GENETICALLY ENGINEERED CELL AND APPLICATION THEREOF

The present application claims priorities of the Chinese patent application CN201810463564.7, the application date of which is May 15, 2018; the Chinese patent application CN201811088090.9, the application date of which is September 18, 2018; the Chinese patent application CN201811552806.6, the application date of which is December 19, 2018; and the Chinese patent application CN201910151930.X, the application date of which is February 28, 2019. The full contents of the Chinese patent applications as said above are incorporated herein.

Technical field

The present invention belongs to the field of cell therapy, and relates to genetically engineered cells and applications thereof. In particular, the present invention relates to cells comprising an exogenous receptor specifically binding to a target antigen and exogenous CCL21.

Background

CAR-T cells can specifically kill tumors in a MHC non-restrictive manner, and exhibit good application prospects in tumor immunotherapy, but there are still many limitations, such as poor efficacy on solid tumors, and a candidate drug showing excellent effects *in vitro* often fails to show corresponding effects *in vivo*.

Adachi et al. tried to use CAR-T cells expressing IL7 and CCL19 (IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. Nature Biotechnology, 2018, 36(4), 346-351), to improve the anti-tumor abilities of CAR-T cells.

Summary of the invention

The purpose of the present invention is to provide a genetically engineered cell.

In the first aspect of the present invention, a genetically engineered cell is provided, wherein the cell comprises an exogenous receptor specifically binding to a target antigen and exogenous CCL21.

In a specific embodiment, the cell expresses an exogenous receptor specifically binding to a target antigen, exogenous CCL21 and a protein promoting the proliferation of the cell. Preferably, the protein promoting the proliferation of the cell is a IL-7R-binding protein or exogenous IL-7.

In a specific embodiment, the IL-7R-binding protein is an exogenous IL-7R-binding protein, that is, the cell comprises an exogenous receptor specifically binding to a target antigen, exogenous CCL21 and exogenous IL-7R-binding protein.

In a specific embodiment, the exogenous IL-7R-binding protein can specifically bind to IL-7R and improve activities of IL-7R.

In a specific embodiment, the exogenous IL-7R-binding protein is selected from IL-7R antibodies. Preferably, the amino acid sequence of the exogenous IL-7R is shown in SEQ ID NO: 19.

In a specific embodiment, the exogenous CCL21 is natural CCL21, or a truncated fragment of natural CCL21, or a mutant of natural CCL21 having the same function as natural CCL21.

In a specific embodiment, the natural CCL21 has at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with the amino acid sequence as shown in SEQ ID NO: 21, or is a truncated fragment of the amino acid sequence as shown in SEQ ID NO: 21; or has at least 80%, 85%, 90%, 95%, 98% or 99% sequence identity with the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 14 or 15, or is a truncated fragment of an amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 14 or 15. In a preferred embodiment, the natural CCL21 is human CCL21, the amino acid sequence of which is shown in SEQ ID NO: 21; or the amino acid sequence of which is encoded by the nucleotide sequence as shown in SEQ ID NO: 14 or 15.

In a specific embodiment, the exogenous CCL21 is constitutively expressed.

In a specific embodiment, the exogenous CCL21 is inducibly expressed. In a preferred embodiment, the inducible expression is initiated by an immune cell inducible promoter. In a preferred embodiment, the immune cell inducible promoter is NFAT promoter.

In a specific embodiment, the exogenous IL-7 is natural IL-7, or a truncated

fragment of natural IL-7, or a mutant of natural IL-7 having the same function as natural IL-7.

In a specific embodiment, the amino acid sequence of the natural IL-7 has at least 90% sequence identity with the amino acid sequence as shown in SEQ ID NO: 18, or is a truncated fragment of the amino acid sequence as shown in SEQ ID NO: 18; or has at least 90% sequence identity with the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 13, or is a truncated fragment of an amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 13.

In a specific embodiment, the exogenous IL-7R-binding protein or exogenous IL-7 is constitutively expressed.

In a preferred embodiment, the exogenous IL-7R-binding protein or exogenous IL-7 is inducibly expressed.

In a preferred embodiment, the inducible expression is initiated by an immune cell inducible promoter. In a preferred embodiment, the immune cell inducible promoter is NFAT promoter.

In a specific embodiment, the cell is an immune effector cell. In a specific embodiment, the immune effector cells are selected from T cells, B cells, natural killer (NK) cells, natural killer T (NKT) cells, mast cells or bone marrow-derived phagocytes or a combination thereof; preferably, the immune effector cells are selected from T cells or NK cells; and more preferably, the immune effector cells are T cells.

In a specific embodiment, the cells are derived from autologous cells; preferably, autologous T cells, autologous NK cells; more preferably, autologous T cells.

In a specific embodiment, the cells are derived from allogeneic cells; preferably, allogeneic T cells or allogeneic NK cells (also including a cell line of NK cells, such as NK92 cells).

In a specific embodiment, the target antigen is a tumor antigen or a pathogen antigen.

In a specific embodiment, the target antigen is a tumor antigen. In a preferred embodiment, the target antigen is selected from: Thyroid-stimulating hormone receptor (TSHR); CD171; CS-1; C-type lectin-like molecule-1; Ganglioside GD3; Tn antigen; CD19; CD20; CD 22; CD 30; CD 70; CD 123; CD 138; CD33; CD44; CD44v7/8; CD38;

CD44v6; B7H3 (CD276), B7H6; KIT (CD117); Interleukin 13 receptor subunit α (IL-13Rα); Interleukin 11 receptor α (IL-11Rα); Prostate Stem Cell Antigen (PSCA); Prostate Specific Membrane Antigen (PSMA); Carcinoembryonic Antigen (CEA); NY-ESO-1; HIV-1 Gag; MART-1; gp100; Tyrosinase; Mesothelin; EpCAM; Protease serine 21 (PRSS21); Vascular endothelial growth factor receptor; Lewis (Y) antigen; CD24; Platelet-derived growth factor receptor β (PDGFR-β); Stage-specific embryonic antigen-4 (SSEA-4); Cell Surface-associated mucin 1 (MUC1), MUC6; epidermal growth factor 20 receptor family and mutants thereof (EGFR, EGFR2, ERBB3, ERBB4, EGFRvIII); nerve cell adhesion molecule (NCAM); carbonic anhydrase IX (CAIX); LMP2; Ephrin A receptor 2 (EphA2); Fucosyl GM1; Sialyl Lewis adhesion molecule (sLe); Ganglioside GM3; TGS5; High molecular weight melanoma associated antigen (HMWMAA); o-acetyl GD2 ganglioside (OAcGD2); folate receptor; tumor vascular endothelial marker 25 1 (TEM1/CD248); tumor vascular endothelial marker 7 related (TEM7R); Claudin 6, Claudin 18.2 (CLD18A2), Claudin18.1; ASGPR1; CDH16; 5T4; 8H9; αvβ6 integrin; B cell maturation antigen (BCMA); CA9; κ light chain (kappa light chain); CSPG4; EGP2, EGP40; FAP; FAR; FBP; embryonic type AchR; HLA-A1, HLA-A2; MAGEA1, MAGE3; KDR; MCSP; NKG2D ligand; PSC1; ROR1; Sp17; SURVIVIN; TAG72; TEM1; Fibronectin; Tenascin; Carcinoembryonic variant of tumor necrosis; G protein-coupled receptor class C group 5-member D (GPRC5D); X chromosome open reading frame 61 (CXORF61); CD97; CD179a; anaplastic lymphoma kinase (ALK); polysialic acid; placental specific 1(PLAC1); hexose moiety of globoH glycoceramide (GloboH); breast differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); hepatitis A virus cell receptor 1 (HAVCR1); adrenergic receptor 5 β3 (ADRB3); pannexin 3 (PANX3); G protein coupled receptor 20 (GPR20); lymphocyte antigen 6 complex locus K9 (LY6K); olfactory receptor 51E2 (OR51E2); TCRγ alternating reading frame protein (TARP); Wilms tumor protein (WT1); ETS translocation variant gene 6 (ETV6-AML); Sperm protein 17 (SPA17); X antigen family member 1A (XAGE1); Angiopoietin-binding cell surface receptor 2 (Tie2); melanoma testis antigen-1 (MAD-CT-1); melanoma testis antigen-2 (MAD-CT-2); Fos-related antigen 1; p53 mutant; human telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoint; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease

serine 2 (TMPRSS2) ETS fusion gene); N-acetylglucosaminyl transferase V (NA17); matching box protein Pax-3 (PAX3); androgen receptor; Cyclin B1; V-myc avian myeloidosis virus oncogene neuroblastoma-derived homolog (MYCN); Ras homolog family member C (RhoC); Cytochrome P450 1B1 (CYP1B1); CCCTC binding factor (zinc finger protein)-like (BORIS); squamous cell carcinoma antigen 3 (SART3) recognized by T cells; paired box protein Pax-5 (PAX5); proacrosin-binding protein sp32 (OYTES1); Lymphocyte specific protein tyrosine kinase; A kinase anchoring protein 4 (AKAP-4); Synovial sarcoma X breakpoint 2 (SSX2); CD79a; CD79b; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); Fc fragment of IgA receptor (FCAR); Leukocyte immunoglobulin-like receptor subfamily member 2 (LILRA2); CD300 molecular-like family member f (CD300LF); C-type lectin domain family 12 member A (CLEC12A); bone marrow stroma Cell antigen 2 (BST2); Mucin-like hormone receptor-like 2 (EMR2) containing EGF-like module; Lymphocyte antigen 75 (LY75); Glypican-3 (GPC3); Fc receptor-like 5 (FCRL5); Immunoglobulin λ-like polypeptide 1 (IGLL1).

In a preferred embodiment, the target antigen is GPC3, EGFR, EGFRvIII or Claudin18.2.

In a specific embodiment, the target antigen is a pathogen antigen. In a preferred embodiment, the pathogen antigen is derived from viruses, bacteria, fungi, protozoa, or parasites. In a specific embodiment, the viral antigen is selected from: cytomegalovirus antigen, Epstein-Barr virus antigen, human immunodeficiency virus antigen or influenza virus antigen.

In a specific embodiment, the exogenous receptor is a chimeric receptor, and the chimeric receptor includes an antigen binding domain, a transmembrane domain and an intracellular domain.

In a specific embodiment, the exogenous receptor is a chimeric receptor selected from the group consisting of chimeric antigen receptor (CAR), modified T cell (antigen) receptor (TCR), T Cell fusion protein (TFP), T cell antigen coupler (TAC) or a combination thereof.

In a preferred embodiment, the exogenous receptor is a chimeric antigen receptor, and the antigen binding domain of the chimeric antigen receptor includes: antibody,

antibody fragment, scFv, Fv, Fab, (Fab')2, single domain antibody (SDAB), VH or VL domain, or camelid VHH domain, or natural ligand of corresponding antigen, or a combination thereof.

In a preferred embodiment, the exogenous receptor is a chimeric antigen receptor, and the transmembrane domain of the chimeric antigen receptor includes a transmembrane domain of a protein selected from the group consisting of: α, β or ζ chain of a T cell receptor, CD28, CD3ε, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154, KIRDS2, OX40, CD2, CD27, LFA-1(CD11a, CD18), ICOS(CD278), 4-1BB(CD137), GITR, CD40, BAFFR, HVEM(LIGHTR), SLAMF7, NKp80(KLRF1), CD160, CD19, IL2Rβ, IL2Rγ, IL7Rα, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1(CD226), SLAMF4(CD244, 2B4), CD84, CD96(Tactile), CEACAM1, CRTAM, Ly9(CD229), CD160(BY55), PSGL1, CD100(SEMA4D), SLAMF6(NTB-A, Ly108), SLAM(SLAMF1, CD150, IPO-3), BLAME(SLAMF8), SELPLG(CD162), LTBR, PAG/Cbp, NKp44, NKp30, NKp46, NKG2D and NKG2C.

In a preferred embodiment, the exogenous receptor is a chimeric antigen receptor, and the intracellular domain of the chimeric antigen receptor includes: a primary signaling domain and/or costimulatory signaling domain, wherein: (1) the primary signaling domain comprises a functional signaling domain of a protein selected from: CD3ζ, CD3γ, CD3δ, CD3ε, common FcRγ (FCER1G), FcRβ (FcεR1b), CD79a, CD79b, FcγRIIa, DAP10, and DAP12, or a combination thereof; and / or (2) the costimulatory signal transduction domain comprises a functional signal transduction domain selected from the following proteins: CD27, CD28, 4-1BB(CD137), OX40, CD30, CD40, PD-1, ICOS, Lymphocyte function related antigen-1(LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3, ligand specifically binding to CD83, CDS, ICAM-1, GITR, BAFFR, HVEM(LIGHTR), SLAMF7, NKp80(KLRF1), CD160, CD19, CD4, CD8α, CD8β, IL2Rβ, IL2Rγ, IL7Rα, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, TRANCE/RANKL,

DNAM1(CD226), SLAMF4(CD244, 2B4), CD84, CD96(Tactile), CEACAM1, CRTAM, Ly9(CD229), CD160(BY55), PSGL1, CD100(SEMA4D), CD69, SLAMF6(NTB-A, Ly108), SLAM(SLAMF1, CD150, IPO-3), BLAME(SLAMF8), SELPLG(CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, NKp44, NKp30, NKp46 and NKG2D, or a combination thereof.

In a specific embodiment, the chimeric antigen receptor includes: (i) an antibody or a fragment thereof specifically binding to a target antigen, a transmembrane domain of CD28 or CD8, a costimulatory signal domain of CD28, and CD3ζ; or (ii) an antibody or a fragment thereof specifically binding to a target antigen, the transmembrane domain of CD28 or CD8, the costimulatory signal domain of 4-1BB, and CD3ζ; or (iii) an antibody or a fragment thereof specifically binding to the target antigen, the transmembrane domain of CD28 or CD8, the costimulatory signal domain of CD28, the costimulatory signal domain of 4-1BB and CD3ζ.

In a specific embodiment, the amino acid sequence of the antigen binding domain of the exogenous receptor has at least 90% identity with the sequence as shown in SEQ ID NO: 2.

In a specific embodiment, the amino acid sequence of the exogenous receptor has at least 90% identity with the sequence as shown in SEQ ID NO: 26, 27 or 35.

In a specific embodiment, the exogenous receptor, and/or exogenous IL-7R binding protein, and/or exogenous CCL21 are expressed by using a viral vector. Preferably, the viral vectors include: lentiviral vectors, retroviral vectors or adenovirus vectors.

In the second aspect of the present invention, an expression construct is provided. The expression construct comprises sequentially connected: an expression cassette 1 for an exogenous receptor specifically binding to a target antigen, an expression cassette 2 for exogenous IL-7R binding protein or exogenous IL-7, and an expression cassette 3 exogenous for CCL21. Preferably, the expression cassettes are connected by tandem fragments, selected from F2A, PA2, T2A, and/or E2A, among which, nucleic acid sequences of F2A and P2A are shown in SEQ ID NO: 11 and SEQ ID NO: 16, respectively.

In the third aspect of the present invention, an expression vector is provided, comprising the expression construct of the second of the present invention.

In the fourth aspect of the present invention, a virus is provided, comprising the expression vector of the third aspect of the present invention.

In the fifth aspect of the present invention, a method for improving the viability of immune response cells is provided, comprising co-expressing in the immune response cells: the chimeric antigen receptor specifically binding to a target antigen of the first aspect of the present invention, exogenous IL-7R binding protein or exogenous IL-7, exogenous CCL21.

In the sixth aspect of the present invention, a use of the cell of the first aspect of the present invention, or the expression construct of the second aspect of the present invention, or the expression vector of the third aspect of the present invention, or the virus of the fourth aspect of the invention is provided for preparing a drug for inhibiting tumors, inhibiting pathogens, or enhancing the immune tolerance of a subject. In a specific embodiment, the use is to prepare a drug for inhibiting tumors. In a preferred embodiment, the prepared drug for inhibiting tumors is used in combination with a chemotherapeutic drug.

In a specific embodiment, the tumor is a hematological tumor.

In a specific embodiment, the tumor is a solid tumor.

In a specific embodiment, the tumor is selected from: colon cancer, rectal cancer, renal cell carcinoma, liver cancer, non-small cell lung cancer, small bowel cancer, esophageal cancer, melanoma, bone cancer, pancreatic cancer, skin cancer, head and neck cancer, skin or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, anal cancer, stomach cancer, testicular cancer, uterine cancer, fallopian tube cancer, endometrial cancer, cervical cancer, vagina cancer, vulva cancer, Hodgkin's disease, non-Hodgkin's lymphoma, endocrine system cancer, thyroid cancer, parathyroid cancer, adrenal cancer, soft tissue sarcoma, urethral cancer, penile cancer, childhood solid tumors, bladder cancer, renal or ureteral cancer, renal pelvis cancer, central nervous system (CNS) tumors, primary CNS lymphoma, tumor angiogenesis, spinal tumor, brainstem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid carcinoma, squamous cell carcinoma, T-cell lymphoma, environmentally induced cancer, a combination thereof and the metastatic foci of the cancer.

In a preferred embodiment, the solid tumor is selected from: colon cancer, rectal

cancer, renal cell carcinoma, liver cancer, non-small cell lung cancer, small intestine cancer, esophageal cancer, melanoma, bone cancer, pancreatic cancer, skin cancer, head and neck cancer, skin or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, anal cancer, stomach cancer, testicular cancer, uterine cancer, fallopian tube cancer, endometrial cancer, cervical cancer, vaginal cancer, vulva cancer, endocrine system cancer, thyroid cancer, parathyroid cancer, adrenal cancer , Soft tissue sarcoma, urethral cancer, penile cancer, bladder cancer, renal or ureteral cancer, renal pelvis cancer, central nervous system (CNS) tumor, primary CNS lymphoma, tumor angiogenesis, spine tumor, brain stem glioma, Pituitary adenoma, Kaposi's sarcoma, epidermoid carcinoma, squamous cell carcinoma.

Preferably, the solid tumor is selected from: colon cancer, rectal cancer, liver cancer, non-small cell lung cancer, small intestine cancer, esophagus cancer, pancreatic cancer, head and neck cancer, skin or intraocular melanoma, uterine cancer, ovarian cancer, rectal cancer, anal cancer, gastric cancer. More preferably, the solid tumor is gastric cancer, pancreatic cancer, or esophageal cancer.

In the seventh aspect of the present invention, a pharmaceutical composition is provided, comprising the cell of the first aspect of the present invention and a pharmaceutically acceptable carrier or excipient.

In the eighth aspect of the present invention, a kit is provided, comprising kit A and kit B. The kit A comprises genetically engineered cells, and the cells comprises the exogenous receptor specifically binding to the target antigen of the first aspect of the present invention. The kit B comprises CCL21, and/or a protein that promotes the proliferation of the cells. Preferably, the protein that promotes the proliferation of the cells includes the IL-7R binding protein or IL-7 of the first aspect of the present invention. More preferably, the kit A and the kit B can be administered in any order.

In a preferred embodiment, the kit A comprises immune effector cells modified by chimeric receptors. Preferably, the chimeric receptor is a chimeric antigen receptor.

In a preferred example, the immune effector cells are T cells, NK cells or NKT cells.

In the ninth aspect of the present invention, a method for suppressing tumors or suppressing pathogens or enhancing the immune tolerance of a subject is provided,

comprising administering the cells of the first aspect of the present invention, or the pharmaceutical composition of the seventh aspect of the present invention or the kit of the eighth aspect of the present invention. Preferably, the method also includes the administration of a chemotherapy drugs.

Beneficial advantages of the invention:

1. The cells provided by the invention can improve cell survival and capacity due to the co-expression of an exogenous receptor specifically binding to the target antigen, exogenous IL-7R binding protein or exogenous IL-7, and exogenous CCL21.

2. The immune effector cells prepared by the technical solution of the present invention have excellent tumor cell killing abilities.

3. During the treatment of cancer, the cells prepared by the technical solution of the present invention can resist the immunosuppression in the cancer micro-environment, thereby significantly enhancing effects on solid tumors. It also has good effects on refractory and progressive cancers.

Description of the drawings

Figure 1A is a plasmid map of MSCV-hu8E5 (2I) -m28Z;

Figure 1B is a plasmid map of MSCV-hu8E5 (2I) -m28Z-F2A-mIL-7-P2A-mCCL21a;

Figure 1C is a plasmid map of MSCV-hu8E5 (2I) -m28Z-F2A-mIL7-P2A-mCCL21b;

Figure 1D is a plasmid map of MSCV-hu8E5 (2I) -mBBZ;

Figure 1E is a plasmid map of MSCV-hu8E5 (2I)-mBBZ-F2A-mIL-7-P2A-mCCL21a;

Figure 1F is a plasmid map of MSCV-hu8E5 (2I) -m28Z-F2A-mIL7-P2A-mCCL21b;

Figure 2 shows the results of *in vitro* cytokine IL7 and CCL21 detection;

Figures 3A and 3B show the secretion of PD-1 by cells in different groups; Figure 3C and 3D show the secretion of LAG-3 by cells in different groups; and Figure 3E and 3F show the secretion of TIM-3 by cells in different groups;

Figure 4A shows the *in vitro* killing results of 28Z; and 4B shows the *in vitro* killing results of BBZ;

Figure 5 shows the results of *in vitro* proliferation test;

Figure 6 shows the results of tumor treatment experiments in mice *in vivo*;

Figure 7 is a plasmid map of mBBZ-7*19;

Figure 8A shows the comparison results of *in vivo* killing effects of CAR-T cells expressing IL7 and CCL21 and CAR-T cells expressing IL7 and CCL19; Figure 8B shows the changes in the body weight of mice; Figure 8C shows the comparison results of tumor weight; Figure 8D shows the number of copies of CAR-T cells after treating PANC02-A2 pancreatic cancer in mice; and Figure 8E shows the immunohistochemical detection results of CD8+ cells in pancreatic cancer in mice;

Figure 9A shows changes in the tumor volume after treating breast cancer E0771-A2 orthotopic xenograft tumor in mice by using CAR-T cells;

Figure 9B shows the tumor weight after treating breast cancer E0771-A2 orthotopic xenograft tumor in mice;

Figure 9C shows the number of copies of CAR-T cells after treating breast cancer in mice;

Figure 9D shows the results of immunohistochemical detection of mouse breast cancer CD8+ cells;

Figure 10A shows changes in the tumor volume after treating Hepal-6-A2 xenograft tumor of liver cancer in mice;

Figure 10B shows the tumor weight after treating the xenograft tumor of Hepa1-6-A2 liver cancer in mice;

Figure 10C shows the number of copies of CAR-T cells after treating liver cancer in mice;

Figure 10D shows the results of immunohistochemical detection of mouse liver cancer CD8+ cells;

Figure 11 shows the results of IFN-γ detection *in vitro*;

Figure 12A shows changes in the tumor volume of the mouse pancreatic cancer subcutaneous tumor lymphocyte-clearing model after CAR-T cell treatment; Figure 12B shows the tumor weight after treating the mouse pancreatic cancer subcutaneous tumor lymphocyte-clearing model; Figure 12C shows the number of copies of CAR-T cells after treating the mouse pancreatic cancer subcutaneous tumor lymphocyte-clearing model; and Figure 12D shows the results of immunohistochemical detection on CD8+ cells in the mouse pancreatic cancer subcutaneous tumor lymphocyte-clearing model.

Figure 13A shows the detection of Tcm in the spleen on day 10 during the CAR-T treatment of mouse pancreatic cancer PANC02-A2 subcutaneous tumor model; and Figure 13B shows the detection of Tcm in the spleen on day 20;

Figure 14A shows the content of Tcm in the bone marrow on day 10 during the CAR-T treatment of mouse pancreatic cancer PANC02-A2 subcutaneous tumor model; and Figure 14B shows the detection of Tcm in the spleen on day 20;

Figure 15 shows that more DC cell infiltration in the mouse tumor tissue on day 10 during the CAR-T treatment of mouse pancreatic cancer PANC02-A2 subcutaneous tumor model;

Figure 16 shows the content of MDSC in the mouse tumor tissue on day 10 during the CAR-T treatment of mouse pancreatic cancer PANC02-A2 subcutaneous tumor model.

Modes for carrying out the invention

In the present invention, it was found that immune effector cells expressing an exogenous receptor targeting a tumor antigen and CCL21 can not only have more excellent killing effects on tumors, but also improve the survival of the immune effector cells in tumor tissues, and show better anti-tumor abilities even for the refractory solid tumors.

Based on the present disclosure, a skilled person should appreciate that many changes or modifications can be made in the disclosed specific embodiments while still obtain the same or similar results without departing from the spirit and scope described herein. The scope of the present invention is not limited to the specific embodiments described herein (which are only intended to exemplify various aspects described herein), and it should be appreciated that functionally equivalent methods and components are still included herein within the stated range.

Unless specifically defined herein, all of the technical and scientific terms uesed herein have the same meaning as commonly understood by ae skilled person in the fields of gene therapy, biochemistry, genetics, and molecular biology. All methods and materials similar or equivalent to those described herein can be used in the practice of or testing the present invention. Unless otherwise stated, traditional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology will be adopted in the practice of the present invention, all of which belong to the technical scope of

the field. Such techniques are explained in detail in the literature. See, for example, Current Protocols in Molecular Biology (FrederickM.AUSUBEL, 2000, Wileyand sonInc, Library of Congress, USA); Molecular Cloning: A Laboratory Manual, Third Edition, (Sambrooketal, 2001, Cold Spring Harbor, NewYork: Cold Spring Harbor Laboratory Press); Oligonucleotide Synthesis (M.J.Gaited., 1984); Mullis et al. U.S. Pat. No. 4,683,195; Nucleic Acid Hybridization (B. D. Harries & S. J. Higginseds. 1984); Transcription And Translation (B. D. Hames & S. J. Higginseds. 1984); Culture Of Animal Cells (R. I. Freshney, Alan R. Liss, Inc., 1987); Immobilized Cells And Enzymes (IRL Press, 1986); B. Perbal, A Practical Guide To Molecular Cloning (1984); the series, Methods In ENZYMOLOGY (J. Abelson 和M. Simon, eds.-in-chief, Academic Press, Inc., New York), especially Vols.154和155 (Wuetal. eds.) and Vol.185, “Gene Expression Technology” (D. Goeddel, ed.); Gene Transfer Vectors For Mammalian Cells (J. H. Miller和M. P. Caloseds., 1987, Cold Spring Harbor Laboratory); Immunochemical Methods In Cell And Molecular Biology (Mayer和 Walker, eds., Academic Press, London, 1987); Hand book Of Experimental Immunology, Vol I-IV (D. M. Weir和C. C. Blackwell, eds., 1986); and Manipulating the Mouse Embryo (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1986).

All of publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be limiting, unless otherwise specified.

The term as used herein "engineered" and other grammatical forms thereof may refer to one or more changes of nucleic acids, such as nucleic acids within the genome of an organism. The term "engineered" may refer to a change, addition and/or deletion of a gene. Engineered cells can also refer to cells that contain added, deleted, and/or changed genes.

The term "genetically engineered cell" as used herein refers to a cell modified by means of genetic engineering. In some embodiments, the cell is an immune effector cell. In some embodiments, the cell is a T cell. In some embodiments, the genetically engineered cell described herein refers to a cell expressing an exogenous receptor that specifically binds to a target antigen. In some embodiments, the genetically engineered cell described herein refers to a cell that expresses an exogenous receptor specifically binding to a target antigen and expresses an exogenous CLL21. In some embodiments, the genetically engineered cell

described herein may also be a T cell co-expressing a chimeric antigen receptor specifically binding to a tumor antigen, CLL21 and a T cell proliferation-promoting protein. In some embodiments, the genetically engineered cell described herein may also be a T cell co-expressing a chimeric antigen receptor specifically binding to a tumor antigen, CLL21 and IL-7R-binding protein or an exogenous IL-7.

The term "immune effector cell" refers to a cell participating in an immune response and producing immune effects, such as a T cell, B cell, natural killer (NK) cells natural killer T (NKT) cell, mast cell, and bone marrow-derived phagocyte. In some embodiments, the immune effector cell is a T cell, NK cell, NKT cell. In some embodiments, the T cell can be an autologous T cell, xenogeneic T cell, or allogeneic T cell. In some specific embodiments, the NK cell may be an allogeneic NK cell.

The terms “peptide”, “polypeptide” and “protein” can be used interchangeably and refer to a compound composed of amino acid residues covalently linked by peptide bonds. The protein or peptide must contain at least two amino acids, and there is no limit to the maximum number of amino acids that can be included in the sequence of a protein or peptide. Polypeptides include any peptide or protein containing two or more amino acids linked to each other by peptide bonds. As used herein, the term refers to short chains (which are also commonly referred to in the art as peptides, oligopeptides, and oligomers, for example) and longer chains (which are also commonly referred to as proteins in the art often with various types). “Polypeptide” includes, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, polypeptide variants, modified polypeptides, derivatives, analogs, fusion proteins, and the like. Polypeptides include natural peptides, recombinant peptides or a combination thereof.

The term “IL-7 (Interleukin7 or IL7)” refers to a protein (preferably from a mammal, such as murine or human) that can interact with (e.g., bind to) IL-7R (preferably from a mammal, such as murine or human IL-7R), and have one of the following characteristics: (i) an amino acid sequence of naturally occurring mammalian IL-7 or a fragment thereof, such as the amino acid sequence as shown in SEQ ID NO: 18 (human) or a fragment thereof; (ii) an amino acid sequence substantially having, for example at least 85%, 90%, 95%, 96%, 97%, 98%, 99% homology to the sequence as shown in SEQ ID NO: 18 (human) or a fragment thereof; (iii) an amino acid sequence encoded by a nucleotide sequence of a naturally occurring mammalian

IL-7 or a fragment thereof (for example, SEQ ID NO: 17 (human) or a fragment thereof); (iv) an amino acid sequence encoded by a nucleotide sequence having, for example, at least 85%, 90% %, 95%, 96%, 97%, 98%, 99% homology to the nucleotide sequence as shown in SEQ ID NO: 17 (human) or a fragment thereof; (v) an amino acid sequence encoded by a degenerate nucleotide sequence from a naturally occurring IL-7 nucleotide sequence or a fragment thereof (for example, SEQ ID NO: 17 (human) or a fragment thereof); or (vi) a nucleotide sequence that hybridizes to one of the aforementioned nucleotide sequences under stringent conditions.

"Exogenous IL-7R-binding protein" refers to all proteins that can specifically bind to IL-7R and enhance the activity of IL-7R. "Enhancing IL-7R activity" should be understood to mean that IL-7R-binding protein can enhance any one or more activities of naturally occurring IL-7R, including but not limited to stimulating the proliferation, cytotoxicity or maturation of NK cells; stimulating the proliferation or differentiation of B cells and T cells; stimulating the production and affinity maturation of antibodies in B cells; stimulating the cytotoxicity of CD8+ T cells; stimulating the production of interferon γin T cells and NK cells; inhibiting the activation and maturation of dendritic cells (DC); inhibiting the release of inflammatory mediators from mast cells; enhancing the phagocytosis of macrophages; inhibiting the production or survival of TReg cells; and stimulating the proliferation of bone marrow progenitor cells.

"CCL21 (Chemokine (CC motif) ligand 21)" is one of the main immunochemokines, expressed in the T cell area of the secondary lymphatic tissues of the spleen and lymph nodes, and is responsible for the recruitment of antigen-activated (mature) dendritic cells (DC), immature DC and naive T cells. In the present invention, CCKL21 has one of the following characteristics: (i) an amino acid sequence of naturally occurring mammalian CCL21 or a fragment thereof, such as the amino acid sequence as shown in SEQ ID NO: 21 (human) or a fragment thereof; (ii) an amino acid sequence substantially having, for example at least 85%, 90%, 95%, 96%, 97%, 98%, 99% homology to the sequence as shown in SEQ ID NO: 21 (human) or a fragment thereof; (iii) an amino acid sequence encoded by a nucleotide sequence of a naturally occurring mammalian CCL21 or a fragment thereof (for example, SEQ ID NO: 20 (human) or a fragment thereof); (iv) an amino acid sequence encoded by a nucleotide sequence having, for example, at least 85%, 90% %, 95%, 96%, 97%, 98%, 99% homology to the nucleotide sequence as shown in SEQ ID NO: 20 (human) or a fragment thereof; (v) an

amino acid sequence encoded by a degenerate nucleotide sequence from a naturally occurring CCL21 nucleotide sequence or a fragment thereof (for example, SEQ ID NO: 20 (human) or a fragment thereof); or (vi) a nucleotide sequence that hybridizes to one of the aforementioned nucleotide sequences under stringent conditions.

The term “amino acid modification” includes amino acid substitutions, additions and / or deletions, and “amino acid substitution” means that an amino acid at a specific position in the parent polypeptide sequence is replaced with another amino acid. “Amino acid insertion” as used herein means that an amino acid is added at a specific position in the parent polypeptide sequence. As used herein, "amino acid deletion" or "deletion" means that an amino acid at a specific position in the parent polypeptide sequence is deleted. As used herein, the term "conservative modification" refers to an amino acid modification that does not significantly affect or change the binding characteristics of an antibody comprising the amino acid sequence. Such conservative modifications include amino acid substitutions, insertions and deletions. Modifications can be introduced into the antibody of the invention by standard techniques known in the art, such as site-directed mutagenesis and PCR-mediated mutagenesis. Conservative amino acid substitutions are substitutions of amino acid residues with amino acid residues having similar side chains. Families of amino acid residues with similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, serine, threonine, tyrosine, cysteine, tryptophan), non-polar side chains (eg, alanine, valine, leucine, isole Amino acid, proline, phenylalanine, methionine), β branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

When referring to a protein and DNA, the terms “wild type”, “parent”, and “natural” as used herein have the same meaning. The term "mutation", "variant" or "mutant" has the same or better biological activities as the natural protein or natural DNA, in which there is one or more substitutions, additions, or deletions of amino acids in the amino acid sequence of the natural protein; or one or more substitutions, additions or deletions of nucleotides in the nucleic acid sequence of natural DNA. In a specific embodiment, the sequence of the mutant herein has at least about 80%, preferably at least about 90%, more preferably at least about 95%, and more preferably at least about 97%, preferably at least about 98%, most preferably at least

about 99% identity to the amino acid sequence of a natural protein or nucleic acid sequence of natural DNA. For example, a “variant of IL-7” generally refers to a polypeptide having similar or better biological activities to IL-7 obtained by amino acid modification of wild-type IL-7. The term “truncated fragment” refers to a non-full-length form of a natural protein or natural DNA, in which there are consecutive or non-contiguous deletions of multiple amino acid residues or nucleotides in the natural amino acid sequence or nucleic acid sequence, and such deletions occur in any position of the sequence, such as head, middle, tail or a combination thereof. In the present invention, the truncated fragment of a protein still retains the same function as the natural protein from which it is derived.

“Consitutive expression”, also known as continuous expression, refers to the continuous expression of genes in cells under almost all physiological conditions. The term "inducible expression" refers to the expression under certain conditions, such as when T cells bind to an antigen.

The terms “effective amount” are used interchangeably herein and refer to the amount of a compound, preparation, substance, or composition which is effective to achieve specific biological results, such as but not limited to an amount or dosage sufficient to promote T cell responses. When indicating “immunologically effective amount”, “anti-tumor effective amount”, “tumor-inhibiting effective amount” or “therapeutically effective amount”, the precise administration dose of the immune effector cells or therapeutic agents described herein can be determined by a physician in consideration of the individual’s age, weight, tumor size, degree of metastasis, and the condition of the patient (subject). An effective amount of immune effector cells refers to, but is not limited to, the number of immune effector cells which can increase, enhance or prolong the anti-tumor activity of the immune effector cells; increase the number of anti-tumor immune effector cells or activated immune effector cells; and promote IFN-γ secretion, tumor regression, tumor shrinkage and tumor necrosis.

The term “promoter” as used herein is a DNA sequence recognized by a synthetic mechanism of a cell or an introduced synthetic mechanism required to initiate the specific transcription of a polynucleotide sequence.

A typical eukaryotic promoter consists of a minimal promoter and other cis elements. The minimal promoter is essentially a TATA box region, where RNA polymerase II (polII), TATA binding protein (TBP) and TBP-related factor (TAF) can be combined to initiate

transcription. It has been found that such sequence elements (e.g., enhancers) increase the overall expression level of adjacent genes, generally in a location and/or orientation-independent manner.

NFAT (Nuclear factor of activated T cells) is a nuclear factor of activated T cells. In some specific embodiments, NFAT plays an important role in the transcription and expression of cytokines during T cell activation. In some embodiments, RUNX3 is inducibly expressed using an inducible promoter. In some embodiments, the inducible promoter is NFAT promoter. In some embodiments, the encoding sequence of RUNX3 is placed under the regulation of the minimal promoter containing NFAT binding motif. In some specific embodiments, the IL2 minimal promoter containing 6 NFAT binding motifs is a promoter composed of 6 NFAT binding sites and IL2 minimal promoter in tandem.

In some embodiments, the antigen-binding receptor described herein refers to a chimeric receptor. “Chimeric receptor” as used herein refers to a fusion molecule formed by linking DNA fragments or cDNAs corresponding to proteins from different sources using gene recombination technology. A chimeric receptor generally includes an extracellular domain, transmembrane domain, and intracellular domain. The chimeric receptor that can be used in the present invention includes but not limited to: chimeric antigen receptor (CAR), modified T cell (antigen) receptor (TCR), T cell fusion protein (TFP), T cell antigen coupler (TAC).

The term “Open Reading Frame (ORF)” is the normal nucleotide sequence of a structural gene. The reading frame from the start codon to the stop codon can encode a complete polypeptide chain without a stop codon which can interrupt the translation.

As used herein, "chimeric antigen receptor" or "CAR" refers to a group of polypeptides that, when present in immune effector cells, render the cells with specificity against target cells (usually cancer cells) and generate intracellular signals. CAR usually includes at least one extracellular antigen binding domain (also named as extracellular region), transmembrane domain (also named as transmembrane region), and cytoplasmic signaling domain (also named herein as “intracellular signaling domain” or “intracellular region”) which includes functional signaling domains derived from stimulatory molecules and/or costimulatory molecules as defined below. In certain aspects, groups of polypeptides are bound to each other. The group of polypeptides includes a dimerization switch that can couple polypeptides to each other in the presence of a dimerization molecule, for example, for coupling an antigen-binding domain to

an intracellular signal transduction domain. In one aspect, the stimulatory molecule is the ζ chain binding to T cell receptor complex. In one aspect, the cytoplasmic signaling domain further comprises one or more functional signaling domains derived from at least one costimulatory molecule as defined below. In one aspect, the costimulatory molecule is selected from the costimulatory molecules described herein, such as 4-1BB (i.e., CD137), CD27 and/or CD28. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen binding domain, transmembrane domain and intracellular signaling domain comprising a functional signaling domain derived from a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen-binding domain, transmembrane domain and a functional signaling domain derived from a co-stimulatory molecule and an intracellular signaling domain derived from a functional signaling domain of a stimulatory molecule. In one aspect, the CAR comprises a chimeric fusion protein comprising an extracellular antigen-binding domain, transmembrane domain, and comprises two functional signaling domains derived from one or more costimulatory molecules.

In one aspect, modifications of the amino acid sequence of a starting antibody or a fragment (e.g., scFv) that can produce a functionally equivalent molecule is contemplated in the present invention. For example, the VH or VL of the antigen-binding domain of the cancer-associated antigen described herein, such as the scFv contained in a CAR, can be modified to retain at least about 70%, 71%, 72% .73%, 74%, 75%, 76%, 77%, 78%, 79%, 80% ,81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the initial VH or VL framework region (e.g. scFv) of the antigen-binding domain of the cancer-associated antigen described herein. Modifications of the entire CAR construct, such as modifications of one or more amino acid sequences of multiple domains of the CAR construct is envisaged in the present invention to produce functionally equivalent molecules. The CAR construct can be modified to retain at least about 70%, 71%, 72% .73%, 74%, 75%, 76%, 77%, 78%, 79%, 80% ,81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% identity with the initial CAR construct.

“Transmembrane domain” (also called as membrane-spanning region) as used herein may include one or more additional amino acids adjacent to the transmembrane region, for example, one or more amino acids associated with the extracellular region of the protein, from

which the transmembrane region is derived (for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids in the extracellular region) and/or one or more additional amino acids associated with the extracellular region of the protein, from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids in the intracellular region). In one aspect, the transmembrane domain is a domain related to one of the other domains of the chimeric receptor. For example, in one embodiment, the transmembrane domain may be derived from the same protein, from which the signaling domain, co-stimulatory domain or hinge domain is derived. In some cases, the transmembrane domain can be selected or modified by amino acid substitutions to prevent such domains from binding to transmembrane domains of the same or different surface membrane proteins, for example, to minimize the interaction with other members of the receptor complex. In one aspect, the transmembrane domain is capable of being subjected to homodimerization with another chimeric receptor on the surface of the cell expressing the chimeric receptor. In a different aspect, the amino acid sequence of the transmembrane domain can be modified or substituted in order to minimize interaction with the binding domain of the natural binding partner present in cells expressing the same chimeric receptor. The transmembrane domain can be derived from natural or recombinant sources. When the source is natural source, the domain can be derived from any membrane-bound protein or transmembrane protein. In one aspect, the transmembrane domain is capable of transmitting a signal to the intracellular domain whenever the chimeric receptor binds to the target antigen. The transmembrane domain, which can be specifically used in the present invention, may include at least the following transmembrane domains: for example, α, β or ζ chains of T cell receptors, CD28, CD27, CD3ε, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. In some embodiments, the transmembrane domain may include at least the following transmembrane regions: for example, KIRDS2, OX40, CD2, CD27, LFA-1(CD11a, CD18), ICOS(CD278), 4-1BB(CD137), GITR, CD40, BAFFR, HVEM(LIGHTR), SLAMF7, NKp80(KLRF1), NKp44, NKp30, NKp46, CD160, CD19, IL2Rβ, IL2Rγ, IL7Rα, ITGA1, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, TNFR2, DNAM1(CD226), SLAMF4(CD244, 2B4), CD84, CD96(Tactile), CEACAM1, CRTAM, Ly9(CD229), CD160(BY55), PSGL1, CD100(SEMA4D), SLAMF6(NTB-A, Ly108),

SLAM(SLAMF1, CD150, IPO-3), BLAME(SLAMF8), SELPLG(CD162), LTBR, PAG/Cbp, NKG2D, NKG2C.

In some cases, the transmembrane domain can be connected to the extracellular region of a CAR, such as the antigen binding domain of the CAR via a hinge (for example, a hinge from a human protein). For example, in one embodiment, the hinge may be a hinge of a human Ig (immunoglobulin) (e.g., IgG4 hinge, IgD hinge), GS linker (e.g., GS linker described herein), KIR2DS2 hinge, or CD8a hinge. In one aspect, the transmembrane domain can be a recombinant transmembrane domain, in which case it will mainly contain hydrophobic residues, such as leucine and valine. In one aspect, a triplet of phenylalanine, tryptophan and valine can be found at each end of the recombinant transmembrane domain. Optionally, short oligopeptide or polypeptide linkers between 2 and 10 amino acids in length can form a bond between the transmembrane domain of the CAR and the cytoplasmic region. Glycine-serine dimer provides a particularly suitable linker.

As used herein, " intracellular domain" (also named as intracellular region) includes intracellular signaling domain. The intracellular signaling domain is generally responsible for the activation of at least one of normal effector functions of an immune cell into which the chimeric receptor has been introduced. The term "effector function" refers to the specialization function of a cell. The effector functions of a T cell can be, for example, cytolytic activity or auxiliary activity, including secretion of cytokines. Therefore, the term “intracellular signaling domain” refers to a part of a protein that transduces effector function signals and guides cells to perform specific functions. The entire intracellular signaling domain can usually be used, however, in many cases, it is not necessary to use the entire chain. When the truncated part of the intracellular signaling domain is used, such a truncated part can be used instead of the complete chain, as long as it transduces the immune effector function signal. Therefore, the term intracellular signaling domain means that a truncated portion of the intracellular signaling domain sufficient to transduce effector function signals is included.

It is well known that the signal generated by TCR alone is not sufficient to fully activate T cells, and secondary and/or costimulatory signals are also required. Therefore, T cell activation can be considered as being mediated by two different kinds of cytoplasmic signaling sequences: those that trigger antigen-dependent primary activation by TCR (primary intracellular signaling domains) and those that act in an antigen-independent manner to provide

secondary or costimulatory signals (secondary cytoplasmic domains, such as costimulatory domains).

The term "stimulation" refers to the binding of a stimulatory molecule (e.g., TCR/CD3 complex or CAR) to its cognate ligand (or a tumor antigen in the case of CAR), thereby mediating the initial response induced by a signal transduction event (for example, but not limited to signal transduction via TCR/CD3 complex or signal transduction via a suitable NK receptor or the signal transduction domain of CAR). Stimulation can mediate the altered expression of certain molecules.

The term "stimulatory molecule" refers to a molecule expressed by immune cells (e.g., T cells, NK cells, B cells) to provide cytoplasmic signal transduction sequences that modulate the activation of immune cells used in at least some aspects of immune cell signaling pathways in a stimulating manner. In one aspect, the signal is a primary signal initiated by, for example, the binding of TCR/CD3 complex and MHC antigen peptide complex, and mediates T cell responses, including, but not limited to, proliferation, activation, differentiation, and the like. The primary cytoplasmic signaling sequence (also named as “primary signaling domain”) that acts in a stimulating manner may contain signaling motif which is named as immunoreceptor tyrosine-based activation motif (ITAM). In particular, examples of ITAM-containing cytoplasmic signaling sequences used herein include, but are not limited to, those derived from CD3ζ, common FcRγ (FCER1G), FcγRIIa, FcRβ (FcEpsilon R1b), CD3γ, CD3δ, CD3ε, CD79a, CD79b, DAP10 and DAP12. In the specific CARs of the present invention, the intracellular signaling domain in any one or more CARs described herein includes intracellular signaling sequences, such as the primary signaling sequence of CD3-ζ. In the specific CARs of the present invention, the primary signaling sequence of CD3-ζ is equivalent residues from human or non-human species, such as mouse, rodent, monkey, ape, etc.

The term “costimulatory molecule” refers to a homologous binding partner on T cells, which specifically binds a costimulatory ligand, thereby mediating the costimulatory response of T cells, such as but not limited to proliferation. Co-stimulatory molecules are cell surface molecules other than antigen receptors or ligands thereof, which promote an effective immune response. Co-stimulatory molecules include but are not limited to MHC class I molecules, BTLA and Toll ligand receptors, and OX40, CD27, CD28, CDS, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278) and 4-1BB (CD137). Further examples of such costimulatory

molecules include CDS, ICAM-1, GITR, BAFFR, HVEM (LIGHTR), SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD160, CD19, CD4, CD8α, CD8β, IL2Rβ, IL2Rγ, IL7Rα, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CD11d, ITGAE, CD103, ITGAL, CD11a, LFA-1, ITGAM, CD11b, ITGAX, CD11c, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100(SEMA4D), CD69, SLAMF6(NTB-A, Ly108), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand specifically binding to CD83.

The costimulatory intracellular signaling domain can be the intracellular part of a costimulatory molecule. The costimulatory molecules can be represented by the following proteins: TNF receptor protein, immunoglobulin-like protein, cytokine receptor, integrin, signaling lymphocyte activation molecule (SLAM protein), and NK cell receptor. Examples of such molecules include CD27, CD28, 4-1BB (CD137), OX40, GITR, CD30, CD40, ICOS, BAFFR, HVEM, ICAM-1, antigen-1 (LFA-1) associated with lymphocyte function, CD2, CDS, CD7, CD287, LIGHT, NKG2C, NKG2D, SLAMF7, NKp80, NKp30, NKp44, NKp46, CD160, B7-H3 and ligands specifically binding to CD83, etc.

The intracellular signaling domain may include all intracellular part or all of the natural intracellular signaling domain of the molecule, or a functional fragment or derivative thereof.

The term “4-1BB” refers to a member of TNFR superfamily with the amino acid sequence provided in GenBank Accession No.AAA62478.2, or equivalent residues from non-human species, such as mice, rodents, monkeys, apes, etc.; and “4-1BB costimulatory domain” is defined as the amino acid residues 214~255 of GenBank Accession No.AAA62478.2, or the equivalent residues from non-human species, such as mouse, rodent, monkey, ape, etc. In one aspect, the “4-1BB costimulatory domain” is equivalent residues from humans or from non-human species, such as mice, rodents, monkeys, apes, and the like.

The term “T cell receptor (TCR)” is a characteristic mark on the surface of all T cells, which binds to CD3 by non-covalent bonds to form a TCR-CD3 complex. TCR is responsible for recognizing antigens bound to major histocompatibility complex molecules. TCR is a heterodimer composed of two different peptide chains, α and β chains, each of which can be divided into several parts, variable region (V region), constant region (C region),

transmembrane region and cytoplasmic region, characterized in that the cytoplasmic region is very short. TCR molecules belong to the immunoglobulin superfamily, and their antigen specificity exists in the V region; each of V regions (Vα, Vβ) has three hypervariable regions CDR1, CDR2, and CDR3, with CDR3 having the largest variation, which directly determines the antigen-binding specificity of TCR. When TCR recognizes the MHC-antigen peptide complex, CDR1 and CDR2 recognize and bind to the side wall of the antigen binding groove of the MHC molecule, and CDR3 directly binds to the antigen peptide. TCR is divided into two categories: TCR1 and TCR2; TCR1 is composed of two chains, γ and δ, and TCR2 is composed of two chains, α and β.

The term "T cell fusion protein (TFP)" includes recombinant polypeptides derived from various polypeptides that constitute TCR, which can bind to the surface antigens of target cells, interact with other polypeptides of the complete TCR complex and usually co-localized on the surface of T cells. TFP consists of a TCR subunit and an antigen binding domain consisting of a human or humanized antibody domain, wherein the TCR subunit includes at least part of the TCR extracellular domain, transmembrane domain, and the stimulation domain of the internal signal domain of the TCR intracellular domain; the TCR subunit and the antibody domain are effectively connected, wherein the extracellular, transmembrane and intracellular signal domains of the TCR subunit are derived from CD3ε or CD3γ, and the TFP integrates into the TCR expressed on T cells.

The term "T cell antigen coupler (TAC)" includes three functional domains: 1. tumor-targeting domain, including single-chain antibodies, designed ankyrin repeat protein (DARPin) or other targeting groups; 2. extracellular domain, a single-chain antibody binding to CD3, so that TAC receptor and TCR receptor are close; 3. transmembrane region and intracellular region of CD4 co-receptor, wherein the intracellular region is connected to the protein kinase LCK to catalyze the phosphorylation of immunoreceptor tyrosine activation motifs (ITAM) of the TCR complex as the initial step of T cell activation.

The term "antibody" refers to a protein or polypeptide sequence derived from an immunoglobulin molecule specifically binding to an antigen. Antibodies can be of polyclonal or monoclonal, multi-chain or single-chain, or whole immunoglobulins, and can be derived from natural sources or recombinant sources. The antibody may be a tetramer of immunoglobulin molecules.

The term “antibody fragment” refers to at least a portion of an antibody that retains the ability to specifically interact with an epitope of an antigen (e.g., through binding, steric hindrance, stabilization/destabilization, spatial distribution). Examples of antibody fragments include, but are not limited to, Fab, Fab’, $F(ab')_2$, Fv fragments, scFv, disulfide-linked Fv (sdFv), Fd fragments composed of VH and CH1 domains, linear antibodies, single domain antibodies (such as sdAb), multispecific antibodies formed by antibody fragments (such as bivalent fragments including two Fab fragments connected by disulfide bonds in the hinge region) and isolated CDRs or other epitope binding fragments of antibodies.

The term “scFv” refers to a fusion protein comprising at least one antibody fragment comprising light chain variable region and at least one antibody fragment comprising heavy chain variable region, wherein the light chain and heavy chain variable regions are contiguous (for example, via a synthetic linker, such as a short flexible polypeptide linker), and can be expressed as a single-chain polypeptide, and wherein the scFv retains the specificity of the intact antibody from which it is derived. Unless specified, as used herein, scFv may have the VL and VH variable regions in any order (for example, relative to the N-terminus and C-terminus of the polypeptide), and the scFv may include VL-linker-VH or may include VH-linker-VL.

The term “antibody heavy chain” refers to the larger of the two polypeptide chains which is present in the antibody molecule in its naturally occurring configuration and usually determines the type of antibody.

The term “antibody light chain” refers to the smaller of the two polypeptide chains which is present in the antibody molecule in its naturally occurring configuration. κ(k) and λ(l) light chains refer to the two main isotypes of antibody light chains.

The term “recombinant antibody” refers to an antibody produced using recombinant DNA technology, such as an antibody expressed by a phage or yeast expression system. The term should also be interpreted as referring to antibodies that have been produced by synthesizing a DNA molecule encoding the antibody (and wherein the DNA molecule expresses the antibody protein) or the amino acid sequence of the specified antibody, wherein the DNA or amino acid sequence has been obtained by recombinant DNA or amino acid sequence technology which is available and well-known in the art.

The term “antigen” refers to a molecule that causes an immune response. The immune

response may involve the production of antibodies or the activation of cells with specific immunity or both. A skilled person should understand that any macromolecule including virtually all proteins or peptides can serve as an antigen. In addition, the antigen can be derived from recombinant or genomic DNA. When the term is used herein, a skilled person should understand that it includes a protein or peptide encoded by any DNA including a nucleotide sequence or part of the nucleotide sequence encoding a protein that causes an immune response. In addition, a skilled person should understand that the antigen need not be encoded only by the full-length nucleotide sequence of the gene. It is obvious that the present invention includes but is not limited to the use of partial nucleotide sequences of more than one gene, and these nucleotide sequences are arranged in different combinations to encode polypeptides that elicit a desired immune response. Moreover, a skilled person should understand that antigens need not be encoded by "genes" at all. It is obvious that the antigen can be synthetically produced, or it can be derived from a biological sample, or it can be a macromolecule other than a polypeptide. Such biological samples may include, but are not limited to tissue samples, tumor samples, cells or fluids containing other biological components.

“Tumor antigen” refers to an antigen that is newly emerged or overexpressed during the occurrence and development of hyperproliferative diseases. In certain aspects, the hyperproliferative disorders described herein refer to tumors.

The tumor antigens described herein can be solid tumor antigens or hematoma antigens.

The tumor antigens described herein include but are not limited to: Thyroid Stimulating Hormone Receptor (TSHR); CD171; CS-1; C-type lectin-like molecule-1; Ganglioside GD3; Tn antigen; CD19; CD20; CD 22 ; CD30; CD70; CD123; CD138; CD33; CD44; CD44v7/8; CD38; CD44v6; B7H3 (CD276), B7H6; KIT (CD117); Interleukin 13 receptor subunit α (IL-13Rα); Interleukin 11 receptor α (IL-11Rα); Prostate Stem Cell Antigen (PSCA); Prostate Specific Membrane Antigen (PSMA); Carcinoembryonic Antigen (CEA); NY-ESO-1; HIV-1 Gag; MART-1; gp100; Tyrosine Enzyme; Mesothelin; EpCAM; Protease Serine 21 (PRSS21); Vascular Endothelial Growth Factor Receptor, Vascular Endothelial Growth Factor Receptor 2 (VEGFR2); Lewis (Y) Antigen; CD24; Platelet Derived Growth Factor Receptor β (PDGFR) -β); stage-specific embryonic antigen-4 (SSEA-4); cell surface-associated mucin 1 (MUC1), MUC6; epidermal growth factor receptor family and its mutants (EGFR, EGFR2, ERBB3, ERBB4, EGFRvIII)); Neural cell adhesion molecule (NCAM); Carbonic anhydrase IX

(CAIX); LMP2; Ephrin A receptor 2 (EphA2); Fucosyl GM1; Sialyl Lewis adhesion molecule (sLe); Ganglioside GM3Galp(1-4)bDGlcp(1-1)Cer; TGS5; high molecular weight melanoma-associated antigen (HMWMAA); o-acetyl GD2 ganglioside (OAcGD2); folate receptor; tumor vascular endothelium Marker 1 (TEM1/CD248); Tumor vascular endothelial marker 7 related (TEM7R); Claudin 6, Claudin 18.2, Claudin 18.1; ASGPR1; CDH16; 5T4; 8H9; αvβ6 integrin; B cell maturation antigen (BCMA); CA9; kappa light chain; CSPG4; EGP2, EGP40; FAP; FAR; FBP; embryonic AchR; HLA-A1, HLA-A2; MAGEA1, MAGE3; KDR; MCSP; NKG2D ligand; PSC1; ROR1 ; Sp17; SURVIVIN; TAG72; TEM1; Fibronectin; Tenascin; Carcinoembryonic variant of tumor necrosis zone; G protein-coupled receptor class C group 5-member D (GPRC5D); X chromosome open reading frame 61 (CXORF61); CD97; CD179a; Anaplastic Lymphoma Kinase (ALK); Polysialic acid; Placenta specific 1 (PLAC1); the hexose part of globoH glycoceramide (GloboH); breast differentiation antigen (NY-BR-1); uroplakin 2 (UPK2); hepatitis A virus cell receptor 1 (HAVCR1); adrenergic receptor β3 (ADRB3); pannexin 3 (PANX3); G protein coupled receptor 20 (GPR20); lymphocyte antigen 6 complex locus K9 (LY6K); olfactory receptor 51E2 (OR51E2); TCRγ alternating reading frame protein (TARP); Wilms tumor protein (WT1); ETS translocation variant gene 6 (ETV6-AML); Sperm protein 17 (SPA17); X antigen family member 1A (XAGE1); Angiopoietin binds to cell surface receptor 2 (Tie2); Melanoma cancer testis antigen-1 (MAD-CT-1); Melanoma cancer testis antigen-2 (MAD-CT-2); Fos-related antigen 1; p53 mutant; human telomerase reverse transcriptase (hTERT); sarcoma translocation breakpoint; melanoma inhibitor of apoptosis (ML-IAP); ERG (transmembrane protease serine 2 (TMPRSS2) ETS fusion gene); N-acetylglucosaminyl transferase V (NA17); Pairing box protein Pax-3 (PAX3); Androgen receptor; Cyclin B1; V-myc avian myeloidosis virus oncogene neuroblastoma-derived homolog (MYCN); Ras homolog Family member C (RhoC); Cytochrome P450 1B1 (CYP1B1); CCCTC binding factor (zinc finger protein)-like (BORIS); Squamous cell carcinoma antigen 3 (SART3) recognized by T cells; Paired box protein Pax-5 (PAX5); proacrosin binding protein sp32 (OYTES1); lymphocyte-specific protein tyrosine kinase (LCK); A kinase anchoring protein 4 (AKAP-4); synovial sarcoma X breakpoint 2 (SSX2); CD79a; CD79b ; CD72; Leukocyte-associated immunoglobulin-like receptor 1 (LAIR1); IgA receptor Fc fragment (FCAR); Leukocyte immunoglobulin-like receptor subfamily member 2 (LILRA2); CD300 molecular-like family member f (CD300LF) ; C-type

lectin domain family 12 member A (CLEC12A); bone marrow stromal cell antigen 2 (BST2); mucin-like hormone receptor-like 2 (EMR2) containing EGF-like module; lymphocyte antigen 75 (LY75); phosphatidyl Inositol proteoglycan-3 (GPC3); Fc receptor-like 5 (FCRL5); immunoglobulin lambda-like polypeptide 1 (IGLL1).

The pathogen antigen is selected from: virus, bacteria, fungus, protozoa, or parasite antigen; and virus antigen is selected from: cytomegalovirus antigen, Epstein-Barr virus antigen, human immunodeficiency virus antigen, or influenza virus antigen.

The term “tumor” refers to a broad category of disorders in which hyperproliferative cell growth occurs *in vitro* (e.g., transformed cells) or *in vivo*. Conditions that can be treated or prevented by the methods described herein include, for example, various neoplasms, including benign or malignant tumors, various hyperplasias, etc. Specific examples of cancer include but are not limited to: breast cancer, prostate cancer, leukemia, lymphoma, nasopharyngeal cancer, glioma, colon cancer, rectal cancer, renal cell carcinoma, liver cancer, non-small cell lung cancer, small bowel cancer, esophageal cancer, melanoma, bone cancer, pancreas Cancer, skin cancer, head and neck cancer, uterine cancer, ovarian cancer, stomach cancer, testicular cancer, fallopian tube cancer, endometrial cancer, cervical cancer, vaginal cancer, thyroid cancer, parathyroid cancer, adrenal cancer, soft tissue sarcoma, urethral cancer, Penile cancer, bladder cancer, ureteral cancer, renal pelvis cancer, central nervous system (CNS) tumor, hemangioma, spine tumor, glioma, astrocytoma, pituitary adenoma, a combination and metastatic foci thereof.

The term “transfected” or “transformed” or “transduced” refers to a process by which exogenous nucleic acid is transferred or introduced into a host cell. A “transfected” or “transformed” or “transduced” cell is a cell that has been transfected, transformed, or transduced with exogenous nucleic acid. The cell includes the cell of the primary subject and a progeny thereof.

The term “specifically binds” refers to an antibody or ligand binding to a binding partner (e.g., tumor antigen) present in a sample, while not substantially recognizing or binding to other molecules in the sample.

As used herein, the term "refractory" refers to a disease, such as a tumor, which does not respond to a treatment. In embodiments, the refractory tumor may be resistant to a treatment before or at the beginning of the treatment. In other embodiments, a refractory tumor can

become resistant during treatment. In the present invention, a refractory tumor includes, but are not limited to, a cancer which is not sensitive to radiotherapy, relapses after radiotherapy, not sensitive to chemotherapy, relapses after chemotherapy, not sensitive to CAR-T treatment, or relapses after CAR-T treatment. The treatment regimens described herein can be used fot the refractory or recurrent malignancies.

As used herein, "relapsed" means that signs and symptoms before the effective treatment re-appear in a patient after a period of improvement, for example, after an effective tumor treatment.

The terms “individual” and “subject” have the same meaning herein, and can be humans and animals from other species.

The term “enhancement” means that the response of a subject or tumor cells to to the treatment disclosed herein is improved. For example, an enhanced response may include 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98% or higher of improvement in response. As used herein, “enhancement” can also refer to increase in the number of subjects responding to treatments such as immune effector cell therapy. For example, an enhanced response can refer to the total percentage of subjects responding to treatment, where the percentages are 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55 %, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 98% more.

In one aspect, the treatment is determined based on clinical results; the increase, enhancement or extension of the anti-tumor activity of T cells; compared with the number before treatment, the increase in the number of anti-tumor T cells or activated T cells, the promotion of IFN-γ secretion, or a combination thereof. In another aspect, the clinical results are tumor regression; tumor shrinkage; tumor necrosis; anti-tumor response through the immune system; tumor enlargement, recurrence or spread, or a combination thereof. In another aspect, the therapeutic effect is predicted by the presence of T cells, the presence of genetic markers indicative of T cell inflammation, promotion of IFN-γ secretion, or a combination thereof.

The immune effector cells as disclosed herein can be administered to an individual via various routes, including, for example, orally or parenterally, such as intravenous, intramuscular, subcutaneous, intraorbital, intrasaccular, intraperitoneal, intrarectal, intracisternal, intratumoral, intravasal, intradermal route, or passive or promoted absorption

through the skin using, for example, skin patches or transdermal iontophoresis, respectively.

When practicing the methods described herein, the total amount of agent to be administered can be administered to the subject as a single dose as a bolus injection or by infusion over a relatively short period of time, or can be administered using a graded treatment regimen, wherein multiple doses are administered over an extended time of period. A skilled person will know that the amount of the composition for treating pathological conditions in a subject depends on many factors, including the age and general health of the subject, as well as the route of administration and the number of treatments to be administered. Taking these factors into account, a technician will adjust the specific dosage as needed. In general, phase I and phase II clinical trials are initially used to determine the formulation of the composition and the route and frequency of administration.

Range: throughout the disclosure, various aspects of the present invention can exist in a range format. It should be understood that the description in range format is only for convenience and brevity, and should not be regarded as an unchangeable limitation on the scope described herein. Therefore, the description of a range should be considered as specifically disclosing all possible subranges and individual values within the range. For example, the description of a range, such as from 1 to 6, should be considered as specifically disclosing subranges, such as 1 to 3, 1 to 4, 1 to 5, 2 to 4, 2 to 6, 3 to 6, etc., and individual values within the range, such as 1, 2, 2.7, 3, 4, 5, 5.3, and 6. For another example, a range, such as 95-99% identity, includes a range with 95%, 96%, 97%, 98%, or 99% identity, and includes a sub-range, such as 96-99%, 96-98%, 96-97%, 97-99%, 97-98% and 98-99% identity. This applies regardless of the width of the range.

Based on the present disclosure, a skilled person should understand that many changes or modifications can be made in the disclosed specific embodiments and the same or similar results can still be obtained without departing from the spirit and scope described herein. The scope of the present invention is not limited to the specific embodiments described herein (which are only intended to exemplify various aspects described herein), and functionally equivalent methods and components are still included within the stated range described herein. In fact, various modifications of the present invention as well as those shown and described herein will become apparent to a skilled person based on the foregoing description.

When CAR-T cells co-expressing IL17 and CCL21 are used in a subject, the

corresponding species can be selected. For example, when used in mice, mouse-derived IL17 and CCL21 is used, and elements for constructing a CAR, such as transmembrane domain and intracelluar domain can also be of murine origin. When the subject is a human, human-derived IL17 and CCL21 as well as human-derived CAR elements are preferred. In some embodiments, the sequence of a CAR used may be as shown in SEQ ID NO: 26, 27 or 34.

In some embodiments, when used for treating a tumor, the cells of the present invention can be used in combination with a chemotherapeutics.

The term “CLD18 (claudin 18)” refers to claudin-18 and includes any variant, conformational variant, isoforms and species homologs of CLD18 (including CLD18A1 (claudin 18.1) and CLD18A2 (claudin 18.2)) that are naturally expressed by cells or expressed by cells transfected with the CLD18 gene. Preferably, "CLD18" refers to human CLD18, particularly CLD18A2 (SEQ ID NO: 22) and/or CLD18A1 (SEQ ID NO: 23), more preferably CLD18A2.

The term “CLD18A1” includes any post-translational modified variants, isoforms and species homologs of human CLD18A1 that are naturally expressed by cells or expressed by cells transfected with the CLD18A1 gene.

The term “CLD18A2” includes any post-translational modified variants, isoforms and species homologs of human CLD18A2 that are naturally expressed by cells or expressed by cells transfected with the CLD18A2 gene.

The term “CLD18 variant” shall includes (i) CLD18 splice variant, (ii) CLD18 post-translational modified variants, especially including variants with different N glycosylation, (iii) CLD18 conformational variants, especially including CLD18-conformation-1, CLD18-conformation-2 and CLD18-conformation-3, (iv) free CLD18 and homo/allo-associated variants at tight junctions between cells, (v) CLD18 cancer-related variants and CLD18 non cancer-related variants.

The chimeric antigen receptor polypeptides described herein can be sequentially linked as follows:

extracellular antigen binding region-CD8 transmembrane region-4-1BB-CD3ζ,

extracellular antigen binding region-CD8 transmembrane region-CD28b-CD3ζ,

extracellular antigen binding region-CD28a-CD28b-CD3ζ,

extracellular antigen binding region-CD28a-CD28b-4-1BB-CD3ζ,

and combinations thereof, where CD28a in the relevant chimeric antigen receptor protein represents the transmembrane region of CD28 molecule, and CD28b represents the intracellular signal region of CD28 molecule. The present invention also includes a nucleic acid encoding the chimeric antigen receptor. The present invention also relates to variants of the aforementioned polynucleotides, which encode polypeptides having the same amino acid sequence as the present invention or polypeptide fragments, analogs and derivatives.

The present invention also provides a vector containing the nucleic acid of the chimeric antigen receptor. The invention also includes viruses comprising the vectors described above. The viruses of the invention include packaged infectious viruses as well as viruses to be packaged that contain the necessary components for packaging into infectious viruses. Other viruses known in the art that can be used to transduce exogenous genes into immune effector cells and their corresponding plasmid vectors are also useful in the present invention.

The present invention further includes a chimeric antigen-modified immune effector cell, which is transduced with a nucleic acid encoding the chimeric antigen receptor or transduced with the recombinant plasmid containing the above-mentioned nucleic acid or a viral system containing the plasmid. Conventional nucleic acid transduction methods in the art, including non-viral and viral transduction methods, can be used in the present invention. Non-viral transduction methods include electroporation and transposon methods. Recently, nucleofector nuclear transfection instrument developed by Amaxa can directly introduce foreign genes into nucleus to achieve highly efficient transduction of target genes. In addition, compared with conventional electroporation, the transduction efficiency of transposon system based on Sleeping Beauty system or PiggyBac transposon was significantly improved. The combination of nucleofector transfection instrument and SB Sleeping Beauty transposon system has been reported [Davies JK., et al. Combining CD19 redirection and alloanergization to generate tumor-specific human T cells for allogeneic cell therapy of B-cell malignancies. Cancer Res, 2010, 70(10): OF1-10.], and high transduction efficiency and site-directed integration of target genes can be achieved by this method. In one embodiment of the invention, the transduction method of a T lymphocyte modified by a chimeric antigen receptor gene is a transduction method based on a virus such as a retrovirus or a lentivirus. The method has the advantages of high transduction efficiency and stable expression of exogenous gene, and the time for in vitro culturing T lymphocytes to clinical level can be shorten. The transduced nucleic acid is

expressed on the surface of the transgenic T lymphocytes by transcription, translation. In vitro cytotoxicity assay performed on various cultured tumor cells demonstrated that the immune effector cells of the present invention have highly specific tumor cell killing effects (also known as cytotoxicity). Therefore, the nucleic acid encoding a chimeric antigen receptor protein of the present invention, a plasmid comprising the nucleic acid, a virus comprising the plasmid, and a transgenic immune effector cells transfected with the nucleic acid, plasmid or virus described above can be effectively used in tumor immunotherapy.

In addition to the chimeric receptor described above, the chimeric antigen-modified immune effector cells of the present invention may also express another chimeric receptor, which does not contain CD3ζ, but contains intracellular signaling domain of CD28 and intracellular signal domain of CD137, or a combination of both.

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

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

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

Advantages of the invention:

1. The immune effector cells provided herein can effectively increase the proliferation, survival and function of the immune effector cells in tumors; reduce the expression of inhibitory immune checkpoints, thereby alleviating the exhaustion of T cells；

2. The immune effector cells provided herein have better killing effects on solid tumor cells and *in vitro* expansion performance.

The present invention will be further described below in conjunction with specific embodiments. It should be understood that these examples are only used to illustrate the present invention and not to limit the scope of the present invention. The experimental methods that do not indicate specific conditions in the following examples are generally performed under conditions described in J. Sambrook et al., Molecular Cloning Experiment Guide, Third Edition, Science Press, 2002, or according to conditions recommended by the manufacturer.

Exemplary antigen receptors of the present invention, including CAR, and methods for engineering and introducing receptors into cells, may refer to, for example, those disclosed in CN107058354A, CN107460201A, CN105194661A, CN105315375A, CN105713881A, CN106146666A, CN106519037A, CN106554414A , CN105331585A, CN106397593A, CN106467573A, CN104140974A, WO2017186121A1, WO2018006882A1, WO2015172339 A8 and WO2018/018958A1.

Example 1. Construction of T cells expressing chimeric antigen receptors

In this example, Claudin 18.2 was selected as the target of CAR-T cells. In order to more accurately verify anti-tumor effects in mice, mouse-derived signal peptide, transmembrane region, intracellular region, and the like were selected. The preparation method was operated in accordance with the conventional CAR-T cell preparation method in the art.

1. Construction of plasmid

Conventional molecular biology methods in the art were used, and the scFv used in this example was an antibody targeting human Claudin 18.2. The nucleic acid sequence was shown in SEQ ID NO: 1, and the used chimeric antigen receptor was the second-generation of chimeric antigen receptor, which has the transmembrane domain of mCD8, intracellular

domain of mCD28 and/or intracellular domain of m4-1BB, and mCD3ζ.

1. MSCV.pBABE 5 (purchased from addgene) was used as a vector to construct a retroviral plasmid MSCV-hu8E5(2I)-28Z expressing the second-generation of chimeric antigen receptor. The nucleic acid sequence of hu8E5(2I)-28Z comprises the signal peptide of CD8α (SEQ ID NO: 3), scFv (SEQ ID NO: 1), hinge region and transmembrane region of mCD8 (SEQ ID NO: 5) and intracellular signal transduction domain of mCD28 (SEQ ID NO: 7) and intracellular segment mCD3ζ of mCD3 (SEQ ID NO: 9). The plasmid map of hu8E5(2I)-28Z is shown in Figure 1A.

The gene of F2A-mIL7-P2A-mCCL21a or F2A-mIL7-P2A-mCCL21b was inserted into the MSCV-hu8E5(2I)-m28Z plasmid to construct the retroviral plasmid MSCV-hu8E5(2I)-m28Z-F2A-mIL7-P2A-mCCL21a (plasmid map shown in Figure 1B) and MSCV-hu8E5(2I)-m28Z-F2A-mIL7-P2A-mCCL21b (plasmid map shown in Figure 1C) expressing CAR, IL7 and CCL21.

F2A-mIL7-P2A-mCCL21a consists of F2A (SEQ ID NO: 11), mouse IL7 (SEQ ID NO: 13), P2A (SEQ ID NO: 16), mouse CCL21a (SEQ ID NO: 14); F2A-mIL7-P2A-mCCL21b consists of F2A (SEQ ID NO: 11), mouse IL7 (SEQ ID NO: 13), P2A (SEQ ID NO: 16), mouse CCL21b (SEQ ID NO: 15).

MSCV.pBABE 5 was used as a vector to construct a retroviral plasmid MSCV-hu8E5(2I)-mBBZ expressing the second-generation of chimeric antigen receptor. The hu8E5(2I)-mBBZ sequence consists of the signal peptide of CD8α (SEQ ID NO: 3), scFv (SEQ ID NO: 1), hinge and transmembrane region of mCD8 (SEQ ID NO: 5), intracellular signal transduction domain (SEQ ID NO: 24) and intracellular segment CD3ζ of mCD3 (SEQ ID NO: 9). The plasmid map is shown in Figure 1D.

F2A-mIL-7-P2A-mCCL21a and F2A-mIL7-P2A-mCCL21b were inserted into the MSCV-hu8E5(2I)-mBBZ plasmid, respectively, so as to construct the retroviral plasmid MSCV-hu8E5(2I)-mBBZ-F2A-mIL-7-P2A-mCCL21a (plasmid map shown in Figure 1E) and MSCV-hu8E5(2I)-mBBZ-F2A-mIL7-P2A-mCCL21b (plasmid map shown in Figure 1F) expressing CAR, IL7 and CCL21.

2. MSCV-hu8E5(2I)-m28Z, MSCV-hu8E5(2I)-m28Z-F2A-mIL-7-P2A-mCCL21a, MSCV-hu8E5(2I)-m28Z-F2A-mIL7-P2A-mCCL21b, MSCV-hu8E5(2I)-mBBZ, MSCV-hu8E5(2I)-mBBZ-F2A-mIL-7-P2A-mCCL21a, MSCV-hu8E5(2I)-mBBZ-F2A-mIL7-P2A-

mCCL21b were transfected into 293T cells, respectively, so as to obtain retroviruses hu8E5(2I)-28Z, IL7-CCL21a-28Z, IL7-CCL21b-28Z, hu8E5(2I)-BBZ, IL7-CCL21a-BBZ, IL7-CCL21b-BBZ.

3. Extraction and activation of mouse T cells: the spleen of C57BL/6 mouse was removed to extract mouse T cells. T cells were cultured and activated, and then infected by the retroviruses hu8E5(2I)-28Z, IL7-CCL21a-28Z, IL7-CCL21b-28Z, hu8E5(2I)-BBZ, IL7-CCL21a-BBZ, IL7-CCL21b-BBZ, respectively, so as to obtain m28Z CAR-T cells, m28Z-7*21A CAR-T cells, m28Z-7*21B CAR-T Cells, mBBZ CAR-T cells, mBBZ-7*21A CAR-T cells, and mBBZ-7*21B CAR-T cells.

Example 2. *In vitro* detection of cytokines

Firstly, mouse pancreatic cancer cells PANC02 (negative expression of claudin18.2, purchased from ATCC) and PANC02-A2 (positive expression of claudin18.2) were pretreated by using mitomycin C (40 μg/ml, 37°C, 2-3h).

Cells were inoculated into a 24-well plate at 2×10^5 cells/ 400ul, and untransduced T cells (UTD), mBBZ CAR-T cells, mBBZ-7*21A CAR-T cells, mBBZ-7*21B CAR-T cells were inoculated into a 24-well plate, respectively. A control group without target cells was set, and the cell supernatant was collected on day 3. The secretion of each cytokine, IL7 and CCL21 was detected by an ELISA kit. The results are shown in Figure 2.

PANC02-A2 cells were constructed by infecting PANC02 cells with pwpt-mclaudin18.2 lentivirus. The pWPT-mclaudin18.2 plasmid was constructed as follows: murine claudin18.2 gene (GeneBank reference sequence number: NM_001194921) was syntheized *in vitro*, and inserted into a lentiviral expression vector pWPT by restriction digestion and ligation, so as to construct the pwpt-mclaudin18.2 plasmid.

Example 3. *In vitro* detection of CAR-T cell phenotype

UTD, mBBZ CAR-T cells, mBBZ-7*21A CAR-T cells, mBBZ-7*21B CAR-T cells were taken and detected for cell surface immune checkpoints: PD-1, LAG-3, TIM-3. Firstly, different CAR-T cells were collected in EP tubes. Each cell is divided into 3 tubes, and washed twice with a pre-cooled flow washing solution (1% NCS + PBS). BV421-labeled anti-PD-1 antibody, APC-labeled anti-LAG-3 antibody, and APC-labeled anti-TIM-3 antibody were

added into different detection tubes, respectively at a ratio of 50: 50, incubated on ice for 45 minutes, washed for 3 times, and detected in flow tube. The results are shown in Figures 3A~3F.

Figure 3A shows the expression of PD-1 in different groups of cells. The results showed that the secretion of PD-1 in the mBBZ group reached 30.2%, the secretion of PD-1 in the mBBZ-7*21A group was only 11.7%, and the secretion of PD-1 in mBBZ-7*21B group was only 9.4%. Figure 3B shows the expression intensity of PD-1. From Figure 3B, the expression of PD-1 in the mBBZ group was higher than that in the mBBZ-7*21A group and the mBBZ-7*21B group.

Figure 3C shows the expression of LAG-3 in different groups of cells. The results showed that the secretion of LAG-3 in the mBBZ group reached 80.7%, the secretion of LAG-3 in the mBBZ-7*21A group was 53.4%, and the secretion of mBBZ-7*21B was 13.7%. Figure 3D shows the expression intensity of LAG-3. From Figure 3D, the expression of LAG-3 in the mBBZ group was higher than that in the mBBZ-7*21A group and the mBBZ-7*21B group.

Figure 3E shows the expression of TIM-3 in different groups of cells. The results showed that the secretion of TIM-3 in the mBBZ group reached 41.3%, the secretion of TIM-3 in the mBBZ-7*21A group was 16.2%, and the secretion of TIM-3 in the mBBZ-7*21B group was 13.2%. Figure 3F shows the expression intensity of TIM-3. From Figure 3F, the expression of TIM-3 in the mBBZ group was higher than that in the mBBZ-7*21A group and mBBZ-7*21B.

In summary, the expressions of PD-1, LAG-3 and TIM-3 in mBBZ-7*21A CAR-T cells and mBBZ-7*21B CAR-T cells are lower than those in mBBZ-CAR-T cells, indicating that the over-expression of cytokines IL7 and CCL21 can reduce the expression of these inhibitory immune checkpoints, thereby alleviating the depletion of T cells.

Example 4. *In vitro* detection of killing toxicity

CytoTox 96 non-radioactive cytotoxicity detection kit (Promega) was used. The specific method may refer to the instructions of CytoTox 96 non-radioactive cytotoxicity detection kit.

Effector cells: UTD cells, m28Z CAR-T cells, 28Z-7*21A CAR-T cells, m28Z-7*21B CAR-T cells, mBBZ CAR-T cells, mBBZ-7*21A CAR-T cells, mBBZ-7*21B CAR-T cells were inoculated into a 96-well plate at an effector target ratio of 3:1, 1:1 or 1:3, respectively.

Target cells: 50 μL of 2×10^5/mL mouse pancreatic cancer cell lines PANC02-A2 and PANC02 cells were inoculated into the corresponding 96-well plates, respectively.

5 replicate wells were set for each group. The plates were placed in a cell incubator for 18 hours.

Each experimental group and each control group were set as follows: experimental group: each target cell + different CAR-T cell; control group 1: maximum release LDH from target cell; control group 2: spontaneous release of LDH from target cell; control group 3: spontaneous release of LDH from effector cell. The calculation formula is: % cytotoxicity = [(experimental group - spontaneous effector cell group - spontaneous target cell group) / (maximum target cell - spontaneous target cell)] * 100. The experimental results are shown in Figures 4A and 4B.

Figure 4A shows that, compared with the control group UTD, m28Z CAR-T cells, m28Z-7*21A CAR-T cells, or m28Z-7*21B CAR-T cells exhibited significant toxic killing effects on PANC02-A2 at both of effector target ratios of 3:1 and 1:1, while no killing effects on PANC02 cells.

Figure 4B shows that, compared with the control group UTD, mBBZ CAR-T cells, mBBZ-7*21A CAR-T cells, or mBBZ-7*21B CAR-T cells exhibited significant toxic killing effects on PANC02-A2 at both of effector target ratios of 3:1 and 1:1, while no killing effects on PANC02 cells.

Example 5. Detection of *in vitro* proliferation

The target cells, PANC02-A2 cells were treated by using Mitomycin C (40 μg/ml, 37°C, 2-3h), and the effector cells, UTD cells, mBBZ CAR-T cells, mBBZ-7*21A CAR-T cells, mBBZ-7*21B CAR-T cells were stained with CFSE, and then incubated for 2 days at a effector target ratio (1×10^6 cells/ml) of 1:1.

Proliferation of CAR-T cells was detected by flow cytometry. The results are shown in Figure 5. mBBZ-7*21A CAR-T cells and mBBZ-7*21B CAR-T cells can proliferate faster than mBBZ CAR-T cells.

Example 6. Tumor treatment of PANC02-A2 pancreatic cancer subcutaneous xenograft tumor model

1) Experimental groups: C57BL/6 mice at 6-8 weeks old (purchased from Shanghai Xipuer-Bikai Experimental Animal Co., Ltd.) were randomly grouped (n = 5-6), that is, UTD cells, mBBZ CAR-T cells, mBBZ-7*21A CAR-T cell, mBBZ-7*21B CAR-T cell treatment groups, respectively.

2) Inoculation of subcutaneous xenograft tumor: PANC02-A2 cells in logarithmic growth phase and good growth state were collected by trypsin digestion method. After washed once with PBS, the cell density was adjusted to 6×10^6/mL. 200 μL of cell suspension was subcutaneously injected into the right abdomen of C57BL/6 mice, that is, each mouse was inoculated with 1.2×10^6 tumor cells, and the inoculation day was recorded as day 0.

3) Reinfusion of CAR-T cells: On day 11 after subcutaneous inoculation of tumor cells, the average tumor volume was about 60 mm^3. Untreated T cells or CAR-T cells were injected with an injection dose of 2.5×10^6/animal.

The results are shown in Figure 6. On day 20 after CAR-T injection, the tumor inhibition rates were as follows: mBBZ CAR-T group: 35.5%, mBBZ-7*21A CAR-T group: 63%, mBBZ-7*21B CAR-T group: 62.4%, indicating that the anti-tumor effects of mBBZ-7*21A CAR-T cells and mBBZ-7*21B CAR-T cell treatment groups are better than mBBZ CAR-T cells ($P<0.05$).

Example 7. Comparison of tumor killings by expressing different chemokines

In this example, CAR-T cells (mBBZ-7*19 CAR-T cells) expressing IL7 and CCL19 were selected as controls. mBBZ-7*19 CAR-T cells were prepared in accordance with Example 1. F2A-mIL7-P2A-mCCL19 was inserted into the MSCV-hu8E5(2I)-mBBZ plasmid, so as to construct the retroviral plasmid expressing CAR, IL7 and CCL19. The plasmid map is shown in Figure 7, and the nucleic acid sequence of mCCL19 is shown in SEQ ID NO: 34. Mouse T cells were infected by the plasmid to obtain mBBZ-7*19 CAR-T cells.

1) Mouse pancreatic cancer subcutaneous xenograft tumor model was prepared according to Example.

When the average tumor volume was about 65 mm^3, mBBZ CAR-T cells, mBBZ-7*19 CAR-T cells, mBBZ-7*21B CAR- T cell were injected in therapy groups, respectively, with an injection dose: 2.5×10^6 cells/mouse. The tumor killing results are shown in Figure 8A, the tumor inhibition rates are: mBBZ CAR-T group: 22.8%, mBBZ-7*19 CAR-T group: 32.7 %,

mBBZ-7*21B CAR-T group: 76.6%. mBBZ-7*21B CAR-T cell treatment group exhibited better anti-tumor effects than mBBZ CAR-T cell and mBBZ-7*19 CAR-T cell group.

At the same time, the changes in body weight of mice in each group were detected (as shown in Figure 8B), and the results showed that there was no significant difference in body weight between mice in each group (ns), indicating that the secretion of cytokines had no toxic effects on mice.

2) On day 31 after tumor inoculation, the mice were euthanized, and the tumors of the mice were removed and weighed. The statistical results are shown in Figure 8C. The results showed that the tumor weight in the mBBZ-7*21B CAR-T treatment group was significantly smaller than that in the mBBZ group ($P < 0.05$), indicating that the chimeric antigen receptor-modified T cells co-expressing IL7 and CCL21 can significantly enhance the inhibition on tumors *in vivo* by T cells.

3) After the CAR-T cells were re-infused, the tumor tissue was removed at the end of the experiment on day 31, and the number of copies of CAR-T cells in the tumor tissue was detected.

1 mg of tumor tissue block was taken and mechanically ground, and then QIAamp® genomic DNA kits was used to extract DNA from the tumor, and the concentration of each sample was measured separately. Real-time quantitative PCR (qPCR) was used to detect the number of copies of CARs. A standard curve was prepared according to the template plasmid, and finally, the number of copies of CARs in each sample was calculated.

The results are shown in Figure 8D, which shows that the number of copies of CAR-T cells in the mBBZ-7*21B CAR-T is higher.

4) Immunohistochemistry detection of tumor infiltration of $CD8^+$ cells

The tumor tissue of the mouse euthanized in step 2) was taken, and prepared into paraffin tissue sections. After routine deparaffinization, the specimen was hydrate. After the hydration is completed, the sections were placed on a shaker and washed with PBS for 3 times. Citric acid buffer was boiled, and then the tissue sections were put into the citric acid buffer for hot-repair of antigen. After the repair was completed, the sections were blocked by using 1% BSA.

The corresponding CD8a antibody (anti-mouse CD8α antibody, purchased from: Cell Signaling) or blank control reagent were added to the blocked sections, incubated overnight at

4°C, washed with 0.5% PBST buffer; and then washed with PBS buffer.

The secondary antibody goat anti-rabbit-HRP was added to the washed sections, and incubated at 37°C for 1 h. The sections were washed twice with 0.5% PBST buffer and once with PBS buffer. DAB (Dako REAL™ EnVision™ Detection System, Peroxidase/DAB+, 1:50 dilution) was used for development.

Hematoxylin was used for counter-stain until the nuclei were stained to deep red, and the counter-stained tissue sections were placed in the differentiation solution of 1% hydrochloric acid ethanol for 3 to 5 seconds; rinsed in tap water for 20 mins, dehydrated to transparent; soaked in 90% ethanol for 1 min; soaked in 100% ethanol I for 1 min; soaked in 100% ethanol II for 1 min; soaked in xylene for 3 min; mounted with neutral gum and air-dried.

The sections were observed under a microscope, the results are shown in Figure 8E. The mBBZ-7*19 group and mBBZ-7*21B group exhibited obvious $CD8^+$ T cell infiltration, however, there was more $CD8^+$ T cell infiltration in the mBBZ-7*21B CAR-T group.

Example 8. Orthotopic xenograft tumor model of breast cancer in mice

A mouse model of breast cancer subcutaneous xenograft tumor was prepared, and E0771-A2 cells in logarithmic growth phase and good growth state were collected by trypsinization method (preparation method: pwpt-mclaudin18.2 plasmid packaging lentivirus was used to infect E0771 cells). The cells were washed with PBS once, and the cell density was adjusted to 2×10^7/mL, and 50 μL of cell suspension was subcutaneously injected into the fourth pair of breasts on the right abdomen of C57BL/6 mice, that is, each mouse was inoculated with 1×10^6 E0771-A2 cells, and the vaccination day was day 0.

Reinfusion of CAR-T: On day 12 after subcutaneous inoculation of tumor cells, the average tumor volume was about 150 mm^3. Untreated T cells or CAR-T cells were injected with an injection dose: 2.5×10^6/animal.

The volume of E0771-A2 xenograft tumor was measured every 3-4 days, changes in the tumor volume in each group of mice were recorded, and the results are shown in Figure 9A. Compared with mBBZ-7*19 CAR-T group, the tumor-killing ability in mBBZ-7*21B CAR T treatment group was significantly enhanced.

On day 31 after tumor inoculation, the mice were euthanized. The tumors of the mice were removed and weighed. The statistical results are shown in Figure 9B. The results showed

that the tumor weight in the mBBZ-7*21B CAR-T treatment group was significantly smaller than that in the mBBZ-7*19 CAR-T group ($P < 0.05$) and mBBZ CAR-T group ($P < 0.001$), indicating that the chimeric antigen receptor-modified T cells co-expressing IL7 and CCL21 can significantly enhance the inhibition on tumors *in vivo* by T cells.

According to step 3) of Example 7, the number of copies of the CAR-T cells in the breast cancer subcutaneous xenograft model was detected. The results are shown in Figure 9C, which showed that the numbers of copies of the CAR-T in mBBZ-7*19 CAR-T and mBBZ-7*21B CAR-T groups are higher than those in the UTD and BBZ groups.

According to step 4) of Example 7, the tumor infiltration of $CD8^+$ cells was detected. The results are shown in Figure 9D. There are obvious tumor infiltration of CD8 T cells in the tumor tissues in the mBBZ-7*19 CAR-T group and mBBZ-7*21B CAR-T group, and there were more infiltration of $CD8^+$ T cells in mBBZ-7*21B CAR-T group.

Example 9. Subcutaneous xenograft tumor model of liver cancer in mice

A mouse model of liver cancer xenograft tumor was prepared, and Hepa1-6-A2 cells in logarithmic growth phase and good growth state were collected by trypsinization method (pwpt-mclaudin18.2 plasmid packaging lentivirus was used to infect Hepa1-6 cells). The cells were washed with PBS once, and the cell density was adjusted to 5×10^7/mL, and 200 μL of cell suspension was subcutaneously injected into the right abdomen of C57BL/6 mice, that is, each mouse was inoculated with 1×10^7 Hepa11-6-A2 liver cancer cells, and the vaccination day was day 0.

Reinfusion of CAR-T cells: On day 7 after subcutaneous inoculation of tumor cells, the average tumor volume was about 300 mm^3. Untreated T cells or CAR-T cells were injected with an injection dose: 1×10^6/animal.

The volume of Hepa11-6-A2 xenograft tumor was measured every 3-4 days, changes in the tumor volume in each group of mice were recorded, and the results are shown in Figure 10A. Compared with mBBZ-7*19 CAR-T group, the tumor-killing ability in mBBZ-7*21B CAR T treatment group was significantly enhanced.

On day 31 after tumor inoculation, the mice were euthanized. The tumors of the mice were removed and weighed. The statistical results are shown in Figure 10B. The results showed that the tumor weight in the mBBZ-7*21B CAR-T treatment group was significantly

smaller than that in the mBBZ-7*19 CAR-T group ($P < 0.01$) and 8E5-2I-mBBZ CAR-T group ($P < 0.05$), indicating that the chimeric antigen receptor-modified T cells co-expressing IL7 and CCL21 can significantly enhance the inhibition on tumors *in vivo* by T cells.

According to step 3) of Example 7, the number of copies of the CAR-T cells was detected. The results are shown in Figure 10C, which showed that the numbers of copies of the CAR-T in mBBZ-7*21B CAR-T group are higher.

According to step 4) of Example 7, the tumor infiltration of $CD8^+$ cells was detected. The results are shown in Figure 10D. There are obvious tumor infiltration of $CD8^+$ T cells in the tumor tissues in the mBBZ-7*19 CAR-T group and mBBZ-7*21B CAR-T group, and there were more infiltration of $CD8^+$ T cells in mBBZ-7*21B CAR-T group.

Example 10. *In vitro* detection of IFN-γ

UTD, 8E5-2I-mBBZ-CAR, mBBZ-7*21B CAR-T, and mBBZ-7*19 CAR-T were incubated with the target cells PANC02-A2 at a ratio of 1:1 for 24 hours, respectively, then the supernatant was collected, and the secretion level of IFN-γ in the supernatant was detected by ELISA. The used ELISA kit is mouse IFN-γ detection kit (LinkTech).

The results are shown in Figure 11, which showed that, after mBBZ-7*21B CAR-T cells were incubated with claudin18.2-positive tumor cells, there was more IFN-γ secretion.

Example 11. Treatment of mouse PANC02-A2 pancreatic cancer subcutaneous tumor lymphocyte-clearing model

According to Example 6, a PANC02-A2 subcutaneous xenograft tumor model of C57BL/6 mouse was prepared, and the vaccination day was day 0. On day 14 after the tumor inoculation, the average tumor volume was about 60 mm^3. Cyclophosphamide was injected into the tail vein at 100 mg/kg. On day 15 after tumor inoculation, untreated T cells or CAR-T cells were injected with an injection dose: 2.5×10^6 / animal.

The volume of PANC02-A2 xenograft tumor was measured every 3-4 days, changes in the tumor volume in each group of mice were recorded, and the results are shown in Figure 12A. On day 38 after tumor inoculation, the mice were euthanized. The tumors of the mice were removed and weighed. The statistical results are shown in Figure 12B.

According to step 3) of Example 7, the number of copies of the CAR-T cells was detected. The results are shown in Figure 12C, which showed that the numbers of copies of the CAR-T in mBBZ-7*19 CAR-T and mBBZ-7*21B CAR-T groups are higher that those in UTD and BBZ group.

According to step 4) of Example 7, the tumor infiltration of $CD8^+$ cells was detected. The results are shown in Figure 12D. There are obvious tumor infiltration of $CD8^+$ T cells in the tumor tissues in the mBBZ-7*19 CAR-T group and mBBZ-7*21B CAR-T group, and there were more infiltration of CD8 T cells in mBBZ-7*21B CAR-T group.

Example 12. Detection and analysis of CAR-T cells in mouse PANC02-A2 pancreatic cancer subcutaneous tumor model

According to Example 6, a pancreatic cancer subcutaneous xenograft tumor model of mouse was prepared. 2×10^6 PANC02-A2 pancreatic cancer cells were subcutaneously injected into the right abdomen of C57BL/6 mice. On day 14 after the subcutaneous inoculation of tumor cells, the average tumor volume was about 60 mm^3. Untreated T cells or CAR-T cells (mBBZ CAR-T cells, mBBZ-7*21A CAR-T cells, and mBBZ-7*19 CAR-T cells) were injected with an injection dose: 4×10^6 / animal.

1. The spleen and bone marrow of mice on day 10 (d10) and day 20 (d20) after CAR-T cell treatment were extracted to detect the content of Tcm (central memory T cells) (2 mice in each treatment group). The experimental method is as follows:

1) Extraction of spleen cells: the mice were sacrificed by cervical dislocation. The spleen was taken, placed in a clean 2 mL EP tube, and washed with PBS to remove blood stains. The spleen cells were ground with a 40 um filter membrane. The spleen cell mixture was centrifuged at 400 g for 5 min to remove the supernatant. 400 μL of mouse red blood cell lysate (1×) was added and stood for 5 min. 1.5 mL of PBS was added to neutralize the reaction, centrifuged, resuspended in PBS, and separated into different tubes for incubation with antibody. Antibodies were marked as CD8 (PerCP), CD44 (BV510), CD62L (APC).

2) Extraction of bone marrow cells: the mice were sacrificed by cervical dislocation. The femurs and tibias of the mouse were taken with the muscles being removed, placed in a clean 2 mL EP tube, and washed with PBS to remove blood stains. 2 mL of PBS was

aspirated by a 2 mL syringe. The needle was punctured along one end of the femur or tibia, and fixed with a tweezers. The piston was squeezed to wash and take the bone marrow cells. The cell mixture was centrifuged at 400 g for 5min to remove the supernatant. 400 μL of mouse red blood cell lysate (1×) was added, and stood for 5min. 400 μL of mouse red blood cell lysate (1×) was added, and stood for 5min. 1.5 mL of PBS was added to neutralize the reaction, centrifuged, resuspended in PBS, and separated into different tubes for incubation with antibody. The antibody was marked as CD8 (PerCP), CD44 (BV510), CD62L (APC).

The detection of Tcm in the spleen on day 10 is shown in Figure 13A, and the detection of Tcm in the spleen on day 20 is shown in Figure 13B. Compared with the conventional CAR-T, the content of Tcm in mBBZ-CAR-T cell group expressing IL7 and CCL21 was significantly increased.

The content of Tcm in the bone marrow on day 10 is shown in Figure 14A, and the detection of Tcm in the spleen on day 20 is shown in Figure 14B. Compared with the conventional CAR-T, after mBBZ-7*21BCAR-T treatment, the content of Tcm in the bone marrow was significantly increased.

2. Detection of DC infiltration

On day 10 (d10) after CAR-T cell treatment, the tumor tissues of mice were frozen and sectioned to detect the infiltration of DC. The results are shown in Figure 15. Compared with the mBBZ cell group, there was more DC cell infiltration in the tumor tissue of mouse in the mBBZ-7*21B cell group.

3. Detection of content of MDSC (inhibiting cells derived from bone marrow)

The tumor tissues of mice in the UTD group, mBBZ group, mBBZ-7*21B group, and mBBZ-7*19 CAR-T group were extracted on day 10 (d10) after CAR-T cell treatment, respectively, and the fat, blood vessels, envelopes and internal necrotic tissues on the tumor surface were removed. The tumor were rinsed with PBS, transferred to a 5 mL of centrifuge tube and 2 mL of culture medium was added. The tumor was cut into about 1×1 mm, and the culture medium was added to 4.7 mL. Digestive enzyme (digestive enzymes used in the separation of tissue: collagenase type I (0.05 mg/ml), collagenase type IV (0.05 mg/ml), hyaluronidase (0.025 mg/ml), DNase I (0.01 mg/ml)) was added in proportion, and placed in a shaker at 37°C for about 30 minutes (during which the sample

was taken out to observe the digestion). After digestion, the suspension was passed through a 70 um cell sieve to a 50 mL tube (operating on ice), a syringe piston was used to gently push the undigested tissue, and a large amount (up to 20 mL) of culture medium was used to wash the sieve and collect cells. The digestion was quenched, the suspension was centrifuged at 400 g for 8 min at 4°C to remove the supernatant. The obtained pellet was washed with PBS, separated into different tubes and incubated with an antibody to detect the contents of $CD45^+$, $CD11b^+$ (FITC) and $Gr\text{-}1^+$ (PE) cells, that is, the content of MDSC. The results are shown in Figure 16: after mBBZ-7*21B CAR-T treatment, the content of MDSC in the tumor tissue was less than that in the mBBZ group.

As an example, CAR-T cells targeting CLD18A2 were selected in the above examples. It should be understood that CAR-T cells acting on other targets, such as GPC3, EGFR, EGFRvIII, CD19, BCMA, and the like will also have the same effect. The used antibodies can be mouse antibodies or humanized, and the used transmembrane domain and intracellular domain can also be of different species according to different purposes, such as human.

As an example, CAR-T cells were used in the above examples, however, the T cells can also express other cytokines that can enhance the function of CAR-T cells, such as CAR-T cells co-expressing CAR and type I interferon, CAR-T cells co-expressing CAR and PD-1, etc. As an example, CAR-T cells were used in the above examples, however, other immune cells, such as NK cells, NK-T cells, can be selected, and specific subtypes of immune cells, such as γ/δ T cells,and the like can also be selected.

The sequences used in the present invention are summarized in the following table:

SEQ ID NO.	Name	Sequence
1	Hu8E5-2I scFv nucleic acid sequence	caggtgcagctgcaggagagcggccccggcctgatcaagcccagccaga ccctgagcctgacctgcaccgtgagcggcggcagcatcagcagcggctac aactggcactggatccggcagccccccggcaagggcctggagtggatcg gctacatccactacaccggcagcaccaactacaaccccgccctgcggagc cgggtgaccatcagcgtggacaccagcaagaaccagttcagcctgaagct gagcagcgtgaccgccgccgacaccgccatctactactgcgcccggatct acaacggcaacagcttcccctactggggccagggcaccaccgtgaccgtg agcagcggtggaggcggttcaggcggaggtggttctggcggtggcggat cggacatcgtgatgacccagagccccgacagcctggccgtgagcctggg cgagcgggccaccatcaactgcaagagcagccagagcctgttcaacagc

		ggcaaccagaagaactacctgacctggtaccagcagaagcccggccagc cccccaagctgctgatctactgggccagcacccgggagagcggcgtgccc gaccggttcagcggcagcggcagcggcaccgacttcaccctgaccatcag cagcctgcaggccgaggacgtggccgtgtactactgccagaacgcctaca gcttcccctacaccttcggcggcggcaccaagctggagatcaagcgg
2	Hu8E5-2I scFv amino acid sequence	qvqlqesgpglikpsqtlsltctvsggsissgynwhwirqppgkglewig yihytgstnynpalrsrvtisvdtsknqfslklssvtaadtaiyycariyngn sfpywgqgttvtvssggggsggggsggggsdivmtqspdslavslgera tinckssqslfnsgnqknyltwyqqkpgqppklliywastresgvpdrfs gsgsgtdftltisslqaedvavyycqnaysfpytfgggtkleikr
3	mouse CD8α signal peptide nucleic acid sequence	atggcctcaccgttgacccgctttctgtcgctgaacctgctgctgctgggtga gtcgattatcctggggagtggagaagct
4	mouse CD8α signal peptide amino acid sequence	maspltrflslnllllgesiilgsgea
5	mouse CD8 hinge region +transmembrane domain nucleic acid sequence	actactaccaagccagtgctgcgaactccctcacctgtgcaccctaccggg acatctcagccccagagaccagaagattgtcggccccgtggctcagtgaag gggaccggattggacttcgcctgtgatatttacatctgggcacccttggccg gaatctgcgtggcccttctgctgtccttgatcatcactctcatctgctaccaca ggagccga
6	mouse CD8 hinge region +transmembrane domain amino acid sequence	tttkpvlrtpspvhptgtsqpqrpedcrprgsvkgtgldfacdiyiwaplag icvalllsliitlicyhrsr
7	mouse CD28 intracellular domain nucleic acid sequence	aatagtagaaggaacagactccttcaaagtgactacatgaacatgactcccc ggaggcctgggctcactcgaaagccttaccagccctacgcccctgccaga gactttgcagcgtaccgcccc
8	mouse CD28 intracellular domain amino acid sequence	nsrrnrllqsdymnmtprrpgltrkpyqpyapardfaayrp
9	Nucleic acid sequence of intracellular segment CD3ξ of mouse CD3	agcaggagtgcagagactgctgccaacctgcaggaccccaaccagctcta caatgagctcaatctagggcgaagagaggaatatgacgtcttggagaagaa gcgggctcgggatccagagatgggaggcaaacagcagaggaggaggaa ccccccaggaaggcgtatacaatgcactgcagaaagacaagatggcagaa gcctacagtgagatcggcacaaaaggcgagaggcggagaggcaaggggg cacgatggcctttaccagggtctcagcactgccaccaaggacacctatgat gccctgcatatgcagaccctggcc
10	amino acid sequence of intracellular	srsaetaanlqdpnqlynelnlgrreeydvlekkrardpemggkqqrrrn pqegvynalqkdkmaeayseigtkgerrrgkghdglyqglstatkdtyd alhmqtla

	segment CD3ξ of mouse CD3	
11	F2A nucleic acid sequence	gtgaaacagactttgaattttgaccttctgaagttggcaggagacgttgagtc caaccctgggccc
12	F2A amino acid sequence	vkqtlnfdllklagdvesnpgp
13	mouse IL7 nucleic acid sequence	atgttccatgtttcttttagatatatctttggaattcctccactgatccttgttctgc tgcctgtcacatcatctgagtgccacattaaagacaaagaaggtaaagcata tgagagtgtactgatgatcagcatcgatgaattggacaaaatgacaggaact gatagtaattgcccgaataatgaaccaaacttttttagaaaacatgtatgtgat gatacaaaggaagctgcttttctaaatcgtgctgctcgcaagttgaagcaattt cttaaaatgaatatcagtgaagaattcaatgtccacttactaacagtatcacaa ggcacacaaacactggtgaactgcacaagtaaggaagaaaaaaacgtaaa ggaacagaaaaagaatgatgcatgtttcctaaagagactactgagagaaat aaaaacttgttggaataaaattttgaagggcagtata
14	mouse CCL21a nucleic acid sequence	atggctcagatgatgactctgagcctccttagcctggtcctggctctctgcat cccctggacccaaggcagtgatggaggggtcaggactgctgccttaagt acagccagaagaaaattccctacagtattgtccgaggctataggaagcaag aaccaagtttaggctgtcccatcccggcaatcctgttctcaccccggaagca ctctaagcctgagctatgtgcaaaccctgaggaaggctgggtgcagaacct gatgcgccgcctggaccagcctccagccccagggaaacaaagccccggc tgcaggaagaaccggggaacctctaagtctggaaagaaaggaaagggct ccaagggctgcaagagaactgaacagacacagccctcaagagga
15	mouse CCL21b nucleic acid sequence	atggctcagatgatgactctgagcctccttagcctggtcctggctctctgcat cccctggacccaaggcagtgatggaggggacaggactgctgccttaagt acagccagaagaaaattccctacagtattgtccgaggctataggaagcaag aaccaagtttaggctgtcccatcccggcaatcctgttcttaccccggaagca ctctaagcctgagctatgtgcaaaccctgaggaaggctgggtgcagaacct gatgcgccgcctggaccagcctccagccccagggaaacaaagccccggc tgcaggaagaaccggggaacctctaagtctggaaagaaaggaaagggct ccaagggctgcaagagaactgaacagacacagccctcaagagga
16	P2A nucleic acid sequence	gctactaacttcagcctgctgaagcaggctggagacgtggaggagaaccct ggacct
17	human IL7 nucleic acid sequence	atgttccatgtttcttttaggtatatctttggacttcctcccctgatccttgttctgtt gccagtagcatcatctgattgtgatattgaaggtaaagatggcaaacaatatg agagtgttctaatggtcagcatcgatcaattattggacagcatgaaagaaatt ggtagcaattgcctgaataatgaatttaacttttttaaaagacatatctgtgatg ctaataaggaaggtatgtttttattccgtgctgctcgcaagttgaggcaatttct taaaatgaatagcactggtgattttgatctccacttattaaaagtttcagaaggc acaacaatactgttgaactgcactggccaggttaaaggaagaaaaccagct gccctgggtgaagcccaaccaacaaagagtttggaagaaaataaatctttaa aggaacagaaaaaactgaatgacttgtgtttcctaaagagactattacaaga gataaaaacttgttggaataaaattttgatgggcactaaagaacactga

18	human IL7 amino acid sequence	mfhvsfryifglpplilvllpvassdcdiegkdgkqyesvlmvsidqllds mkeigsnclnnefnffkrhicdankegmflfraarklrqflkmnstgdfdl hllkvsegttillnctgqvkgrkpaalgeaqptksleenkslkeqkklndlc flkrllqeiktcwnkilmgtkeh
19	human IL7R amino acid sequence	mtilgttfgmvfsllqvvsgesgyaqngdledaelddysfscysqlevng sqhsltcafedpdvnitnlefeicgalvevkclnfrklqeiyfietkkflligk snicvkvgeksltckkidlttivkpeapfdlsvvyregandfvvtfntshlq kkyvkvlmhdvayrqekdenkwthvnlsstkltllqrklqpaamyeikv rsipdhyfkgfwsewspsyyfrtpeinnssgemdpilltisilsffsvallvi lacvlwkkrikpivwpslpdhkktlehlckkprknlnvsfnpesfldcqi hrvddiqardevegflqdtfpqqleesekqrlggdvqspncpsedvvitp esfgrdssltclagnvsacdapilsssrsldcresgkngphvyqdlllslgtt nstlpppfslqsgiltlnpvaqgqpiltslgsnqeeayvtmssfyqnq
20	human CCL21 nucleic acid sequence	atggctcagtcactggctctgagcctccttatcctggttctggcctttggcatc cccaggacccaaggcagtgatggaggggctcaggactgttgcctcaagta cagccaaaggaagattcccgccaaggttgtccgcagctaccggaagcagg aaccaagcttaggctgctccatcccagctatcctgttcttgccccgcaagcg ctctcaggcagagctatgtgcagacccaaaggagctctgggtgcagcagct gatgcagcatctggacaagacaccatccccacagaaaccagcccagggct gcaggaaggacaggggggcctccaagactggcaagaaaggaaagggct ccaaaggctgcaagaggactgagcggtcacagacccctaaagggccata g
21	human CCL21 amino acid sequence	maqslalsllilvlafgiprtqgsdggaqdcclkysqrkipakvvrsyrkqe pslgcsipailflprkrsqaelcadpkelwvqqlmqhldktpspqkpaqg crkdrgasktgkkgkgskgckrtersqtpkgp
22	Claudin18.2 amino acid sequence	mavtacqglgfvvsligiagiiaatcmdqwstqdlynnpvtavfnyqgl wrscvressgftecrgyftllglpamlqavralmivgivlgaigllvsifalk cirigsmedsakanmtltsgimfivsglcaiagvsvfanmlvtnfwmsta nmytgmggmvqtvqtrytfgaalfvgwvaggltliggvmmciacrgla peetnykavsyhasghsvaykpggfkastgfgsntknkkiydggarted evqsypskhdyv
23	Claudin18.1 amino acid sequence	mstttcqvvafllsilglagciaatgmdmwstqdlydnpvtsvfqyeglw rscvrqssgftecrpyftilglpamlqavralmivgivlgaigllvsifalkci rigsmedsakanmtltsgimfivsglcaiagvsvfanmlvtnfwmstan mytgmggmvqtvqtrytfgaalfvgwvaggltliggvmmciacrglap eetnykavsyhasghsvaykpggfkastgfgsntknkkiydggartede vqsypskhdyv
24	mouse 4-1BB intracellular domain nucleic acid sequence	aaatggatcaggaaaaaattcccccacatattcaagcaaccatttaagaaga ccactggagcagctcaagaggaagatgcttgtagctgccgatgtccacagg aagaagaaggaggaggaggaggctatgagctg
25	mouse4-1BB intracellular domain amino acid sequence	kwirkkfphifkqpfkkttgaaqeedacscrcpqeeeggggggyel
26	Hu8E5-28ZCAR amino acid sequence	QVQLQESGPGLIKPSQTLSLTCTVSGGSISSGYNW HWIRQPPGKGLEWIGYIHYTGSTNYNPALRSRVTI SVDTSKNQFSLKLSSVTAADTAIYYCARIYNGNSF

	(human)	PYWGQGTTVTVSSGGGGSGGGGSGGGGSDIVMT QSPDSLAVSLGERATINCKSSQSLFNSGNQKNYLT WYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYCQNAYSFPYTFGGGTK LEIKRTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDFWVLVVVGGVLACYSLLVTV AFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHY QPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQL YNELNLGRREEYDVLDKRRGRDPEMGGKPQRRK NPQEGLYNELQKDKMAEAYSEIGMKGERRRGKG HDGLYQGLSTATKDTYDALHMQALPPR
27	Hu8E5-BBZC AR amino acid sequence (human)	QVQLQESGPGLIKPSQTLSLTCTVSGGSISSGYNW HWIRQPPGKGLEWIGYIHYTGSTNYNPALRSRVTI SVDTSKNQFSLKLSSVTAADTAIYYCARIYNGNSF PYWGQGTTVTVSSGGGGSGGGGSGGGGSDIVMT QSPDSLAVSLGERATINCKSSQSLFNSGNQKNYLT WYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYCQNAYSFPYTFGGGTK LEIKRTTTPAPRPPTPAPTIASQPLSLRPEACRPAAG GAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITL YCKRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRF PEEEEGGCELRVKFSRSADAPAYQQGQNQLYNEL NLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQE GLYNELQKDKMAEAYSEIGMKGERRRGKGHDGL YQGLSTATKDTYDALHMQALPPR
28	human CD8α signal peptide amino acid sequence	malpvtalllplalllhaarp
29	human CD8 hinge region amino acid sequence	tttpaprpptpaptiasqplslrpeacrpaaggavhtrgldfacd
30	human CD28 transmembrane region amino acid sequence	fwvlvvvggvlacysllvtvafiifwv
31	humanCD28 intracellular domain amino acid sequence	rskrsrllhsdymnmtprrpgptrkhyqpyapprdfaayrs
32	human4-1BB intracellular domain amino acid sequence	krgrkkllyifkqpfmrpvqttqeedgcscrfpeeeeggcel
33	amino acid sequence of intracellular segment CD3ξ of human CD3	rvkfsrsadapayqqgqnqlynelnlgrreeydvldkrrgrdpemggkp qrrknpqeglynelqkdkmaeayseigmkgerrrgkghdglyqglstat kdtydalhmqalppr

34	mouse CCL19 nucleic acid sequence	atggccccccgtgtgaccccactcctggccttcagcctgctggttctctgga ccttcccagccccaactctgggggggtgctaatgatgcggaagactgctgcc tgtctgtgacccagcgccccatccctgggaacatcgtgaaagccttccgcta ccttcttaatgaagatggctgcagggtgcctgctgttgtgttcaccacactaa ggggctatcagctctgtgcacctccagaccagccctgggtggatcgcatca tccgaagactgaagaagtcttctgccaagaacaaaggcaacagcaccaga aggagccctgtgtct
35	28BBZ amino acid sequence	QVQLQESGPGLIKPSQTLSLTCTVSGGSISSGYNW HWIRQPPGKGLEWIGYIHYTGSTNYNPALRSRVTI SVDTSKNQFSLKLSSVTAADTAIYYCARIYNGNSF PYWGQGTTVTVSSGGGGSGGGGSGGGGSDIVMT QSPDSLAVSLGERATINCKSSQSLFNSGNQKNYLT WYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSG TDFTLTISSLQAEDVAVYYCQNAYSFPYTFGGGT KLEIKRTTTPAPRPPTPAPTIASQPLSLRPEACRPA AGGAVHTRGLDFACDFWVLVVVGGVLACYSLL VTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTR KHYQPYAPPRDFAAYRSKRGRKKLLYIFKQPFMR PVQTTQEEDGCSCRFPEEEEGGCELRVKFSRSAD APAYQQGQNQLYNELNLGRREEYDVLDKRRGR DPEMGGKPQRRKNPQEGLYNELQKDKMAEAYS EIGMKGERRRGKGHDGLYQGLSTATKDTYDALH MQALPPR

All documents mentioned herein are cited as references in the present application, as if each document is individually cited as a reference. In addition, it should be understood that after reading the above teachings of the present invention, a skilled person can make various changes or modifications to the present invention, and these equivalent forms also fall within the scope defined by the appended claims of the present application.

SEQUENCE LISTING

<110> CARSGEN THERAPEUTICS CO., LTD.
SHANGHAI CANCER INSTITUTE

<120> GENETICALLY ENGINEERED CELL AND APPLICATION THEREOF

<130> P19401638C

<150> CN201810463564.7
<151> 2018-05-15

<150> CN201811088090.9
<151> 2018-09-18

<150> CN201811552806.6
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<150> CN201910151930.X
<151> 2019-02-28

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acctgcaccg tgagcggcgg cagcatcagc agcggctaca actggcactg gatccggcag 120

ccccccggca agggcctgga gtggatcggc tacatccact acaccggcag caccaactac 180

aaccccgccc tgcggagccg ggtgaccatc agcgtggaca ccagcaagaa ccagttcagc 240

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cccgacagcc tggccgtgag cctgggcgag cgggccacca tcaactgcaa gagcagccag 480

agcctgttca acagcggcaa ccagaagaac tacctgacct ggtaccagca gaagcccggc 540

cagccccca agctgctgat ctactgggcc agcacccggg agagcggcgt gcccgaccgg 600

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<210> 2
<211> 247
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<400> 2

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Ile Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
20 25 30

Tyr Asn Trp His Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile His Tyr Thr Gly Ser Thr Asn Tyr Asn Pro Ala Leu
50 55 60

Arg Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Ile Tyr Asn Gly Asn Ser Phe Pro Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu
130 135 140

Ala Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln

145 150 155 160

Ser Leu Phe Asn Ser Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln
165 170 175

Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr
180 185 190

Arg Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
195 200 205

Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val
210 215 220

Tyr Tyr Cys Gln Asn Ala Tyr Ser Phe Pro Tyr Thr Phe Gly Gly Gly
225 230 235 240

Thr Lys Leu Glu Ile Lys Arg
245

<210> 3
<211> 81
<212> DNA
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<220>
<223> mouse CD8 alpha signal peptide nucleic acid sequence

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atcctgggga gtggagaagc t 81

<210> 4
<211> 27
<212> PRT
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<223> mouse CD8 alpha signal peptide nucleic acid sequence

<400> 4

Met Ala Ser Pro Leu Thr Arg Phe Leu Ser Leu Asn Leu Leu Leu Leu
1 5 10 15

Gly Glu Ser Ile Ile Leu Gly Ser Gly Glu Ala
20 25

<210> 5
<211> 216
<212> DNA
<213> Artificial Sequence

<220>
<223> mouse CD8 hinge region+transmembrane domain nucleic acid sequence

<400> 5
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ccccagagac cagaagattg tcggccccgt ggctcagtga aggggaccgg attggacttc 120

gcctgtgata tttacatctg ggcacccttg gccggaatct gcgtggccct tctgctgtcc 180

ttgatcatca ctctcatctg ctaccacagg agccga 216

<210> 6
<211> 72
<212> PRT
<213> Artificial Sequence

<220>
<223> mouse CD8 hinge region+transmembrane domain nucleic acid sequence

<400> 6

Thr Thr Thr Lys Pro Val Leu Arg Thr Pro Ser Pro Val His Pro Thr
1 5 10 15

Gly Thr Ser Gln Pro Gln Arg Pro Glu Asp Cys Arg Pro Arg Gly Ser
20 25 30

Val Lys Gly Thr Gly Leu Asp Phe Ala Cys Asp Ile Tyr Ile Trp Ala
35 40 45

Pro Leu Ala Gly Ile Cys Val Ala Leu Leu Leu Ser Leu Ile Ile Thr
50 55 60

Leu Ile Cys Tyr His Arg Ser Arg
65 70

<210> 7

<211> 123
<212> DNA
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<220>
<223> mouse CD28 intracellular domain nucleic acid sequence

<400> 7
aatagtagaa ggaacagact ccttcaaagt gactacatga acatgactcc ccggaggcct 60

gggctcactc gaaagcctta ccagccctac gcccctgcca gagactttgc agcgtaccgc 120

ccc 123

<210> 8
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> mouse CD28 intracellular domain nucleic acid sequence

<400> 8

Asn Ser Arg Arg Asn Arg Leu Leu Gln Ser Asp Tyr Met Asn Met Thr
1 5 10 15

Pro Arg Arg Pro Gly Leu Thr Arg Lys Pro Tyr Gln Pro Tyr Ala Pro
20 25 30

Ala Arg Asp Phe Ala Ala Tyr Arg Pro
35 40

<210> 9
<211> 321
<212> DNA
<213> Artificial Sequence

<220>
<223> intracellular segment CD3xi nucleic acid sequence of mouse CD3

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aatctagggc gaagagagga atatgacgtc ttggagaaga agcgggctcg ggatccagag 120

atgggaggca aacagcagag gaggaggaac ccccaggaag gcgtatacaa tgcactgcag 180

aaagacaaga tggcagaagc ctacagtgag atcggcacaa aaggcgagag gcggagaggc 240

aaggggcacg atggccttta ccagggtctc agcactgcca ccaaggacac ctatgatgcc 300

ctgcatatgc agaccctggc c 321

<210> 10
<211> 107
<212> PRT
<213> Artificial Sequence

<220>
<223> intracellular segment CD3xi amino acid sequence of mouse CD3

<400> 10

Ser Arg Ser Ala Glu Thr Ala Ala Asn Leu Gln Asp Pro Asn Gln Leu
1 5 10 15

Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Glu
20 25 30

Lys Lys Arg Ala Arg Asp Pro Glu Met Gly Gly Lys Gln Gln Arg Arg
35 40 45

Arg Asn Pro Gln Glu Gly Val Tyr Asn Ala Leu Gln Lys Asp Lys Met
50 55 60

Ala Glu Ala Tyr Ser Glu Ile Gly Thr Lys Gly Glu Arg Arg Arg Gly
65 70 75 80

Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp
85 90 95

Thr Tyr Asp Ala Leu His Met Gln Thr Leu Ala
100 105

<210> 11
<211> 66
<212> DNA
<213> Artificial Sequence

<220>
<223> F2A nucleic acid sequence

<400> 11
gtgaaacaga ctttgaattt tgaccttctg aagttggcag gagacgttga gtccaaccct 60

gggccc 66

<210> 12
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<223> F2A amino acid sequence

<400> 12

Val Lys Gln Thr Leu Asn Phe Asp Leu Leu Lys Leu Ala Gly Asp Val
1 5 10 15

Glu Ser Asn Pro Gly Pro
20

<210> 13
<211> 462
<212> DNA
<213> Artificial Sequence

<220>
<223> mouse IL7 nucleic acid sequence

<400> 13
atgttccatg tttcttttag atatatcttt ggaattcctc cactgatcct tgttctgctg 60

cctgtcacat catctgagtg ccacattaaa gacaaagaag gtaaagcata tgagagtgta 120

ctgatgatca gcatcgatga attggacaaa atgacaggaa ctgatagtaa ttgcccgaat 180

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aatcgtgctg ctcgcaagtt gaagcaattt cttaaaatga atatcagtga agaattcaat 300

gtccacttac taacagtatc acaaggcaca caaacactgg tgaactgcac aagtaaggaa 360

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gaaataaaaa cttgttggaa taaaattttg aagggcagta ta 462

<210> 14
<211> 399
<212> DNA
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<220>
<223> mouse CCL21a nucleic acid sequence

<400> 14
atggctcaga tgatgactct gagcctcctt agcctggtcc tggctctctg catcccctgg 60

acccaaggca gtgatggagg gggtcaggac tgctgcctta agtacagcca gaagaaaatt 120

ccctacagta ttgtccgagg ctataggaag caagaaccaa gtttaggctg tcccatcccg 180

gcaatcctgt tctcaccccg gaagcactct aagcctgagc tatgtgcaaa ccctgaggaa 240

ggctgggtgc agaacctgat gcgccgcctg gaccagcctc cagccccagg gaaacaaagc 300

cccggctgca ggaagaaccg gggaacctct aagtctggaa agaaaggaaa gggctccaag 360

ggctgcaaga gaactgaaca gacacagccc tcaagagga 399

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<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> mouse CCL21b nucleic acid sequence

<400> 15
atggctcaga tgatgactct gagcctcctt agcctggtcc tggctctctg catcccctgg 60

acccaaggca gtgatggagg gggacaggac tgctgcctta agtacagcca gaagaaaatt 120

ccctacagta ttgtccgagg ctataggaag caagaaccaa gtttaggctg tcccatcccg 180

gcaatcctgt tcttaccccg gaagcactct aagcctgagc tatgtgcaaa ccctgaggaa 240

ggctgggtgc agaacctgat gcgccgcctg gaccagcctc cagccccagg gaaacaaagc 300

cccggctgca ggaagaaccg gggaacctct aagtctggaa agaaaggaaa gggctccaag 360

ggctgcaaga gaactgaaca gacacagccc tcaagagga 399

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<211> 57
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<210> 17

<211> 534
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<400> 17
atgttccatg tttcttttag gtatatcttt ggacttcctc ccctgatcct tgttctgttg 60

ccagtagcat catctgattg tgatattgaa ggtaaagatg gcaaacaata tgagagtgtt 120

ctaatggtca gcatcgatca attattggac agcatgaaag aaattggtag caattgcctg 180

aataatgaat ttaacttttt taaaagacat atctgtgatg ctaataagga aggtatgttt 240

ttattccgtg ctgctcgcaa gttgaggcaa tttcttaaaa tgaatagcac tggtgatttt 300

gatctccact tattaaaagt ttcagaaggc acaacaatac tgttgaactg cactggccag 360

gttaaaggaa gaaaaccagc tgccctgggt gaagcccaac caacaaagag tttggaagaa 420

aataaatctt taaaggaaca gaaaaaactg aatgacttgt gtttcctaaa gagactatta 480

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<211> 177
<212> PRT
<213> Artificial Sequence

<220>
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<400> 18

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1 5 10 15

Leu Val Leu Leu Pro Val Ala Ser Ser Asp Cys Asp Ile Glu Gly Lys
20 25 30

Asp Gly Lys Gln Tyr Glu Ser Val Leu Met Val Ser Ile Asp Gln Leu
35 40 45

Leu Asp Ser Met Lys Glu Ile Gly Ser Asn Cys Leu Asn Asn Glu Phe
50 55 60

Asn Phe Phe Lys Arg His Ile Cys Asp Ala Asn Lys Glu Gly Met Phe

65 70 75 80

Leu Phe Arg Ala Ala Arg Lys Leu Arg Gln Phe Leu Lys Met Asn Ser
85 90 95

Thr Gly Asp Phe Asp Leu His Leu Leu Lys Val Ser Glu Gly Thr Thr
100 105 110

Ile Leu Leu Asn Cys Thr Gly Gln Val Lys Gly Arg Lys Pro Ala Ala
115 120 125

Leu Gly Glu Ala Gln Pro Thr Lys Ser Leu Glu Glu Asn Lys Ser Leu
130 135 140

Lys Glu Gln Lys Lys Leu Asn Asp Leu Cys Phe Leu Lys Arg Leu Leu
145 150 155 160

Gln Glu Ile Lys Thr Cys Trp Asn Lys Ile Leu Met Gly Thr Lys Glu
165 170 175

His

<210> 19
<211> 459
<212> PRT
<213> Artificial Sequence

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<223> human IL7R amino acid sequence

<400> 19

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1 5 10 15

Val Val Ser Gly Glu Ser Gly Tyr Ala Gln Asn Gly Asp Leu Glu Asp
20 25 30

Ala Glu Leu Asp Asp Tyr Ser Phe Ser Cys Tyr Ser Gln Leu Glu Val
35 40 45

Asn Gly Ser Gln His Ser Leu Thr Cys Ala Phe Glu Asp Pro Asp Val

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    50                  55                  60

Asn Ile Thr Asn Leu Glu Phe Glu Ile Cys Gly Ala Leu Val Glu Val
65                  70                  75                  80

Lys Cys Leu Asn Phe Arg Lys Leu Gln Glu Ile Tyr Phe Ile Glu Thr
                85                  90                  95

Lys Lys Phe Leu Leu Ile Gly Lys Ser Asn Ile Cys Val Lys Val Gly
            100                 105                 110

Glu Lys Ser Leu Thr Cys Lys Lys Ile Asp Leu Thr Thr Ile Val Lys
        115                 120                 125

Pro Glu Ala Pro Phe Asp Leu Ser Val Val Tyr Arg Glu Gly Ala Asn
    130                 135                 140

Asp Phe Val Val Thr Phe Asn Thr Ser His Leu Gln Lys Lys Tyr Val
145                 150                 155                 160

Lys Val Leu Met His Asp Val Ala Tyr Arg Gln Glu Lys Asp Glu Asn
                165                 170                 175

Lys Trp Thr His Val Asn Leu Ser Ser Thr Lys Leu Thr Leu Leu Gln
            180                 185                 190

Arg Lys Leu Gln Pro Ala Ala Met Tyr Glu Ile Lys Val Arg Ser Ile
        195                 200                 205

Pro Asp His Tyr Phe Lys Gly Phe Trp Ser Glu Trp Ser Pro Ser Tyr
    210                 215                 220

Tyr Phe Arg Thr Pro Glu Ile Asn Asn Ser Ser Gly Glu Met Asp Pro
225                 230                 235                 240

Ile Leu Leu Thr Ile Ser Ile Leu Ser Phe Phe Ser Val Ala Leu Leu
                245                 250                 255

Val Ile Leu Ala Cys Val Leu Trp Lys Lys Arg Ile Lys Pro Ile Val
            260                 265                 270
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Trp Pro Ser Leu Pro Asp His Lys Lys Thr Leu Glu His Leu Cys Lys
        275                 280                 285

Lys Pro Arg Lys Asn Leu Asn Val Ser Phe Asn Pro Glu Ser Phe Leu
    290                 295                 300

Asp Cys Gln Ile His Arg Val Asp Asp Ile Gln Ala Arg Asp Glu Val
305                 310                 315                 320

Glu Gly Phe Leu Gln Asp Thr Phe Pro Gln Gln Leu Glu Glu Ser Glu
                325                 330                 335

Lys Gln Arg Leu Gly Gly Asp Val Gln Ser Pro Asn Cys Pro Ser Glu
            340                 345                 350

Asp Val Val Ile Thr Pro Glu Ser Phe Gly Arg Asp Ser Ser Leu Thr
        355                 360                 365

Cys Leu Ala Gly Asn Val Ser Ala Cys Asp Ala Pro Ile Leu Ser Ser
    370                 375                 380

Ser Arg Ser Leu Asp Cys Arg Glu Ser Gly Lys Asn Gly Pro His Val
385                 390                 395                 400

Tyr Gln Asp Leu Leu Leu Ser Leu Gly Thr Thr Asn Ser Thr Leu Pro
                405                 410                 415

Pro Pro Phe Ser Leu Gln Ser Gly Ile Leu Thr Leu Asn Pro Val Ala
            420                 425                 430

Gln Gly Gln Pro Ile Leu Thr Ser Leu Gly Ser Asn Gln Glu Glu Ala
        435                 440                 445

Tyr Val Thr Met Ser Ser Phe Tyr Gln Asn Gln
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cccgccaagg ttgtccgcag ctaccggaag caggaaccaa gcttaggctg ctccatccca 180

gctatcctgt tcttgccccg caagcgctct caggcagagc tatgtgcaga cccaaaggag 240

ctctgggtgc agcagctgat gcagcatctg gacaagacac catccccaca gaaaccagcc 300

cagggctgca ggaaggacag gggggcctcc aagactggca agaaaggaaa gggctccaaa 360

ggctgcaaga ggactgagcg gtcacagacc cctaaagggc catag 405

<210> 21
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<400> 21

Met Ala Gln Ser Leu Ala Leu Ser Leu Leu Ile Leu Val Leu Ala Phe
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Gly Ile Pro Arg Thr Gln Gly Ser Asp Gly Gly Ala Gln Asp Cys Cys
20 25 30

Leu Lys Tyr Ser Gln Arg Lys Ile Pro Ala Lys Val Val Arg Ser Tyr
35 40 45

Arg Lys Gln Glu Pro Ser Leu Gly Cys Ser Ile Pro Ala Ile Leu Phe
50 55 60

Leu Pro Arg Lys Arg Ser Gln Ala Glu Leu Cys Ala Asp Pro Lys Glu
65 70 75 80

Leu Trp Val Gln Gln Leu Met Gln His Leu Asp Lys Thr Pro Ser Pro
85 90 95

Gln Lys Pro Ala Gln Gly Cys Arg Lys Asp Arg Gly Ala Ser Lys Thr

100 105 110

Gly Lys Lys Gly Lys Gly Ser Lys Gly Cys Lys Arg Thr Glu Arg Ser
115 120 125

Gln Thr Pro Lys Gly Pro
130

<210> 22
<211> 261
<212> PRT
<213> Artificial Sequence

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<400> 22

Met Ala Val Thr Ala Cys Gln Gly Leu Gly Phe Val Val Ser Leu Ile
1 5 10 15

Gly Ile Ala Gly Ile Ile Ala Ala Thr Cys Met Asp Gln Trp Ser Thr
20 25 30

Gln Asp Leu Tyr Asn Asn Pro Val Thr Ala Val Phe Asn Tyr Gln Gly
35 40 45

Leu Trp Arg Ser Cys Val Arg Glu Ser Ser Gly Phe Thr Glu Cys Arg
50 55 60

Gly Tyr Phe Thr Leu Leu Gly Leu Pro Ala Met Leu Gln Ala Val Arg
65 70 75 80

Ala Leu Met Ile Val Gly Ile Val Leu Gly Ala Ile Gly Leu Leu Val
85 90 95

Ser Ile Phe Ala Leu Lys Cys Ile Arg Ile Gly Ser Met Glu Asp Ser
100 105 110

Ala Lys Ala Asn Met Thr Leu Thr Ser Gly Ile Met Phe Ile Val Ser
115 120 125

Gly Leu Cys Ala Ile Ala Gly Val Ser Val Phe Ala Asn Met Leu Val

130 135 140

Thr Asn Phe Trp Met Ser Thr Ala Asn Met Tyr Thr Gly Met Gly Gly
145 150 155 160

Met Val Gln Thr Val Gln Thr Arg Tyr Thr Phe Gly Ala Ala Leu Phe
165 170 175

Val Gly Trp Val Ala Gly Gly Leu Thr Leu Ile Gly Gly Val Met Met
180 185 190

Cys Ile Ala Cys Arg Gly Leu Ala Pro Glu Glu Thr Asn Tyr Lys Ala
195 200 205

Val Ser Tyr His Ala Ser Gly His Ser Val Ala Tyr Lys Pro Gly Gly
210 215 220

Phe Lys Ala Ser Thr Gly Phe Gly Ser Asn Thr Lys Asn Lys Lys Ile
225 230 235 240

Tyr Asp Gly Gly Ala Arg Thr Glu Asp Glu Val Gln Ser Tyr Pro Ser
245 250 255

Lys His Asp Tyr Val
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<400> 23

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1 5 10 15

Gly Leu Ala Gly Cys Ile Ala Ala Thr Gly Met Asp Met Trp Ser Thr
20 25 30

Gln Asp Leu Tyr Asp Asn Pro Val Thr Ser Val Phe Gln Tyr Glu Gly

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        35                  40                  45

Leu Trp Arg Ser Cys Val Arg Gln Ser Ser Gly Phe Thr Glu Cys Arg
    50                  55                  60

Pro Tyr Phe Thr Ile Leu Gly Leu Pro Ala Met Leu Gln Ala Val Arg
65                  70                  75                  80

Ala Leu Met Ile Val Gly Ile Val Leu Gly Ala Ile Gly Leu Leu Val
                85                  90                  95

Ser Ile Phe Ala Leu Lys Cys Ile Arg Ile Gly Ser Met Glu Asp Ser
            100                 105                 110

Ala Lys Ala Asn Met Thr Leu Thr Ser Gly Ile Met Phe Ile Val Ser
        115                 120                 125

Gly Leu Cys Ala Ile Ala Gly Val Ser Val Phe Ala Asn Met Leu Val
    130                 135                 140

Thr Asn Phe Trp Met Ser Thr Ala Asn Met Tyr Thr Gly Met Gly Gly
145                 150                 155                 160

Met Val Gln Thr Val Gln Thr Arg Tyr Thr Phe Gly Ala Ala Leu Phe
                165                 170                 175

Val Gly Trp Val Ala Gly Gly Leu Thr Leu Ile Gly Gly Val Met Met
            180                 185                 190

Cys Ile Ala Cys Arg Gly Leu Ala Pro Glu Glu Thr Asn Tyr Lys Ala
        195                 200                 205

Val Ser Tyr His Ala Ser Gly His Ser Val Ala Tyr Lys Pro Gly Gly
    210                 215                 220

Phe Lys Ala Ser Thr Gly Phe Gly Ser Asn Thr Lys Asn Lys Lys Ile
225                 230                 235                 240

Tyr Asp Gly Gly Ala Arg Thr Glu Asp Glu Val Gln Ser Tyr Pro Ser
                245                 250                 255
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Lys His Asp Tyr Val
260

<210> 24
<211> 135
<212> DNA
<213> Artificial Sequence

<220>
<223> mouse4-1BB intracellular domain nucleic acid sequence

<400> 24
aaatggatca ggaaaaaatt cccccacata ttcaagcaac catttaagaa gaccactgga 60

gcagctcaag aggaagatgc ttgtagctgc cgatgtccac aggaagaaga aggaggagga 120

ggaggctatg agctg 135

<210> 25
<211> 45
<212> PRT
<213> Artificial Sequence

<220>
<223> mouse4-1BB intracellular domain amino acid sequence

<400> 25

Lys Trp Ile Arg Lys Lys Phe Pro His Ile Phe Lys Gln Pro Phe Lys
1 5 10 15

Lys Thr Thr Gly Ala Ala Gln Glu Glu Asp Ala Cys Ser Cys Arg Cys
20 25 30

Pro Gln Glu Glu Glu Gly Gly Gly Gly Gly Tyr Glu Leu
35 40 45

<210> 26
<211> 473
<212> PRT
<213> Artificial Sequence

<220>
<223> Hu8E5-28ZCAR amino acid sequence (human)

<400> 26

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Ile Lys Pro Ser Gln

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1               5                   10                  15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
            20                  25                  30

Tyr Asn Trp His Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
        35                  40                  45

Ile Gly Tyr Ile His Tyr Thr Gly Ser Thr Asn Tyr Asn Pro Ala Leu
    50                  55                  60

Arg Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65                  70                  75                  80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ile Tyr Tyr Cys
                85                  90                  95

Ala Arg Ile Tyr Asn Gly Asn Ser Phe Pro Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125

Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu
    130                 135                 140

Ala Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln
145                 150                 155                 160

Ser Leu Phe Asn Ser Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln
                165                 170                 175

Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr
            180                 185                 190

Arg Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
        195                 200                 205

Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val
    210                 215                 220
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Tyr Tyr Cys Gln Asn Ala Tyr Ser Phe Pro Tyr Thr Phe Gly Gly Gly
225                 230                 235                 240

Thr Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro Ala Pro Arg Pro Pro
                245                 250                 255

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
            260                 265                 270

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
        275                 280                 285

Phe Ala Cys Asp Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala
    290                 295                 300

Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg
305                 310                 315                 320

Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro
                325                 330                 335

Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro
            340                 345                 350

Arg Asp Phe Ala Ala Tyr Arg Ser Arg Val Lys Phe Ser Arg Ser Ala
        355                 360                 365

Asp Ala Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu
    370                 375                 380

Asn Leu Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly
385                 390                 395                 400

Arg Asp Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln
                405                 410                 415

Glu Gly Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr
            420                 425                 430

Ser Glu Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp
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435 440 445

Gly Leu Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala
450 455 460

Leu His Met Gln Ala Leu Pro Pro Arg
465 470

<210> 27
<211> 471
<212> PRT
<213> Artificial Sequence

<220>
<223> Hu8E5-BBZCAR amino acid sequence (human)

<400> 27

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Ile Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
20 25 30

Tyr Asn Trp His Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
35 40 45

Ile Gly Tyr Ile His Tyr Thr Gly Ser Thr Asn Tyr Asn Pro Ala Leu
50 55 60

Arg Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65 70 75 80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ile Tyr Tyr Cys
85 90 95

Ala Arg Ile Tyr Asn Gly Asn Ser Phe Pro Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
115 120 125

Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu

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    130                 135                 140

Ala Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln
145                 150                 155                 160

Ser Leu Phe Asn Ser Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln
                165                 170                 175

Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr
            180                 185                 190

Arg Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
        195                 200                 205

Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val
    210                 215                 220

Tyr Tyr Cys Gln Asn Ala Tyr Ser Phe Pro Tyr Thr Phe Gly Gly Gly
225                 230                 235                 240

Thr Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro Ala Pro Arg Pro Pro
                245                 250                 255

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
            260                 265                 270

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
        275                 280                 285

Phe Ala Cys Asp Ile Tyr Ile Trp Ala Pro Leu Ala Gly Thr Cys Gly
    290                 295                 300

Val Leu Leu Leu Ser Leu Val Ile Thr Leu Tyr Cys Lys Arg Gly Arg
305                 310                 315                 320

Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln
                325                 330                 335

Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu
            340                 345                 350
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Glu Gly Gly Cys Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala
        355                 360                 365

Pro Ala Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu
    370                 375                 380

Gly Arg Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
385                 390                 395                 400

Pro Glu Met Gly Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly
                405                 410                 415

Leu Tyr Asn Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu
            420                 425                 430

Ile Gly Met Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu
        435                 440                 445

Tyr Gln Gly Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His
    450                 455                 460

Met Gln Ala Leu Pro Pro Arg
465                 470
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<210> 28
<211> 21
<212> PRT
<213> Artificial Sequence

<220>
<223> human CD8 alpha signal peptide amino acid sequence

<400> 28

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Met Ala Leu Pro Val Thr Ala Leu Leu Leu Pro Leu Ala Leu Leu Leu
1               5                   10                  15

His Ala Ala Arg Pro
            20
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<210> 29
<211> 45
<212> PRT

<213> Artificial Sequence

<220>
<223> humanCD8 hinge region amino acid sequence

<400> 29

Thr Thr Thr Pro Ala Pro Arg Pro Pro Thr Pro Ala Pro Thr Ile Ala
1 5 10 15

Ser Gln Pro Leu Ser Leu Arg Pro Glu Ala Cys Arg Pro Ala Ala Gly
20 25 30

Gly Ala Val His Thr Arg Gly Leu Asp Phe Ala Cys Asp
35 40 45

<210> 30
<211> 27
<212> PRT
<213> Artificial Sequence

<220>
<223> human CD28 Transmembrane amino acid sequence

<400> 30

Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala Cys Tyr Ser Leu
1 5 10 15

Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val
20 25

<210> 31
<211> 41
<212> PRT
<213> Artificial Sequence

<220>
<223> human CD28 intracellular domain amino acid sequence

<400> 31

Arg Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr
1 5 10 15

Pro Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro
20 25 30

Pro Arg Asp Phe Ala Ala Tyr Arg Ser
35 40

<210> 32
<211> 42
<212> PRT
<213> Artificial Sequence

<220>
<223> human 4-1BB intracellular domain amino acid sequence

<400> 32

Lys Arg Gly Arg Lys Lys Leu Leu Tyr Ile Phe Lys Gln Pro Phe Met
1 5 10 15

Arg Pro Val Gln Thr Thr Gln Glu Glu Asp Gly Cys Ser Cys Arg Phe
20 25 30

Pro Glu Glu Glu Glu Gly Gly Cys Glu Leu
35 40

<210> 33
<211> 113
<212> PRT
<213> Artificial Sequence

<220>
<223> intracellular segment CD3 xi amino acid sequence of human CD3

<400> 33

Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln Gln Gly
1 5 10 15

Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu Glu Tyr
20 25 30

Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly Gly Lys
35 40 45

Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu Leu Gln
50 55 60

Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys Gly Glu

65 70 75 80

Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu Ser Thr
85 90 95

Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu Pro Pro
100 105 110

Arg

<210> 34
<211> 324
<212> DNA
<213> Artificial Sequence

<220>
<223> mouse CCL19 nucleic acid sequence

<400> 34
atggcccccc gtgtgacccc actcctggcc ttcagcctgc tggttctctg gaccttccca 60

gccccaactc tgggggggtgc taatgatgcg gaagactgct gcctgtctgt gacccagcgc 120

cccatccctg ggaacatcgt gaaagccttc cgctaccttc ttaatgaaga tggctgcagg 180

gtgcctgctg ttgtgttcac cacactaagg ggctatcagc tctgtgcacc tccagaccag 240

ccctgggtgg atcgcatcat ccgaagactg aagaagtctt ctgccaagaa caaaggcaac 300

agcaccagaa ggagccctgt gtct 324

<210> 35
<211> 515
<212> PRT
<213> Artificial Sequence

<220>
<223> 28BBZ amino acid sequence

<400> 35

Gln Val Gln Leu Gln Glu Ser Gly Pro Gly Leu Ile Lys Pro Ser Gln
1 5 10 15

Thr Leu Ser Leu Thr Cys Thr Val Ser Gly Gly Ser Ile Ser Ser Gly
20 25 30

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Tyr Asn Trp His Trp Ile Arg Gln Pro Pro Gly Lys Gly Leu Glu Trp
        35                  40                  45

Ile Gly Tyr Ile His Tyr Thr Gly Ser Thr Asn Tyr Asn Pro Ala Leu
    50                  55                  60

Arg Ser Arg Val Thr Ile Ser Val Asp Thr Ser Lys Asn Gln Phe Ser
65                  70                  75                  80

Leu Lys Leu Ser Ser Val Thr Ala Ala Asp Thr Ala Ile Tyr Tyr Cys
                85                  90                  95

Ala Arg Ile Tyr Asn Gly Asn Ser Phe Pro Tyr Trp Gly Gln Gly Thr
            100                 105                 110

Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
        115                 120                 125

Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser Pro Asp Ser Leu
    130                 135                 140

Ala Val Ser Leu Gly Glu Arg Ala Thr Ile Asn Cys Lys Ser Ser Gln
145                 150                 155                 160

Ser Leu Phe Asn Ser Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln
                165                 170                 175

Gln Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr
            180                 185                 190

Arg Glu Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr
        195                 200                 205

Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Ala Glu Asp Val Ala Val
    210                 215                 220

Tyr Tyr Cys Gln Asn Ala Tyr Ser Phe Pro Tyr Thr Phe Gly Gly Gly
225                 230                 235                 240

Thr Lys Leu Glu Ile Lys Arg Thr Thr Thr Pro Ala Pro Arg Pro Pro
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                245                 250                 255

Thr Pro Ala Pro Thr Ile Ala Ser Gln Pro Leu Ser Leu Arg Pro Glu
            260                 265                 270

Ala Cys Arg Pro Ala Ala Gly Gly Ala Val His Thr Arg Gly Leu Asp
        275                 280                 285

Phe Ala Cys Asp Phe Trp Val Leu Val Val Val Gly Gly Val Leu Ala
    290                 295                 300

Cys Tyr Ser Leu Leu Val Thr Val Ala Phe Ile Ile Phe Trp Val Arg
305                 310                 315                 320

Ser Lys Arg Ser Arg Leu Leu His Ser Asp Tyr Met Asn Met Thr Pro
                325                 330                 335

Arg Arg Pro Gly Pro Thr Arg Lys His Tyr Gln Pro Tyr Ala Pro Pro
            340                 345                 350

Arg Asp Phe Ala Ala Tyr Arg Ser Lys Arg Gly Arg Lys Lys Leu Leu
        355                 360                 365

Tyr Ile Phe Lys Gln Pro Phe Met Arg Pro Val Gln Thr Thr Gln Glu
    370                 375                 380

Glu Asp Gly Cys Ser Cys Arg Phe Pro Glu Glu Glu Glu Gly Gly Cys
385                 390                 395                 400

Glu Leu Arg Val Lys Phe Ser Arg Ser Ala Asp Ala Pro Ala Tyr Gln
                405                 410                 415

Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg Arg Glu
            420                 425                 430

Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu Met Gly
        435                 440                 445

Gly Lys Pro Gln Arg Arg Lys Asn Pro Gln Glu Gly Leu Tyr Asn Glu
    450                 455                 460
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Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser Glu Ile Gly Met Lys
465                 470                 475                 480

Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly Leu
                485                 490                 495

Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala Leu
            500                 505                 510

Pro Pro Arg
        515
```

Claims

1. A genetically engineered cell, wherein the cell expresses an exogenous receptor specifically binding to a target antigen and exogenous CCL21; and preferably, further expresses a protein promoting the proliferation of the cell; and more preferably, the protein promoting the proliferation of the cell is a IL-7R-binding protein or exogenous IL-7.

2. The cell of claim 1, wherein the IL-7R-binding protein is an exogenous IL-7R-binding protein, and the exogenous IL-7R-binding protein can specifically bind to IL-7R and improve activities of IL-7R;

preferably, the amino acid sequence of the exogenous IL-7R is shown in SEQ ID NO: 19.

3. The cell of claim 1 or 2, wherein the exogenous CCL21 is natural CCL21, or a truncated fragment of natural CCL21, or a mutant of natural CCL21 having the same function as natural CCL21;

and preferably, the natural CCL21 has at least 90% sequence identity with the amino acid sequence as shown in SEQ ID NO: 21, or is a truncated fragment of the amino acid sequence as shown in SEQ ID NO: 21; or has at least 90% sequence identity with the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 14 or 15, or is a truncated fragment of an amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 14 or 15.

4. The cell of any one of claims 1-3, wherein the exogenous CCL21 is constitutively expressed.

5. The cell of any one of claims 1-3, wherein the exogenous CCL21 is inducibly expressed; preferably, the inducible expression is initiated by an immune cell inducible promoter; and more preferably, the immune cell inducible promoter is NFAT promoter.

6. The cell of any one of claims 1-5, wherein the exogenous IL-7 is natural IL-7, or

a truncated fragment of natural IL-7, or a mutant of natural IL-7 having the same function as natural IL-7;

preferably, the natural IL-7 has at least 90% sequence identity with the amino acid sequence as shown in SEQ ID NO: 18, or is a truncated fragment of the amino acid sequence as shown in SEQ ID NO: 18; or has at least 90% sequence identity with the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 13, or is a truncated fragment of an amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 13.

7. The cell of any one of claims 1-5, wherein the exogenous IL-7R-binding protein or exogenous IL-7 is constitutively expressed.

8. The cell of any one of claims 1-5, wherein the exogenous IL-7R-binding protein or exogenous IL-7 is inducibly expressed; preferably, the inducible expression is initiated by an immune cell inducible promoter; and more preferably, the immune cell inducible promoter is NFAT promoter.

9. The cell of any one of claims 1-8, wherein the cell is an immune effector cell;

preferably, the immune effector cells are T cells, NK cells or NKT cells;

and more preferably, the immune effector cells are T cells.

10. The cell of claim 1, wherein the target antigen is a tumor antigen and / or a pathogen antigen; preferably, a tumor antigen.

11. The cell of claim 10, wherein the target antigen is a solid tumor antigen;

and preferably, the solid tumor antigen is GPC3, EGFR or Claudin18.2; and more preferably, the solid tumor antigen is Claudin18.2.

12. The cell of any one of claims 1-11, wherein the exogenous receptor is a chimeric receptor, which includes an antigen-binding domain specifically binding to a target antigen, a transmembrane domain and an intracellular domain.

13. The cell of claim 12, wherein the exogenous receptor is selected from the group consisting of chimeric antigen receptor (CAR), modified T cell (antigen) receptor (TCR), T Cell fusion protein (TFP), T cell antigen coupler (TAC) or a combination thereof;

and preferably, the exogenou s receptor is a chimeric antigen receptor.

14. The cell of claim 13, wherein the chimeric antigen receptor includes:

(i) an antibody or a fragment thereof specifically binding to a target antigen, a transmembrane domain of CD28 or CD8, a costimulatory signal domain of CD28, and CD3ζ; or

(ii) an antibody or a fragment thereof specifically binding to a target antigen, the transmembrane domain of CD28 or CD8, the costimulatory signal domain of 4-1BB, and CD3ζ; or

(iii) an antibody or a fragment thereof specifically binding to the target antigen, the transmembrane domain of CD28 or CD8, the costimulatory signal domain of CD28, the costimulatory signal domain of 4-1BB and CD3ζ.

15. The cell of claim 12, wherein the amino acid sequence of the antigen binding domain of the exogenous receptor has at least 90% identity with the sequence as shown in SEQ ID NO: 2.

16. The cell of claim 15, wherein the amino acid sequence of the exogenous receptor has at least 90% identity with the sequence as shown in SEQ ID NO: 26, 27 or 35.

17. The cell of any one of claims 1-16, wherein the exogenous receptor, and/or exogenous IL-7R binding protein or exogenous IL-7, and/or exogenous CCL21 are expressed by using a viral vector;

and preferably, the viral vectors include: lentiviral vectors, retroviral vectors or adenovirus vectors.

18. An expression construct, comprising sequentially connected: an expression cassette 1 for an exogenous receptor specifically binding to a target antigen, an expression cassette 2 for exogenous IL-7R binding protein or exogenous IL-7, and an expression cassette 3 exogenous for CCL21; and preferably, the expression cassettes are connected by tandem fragments, selected from F2A, PA2, T2A, and/or E2A.

19. The expression construct of claim 18. wherein said expression cassette 2 comprises a nucleic acid sequence as shown in SEQ ID NO: 17.

20. The expression construct of claim 18. wherein said expression cassette 3 comprises a nucleic acid sequence as shown in SEQ ID NO: 20.

21. An expression vector, comprising the expression construct of any one of claims 18-20.

22. A virus, comprising the expression vector of claim 21.

23. A method for improving the viability of immune response cells in an individual, wherein an exogenous CCL21, exogenous IL-7R binding protein or exogenous IL-7 are co-expressed in the immune response cells; and preferably, thw chimeric receptor is an chimeric antigen receptor.

24. The method of claim 23, wherein the exogenous IL-7R binding protein can specifically bind to IL-7R and enhance activities of IL-7R;

and preferably, the amino acid sequence of the exogenous IL-7R is shown in SEQ ID NO: 19.

25. The method of claim 23 or 24, wherein the exogenous CCL21 is natural CCL21, or a truncated fragment of natural CCL21, or a mutant of natural CCL21 having the same function as natural CCL21;

and preferably, the natural CCL21 has at least 90% sequence identity with the amino acid sequence as shown in SEQ ID NO: 21, or is a truncated fragment of the amino acid sequence as shown in SEQ ID NO: 21; or has at least 90% sequence identity with the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 14 or 15, or is a truncated fragment of an amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 14 or 15.

26. The method of any one of claims 23-25, wherein the exogenous CCL21 is constitutively expressed.

27. The method of any one of claims 23-25, wherein the exogenous CCL21 is inducibly expressed; preferably, the inducible expression is initiated by an immune cell inducible promoter; and more preferably, the immune cell inducible promoter is NFAT promoter.

28. The method of any one of claims 23-27, wherein the exogenous IL-7 is natural IL-7, or a truncated fragment of natural IL-7, or a mutant of natural IL-7 having the same function as natural IL-7;

and preferably, the natural IL-7 has at least 90% sequence identity with the amino acid sequence as shown in SEQ ID NO: 18, or is a truncated fragment of the amino acid

sequence as shown in SEQ ID NO: 18; or has at least 90% sequence identity with the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 13, or is a truncated fragment of an amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 13.

29. The method of any one of claims 23-27, wherein the exogenous IL-7R-binding protein or exogenous IL-7 is constitutively expressed.

30. The method of any one of claims 23-27, wherein the exogenous IL-7R-binding protein or exogenous IL-7 is inducibly expressed; preferably, the inducible expression is initiated by an immune cell inducible promoter; and more preferably, the immune cell inducible promoter is NFAT promoter.

31. The method of any one of claims 23-30, wherein the immune effector cells are T cells, NK cells or NKT cells.

32. Use of the cell of any one of claims 1-17, or the expression construct of any one of claims 18-20, or the expression vector of claim 21, or the virus of claim 22 for preparing a drug for inhibiting tumors, inhibiting pathogens; preferably, a drug for inhibiting tumors.

33. The use of claim 32, wherein the drug for inhibiting tumors is used in combination with a chemotherapeutic drug.

34. A pharmaceutical composition, comprising the cell of any one of claims 1-17 and a pharmaceutically acceptable carrier or excipient.

35. A kit, comprising kit A and kit B, wherein the kit A comprises genetically engineered cells, and the cells expresses an exogenous receptor specifically binding to a target antigen; and the kit B comprises CCL21, and/or a protein that promotes the proliferation of the cells; preferably, the protein that promotes the proliferation of the cells is IL-7R binding protein or IL-7; and more preferably, the kit A and the kit B are administered in any order.

36. The kit of claim 35, wherein the IL-7R binding protein can specifically bind to IL-7R and enhance activities of IL-7R; and preferably, the amino acid sequence of the exogenous IL-7R is shown in SEQ ID NO: 19.

37. The kit of claim 35 or 36, wherein the CCL21 is natural CCL21, or a truncated fragment of natural CCL21, or a mutant of natural CCL21 having the same function as

natural CCL21;

and preferably, the natural CCL21 has at least 90% sequence identity with the amino acid sequence as shown in SEQ ID NO: 21, or is a truncated fragment of the amino acid sequence as shown in SEQ ID NO: 21; or has at least 90% sequence identity with the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 14 or 15, or is a truncated fragment of an amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 14 or 15.

38. The kit of any one of claims 35-37, wherein the IL-7 is natural IL-7, or a truncated fragment of natural IL-7, or a mutant of natural IL-7 having the same function as natural IL-7;

and preferably, the natural IL-7 has at least 90% sequence identity with the amino acid sequence as shown in SEQ ID NO: 18, or is a truncated fragment of the amino acid sequence as shown in SEQ ID NO: 18; or has at least 90% sequence identity with the amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 13, or is a truncated fragment of an amino acid sequence encoded by the nucleotide sequence as shown in SEQ ID NO: 13.

39. The kit of claim 35, wherein the kit A comprises immune effector cells modified by chimeric receptors;

and preferably, the chimeric receptor is a chimeric antigen receptor.

40. The kit of claim 35, wherein the immune effector cells are T cells, NK cells or NKT cells.