into female NSG mice via tail intravenous injection at a volume of 2×10^5 cells/0.2 ml per mouse to establish CCRF-CEM-GFP-Luc T acute lymphoblastic leukemia animal model. On Day 4 after cell inoculation, the mice were randomly divided into groups according to tumor imaging signal intensity, the corresponding cells were infused via tail intravenous injection. The experiment was divided into totaling 8 groups (the grouping information was shown in the table below), including: PBS group, untransduced and non-knockout T cell group (T cell group), CD7/TRAC double-knockout and untransduced T cell group (DKO-T cell group), positive control 3A1e group (as mentioned in Example 5), murine-derived Th69 CAR-T cell group (mTh69 KO group), humanized hu01 CAR-T cell group (hu01 KO group), humanized hu10 CAR-T cell group (hu10 KO group), and humanized hu21 CAR-T cell group (hu21 KO group). The T cells in the above groups 3-8 were all derived from CD7/TRAC double-knockout T cells. The CAR-T positive proportion of each group was similar, after adjusting to same positive proportion, CAR-T cells were infused via single-time tail intravenous injection at a dose of 3×10^6 CAR-T cells /0.2ml per mouse. From Day 0 to Day 14 after infusion, living imaging was performed twice a week and

blood was taken for flow cytometry twice a week, the frequency was changed to once a week after Day 14. Mouse body weight and tumor imaging signal values were recorded to monitor and evaluate the tumor clearance effects of humanized hu01, hu10, and hu21 CAR-T cells in animals *in vivo*.

Table 3. Grouping and doses for each group in the mouse experiment

Groups		Doses (same positive proportion, same doses)	Number of animals
1	PBS group	NA	3
2	T cell group	Counted based on the group with the largest total cell number of the test sample	3
3	DKO-T cell group	Counted based on the group with the largest total cell number of the test sample	3
4	3A1e group	3×10^6	4
5	mTh 69 KO group	3×10^6	4
6	hu01 KO group	3×10^6	4
7	hu10 KO group	3×10^6	4
8	hu21 KO group	3×10^6	4

Note: Counted based on the total number of viable cells after recovery, and the cell concentration was adjusted to the administration concentration with PBS.

The results of the mice experiments are shown in Table 4, FIG. 5, and FIG. 6, respectively. The results in Table 4 and FIG. 5 showed the survival status of the mice. The survival times of mice in 3A1e group, mTh69 group, hu01 KO group and hu10 KO group were longer than those of PBS group, T cell group and DKO-T cell group, and the survival time of mice in hu21 KO group was the longest (as shown in FIG. 5), with a median survival time of 34.5 days (as shown in Table 4).

Table 4

	PBS group	T cell group	DKO-T cell group	3A1e group	mTh 69 group	hu01 KO group	hu10 KO group	hu21 KO group
Median survival time/Days	27	26	26	31	31.5	32	31	34.5

The results of mice *in vivo* tumor imaging signal value were shown in FIG. 6. Compared with PBS group, T cell group and DKO-T cell group, CAR-T cells in other groups all significantly inhibited tumor growth at a tested dosage, wherein the anti-tumor effect of hu21 KO group was the strongest.

By comprehensively comparing the anti-tumor effect of CAR-T cells and the survival period of mice in each group, the *in vivo* pharmacodynamic results demonstrated that the tumor-killing activity of humanized sequence hu21 was stronger than that of mTh69, hu-10 KO, hu-21 KO and 3A1e groups, and the survival time of mice with humanized

sequence hu21 was prolonged by 7.5 days compared with that of PBS control group, and by 3 days compared with that of murine-derived Th69 CAR-T group.

Sequence Description

SEQ ID NO: 1---The amino acid sequence of heavy chain variable region of scFv in Th69 VH-1, hu01/11/21 CAR

EVQLVESGGGLVKPGGSLRLSCAASGLTFSSYAMSWVRQAPEKRLEWVSSISS
GGFTYYPD SVKGRFTISRDN AKNSLYLQMNSLRAEDTAVYYCARDEV RGYLD
VWGQGTTVTVSS

SEQ ID NO: 2---The amino acid sequence of light chain variable region of scFv in Th69 VL-3, hu21 CAR

DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQKPD KTVKLLIYYTSSL
HSGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSKLPYTFGGG TKLEIK

SEQ ID NO: 3---The amino acid sequence of scFv in hu21 CAR

DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQKPD KTVKLLIYYTSSL
HSGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQYSKLPYTFGGG TKLEIKG
GGGSGGGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGLTFSSYAMSWVR
QAPEKRLEWVSSISSGGFTYYPD SVKGRFTISRDN AKNSLYLQMNSLRAEDTA
VYYCARDEV RGYLDVWGQGTTVTVSS

SEQ ID NO: 4---The nucleotide sequence encoding the amino acid sequence of heavy chain variable region as shown in SEQ ID NO: 1

GAGGTCCA ACTGGTGGAGTCCGGCGGGGGCCTCGTGAAGCCTGGCGGCA
GCCTGAGACTGAGCTGCGCCGCCTCCGGCCTGACCTTCAGCAGCTACGCC
ATGAGCTGGGTGAGACAAGCCCCGAGAAGAGACTGGAGTGGGTGAGCA
GCATCAGCAGCGGCGGCTTCACCTACTACCCCGACAGCGTGAAGGGCAGA
TTCACCATCAGCAGAGACAACGCCAAGAACAGCCTGTACCTGCAGATGAA
CAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGCTAGAGACGAGG
TGAGAGGCTACCTGGACGTGTGGGGCCAAGGCACCACCGTGACCGTGAGC
AGC

SEQ ID NO: 5---The nucleotide sequence encoding the amino acid sequence of light chain variable region as shown in SEQ ID NO: 2

GACATTCAGATGACACAGAGCCCTAGCAGCCTGAGCGCCTCCGTGGGCGA
CAGAGTGACCATCACCTGCAGCGCCTCCCAAGGCATCAGCAACTACCTGA
ACTGGTATCAGCAGAAGCCCGACAAGACCGTGAAGCTGCTGATCTACTACA
CAAGCAGCCTGCACAGCGGCGTGCCTAGCAGATTCAGCGGCAGCGGCAGC
GGCACCGACTACACCCTGACCATCAGCAGCCTGCAGCCTGAGGACTTCGC
CACCTACTACTGTCAGCAGTACAGCAAGCTGCCCTACACATTTGGGGGCGG
GACCAA ACTCGAGATTAAG

SEQ ID NO: 6---Linker sequence
GGGGS GGGGS GGGGS

SEQ ID NO: 7---Linker sequence
GSTSGSGKPGSGEGSTKG

SEQ ID NO: 8---Linker sequence
GGGGS

SEQ ID NO: 9---The amino acid sequence of the hinge region
TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD

SEQ ID NO: 10---The amino acid sequence of the transmembrane region
IYIWAPLAGTCGVLLLSLVITLYC

SEQ ID NO: 11---The amino acid sequence of the intracellular signal transduction region
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPRR
KNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYD
ALHMQALPPR

SEQ ID NO: 12---The amino acid sequence of the costimulatory signal transduction region
KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL

SEQ ID NO: 13---The amino acid sequence of the guiding peptide
MALPVTALLPLALLHAARP

SEQ ID NO: 14---The amino acid sequence of hu21 CAR
MALPVTALLPLALLHAARPDIQMTQSPSSLSASVGDRVTITCSASQGISNYL
NWYQQKPKDKTVKLLIYTTSSLHSGVPSRFSGSGGTDYTLTISSLQPEDFATYY
CQQYSKLPYTFGGGTKLEIKGGGGSGGGSGGGGSEVQLVESGGGLVKPGGG
LRLSCAASGLTFSSYAMSWVRQAPEKRLVWVSSISSGGFTYYPDSVKGRFTIS
RDNAKNSLYLQMNSLRAEDTAVYYCARDEVRGYLDVWGQGTTVTVSSTTTP
APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWAPLAGTCGV
LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA
LHMQALPPR

SEQ ID NO: 15---The nucleotide sequence encoding the amino acid sequence of hu21 CAR
ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCAC
GCCGCCAGGCCGGACATTCAGATGACACAGAGCCCTAGCAGCCTGAGCGC

CTCCGTGGGCGACAGAGTGACCATCACCTGCAGCGCCTCCCAAGGCATCA
GCAACTACCTGAACTGGTATCAGCAGAAGCCCGACAAGACCGTGAAGCTG
CTGATCTACTACACAAGCAGCCTGCACAGCGGCGTGCCTAGCAGATTGAGC
GGCAGCGGCAGCGGCACCGACTACACCCTGACCATCAGCAGCCTGCAGCC
TGAGGACTTCGCCACCTACTACTGTCAGCAGTACAGCAAGCTGCCCTACAC
ATTTGGGGGCGGGACCAAACCTCGAGATTAAGGGCGGGGGCGGGAGCGGC
GGGGGCGGGTCCGGGGGCGGGGGCAGCGAGGTCCAACCTGGTGGAGTCCG
GCGGGGGCCTCGTGAAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCC
TCCGGCCTGACCTTCAGCAGCTACGCCATGAGCTGGGTGAGACAAGCCCC
CGAGAAGAGACTGGAGTGGGTGAGCAGCATCAGCAGCGGCGGCTTCACCT
ACTACCCCGACAGCGTGAAGGGCAGATTCACCATCAGCAGAGACAACGCC
AAGAACAGCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGC
CGTGTACTACTGCGCTAGAGACGAGGTGAGAGGCTACCTGGACGTGTGGG
GCCAAGGCACCACCGTGACCGTGAGCAGCACCACGACGCCAGCGCCGCG
ACCACCAACACCGGCGCCACCATCGCGTCGCAGCCCCTGTCCCTGCGCC
CAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCT
GGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGG
GGTCCTTCTCCTGTCACTGGTTATCACCCCTTTACTGCAAACGGGGCAGAAA
GAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAACTACT
CAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAG
GATGTGAAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTAC
CAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGA
GGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGG
GAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCA
GAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGC
GCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCC
ACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC

SEQ ID NO: 16---The amino acid sequence of hu01 CAR

MALPVTALLLPLALLHAARPDIQMTQSPSSLSASVGDRVTITCSASQGISNYL
NWYQQKPGKAPKLLIYYTSSLHSGVPSRFSGSGSGTDFTLTISLQPEDFATYY
CQQYSKLPYTFGGGTKLEIKGGGGSGGGGSGGGGSEVQLVESGGGLVKPGGG
LRLSCAASGLTFSSYAMSWVRQAPEKRLVWVSSISSGGFTYYPDSVKGRFTIS
RDNAKNSLYLQMNSLRAEDTAVYYCARDEVRYLDVWGQGTTVTVSSTTTP
APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGV
LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR
VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA
LHMQUALPPR

SEQ ID NO: 17---The amino acid sequence of hu10 CAR

MALPVTALLLPLALLHAARPDIQMTQSPSSLSASVGDRVTITCSASQGISNYL
NWYQQKPGKTVKLLIYYTSSLHSGVPSRFSGSGSGTDFTLTISLQPEDFATYY
CQQYSKLPYTFGGGTKLEIKGGGGSGGGGSGGGGSEVQLVESGGGLVKPGGG

LRLSCAASGLTFSSYAMSWVRQAPGKGLEWVSSISSGGFTYYPDSVKGRFTIS
RDNAKNSLYLQMNSLRAEDTAVYYCARDEVIRGYLDVWGQGTTVTVSSTTTP
APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGV
LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR
VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDA
LHMQALPPR

SEQ ID NO: 18---The amino acid sequence of hu11 CAR

MALPVTALLPLALLHAARPDIQMTQSPSSLSASVGDRVTITCSASQGISNYL
NWYQQKPGKTVKLLIYYTSSLHSGVPSRFSGSGSGTDFTLTISLQPEDFATYY
CQQYSKLPYTFGGGTKLEIKGGGGSGGGGSGGGGSEVQLVESGGGLVKPGGS
LRLSCAASGLTFSSYAMSWVRQAPEKRLEWVSSISSGGFTYYPDSVKGRFTIS
RDNAKNSLYLQMNSLRAEDTAVYYCARDEVIRGYLDVWGQGTTVTVSSTTTP
APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGV
LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR
VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDA
LHMQALPPR

SEQ ID NO: 19---The amino acid sequence of hu12 CAR

MALPVTALLPLALLHAARPDIQMTQSPSSLSASVGDRVTITCSASQGISNYL
NWYQQKPGKTVKLLIYYTSSLHSGVPSRFSGSGSGTDFTLTISLQPEDFATYY
CQQYSKLPYTFGGGTKLEIKGGGGSGGGGSGGGGSEVQLVESGGGLVKPGGS
LRLSCAASGLTFSSYAMSWVRQAPEKRLEWVASISSGGFTYYPDSVKGRFTIS
RDNAKNSLYLQMNSLRAEDTAVYYCARDEVIRGYLDVWGAGTTVTVSSTTTP
APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGV
LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR
VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDA
LHMQALPPR

SEQ ID NO: 20---The amino acid sequence of Th69 CAR

MALPVTALLPLALLHAARPDIQMTQTSSLSASLGDRVTISCSASQGISNYL
NWYQQKPDGTVKLLIYYTSSLHSGVPSRFSGSGSGTDYSLTISNLEPEDIATYY
CQQYSKLPYTFGGGTKLEIKRGGGGSGGGGSGGGGSEVQLVESGGGLVKPGG
SLKLSAASGLTFSSYAMSWVRQTPEKRLEWVASISSGGFTYYPDSVKGRFTIS
RDNARNILYLQMSSLRSEDTAMYYCARDEVIRGYLDVWGAGTTVTVSSTTTP
APRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYWAPLAGTCGV
LLLSLVITLYCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCELR
VKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRK
NPQEGLYNELQKDKMAEAYSEIGMKGERRRRGKGHDGLYQGLSTATKDTYDA
LHMQALPPR

SEQ ID NO: 21---The amino acid sequence of VH CDR1 of Th69 antibody
SYAMS

SEQ ID NO: 22---The amino acid sequence of VH CDR2 of Th69 antibody
SISSGGFTYYPDSVKG

SEQ ID NO: 23---The amino acid sequence of VH CDR3 of Th69 antibody
DEVRGYLDV

SEQ ID NO: 24---The amino acid sequence of VL CDR1 of Th69 antibody
SASQGISNYLN

SEQ ID NO: 25---The amino acid sequence of VL CDR2 of Th69 antibody
YTSSLHS

SEQ ID NO:26---The amino acid sequence of VL CDR3 Th69 antibody
QQYSKLPYT

SEQ ID NO: 27---The amino acid sequence of heavy chain variable region of scFv in
Th69 VH-2, hu10 CAR
EVQLVESGGGLVKPGGSLRLSCAASGLTFSSYAMSWVRQAPGKGLEWVSSISS
GGFTYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARDEVRYLD
VWGQGTTVTVSS

SEQ ID NO: 28---The amino acid sequence of heavy chain variable region of scFv in
Th69 VH-3, hu12 CAR
EVQLVESGGGLVKPGGSLRLSCAASGLTFSSYAMSWVRQAPEKRLEWVASISS
GGFTYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARDEVRYLD
VWGAGTTVTVSS

SEQ ID NO: 29---The amino acid sequence of light chain variable region of scFv in
Th69 VL-1, hu01 CAR
DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQKPGKAPKLLIYYTSSL
HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSKLPYTFGGGTKLEIK

SEQ ID NO: 30---The amino acid sequence of light chain variable region of scFv in
Th69 VL-2, hu10/11/12 CAR
DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQKPGKTVKLLIYYTSSL
HSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYSKLPYTFGGGTKLEIK

SEQ ID NO: 31---The amino acid sequence of heavy chain variable region of murine-
derived antibody Th69
EVQLVESGGGLVKPGGSLKLSAASGLTFSSYAMSWVRQTPEKRLEWVASISS
GGFTYYPDSVKGRFTISRDNARNILYLQMSSLRSEDAMYYCARDEVRYLD

VWGAGTTVTVSS

SEQ ID NO: 32---The amino acid sequence of light chain variable region of murine-derived antibody Th69

DIQMTQTTSSLSASLGDRVTISCSASQGISNYLNWYQQKPDGTVKLLIYYTSSL
HSGVPSRFSGSGSGTDYSLTISNLEPEDIATYYCQQYSKLPYTFGGGGTKLEIKR

SEQ ID NO: 33---CD7 sgRNA

CGGAACCGUCUGUCCGUAGU

SEQ ID NO: 34---TRAC chRDNA

AGAGTCUCTCAGCUGGUACA

SEQ ID NO: 35---The amino acid sequence of scFv in hu11 CAR

DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQKPGKTVKLLIYYTSSL
HSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYSKLPYTFGGGGTKLEIKG
GGGSGGGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGLTFSSYAMSWVR
QAPEKRLEWVSSISSGGFTYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTA
VYYCARDEVIRGYLDVWGQGTTVTVSS

SEQ ID NO: 36---The amino acid sequence of scFv in hu12 CAR

DIQMTQSPSSLSASVGDRVTITCSASQGISNYLNWYQQKPGKTVKLLIYYTSSL
HSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQYSKLPYTFGGGGTKLEIKG
GGGSGGGGSGGGGSEVQLVESGGGLVKPGGSLRLSCAASGLTFSSYAMSWVR
QAPEKRLEWVASISSGGFTYYPDSVKGRFTISRDNKNSLYLQMNSLRAEDTA
VYYCARDEVIRGYLDVWGAGTTVTVSS

SEQ ID NO: 37---The nucleotide sequence encoding the amino acid sequence of heavy chain variable region as shown in SEQ ID NO: 28

GAGGTCCAACCTGGTGGAGTCCGGCGGGGGCCTCGTGAAGCCTGGCGGCA
GCCTGAGACTGAGCTGCGCCGCTCCGGCCTGACCTTCAGCAGCTACGCC
ATGAGCTGGGTGAGACAAGCCCCGAGAAGAGACTGGAGTGGGTGGCCT
CCATCAGCAGCGGCGGCTTCACCTACTACCCCGACAGCGTGAAGGGCAGA
TTCACCATCAGCAGAGACAACGCCAAGAACAGCCTGTACCTGCAGATGAA
CAGCCTGAGAGCCGAGGACACCGCCGTGTACTACTGCGCTAGAGACGAGG
TGAGAGGCTACCTGGACGTGTGGGGCGCCGGCACCAACCGTGACCGTGAGC
AGC

SEQ ID NO: 38---The nucleotide sequence encoding the amino acid sequence of light chain variable region as shown in SEQ ID NO: 30

GACATTCAGATGACACAGAGCCCTAGCAGCCTGAGCGCCTCCGTGGGCGA
CAGAGTGACCATCACCTGCAGCGCCTCCCAAGGCATCAGCAACTACCTGA
ACTGGTATCAGCAGAAGCCCGGCAAGACCGTGAAGCTGCTGATCTACTACA
CAAGCAGCCTGCACAGCGGCGTGCCTAGCAGATTCAGCGGCAGCGGCAGC

GGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCCTGAGGACTTCGC
CACCTACTACTGTCAGCAGTACAGCAAGCTGCCCTACACATTTGGGGGCGG
GACCAAACCTCGAGATTAAG

SEQ ID NO: 39---The nucleotide sequence encoding the amino acid sequence of hu11
CAR

ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCAC
GCCGCCAGGCCGGACATTCAGATGACACAGAGCCCTAGCAGCCTGAGCGC
CTCCGTGGGCGACAGAGTGACCATCACCTGCAGCGCCTCCCAAGGCATCA
GCAACTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGACCGTGAAGCTG
CTGATCTACTACACAAGCAGCCTGCACAGCGGCGTGCCTAGCAGATTCAGC
GGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCC
TGAGGACTTCGCCACCTACTACTGTCAGCAGTACAGCAAGCTGCCCTACAC
ATTTGGGGGCGGGACCAAACCTCGAGATTAAGGGCGGGGGCGGGAGCGGC
GGGGGCGGGTCCGGGGGGCGGGGGCAGCGAGGTCCAACCTGGTGGAGTCCG
GCGGGGGCCTCGTGAAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCC
TCCGGCCTGACCTTCAGCAGCTACGCCATGAGCTGGGTGAGACAAGCCCC
CGAGAAGAGACTGGAGTGGGTGAGCAGCATCAGCAGCGGCGGCTTCACCT
ACTACCCCGACAGCGTGAAGGGCAGATTCACCATCAGCAGAGACAACGCC
AAGAACAGCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGC
CGTGTACTACTGCGCTAGAGACGAGGTGAGAGGCTACCTGGACGTGTGGG
GCCAAGGCACCACCGTGACCGTGAGCAGCACCACGACGCCAGCGCCGCG
ACCACCAACACCGGCGCCACCATCGCGTCGCAGCCCCCTGTCCCTGCGCC
CAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCT
GGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGG
GGTCCTTCTCCTGTCACTGGTTATCACCTTTACTGCAAACGGGGCAGAAA
GAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACT
CAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAG
GATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTAC
CAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGA
GGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGG
GAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCA
GAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGC
GCCGGAGGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCC
ACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC

SEQ ID NO: 40---The nucleotide sequence encoding the amino acid sequence of hu12
CAR

ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCAC
GCCGCCAGGCCGGACATTCAGATGACACAGAGCCCTAGCAGCCTGAGCGC
CTCCGTGGGCGACAGAGTGACCATCACCTGCAGCGCCTCCCAAGGCATCA
GCAACTACCTGAACTGGTATCAGCAGAAGCCCGGCAAGACCGTGAAGCTG
CTGATCTACTACACAAGCAGCCTGCACAGCGGCGTGCCTAGCAGATTCAGC
GGCAGCGGCAGCGGCACCGACTTCACCCTGACCATCAGCAGCCTGCAGCC

TGAGGACTTCGCCACCTACTACTGTCAGCAGTACAGCAAGCTGCCCTACAC
ATTTGGGGGCGGGACCAAACCTCGAGATTAAGGGCGGGGGCGGGAGCGGC
GGGGGCGGGTCCGGGGGCGGGGGCAGCGAGGTCCAACCTGGTGGAGTCCG
GCGGGGGCCTCGTGAAGCCTGGCGGCAGCCTGAGACTGAGCTGCGCCGCC
TCCGGCCTGACCTTCAGCAGCTACGCCATGAGCTGGGTGAGACAAGCCCC
CGAGAAGAGACTGGAGTGGGTGGCCTCCATCAGCAGCGGCGGCTTCACCT
ACTACCCCGACAGCGTGAAGGGCAGATTCACCATCAGCAGAGACAACGCC
AAGAACAGCCTGTACCTGCAGATGAACAGCCTGAGAGCCGAGGACACCGC
CGTGTACTACTGCGCTAGAGACGAGGTGAGAGGCTACCTGGACGTGTGGG
GCGCCGGCACCAACCGTGACCGTGAGCAGCACCAACGACGCCAGCGCCGCG
ACCACCAACACCGGCGCCCAACATCGCGTCGCAGCCCCTGTCCCTGCGCC
CAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGACACAGAGGGGGCT
GGACTTCGCCTGTGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGG
GGTCCTTCTCCTGTCACTGGTTATCACCTTTACTGCAAACGGGGCAGAAA
GAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAACTACT
CAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAGAAGAAGGAG
GATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTAC
CAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGA
GGAGTACGATGTTTTGGACAAGAGACGTGGCCGGGACCCTGAGATGGGGG
GAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCA
GAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCGAGC
GCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCC
ACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC

Claims

1. A CD7-targeting humanized antibody or antigen-binding fragment thereof, comprising: the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2;

or, the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30;

or, the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30.

2. The humanized antibody or antigen-binding fragment thereof according to claim 1, which is a scFv antibody, a sc(Fv)₂ antibody, or a [sc(Fv)₂]₂ antibody.

3. The humanized antibody or antigen-binding fragment thereof according to claim 1 or 2, which is the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 3;

or, which is the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 35;

or, which is the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 36.

4. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the humanized antibody or antigen-binding fragment thereof according to any one of claims 1-3.

5. The nucleic acid molecule according to claim 4, wherein: the nucleotide sequence encoding the amino acid sequences of the heavy chain variable region as shown in SEQ ID NO: 1, is shown in SEQ ID NO: 4;

and/or, the nucleotide sequence encoding the amino acid sequences of the light chain variable region as shown in SEQ ID NO: 2, is shown in SEQ ID NO: 5;

and/or, the nucleotide sequence encoding the amino acid sequences of the heavy chain variable region as shown in SEQ ID NO: 28, is shown in SEQ ID NO: 37;

and/or, the nucleotide sequence encoding the amino acid sequences of the light chain variable region as shown in SEQ ID NO: 30, is shown in SEQ ID NO: 38.

6. A vector comprising the nucleic acid molecule according to claim 4 or 5.
7. A cell comprising the humanized antibody or antigen-binding fragment thereof according to any one of claims 1-3, the isolated nucleic acid molecule according to claim 4 or 5, or the vector according to claim 6.
8. A pharmaceutical composition comprising the humanized antibody or antigen-binding fragment thereof according to any one of claims 1-3, the isolated nucleic acid molecule according to claim 4 or 5, the vector according to claim 6, or the cell according to claim 7, and a pharmaceutically acceptable adjuvant.
9. Use of the humanized antibody or antigen-binding fragment thereof according to any one of claims 1-3, the isolated nucleic acid molecule according to claim 4 or 5, the vector according to claim 6, or the cell according to claim 7 in the preparation of a detection reagent for diagnosing diseases or conditions related to the expression of CD7.
10. The use according to claim 9, wherein the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor; optionally, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.
11. The use according to claim 10, wherein the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia; and/or, wherein the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.
12. Use of the humanized antibody or antigen-binding fragment thereof according to any one of claims 1-3, the isolated nucleic acid molecule according to claim 4 or 5, the vector according to claim 6, or the cell according to claim 7 in the preparation of a medicament for treating diseases or conditions related to the expression of CD7.
13. The use according to claim 12, wherein the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor; optionally, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.
14. The use according to claim 13, wherein the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia; and/or, wherein the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous

T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.

15. An antibody drug comprising the humanized antibody or antigen-binding fragment thereof according to any one of claims 1-3.

16. An antibody-drug conjugate comprising the humanized antibody or antigen-binding fragment thereof according to any one of claims 1-3.

17. A CD7-targeting chimeric antigen receptor, comprising a CD7-targeting extracellular antigen recognition domain, a hinge region, a transmembrane region, and an intracellular domain, wherein the CD7-targeting extracellular antigen recognition domain comprises:

the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2;

or, the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30;

or, the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28 and the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30.

18. The chimeric antigen receptor according to claim 17, wherein the extracellular antigen recognition domain comprises any one structure selected from the group consisting of: (the amino acid sequence as shown in SEQ ID NO: 1)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 2), (the amino acid sequence as shown in SEQ ID NO: 2)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 1)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 30), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 28), (the amino acid sequence as shown in SEQ ID NO: 28)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 30), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 28);

optionally, the extracellular antigen recognition domain comprises any one structure selected from the group consisting of: (the amino acid sequence as shown in SEQ ID NO: 2)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 28);

further optionally, the extracellular antigen recognition domain is any one structure selected from the group consisting of: (the amino acid sequence as shown in SEQ ID NO: 2)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 1), (the amino acid sequence as shown in SEQ ID NO: 30)-(a linker sequence)-(the amino acid sequence as shown in SEQ ID NO: 28).

19. The chimeric antigen receptor according to claim 18, wherein the linker sequence is selected from one or more of the following sequences: SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8.

20. The chimeric antigen receptor according to claim 19, wherein the extracellular antigen recognition domain comprises: the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 3, or the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 35, or the scFv antibody with the amino acid sequence as shown in SEQ ID NO: 36;

optionally, the amino acid sequence of the extracellular antigen recognition domain is shown in SEQ ID NO: 3, or is shown in SEQ ID NO: 35, or is shown in SEQ ID NO: 36.

21. The chimeric antigen receptor according to any one of claims 17-20, wherein the hinge region is derived from one or more of IgG1, IgG4, CD4, CD7, CD28, CD84, CD8 α ; optionally, the amino acid sequence of the hinge region is derived from CD8 α ; further optionally, the amino acid sequence of the hinge region comprises the amino acid sequence as shown in SEQ ID NO: 9.

22. The chimeric antigen receptor according to any one of claims 17-20, wherein the transmembrane region is derived from one or more of CD3, CD4, CD7, CD8 α , CD28, CD80, CD86, CD88, 4-1BB, CD152, OX40, Fc70; optionally, the amino acid sequence of the transmembrane region is derived from CD8 α ; further optionally, the amino acid sequence of the transmembrane region comprises the amino acid sequence as shown in SEQ ID NO: 10.

23. The chimeric antigen receptor according to any one of claims 17-20, wherein the intracellular domain comprises an intracellular signal transduction region; optionally, the intracellular domain further comprises a costimulatory signal transduction region.

24. The chimeric antigen receptor according to claim 23, wherein the intracellular signal transduction region is derived from one or more of CD3 ζ , CD3 γ , CD3 δ , CD3 ϵ , CD5, CD22, CD79a, CD79b, FcR γ , FcR β , CD66d, DAP10, DAP12, Syk; optionally, the intracellular signal transduction region is derived from CD3 ζ ; further optionally, the amino acid sequence of the intracellular signal transduction region comprises the amino

acid sequence as shown in SEQ ID NO: 11.

25. The chimeric antigen receptor according to claim 23, wherein the costimulatory signal transduction region is derived from one, two, or more of CD2, CD3, CD7, CD27, CD28, CD30, CD40, CD83, CD244, 4-1BB, OX40, LFA-1, ICOS, LIGHT, NKG2C, NKG2D, DAP10, B7-H3, MyD88; optionally, the costimulatory signal transduction region is derived from CD28 or 4-1BB; further optionally, the amino acid sequence of the costimulatory signal transduction region comprises the amino acid sequence as shown in SEQ ID NO: 12.

26. The chimeric antigen receptor according to any one of claims 17-20, which further comprises a guiding peptide located at the N-terminus of the amino acid sequence of the chimeric antigen receptor; optionally, the guiding peptide is derived from CD8 α ; further optionally, the amino acid sequence of the guiding peptide comprises the amino acid sequence as shown in SEQ ID NO: 13.

27. The chimeric antigen receptor according to any one of claims 17-20, wherein the chimeric antigen receptor comprises any one of the following sequences: the amino acid sequence as shown in SEQ ID NO: 14, or the amino acid sequence as shown in SEQ ID NO: 18; or the amino acid sequence as shown in SEQ ID NO: 19;

optionally, the amino acid sequence of the chimeric antigen receptor is shown in SEQ ID NO: 14, or is shown in SEQ ID NO: 18, or is shown in SEQ ID NO: 19.

28. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the chimeric antigen receptor according to any one of claims 17-20.

29. The nucleic acid molecule according to claim 28, wherein the nucleotide sequence comprises:

a nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1, which is shown in SEQ ID NO: 4; and a nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 2, which is shown in SEQ ID NO: 5;

or, a nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 1, which is shown in SEQ ID NO: 4; and a nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30, which is shown in SEQ ID NO: 38;

or, a nucleotide sequence encoding the amino acid sequence of the heavy chain variable region as shown in SEQ ID NO: 28, which is shown in SEQ ID NO: 37; and a nucleotide sequence encoding the amino acid sequence of the light chain variable region as shown in SEQ ID NO: 30, which is shown in SEQ ID NO: 38.

30. The nucleic acid molecule according to claim 28, wherein the nucleotide sequence comprises:

a nucleotide sequence encoding the amino acid sequence as shown in SEQ ID NO: 14, which is shown in SEQ ID NO: 15;

or, a nucleotide sequence encoding the amino acid sequence as shown in SEQ ID NO: 18, which is shown in SEQ ID NO: 39;

or, a nucleotide sequence encoding the amino acid sequence as shown in SEQ ID NO: 19, which is shown in SEQ ID NO: 40.

31. A vector comprising the isolated nucleic acid molecule according to any one of claims 28-30; optionally, the vector is an expression vector.

32. The vector according to claim 31, the vector is a viral vector; optionally, the vector is a lentiviral vector.

33. An engineered immune effector cell comprising the chimeric antigen receptor according to any one of claims 17-27, the isolated nucleic acid molecule according to any one of claims 28-30, or the vector according to any one of claims 31-32.

34. The engineered immune effector cell according to claim 33, which is selected from one or more of a T lymphocyte, natural killer cell, peripheral blood mononuclear cell, induced pluripotent stem cell, T cell differentiated from an induced pluripotent stem cell, NK cell differentiated from an induced pluripotent stem cell and embryonic stem cell; optionally, which is selected from T lymphocyte; further optionally, the source of the T lymphocyte is an autologous T lymphocyte or allogeneic T lymphocyte; even further optionally, the allogeneic T lymphocyte comprises gene-edited *CD7/TRAC* double-negative cell; yet even further optionally, the allogeneic T lymphocyte comprises more than 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 95%, 95%, 96%, 97%, 98%, 99% gene-edited *CD7/TRAC* double-negative cell.

35. The engineered immune effector cell according to claim 34, wherein the T lymphocyte is an $\alpha\beta$ T lymphocyte or a $\gamma\delta$ T lymphocyte.

36. A pharmaceutical composition comprising the engineered immune effector cell according to any one of claims 33-35, and a pharmaceutically acceptable adjuvant.

37. The pharmaceutical composition according to claim 36, wherein the pharmaceutically acceptable adjuvant includes a protective agent.

38. The pharmaceutical composition according to claim 37, wherein the

pharmaceutically acceptable adjuvant includes a cell cryopreservation solution.

39. A method for preparing the engineered immune effector cell, including the following steps: introducing a nucleotide sequence encoding the chimeric antigen receptor according to any one of claims 26-36 into an immune effector cell.

40. The method according to claim 39, wherein the immune effector cell is selected from one or more of a T lymphocyte, natural killer cell, peripheral blood mononuclear cell, induced pluripotent stem cell, T cell differentiated from an induced pluripotent stem cell, NK cell differentiated from an induced pluripotent stem cell and embryonic stem cell; optionally, the immune effector cell is a T lymphocyte; further optionally, the source of the T lymphocyte is an autologous T lymphocyte or allogeneic T lymphocyte; even further optionally, the allogeneic T lymphocyte comprises gene-edited *CD7/TRAC* double-negative cell; yet even further optionally, the allogeneic T lymphocyte comprises more than 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 95%, 95%, 96%, 97%, 98%, 99% gene-edited *CD7/TRAC* double-negative cell.

41. The method according to claim 39, wherein the T lymphocyte is an $\alpha\beta$ T lymphocyte or a $\gamma\delta$ T lymphocyte.

42. The method according to claim 40, wherein *Trac* gene and *CD7* gene of T cell are gene-edited with gene editing tool to obtain *CD7/TRAC* double-negative cell; wherein the gene editing tool is selected from the group consisting of CRISPR/Cas system, zinc-finger nuclease system, transcription activator-like effector nuclease system; optionally, the gene editing tool is selected from CRISPR/Cas system; further optionally, the CRISPR/Cas system comprises Cas9 protein and sgRNA.

43. The method according to claim 39, wherein the sgRNA of the CRISPR/Cas system targeting *CD7* gene is shown in SEQ ID NO: 33; and/or, the chRDNA of the CRISPR/Cas system targeting *Trac* gene is shown in SEQ ID NO: 34.

44. The method according to any one of claims 39-43, wherein the method of introducing the nucleotide sequence encoding the chimeric antigen receptor according to any one of claims 26-36 into the immune effector cell is selected from one or more of: the method of using virus or the method of using non-virus; optionally, the method of using virus comprises using one or more of the following viral vectors: γ -retroviral vector, lentiviral vector, adenovirus-associated viral vector; the method of using non-virus comprises one or more of the following methods: gene transfer with transposon, gene transduction with mRNA-mediating, electrotransformation.

45. Use of the chimeric antigen receptor according to any one of claims 17-27, the isolated nucleic acid molecule according to any one of claims 28-30, the vector according to any one of claims 31-32, or the engineered immune effector cell according to any one of claims 33-35 in the preparation of a medicament, wherein the medicament

is used for treating diseases or conditions related to the expression of CD7.

46. The use according to claim 45, wherein the diseases or conditions related to the expression of CD7 is a CD7⁺ hematological tumor; optionally, the CD7⁺ hematological tumor is selected from one or more of CD7⁺ T acute lymphoblastic leukemia, CD7⁺ T-cell lymphoma, CD7⁺ acute myelocytic leukemia.

47. The use according to claim 46, wherein the CD7⁺ T acute lymphoblastic leukemia comprises CD7⁺ early T cell precursor acute lymphoblastic leukemia and other T acute lymphoblastic leukemia; and/or wherein the CD7⁺ T-cell lymphoma is selected from one or more of CD7⁺ T-lymphoblastic lymphoma, CD7⁺ extranodal NK/T-cell lymphoma, CD7⁺ enteropathy-associated T-cell lymphoma, CD7⁺ primary cutaneous T-cell lymphoma and CD7⁺ peripheral T-cell lymphoma.