Improved Lentiviral Vector

Technical Field

The invention belongs to the field of biomedicine. Specifically, the present invention relates to improved t lentiviral vector, and preparation method and uses thereof. Specifically, the present invention relates to a lentiviral vector especially suitable for preparing a therapeutic T cell.

Background

T cells are the key immune cells that kill tumor cells and virus-infected cells in the body. In recent years, T cells, including antigen-specific T cells derived from in vitro induced or tumor infiltrating lymphocytes, genetically modified chimeric antigen receptor T cells (CAR-T cells), and genetically modified T cell receptor T cells (TCR-T cells), have been used for the treatment of malignant tumors, showing significant tumor clearance and control effects in some clinical patients. However, due to the immune escape effect of tumor in patients, some tumor patients have resistance to the infused T cells, resulting in T cells not being able to exert their anti-tumor effects.

Both in vivo and in vitro studies have shown that TGF-β is an important T cell inhibitory factor, leading to the weakening or loss of the killing effect of T cells on target cells. Clinically, TGF-β is widely expressed in a variety of tumor tissues, and significantly inhibits the killing activity of tumor-specific T cells on tumor cells, which is an important reason for the failure of immunotherapy. The dominant negative TGF-β receptor type II (DNRII) is a negative regulatory receptor of TGF-β, which can inhibit the inhibitory effect of TGF-β on T cells. In animals, the killing effect of T cells on tumors can be significantly increased by administering or expressing T cell-specific DNRII, or administering soluble TGF-β RII, to interfere with the TGF-β signaling pathway. The research team led by Catherin M Bollard of Baylor College of Medicine found that giving patients EBV-specific T cells (EBV-CTL) treatment has a certain effect on Hodgkin and non-Hodgkin's lymphoma caused by EBV infection. However, in these diseases, the efficacy of EBV-CTL is disturbed due to the expression of TGF-β in tumor tissues. The research team used gene transduction to express DNRII on the surface of EBV-CTL cells for the treatment of relapsed Hodgkin's lymphoma. Among the 7 patients who can be evaluated, 4 patients achieved complete remission, of which 2 patients had complete remission lasting for 4 years, and one of them was the patient who failed to obtain complete remission after treatment with EBV-CTL without DNRII gene modification. However, these EBV-CTLs only express one exogenous protein, namely

DNRII.

In the currently applied clinical treatment with CAR-T cells and TCR-T cells, the same issue remains that tumor cells express TGF-β which leads to the inhibition of CAR-T cells and TCR-T cell functions. It is desired in the art to introduce DNRII into CAR-T cells or TCR-T cells. However, so far, there has not been a report about the co-expression of CAR/TCR and DNRII in the same T cell for the treatment of tumors. This may be due to the low co-expression efficiency of the two proteins, which is difficult to meet clinical needs.

To solve this problem, there is a need of an improved expression vector, such as an improved lentiviral vector, which is especially suitable for co-expressing two or more proteins efficiently.

Brief Description of Drawings

Figure 1 shows the genome of the lentiviral vector for expressing CAR-19 and the strategy for identifying integrity thereof. (A) The old vector pPVLV1 containing $P_{EF1\alpha}$-L (long promoter, 531bp); (B) the new vector pPVLV2 containing $P_{EF1\alpha}$-S (short promoter, 212bp). The integrity of the viral vector genome was identified by generating expected PCR products (F1-F5: PCR fragmenta) from cDNA reverse-transcribed using random hexamer primers.

Figure 2 shows the difference between pPVLV1 and pPVLV2. (A) The expected DNA fragments were amplified from the reverse-transcribed cDNA of viral genome. Defective gene site was observed in the $P_{EF1\alpha}$-L (long promoter) containing viral gene fragment. DNA fragment with unexpected size was indicated by arrows (left panel). (B) Comparison of the percentages of CAR-19 expressing cells, and (C) the titer of each vector 48 hours after transduction into 293T cells.

Figure 3 shows the structure and luciferase activity of the CAR-19-Fluc. (A) and (B) Bicistronic constructs encoding the CAR-19 cloned upstream of the P2A-Fluc cassette were used in this experiment. (C) Schematic representation of CAR-19 and Fluc molecules. (D) Luciferase activity of lentiviral vectors was determined 48 hrs after transduction of 293T cells.

Figure 4 shows the structure and viral vector of CAR-19-DNRII. (A) and (B) show the vector map of CAR-19 co-expressing truncated TGFBRII (DNRII). (C) Schematic diagram of the co-expressed CAR-19 and DNRII molecules.

Figure 5 shows the transduction efficiency of CAR-19 and DNRII expression in transduced 293T cells. The numbers in the figure represent the percentage of CAR-19 (top) or DNRII (bottom) positive cells relative to the negative control of un-transduced 293T cells. The results of representative experiments from ten independent experiments are presented.

Figure 6 shows the expression of CAR-19 and DNRII in transduced T cells. The activated T cells were transduced with lentiviral vectors to express CAR-19 or CAR-19-DNRII, and evaluated by flow cytometry. The numbers in the figure represent the percentage of CAR-19 (top) or DNRII (bottom) positive cells relative to the negative control of un-transduced T cells. The results are representative of three independent experiments.

Figure 7 shows the cell viability and counts after transduction with CAR-19 or CAR-19-DNRII vector. Data are expressed as mean ± SD.

Figure 8 shows that DNRII reduced TGF-β1-induced phosphorylation of SMAD2.

Figure 9 shows the mRNA levels of IFN-γ and TNF-α in CAR-T-19 and CAR-T-19-DNRII cells. Data are expressed as mean ± SEM

Figure 10 shows the antigen-specific killing of CD19+ tumor cells by CAR-T-19 and CAR-T-19-DNRII cells in the presence of TGF-β1. Twelve days after the initial activation of CAR T cells, the cell lysis activity was measured by the DELFIA® EuTDA cytotoxicity assay. T cells were collected 3 days before the measurement and cultured with rhTGF-β1 (10ng/ml) for 72 hours. The target cells were labeled with BATDA reagent for 15 minutes, and then transduced T cells as effector cells were added at the specified E:T ratio. Lysis was measured after 4 hours of incubation.

Detailed Description of the Invention

Unless otherwise indicated or defined, all the terms used have their usual meanings in the art, which will be understood by those skilled in the art. Reference is made to, for example, standard manuals such as Sambrook et al., "Molecular Cloning: A Laboratory Manual"; Lewin, " Genes VIII"; and Roitt et al., " Immunology" (Version 8), and general prior art cited in this specification. In addition, unless otherwise described, all methods, steps, technologies, and operations that are not specifically detailed can be and have been performed in a manner known per se, which will be understood by those skilled in the art. Reference is also made to, for example, the standard manual, the above-mentioned general prior art and other references cited therein.

In a first aspect, the present invention provides a lentiviral vector comprising a truncated EF1α promoter for directing the expression of a nucleotide sequence encoding a polypeptide of interest in a host cell. In some embodiments, the truncated EF1α promoter is an EF1α core promoter comprising the nucleotide sequence setting forth in SEQ ID NO: 13.

Within the scope of the present invention, "lentiviral vector" refers to a non-replicating vector, which is used to transduce a transgene containing a cis-acting lentiviral RNA or DNA sequence to a host cell, where lentiviral proteins (for example, Gag, Pol and/or Env) need to

be provided in trans form. Lentiviral vectors lack the coding sequences for functional Gag, Pol and Env proteins. Lentiviral vectors can exist in the form of RNA or DNA molecules, depending on the stage of production or development of the viral vector.

The lentiviral vector may be in the form of a recombinant DNA molecule, such as a plasmid (e.g., a transfer plasmid vector). The lentiviral vector may be in the form of a lentiviral particle vector, such as an RNA molecule in a complex of lentivirus and other proteins. Generally, a lentiviral vector corresponding to a modified or recombined lentiviral particle contains a genome composed of two copies of single-stranded RNA. These RNA sequences can be obtained by transcription from a double-stranded DNA sequence (proviral vector DNA) inserted into the genome of a host cell, or can be obtained by transient expression of plasmid DNA (plasmid vector DNA) in a transformed host cell. Lentiviral vector can also refer to a DNA sequence integrated into a host cell.

Lentiviral vector can be derived from lentiviruses, especially human immunodeficiency virus (HIV-1 or HIV-2), simian immunodeficiency virus (SIV), equine infectious encephalitis virus (EIAV), goat arthritis encephalitis virus (CAEV), bovine immunodeficiency virus (BIV) and feline immunodeficiency virus (FIV), which is modified to remove genetic determinants involved in pathogenicity and introduced with exogenous expression cassette.

In some embodiments, the lentiviral vector further comprises at least one element selected from the group consisting of a 5'LTR, a ψ sequence, an RRE sequence, a cPPT/CTS sequence, a multiple cloning site for inserting a nucleotide sequence encoding the polypeptide of interest, a WPRE sequence, and a 3'LTR.

In some embodiments, the lentiviral vector comprises a 5'LTR, a ψ element, an RRE element, a cPPT/CTS element, the truncated EF1α promoter, aWPRE component, a 3'LTR, and optionally, a multiple cloning site for inserting a nucleotide sequence encoding the polypeptide of interest, which are operably linked.

In some specific embodiments, the 5'LTR comprises the nucleotide sequence shown in SEQ ID NO: 3 or 11; the ψ element comprises the nucleotide sequence shown in SEQ ID NO: 4 or 12; the RRE element comprises the nucleotide sequence shown in SEQ ID NO: 5; the cPPT/CTS element comprises the nucleotide sequence shown in SEQ ID NO: 6; the WPRE element comprises the nucleotide sequence shown in SEQ ID NO: 9 or 14; the 3'LTR comprises the nucleotide sequence shown in SEQ ID NO: 10 or 15.

In some embodiments, the lentiviral vector comprises a 5'LTR comprising the nucleotide sequence shown in SEQ ID NO: 11, a ψ element comprising the nucleotide sequence shown in SEQ ID NO: 12, and an RRE element of the nucleotide sequence shown in SEQ ID NO: 5, a cPPT/CTS element including the nucleotide sequence shown in SEQ ID NO: 6, a truncated

EF1α promoter of the nucleotide sequence shown in SEQ ID NO: 13, a WPRE element comprising the nucleotide sequence shown in SEQ ID NO: 14, a 3'LTR of the nucleotide sequence shown in SEQ ID NO: 15, and optionally, a multiple cloning site for inserting a nucleotide sequence encoding the polypeptide of interest, which are operably linked.

In some embodiments, the lentiviral vector is derived from SEQ ID NO: 2, wherein the nucleotide sequence from position 2,042 to position 3,499 of SEQ ID NO: 2 encoding CAR-19 can be replaced by a nucleotide sequence encoding t the polypeptide of interest.

In some embodiments, the lentiviral vector further comprises a nucleotide sequence encoding the polypeptide of interest.

In some embodiments, the polypeptide of interest is a fusion polypeptide comprising a plurality of proteins, the plurality of proteins in the fusion polypeptide being separated by a self-cleavable peptide.

In some embodiments, the polypeptide of interest is a fusion polypeptide comprising a first protein and a second protein, and the fusion polypeptide comprises a self-cleavable peptide between the first protein and the second protein.

Therefore, in some embodiments, the lentiviral vector further comprises a nucleotide sequence encoding a self-cleavable peptide. The coding nucleotide sequence of the self-cleavable peptide is used for co-expression of two or more different proteins by the lentiviral vector.

As used herein, "self-cleavable peptide" refers to a peptide that can achieve self-cleavage within a cell. For example, the self-cleavable peptide may include a protease recognition site, so that it can be recognized and specifically cleaved by the protease in the cell.

Alternatively, the self-cleaving peptide may be a 2A polypeptide. 2A polypeptide is a type of short peptides derived from viruses, and its self-cleavage occurs during translation. When 2A polypeptide is used to connect two different target proteins and expressed in the same reading frame, the two target proteins are almost produced at a ratio of 1:1. Commonly used 2A polypeptides can be P2A from porcine techovirus-1, T2A from Thosea asigna virus, and E2A from equine rhinitis A virus, and F2A from foot-and-mouth disease virus. Among them, P2A has the highest cutting efficiency and is therefore preferred. A variety of functional variants of these 2A polypeptides are also known in the art, and these variants can also be used in the present invention.

Separating the first protein and the second protein by the 2A polypeptide, placing them in a same open reading frame, and driving the expression by the same promoter, can maximize the possibility that the transduced cells express both proteins. Because if the two proteins are separately transduced into the cells in different vectors, some cells may only

express the first protein, while some cells only express the second protein. The proportion of cells co-expressing the two proteins will be low. In addition, if the expression of two proteins is driven by different promoters in the same vector, due to the difference in promoter efficiency, the proportion of cells expressing the two proteins will also be reduced.

In some embodiments, the first protein is a cancer-associated antigen-specific receptor protein. In some embodiments, the second protein is a dominant negative TGF-β type II receptor.

As used in the present invention, "dominant negative TGF-β type II receptor" means a variant of the TGF-β type II receptor that can compete with TGF-β RII for binding to the TGF-β ligand (such as TGF-β1), but cannot perform TGF-β RII signal transduction function. In some embodiments, the intracellular signaling domain of the dominant negative TGF-β type II receptor is mutated, thereby losing the ability of intracellular signaling. In some embodiments, the dominant negative TGF-β type II receptor lacks the intracellular signaling domain of the TGF-β type II receptor. In some specific embodiments, the dominant negative TGF-β type II receptor comprises the amino acid sequence shown in SEQ ID NO:18.

The " cancer-associated antigen-specific receptor protein" of the present invention can be an exogenous T cell receptor (TCR) or a chimeric antigen receptor (CAR).

The cancer-associated antigen of the present invention includes but is not limited to CD16, CD64, CD78, CD96, CLL1, CD116, CD117, CD71, CD45, CD71, CD123, CD138, ErbB2 (HER2/neu), carcinoembryonic antigen (CEA), epithelial cell adhesion molecule (EpCAM) , epidermal growth factor receptor (EGFR), EGFR variant III (EGFRvIII), CD19, CD20, CD30, CD40, disialylganglioside GD2, ductal epithelial mucin, gp36, TAG-72, glycosphingolipid, glioma-related antigens, β-human chorionic gonadotropin, α-fetoglobulin (AFP), lectin-responsive AFP, thyroglobulin, RAGE-1, MN-CA IX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostase, prostatase specific antigen (PSA), PAP, NY-ESO-1, LAGA-1a, p53, Prostein, PSMA, survival and telomerase, prostate cancer tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrin B2, CD22, insulin growth factor (IGF1)-I, IGF-II, IGFI receptor, mesothelin, major histocompatibility complex (MHC) molecules that present tumor-specific peptide epitopes, 5T4, ROR1, Nkp30, NKG2D, tumor stromal antigen, fibronectin extra domain A (EDA) and extra domain B (EDB), tenascin-C A1 domain (TnC A1), fibroblast-associated protein (fap), CD3, CD4, CD8, CD24 , CD25, CD33, CD34, CD133, CD138, Foxp3, B7-1 (CD80), B7-2 (CD86), GM-CSF, cytokine receptor, endothelial factor, BCMA (CD269, TNFRSF17), TNFRSF17 (UNIPROT Q02223), SLAMF7 (UNIPROT Q9NQ25), GPRC5D (UNIPROT Q9NZD1), FKBP11 (UNIPROT Q9NYL4), KAMP3, ITGA8 (UNIPROT P53708)

and FCRL5 (UNIPROT Q68SN8).

"T cell receptor (TCR)", also known as T cell antigen receptor, is a molecular structure of T cell that specifically recognizes and binds antigen peptide-MHC molecules, and usually exists on the surface of T cell in the form of a complex with CD3 molecules. The TCR of most T cells is composed of α and β peptide chains, while the TCR of a few T cells is composed of γ and δ peptide chains.

"Chimeric antigen receptor (CAR)", also known as artificial T cell receptor, chimeric T cell receptor, or chimeric immune receptor, is an artificially designed receptor that can confer certain specificity to immune effector cells. Generally, this technology is used to confer T cells the ability to specifically recognize tumor surface antigens. In this way, a large number of targeting tumor killer cells can be produced.

The CAR may include an extracellular antigen binding domain against the cancer-associated antigen. The extracellular antigen binding domain may be, for example, a monoclonal antibody, a synthetic antibody, a human antibody, a humanized antibody, a single domain antibody, an antibody single-chain variable fragment (scFV), and an antigen-binding fragment thereof. For example, the extracellular antigen binding domain may be derived from one or more known antibodies including any commercially available antibody, such as FMC63, rituximab, alemtuzumab, epratuzumab, trastuzumab, bivatuzumab, cetuximab, labetuzumab, palivizumab, sevirumab, tuvirumab, basiliximab, daclizumab, infliximab, omalizumab, efalizumab, Keliximab, siplizumab, natalizumab, clenoliximab, pemtumomab, Edrecolomab, Cantuzumab, and the like.

In some embodiments of various aspects of the present invention, the CAR further includes a transmembrane domain and an intracellular signal transduction domain. The intracellular signal transduction domain of the CAR according to the present invention is responsible for the intracellular signal transduction after the extracellular ligand binding domain binds to the target, leading to the activation of immune cells and immune response. The intracellular signal transduction domain has the capability to activate at least one normal effector function of immune cells expressing the CAR. For example, the effector function of T cells may be cytolytic activity or auxiliary activity, including the secretion of cytokines.

The intracellular signal transduction domain of a CAR may be a cytoplasmic sequence, such as but not limited to the cytoplasmic sequence of T cell receptors and co-receptors (which act in concert to initiate signal transduction after antigen receptor binding), and any derivative or variant of these sequences and any synthetic sequence with the same functional capability. The Intracellular signal transduction domain includes two different types of cytoplasmic signal transduction sequences: the sequences that initiate antigen-dependent

primary activation, and the sequences that act in an antigen-independent manner to provide secondary or co-stimulatory signals. The primary cytoplasmic signal transduction sequence may include a signal transduction motif referred to as the immunoreceptor tyrosine activation motif, ITAM. Non-limiting examples of the ITAM used in the present invention may include those derived from TCRζ, FcRγ, FcRβ, FcRε, CD3γ, CD3δ, CD3ε, CD5, CD22, CD79a, CD79b, and CD66d. In some embodiments, the intracellular signal transduction domain of the CAR may include the CD3ζ signal transduction domain. In some embodiments, the intracellular signal transduction domain of the CAR of the present invention further includes a costimulatory domain. In some embodiments, the costimulatory domain is selected from the 41BB costimulatory domain or the CD28 costimulatory domain.

CAR is expressed on the surface of cells. Therefore, the CAR may include a transmembrane domain. The suitable transmembrane domain of the CAR of the present invention has the following capabilities: (a) expression on the cell surface, preferably immune cells, such as but not limited to lymphocytes or natural killer (NK) cells, and (b) interacting with the ligand binding domain and intracellular signal transduction domain to guide the cellular response of immune cells to predetermined target cells. The transmembrane domain may be derived from natural or synthetic sources. The transmembrane domain may be derived from any membrane-binding protein or transmembrane protein. As a non-limiting example, the transmembrane domain may be derived from subunits of T cell receptors such as α subunits, β subunits, γ or δ subunits, polypeptides constituting the CD3 complex, and p55(α chain), p75 (β chain) or γ of IL-2 receptors, a subunit chain of Fc receptors, especially Fcγ receptor III or CD protein. Alternatively, the transmembrane domain may be synthetic, and may mainly include hydrophobic residues such as leucine and valine. In some embodiments, the transmembrane domain is derived from a human CD8 α chain. The transmembrane domain may further include a hinge region located between the extracellular ligand binding domain and the transmembrane domain. The hinge region is, for example, derived from the extracellular region of CD8, CD4 or CD28. In some embodiments, the hinge region is part of a human CD8 α chain.

In some specific embodiments of various aspects of the present invention, the CAR used in the present invention may include an extracellular antigen binding domain that specifically binds cancer-associated antigens (e.g., scFv), a CD8α hinge and a transmembrane domain, a CD3ζ signal transduction domain, and a 4-1BB costimulatory domain.

In some specific embodiments, the CAR comprises an extracellular antigen binding domain against CD19. In some specific embodiments, the CAR comprises the amino acid sequence shown in SEQ ID NO:16.

Co-expression of T cell receptor (TCR) or chimeric antigen receptor (CAR) and a dominant negative TGF-β type II receptor in T cells can relieve the inhibition of TGF-β on T cells and significantly increase the activity of TCR-T cells or CAR-T cells, such as tumor killing activity. As shown in the examples, the lentiviral vector of the present invention is particularly suitable for co-expression of T cell receptor (TCR) or chimeric antigen receptor (CAR) and the dominant negative TGF-β type II receptor in T cells.

In another aspect, the present invention provides a method for preparing a lentiviral vector particle, the method comprising:

a) co-transfect a suitable host cell with the lentiviral vector of the present invention, one or more packaging vectors expressing Gag and/or Pol, and an envelope vector expressing an envelope protein such as VSV-G;

b) culturing the transfected host cell to package the lentiviral vector into a lentiviral vector particle; and

c) harvesting the lentiviral vector particle produced in step b).

It is known in the art and those skilled in the art can easily obtain suitable one or more packaging vectors for expressing Gag and/or Pol, and envelope vectors for expressing envelope proteins such as VSV-G. In some embodiments, the vector is a plasmid.

Suitable host cells for preparing lentiviral vector particles include but are not limited to 293T cells.

In another aspect, the present invention provides a lentiviral vector particle, which comprises the lentiviral vector of the present invention or is prepared by the above-mentioned method of the present invention.

In another aspect, the present invention provides the use of the lentiviral vector particle of the present invention in the preparation of a therapeutic T cell, wherein the therapeutic T cell expresses a cancer-related antigen-specific receptor protein, such as a T cell receptor (TCR) or a chimeric antigen receptor (CAR), and optionally a dominant negative TGF-β type II receptor.

In another aspect, the present invention provides a method for preparing a therapeutic T cell, which includes transducing the T cell with the lentiviral vector particle of the present invention. The transduction of the lentiviral vector particle will cause the therapeutic T cell to express the cancer-related antigen-specific receptor protein, such as a T cell receptor (TCR) or chimeric antigen receptor (CAR), and optionally a dominant negative TGF-β type II receptor.

The T cells of the present invention can be obtained from many non-limiting sources by various non-limiting methods, including peripheral blood mononuclear cells, bone marrow, lymph node tissues, umbilical cord blood, thymus tissues, ascites, pleural effusions, spleen

tissues and tumors. In some embodiments, cell lines available and known to those skilled in the art can be used. In some embodiments, the cells may be derived from a healthy donor or from a patient diagnosed with cancer. In some embodiments, the cells may be part of a mixed population of cells exhibiting different phenotypic characteristics. For example, the T cells can be obtained by isolating peripheral blood mononuclear cells (PBMC), then activating and expanding by using specific antibodies.

In some embodiments of various aspects of the present invention, the T cells are derived from autologous cells of the subject. As used herein, "autologous" refers to that cells, cell lines, or cell populations used to treat the subject are derived from the subject. In some embodiments, the T cells are derived from allogeneic cells, such as from a donor compatible with the subject's human leukocyte antigen (HLA). Standard schemes can be used to convert cells from a donor into non-alloreactive cells and to replicate the cells as required, generating cells that can be administered to one or more patients.

In another aspect, the present invention provides a kit for producing a lentiviral vector particle, which comprises the lentiviral vector of the present invention, a suitable packaging vector, a suitable envelope vector and/or a suitable host cell such as 293T cell. The kit may also include a cell transfection reagent. In another aspect, the present invention provides a kit for expressing a polypeptide of interest in a cell, which comprises the lentiviral vector particle of the present invention.

Examples

Statistical analysis in the examples was performed using GraphPad software (GraphPad Prism v5.0; GraphPad Software, San Diego, CA, USA). Data were analyzed by Paired t-test followed by the Newman-Keuls test. Results were expressed as the mean ± SEM. A p-value of <0.05 was considered significant.

Example 1. Optimization of lentiviral vector for expression of CAR

The lentiviral vector used to transduce CAR should contain the required CAR transgene and be able to express CAR in the cell. Two third-generation lentiviral vectors for expressing CAR were designed, namely the old vector pPVLV1 (Figure 1A) and the new vector pPVLV2 (Figure 1B). pPVLV1 contains a 531 bp long human elongation factor 1α (EF1α) promoter, and pPVLV2 contains a 212 bp truncated human EF1α promoter. The various elements contained in the two vectors and their descriptions are shown in Table 1 below.

The CAR to be expressed in the examples of the application includes the scFv targeting CD19, the hinge and transmembrane domain of human CD8, the intracellular domain 4-1BB

and CD3ζ. The amino acid of the CAR targeting CD19 is shown in SEQ ID NO: 16, and the nucleotide sequence is shown in SEQ ID NO: 8.

Table 1. Related elements on pPVLV1 and pPVLV2 lentiviral vectors

Feature	Location (size, bp)		description
	pPVLV1[1)]	pPVLV2[2)]	
5' LTR	1-675 (675) 5'HIV R-U5- Δ gag (SEQ ID NO:3)	1-181 (181) truncated (SEQ ID NO:11)	Truncated 5' LTR from HIV-1. Essential for viral transcription, reverse transcription, and integration
HIV-psi (ψ)	703-1,560 (858) (SEQ ID NO:4)	228-353 (126) (SEQ ID NO:12)	Packaging signal of HIV-1. Essential for transfer plasmid packaging.
RRE	850-1,083 (234) (SEQ ID NO:5)	846-1,079 (234) (SEQ ID NO:5)	Essential for Rev-dependent mRNA export from the nucleus to the cytoplasm of viral transcripts.
cPPT/CTS	1,610,-1,727 (118) (SEQ ID NO:6)	1,606-1,723 (118) (SEQ ID NO:6)	cPPT/CTS of HIV-1. Improves vector integration and transduction efficiency.
EF1α 启动子	1,827-2,357 (531) (SEQ ID NO:7)	1,817-2,028 (212) (SEQ ID NO:13)	Promoter that drives ubiquitous expression of the transgenes.
CAR-19	2,507-3,964 (1,458) (SEQ ID NO:8)	2,042-3,499 (1,458) (SEQ ID NO:8)	transgene; CD19 targeting chimeric antigen receptor.
WPRE	4,022-4,611 (590) (SEQ ID NO:9)	3,524-4,112 (589) (SEQ ID NO:14)	Improves transgene expression by facilitating mRNA transcript maturation.
3' LTR	4,631-5,320 (690) 3' SIN LTR (SEQ ID NO:10)	4,184-4,417 (234) Δ -U3 (SEQ ID NO:15)	self-inactivating 3' LTR from HIV-1. Essential for viral transcription, reverse transcription and integration. Contains a safety measure to prevent viral replication.

1) PPVLV1 vector including EF1α long promoter (5,320 bp，SEQ ID NO:1)

2) PPVLV2 vector including EF1α short promoter (4,417 bp，SEQ ID NO:2)

Lentiviral supernatant was created through transfection of 293T cells with gag/pol packaging plasmid, VSV-G envelope plasmid, and the transfer construct comprising the above-mentioned lentiviral vector sequences. Briefly, DNA mixtures were mixed in Opti-MEM (Life Technologies, Gaithersburg, MD, USA) and combined with equal volume of Opti-MEM containing Lipofectamine 3000 (Life Technologies). The resulting mixture was applied to 293T cells after 15 mins incubation at room temperature. Lentivirus-containing medium was collected at 24 hours post-transfection. After each collection, the supernatant was filtered through PVDF membrane (0.45 μm pore). Lentivirus harvests were combined and stored at 4°C before ultracentrifugation for 1 hour 30 mins at 20,000 x g. Lentiviral pellets were re-suspended in PBS.

Figure 1 shows a schematic diagram of the structure of two lentiviral vectors and a strategy for checking the integrity of the lentivirus by overlapping PCR products. Appropriate primers were designed to amplify overlapping fragments F1-F5 from cDNA

reverse-transcribed using random primers. The PCR product with the expected size can prove the integrity of the lentivirus.

Figure 2A shows each DNA fragment amplified from cDNA reverse-transcribed from the viral genome. Unexpectedly, defective gene sites were observed in viral gene fragments containing $P_{EF1\alpha}$-L (long promoter). The arrow indicates the presence of unexpected DNA fragments (left). This phenomenon was not observed in viral gene fragments containing $P_{EF1\alpha}$-S (short promoter). Such defective viral genome may affect the titer and transduction efficiency.

To this end, the titers and transduction efficiency of the two lentiviruses were tested. For lentivirus titration, 2 x 10^6 293T cells were plated into each well of a 6-well plate and transduced with a range of volumes of the concentrated lentivirus. After 48 hours post-transduction, 293T cells were detached from plate. The presence of the CAR was detected through flow cytometry using a Alexa Fluor 488-labeled goat anti-human IgG $F(ab)_2$. Viral genomic RNA from 5' LTR to 3' LTR was checked using conventional PCR.

The results are shown in Figures 2B and C. The transduction efficiency (proportion of CAR-expressing cells) of the virus with $P_{EF1\alpha}$-L (based on the pPVLV1 vector) is only 9.95%, which is much lower than the 70.4% of the virus with $P_{EF1\alpha}$-S (based on the pPVLV2 vector). In addition, the titer of the virus with $P_{EF1\alpha}$-L (based on the pPVLV1 vector) is also significantly lower than the lentivirus with $P_{EF1\alpha}$-S (based on the pPVLV2 vector). It shows that pPVLV2 vector is better than pPVLV1 vector, and this may be caused by the different length of EF1α promoter.

Example 2. The promoter affects the transduction and expression of CAR gene

In order to further prove the influence of different promoters, two CAR-luciferase reporter vectors shown in Figure 3 were constructed based on pPVLV2. The difference is only in the promoters for driving transgene expression, in which CAR-19 was cloned upstream of the P2A-Fluc (Firefly Fluorescence) cassette, thereby a bicistron is formed (Figure 3A and B).

After the vectors are transduced into the cell, due to the presence of the coding sequence of the P2A self-cleavable peptide, two molecules, CAR-19 and luciferase, will be expressed in the same cell at a ratio of approximately 1:1, where the fluorescence intensity can reflect the transduction efficiency of CAR-19 (see schematic diagram in Figure 3C). Figure 3D shows the luciferase activity measured 48 hours post transduction of the two lentiviral vectors into 293T cells. The results showed that the fluorescence of cells transduced with the lentiviral vector with $P_{EF1\alpha}$-S was significantly stronger than that of the cells transduced with the lentiviral vector with $P_{EF1\alpha}$-L. It is further proved that $P_{EF1\alpha}$-S significantly improved the

expression of transgene in cells.

This example proves that the conventional strong promoter, the 531bp EF1α promoter, when used for protein expression in a lentiviral vector, will unexpectedly lead to low transduction efficiency. By using the truncated EF1α promoter (212bp), the transduction efficiency can be significantly improved, and the expression of foreign proteins such as CAR in cells can be improved.

Example 3. Co-expression of CAR and DNRII in cells

TGF-β is an important T cell inhibitory factor, which may lead to the weakening or loss of the killing effect of therapeutic T cells on target cells. Clinically, TGF-β is widely expressed in a variety of tumor tissues, and significantly inhibits the killing activity of tumor-specific T cells on tumor cells, which is an important reason for the failure of immunotherapy. The dominant negative TGF-β receptor type II (DNRII) is the negative regulatory receptor of TGF-β, which can inhibit the inhibitory effect of TGF-β on T cells. The following examples study the effect of co-expression of CAR and DNRII in T cells. The amino acid sequence of DNRII is shown in SEQ ID NO: 17, and its nucleotide sequence is shown in SEQ ID NO: 18.

First, similar to Example 2, wo CAR-19-DNRII vectors as shown in Figure 4 (A and B) were constructed based on pPVLV2. The difference is only in the promoters driving the expression of the transgenes. CAR-19 and DNRII were in the same open reading frame, with the 2A polypeptide coding sequence therebetween. Figure 4C shows a schematic diagram of the structure of CAR-19 and DNRII molecules. DNRII lacks the intracellular serine/threonine kinase domain of TGFBRII and cannot transmit signals downstream.

The CAR-19 and DNRII coding sequences are separated by the 2A coding sequence, placed in the same open reading frame, and expressed by the same promoter, which can ensure that the obtained transduced cells express both CAR-19 and DNRII. This is because if CAR-19 and DNRII are separately transduced into cells in different vectors, some cells may only express CAR-19 and some cells only express DNRII, and the proportion of cells co-expressing the two proteins will be very low. In addition, if the expression of two proteins is driven by different promoters in the same vector, due to the difference in promoter efficiency, the proportion of cells co-expressing both two proteins will also be reduced.

Two CAR-19-DNRII lentiviral vectors were transduced into 293T cells with equal MOI (multiplicity of infection). The expression of CAR or DNRII was detected with labeled goat anti-human IgG $F(ab)_2$ or anti-DNRII antibody by flow cytometry using MACSQuant analyzer 10, and the data was analyzed with FlowJo software.

The results are shown in Fig. 5. The expression of CAR-19 and DNRII in 293T cells transduced with the lentiviral vector with $P_{EF1\alpha}$-S was significantly higher than that in 293T cells transduced with tje lentiviral vector with $P_{EF1\alpha}$-L.

In addition, two CAR-19 lentiviral vectors and two CAR-19-DNRII lentiviral vectors were tested for the expression of CAR-19 and DNRII after transduction into T cells.

Human peripheral blood mononuclear cells (PBMC) from healthy donors were activated with anti-CD3/CD28 Dynabeads magnetic beads for 2 days (beads: cells=3:1), and resuspended at 1×10^6 cells/ml supplemented with rhIL- 2 (200IU/mL) in IMSF100 serum-free medium (LONZA, Belgium). CAR-19 and CAR-19-DNRII lentiviral supernatants were added respectively for transduction, then centrifuged at 1,200×g at 32°C for 2 hours. After 24 hours, the supernatant containing the viral vector was removed. The cells were suspended in a medium containing rhIL-2 (200IU/mL) at 3×10^5 cells/ml, and expanded and cultured with medium replacing every 2 to 3 days for 12 days to obtain CAR T-19 cells expressing CAR-19 molecules and CAR-T-19-DNRII cells co-expressing CAR-19 molecules and DNRII molecules. PBMCs cultured under the same culture conditions but not transduced were used as controls (NC). Flow cytometry was used to detect the expression of each protein molecule of the CAR-T cells obtained after transduction. Cells were stained with propidium iodide (PI) every 2-3 days, and cell viability was detected by flow cytometry. During the cell culture process, trypan blue staining was used to count the cells every 2-3 days (three replicates for each sample), and the number of cells was calculated (mean ± SD).

The results are shown in Figure 6, the non-transduced cells (NC) did not express CAR-19 or DNRII. CAR-T-19 using the pPVLV2 vector containing $P_{EF1\alpha}$-S expressed CAR-19 (expression rate 67.4%); CAR-T-19-DNRII cells expressed both CAR-19 (expression rate 62.9%) and DNRII (the expression rate is 62.3%). It shows that CAR-19 and DNRII were co-expressed in CAR-T-19-DNRII cells, and the transduction efficiency is equivalent to that of CAR-19 alone. When using $P_{EF1\alpha}$-L vector, CAR-19 and DNRII were also co-expressed in CAR-T-19-DNRII cells, but the expression rate was significantly reduced; while in CAR-T-19 cells, the expression rate of CAR-19 was also significant reduce.

In addition, as shown in Figure 7, there is no difference of cell viability and cell number between CAR-T-19-DNRII cells and CAR-T-19 cells.

Therefore, this example determined that the backbone of the pPVLV2 vector (comprsing $P_{EF1\alpha}$-S) is particularly suitable for the expression of CAR in cells such as T cells, and is particularly suitable for co-expression of CAR and other proteins such as DNRII. In addition, placing CAR and DNRII coding sequences in the same open reading frame can achieve high co-expression rate of the two molecules.

Example 4. The expression of DNRII reduces the phosphorylation of SMAD2 molecules induced by TFG-β1

The inhibitory effect of TFG-β on T cells is achieved by phosphorylation of SMAD2 molecules after TFG-β binds to its receptor.

After 9 days of transduction, CAR-T-19 cells and CAR-T-19-DNRII cells were incubated with recombinant human TFG-β1 (10ng/ml) for 24 hours to determine the expression level of phosphorylated SMAD2 (pSMAD2). With GAPDH and unphosphorylated SMAD2 molecules as controls, the relative quantification of pSMAD2 molecules was performed by western blot.

Specifically, protein concentrations of whole cell lysates were measured using a Bradford assay kit (Sigma-Aldrich). Equal amounts of protein were loaded into the wells of a SDS-PAGE gel and the separated proteins transferred to PVDF membranes (Thermo Scientific). The membranes were blocked with 10% (w/v) skim milk in TBST and then incubated with primary antibody (anti-pSMAD2 and -SMAD2 (Cell signaling Technologies, Danvers, MA, USA); all diluted 1:1000) overnight at 4°C. The membranes were then washed with TBST and incubated with an HRP-conjugated goat anti-rabbit IgG (diluted 1:2000; Cell Signaling Technologies) for 2 hours at room temperature. The membrane was then exposed to ECL reagents (Thermo Scientific) and the resulting signals detected using a Luminescent image analyzer (LAS-4000; Fuji Film, Tokyo, Japan)

The results are shown in Figure 8. The level of pSAMD2 in CAR-T-19-DNRII cells was significantly lower than that in CAR-T-19 cells. It shows that the expression of DNRII inhibits the phosphorylation of SMAD2, a key signaling molecule in the TGF-β signaling pathway.

Example 5　Expression of IFN-γ and TNF-α in CAR-T-19-DNRII cells and CAR-T-19 cells treated with recombinant human TGF-β1

IFN-γ and TNF-α are the hallmark cytokines for T cells to kill target cells. The high expression levels of these two cytokines indicate that T cells have high killing potential to target cells, and vice versa.

Transduced-T cells were cultured with or without 10 ng/ml rhTGF-β1 for 24 hours following 9 days after post transduction. Then, each transduced-T cells were mixed CD19+-K562 for 24 hours, respectively. To determine the amounts of IFN-γ and TNF-α mRNA levels, each mixed cells were harvested and extracted the total RNA using PureLink RNA Mini kit (Thermo Scientific, Waltham, MA, USA). After DNase digestion and

concentration determination using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, USA), total RNA samples were subjected to real-time quantitative RT-PCR analysis with specific primers and One-step SensiFAST SYBR Low-ROX kit (Bioline, Maryland, USA), using a QuantStudio3 Real-Time PCR detection system (Applied Biosystems, Foster City, CA, USA). The 18s rRNA was amplified as an internal control. Expression level was calculated by ΔΔCt method, and fold expression were obtained using the formula 2-ΔΔCt. All experiments were run in triplicate.

The results showed that after treatment with recombinant human TGF-β1, the expression of IFN-γ and TNF-α in CAR-T-19-DNRII cells was significantly higher than that in CAR-T-19 cells (Figure 9).

Example 6. Specifically killing of tumor target cells by CAR-T-19-DNRII cells and CAR-T-19 cells treated with recombinant human TGF-β1

Target cell killing experiments were performed using CAR-T-19 cells and CAR-T-19-DNRII cells 12 days post transduction .

TDA release assay was performed to determine the cytotoxic activity of CAR-T-19 cells and CAR-T-19-DNRII cells against K562 or CD19+-K562 in the presence of TGF-β1. CAR-T-19 cells and CAR-T-19-DNRII cells were incubated with recombinant human TGF-β1 (10ng/ml) for 72 hours. The target cells were labeled with BA-TDA (Perkin Elmer, Norwalk, Connecticut, USA) for 15 minutes, and mixed with effector cells according to the effector cell (T cell) : target cell (tumor cell) ratio of 20:1, 10:1, 5:1, and 2.5:1 respectively, and TDA release (target cell lysis) was detected after 4 hours of co-incubation. A time-resolved fluorescence (TRF) reader (Thermo Scientific) was used to detect the TDA release of the assay supernatant. The specific lysis is calculated as follows: % lysis = (experimental lysis-spontaneous lysis)/(maximum lysis-spontaneous lysis)×100.

The results are shown in Figure 10. After treatment with recombinant human TGF-β1, the killing effect of CAR-T-19 cells on K562 target cells expressing CD19 was reduced to the background (without CAR-T cells) level (Figure 10A). The killing effect of CAR-T-19-DNRII cells on K562 target cells expressing CD19 nearly did not decrease, which was significantly different from the killing effect without the addition of CAR-T cells (Figure 10B). It shows that DNRII effectively reversed the inhibitory effect of TGF-β on T cell killing.

Sequence listing

SEQ ID NO:1 pPVLV1 vector containing CAR-19 coding sequence

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SEQ ID NO:2 pPVLV2 vector containing CAR-19 coding sequence

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AATTACAAAAACAAATTACAAAAATTCAAAATTTT

SEQ ID NO:7 EF1α promoter long
TCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGAACCGGTGC
CTAGAGAAGGTGGCGCGGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGT
ATATAAGTGCAGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGC
ATCTCTCCTTCACGCGCCCGCCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCCTCCCGCCTGTGG
TGCCTCCTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGGAGCC
TACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGCTCAACTCTACGTCTTTGTTTCGTTTTCTGTTCTGCGC
CGTTACAGATCCAAGCTGTGACCGGCGCCTACG

SEQ ID NO:8 CAR-19 coding sequence
ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGCCAGGCCGGACATCCAGATGACACAGAC
TACATCCTCCCTGTCTGCCTCTCTGGGAGACAGAGTCACCATCAGTTGCAGGGCAAGTCAGGACATTAGTAAATATTTAAATT
GGTATCAGCAGAAACCAGATGGAACTGTTAAACTCCTGATCTACCATACATCAAGATTACACTCAGGAGTCCCATCAAGGTTC
AGTGGCAGTGGGTCTGGAACAGATTATTCTCTCACCATTAGCAACCTGGAGCAAGAAGATATTGCCACTTACTTTTGCCAACA
GGGTAATACGCTTCCGTACACGTTCGGAGGGGGGACCAAGCTGGAGATCACAGGTGGCGGTGGCTCGGGCGGTGGTGGGTCGG
GTGGCGGCGGATCTGAGGTGAAACTGCAGGAGTCAGGACCTGGCCTGGTGGCGCCCTCACAGAGCCTGTCCGTCACATGCACT
GTCTCAGGGGTCTCATTACCCGACTATGGTGTAAGCTGGATTCGCCAGCCTCCACGAAAGGGTCTGGAGTGGCTGGGAGTAAT
ATGGGGTAGTGAAACCACATACTATAATTCAGCTCTCAAATCCAGACTGACCATCATCAAGGACAACTCCAAGAGCCAAGTTT
TCTTAAAAATGAACAGTCTGCAAACTGATGACACAGCCATTTACTACTGTGCCAAACATTATTACTACGGTGGTAGCTATGCT
ATGGACTACTGGGGCCAAGGAACCTCAGTCACCGTCTCCTCAACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCAC
CATCGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCACACGAGGGGGCTGGACT
TCGCCTGTGATATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTTTACTGC
AAACGGGGCAGAAAGAAACTCCTGTATATATTCAAACAACCATTTATGAGACCAGTACAAACTACTCAAGAGGAAGATGGCTG
TAGCTGCCGATTTCCAGAAGAAGAAGAAGGAGGATGTGAACTGAGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACA
AGCAGGGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAGAGACGTGGCCGG
GACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGA
GGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCA
CCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGC

SEQ ID NO:9 WPRE
TAATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACG
CTGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCT
CTTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGG
CATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCC
TTGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTGG
CTGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCC
TTCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCG
CCTCCCCGC

SEQ ID NO:10 3' SIN LTR

ATCGAGACCTAGAAAAACATGGAGCAATCACAAGTAGCAATACAGCAGCTACCAATGCTGATTGTGCCTGGCTAGAAGCACAA
GAGGAGGAGGAGGTGGGTTTTCCAGTCACACCTCAGGTACCTTTAAGACCAATGACTTACAAGGCAGCTGTAGATCTTAGCCA
CTTTTTAAAAGAAAAGGGGGGACTGGAAGGGCTAATTCACTCCCAACGAAGACAAGATATCCTTGATCTGTGGATCTACCACA
CACAAGGCTACTTCCCTGATTGGCAGAACTACACACCAGGGCCAGGGATCAGATATCCACTGACCTTTGGATGGTGCTACAAG
CTAGTACCAGTTGAGCAAGAGAAGGTAGAAGAAGCCAATGAAGGAGAGAACACCCGCTTGTTACACCCTGTGAGCCTGCATGG
GATGGATGACCCGGAGAGAGAAGTATTAGAGTGGAGGTTTGACAGCCGCCTAGCATTTCATCACATGGCCCGAGAGCTGCATC
CGGACTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCT
CAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTT
TTAGTCAGTGTGGAAAATCTCTAGCA

SEQ ID NO:11 truncated 5'LTR

GGGTCTCTCTGGTTAGACCAGATCTGAGCCTGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTT
GCCTTGAGTGCTTCAAGTAGTGTGTGCCCGTCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGT
GGAAAATCTCTAGCA

SEQ ID NO:12 HIV-psi (ψ)

CTCTCTCGACGCAGGACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGCGGCGACTGGTGAGTACGCCAAAAATTTT
GACTAGCGGAGGCTAGAAGGAGAGAGATGGGTGCGAGAGCGTC

SEQ ID NO:13 EF1α promoter short

GGGCAGAGCGCACATCGCCCACAGTCCCCGAGAAGTTGGGGGGAGGGGTCGGCAATTGATCCGGTGCCTAGAGAAGGTGGCGC
GGGGTAAACTGGGAAAGTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGT
CGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAG

SEQ ID NO:14 WPRE

AATCAACCTCTGGATTACAAAATTTGTGAAAGATTGACTGGTATTCTTAACTATGTTGCTCCTTTTACGCTATGTGGATACGC
TGCTTTAATGCCTTTGTATCATGCTATTGCTTCCCGTATGGCTTTCATTTTCTCCTCCTTGTATAAATCCTGGTTGCTGTCTC
TTTATGAGGAGTTGTGGCCCGTTGTCAGGCAACGTGGCGTGGTGTGCACTGTGTTTGCTGACGCAACCCCCACTGGTTGGGGC
ATTGCCACCACCTGTCAGCTCCTTTCCGGGACTTTCGCTTTCCCCCTCCCTATTGCCACGGCGGAACTCATCGCCGCCTGCCT
TGCCCGCTGCTGGACAGGGGCTCGGCTGTTGGGCACTGACAATTCCGTGGTGTTGTCGGGGAAATCATCGTCCTTTCCTTGGC
TGCTCGCCTGTGTTGCCACCTGGATTCTGCGCGGGACGTCCTTCTGCTACGTCCCTTCGGCCCTCAATCCAGCGGACCTTCCT
TCCCGCGGCCTGCTGCCGGCTCTGCGGCCTCTTCCGCGTCTTCGCCTTCGCCCTCAGACGAGTCGGATCTCCCTTTGGGCCGC
CTCCCCGC

SEQ ID NO:15 3' LTR Δ-U3

TGGAAGGGCTAATTCACTCCCAACGAAGACAAGATCTGCTTTTTGCTTGTACTGGGTCTCTCTGGTTAGACCAGATCTGAGCC
TGGGAGCTCTCTGGCTAACTAGGGAACCCACTGCTTAAGCCTCAATAAAGCTTGCCTTGAGTGCTTCAAGTAGTGTGTGCCCG
TCTGTTGTGTGACTCTGGTAACTAGAGATCCCTCAGACCCTTTTAGTCAGTGTGGAAAATCTCTAGCA

SEQ ID NO:16 CAR-19vamino acid sequence

MALPVTALLL PLALLLHAAR PDIQMTQTTS SLSASLGDRV TISCRASQDI SKYLNWYQQK PDGTVKLLIY HTSRLHSGVP
SRFSGSGSGT DYSLTISNLE QEDIATYFCQ QGNTLPYTFG GGTKLEITGG GGSGGGGSGG GGSEVKLQES GPGLVAPSQS
LSVTCTVSGV SLPDYGVSWI RQPPRKGLEW LGVIWGSETT YYNSALKSRL TIIKDNSKSQ VFLKMNSLQT DDTAIYYCAK
HYYYGGSYAM DYWGQGTSVT VSSTTTPAPR PPTPAPTIAS QPLSLRPEAC RPAAGGAVHT RGLDFACDIY IWAPLAGTCG
VLLLSLVITL YCKRGRKKLL YIFKQPFMRP VQTTQEEDGC SCRFPEEEEG GCELRVKFSR SADAPAYQQG QNQLYNELNL
GRREEYDVLD KRRGRDPEMG GKPRRKNPQE GLYNELQKDK MAEAYSEIGM KGERRRGKGH DGLYQGLSTA TKDTYDALHM
QALPPR

SEQ ID NO:17 amino acid sequence of DNRII

MGRGLLRGLW PLHIVLWTRI ASTIPPHVQK SVNNDMIVTD NNGAVKFPQL CKFCDVRFST CDNQKSCMSN CSITSICEKP
QEVCVAVWRK NDENITLETV CHDPKLPYHD FILEDAASPK CIMKEKKKPG ETFFMCSCSS DECNDNIIFS EEYNTSNPDL
LLVIFQVTGI SLLPPLGVAI SVIIIFYCYR VNRQQKLSST WETGKTRKLM EFSEHCAII

SEQ ID NO:18 nucleotide sequence of DNRII

ATGGGTCGGG GGCTGCTCAG GGGCCTGTGG CCGCTGCACA TCGTCCTGTG GACGCGTATC GCCAGCACGA TCCCACCGCA
CGTTCAGAAG TCGGTTAATA ACGACATGAT AGTCACTGAC AACAACGGTG CAGTCAAGTT TCCACAACTG TGTAAATTTT
GTGATGTGAG ATTTTCCACC TGTGACAACC AGAAATCCTG CATGAGCAAC TGCAGCATCA CCTCCATCTG TGAGAAGCCA
CAGGAAGTCT GTGTGGCTGT ATGGAGAAAG AATGACGAGA ACATAACACT AGAGACAGTT TGCCATGACC CCAAGCTCCC
CTACCATGAC TTTATTCTGG AAGATGCTGC TTCTCCAAAG TGCATTATGA AGGAAAAAAA AAAGCCTGGT GAGACTTTCT
TCATGTGTTC CTGTAGCTCT GATGAGTGCA ATGACAACAT CATCTTCTCA GAAGAATATA ACACCAGCAA TCCTGACTTG
TTGCTAGTCA TATTTCAAGT GACAGGCATC AGCCTCCTGC CACCACTGGG AGTTGCCATA TCTGTCATCA TCATCTTCTA
CTGCTACCGC GTTAACCGGC AGCAGAAGCT GAGTTCAACC TGGGAAACCG GCAAGACGCG GAAGCTCATG GAGTTCAGCG
AGCACTGTGC CATCATC

What we claim is:

1. A lentiviral vector comprising a truncated EF1α promoter for directing the expression of a nucleotide sequence encoding a polypeptide of interest in a host cell, for example, the truncated EF1α promoter is an EF1α core promoter comprising the nucleotide sequence setting forth in SEQ ID NO: 13.

2. The lentiviral vector of claim 1, wherein the lentiviral vector is a non-replicating lentiviral vector.

3. The lentiviral vector of claim 1 or 2, further comprises an element selecting from the group consisting of a 5'LTR, a ψ element, an RRE element, a cPPT/CTS element, a truncated EF1α promoter, a WPRE element, a multiple cloning site for inserting the nucleotide sequence encoding the polypeptide of interest, and a 3'LTR.

4. The lentiviral vector of any one of claim 1-3, which comprises a 5'LTR, a ψ element, an RRE element, a cPPT/CTS element, a truncated EF1α promoter, a WPRE element, a 3'LTR, and optionally, a multiple cloning site for inserting the nucleotide sequence encoding the polypeptide of interest, which are operably linked.

5. The lentiviral vector of claim 3 or 4, wherein the 5'LTR comprises the nucleotide sequence set forth in SEQ ID NO: 3 or 11; the ψ element comprises the nucleotide sequence set forth in SEQ ID NO: 4 or 12; the RRE element comprises the nucleotide sequence set forth in SEQ ID NO: 5; the cPPT/CTS element comprises the nucleotide sequence set forth in SEQ ID NO: 6; the WPRE element comprises the nucleotide sequence set forth in SEQ ID NO: 9 or 14; the 3'LTR comprises the nucleotide sequence set forth in SEQ ID NO: 10 or 15.

6. The lentiviral vector of any one of claims 1-5, which comprises a 5'LTR comprising the nucleotide sequence set forth in SEQ ID NO: 11, a ψ element comprising the nucleotide sequence set forth in SEQ ID NO: 12, an RRE element comprising the nucleotide sequence set forth in SEQ ID NO: 5, a cPPT/CTS element comprising the nucleotide sequence set forth in SEQ ID NO: 6, a truncated EF1α promoter comprising the nucleotide sequence set forth in SEQ ID NO: 13, a WPRE element comprising the nucleotide sequence set forth in SEQ ID NO: 14, a 3'LTR comprising the nucleotide sequence set forth in SEQ ID NO: 15, and optionally, a multiple cloning site for inserting the nucleotide sequence encoding the polypeptide of interest, which are operably linked.

7. The lentiviral vector of any one of claims 1 to 6, further comprising a nucleotide sequence encoding a polypeptide of interest.

8. The lentiviral vector of any one of claims 1 to 7, wherein the polypeptide of interest is a fusion polypeptide comprising a plurality of proteins, and the plurality of proteins in the fusion polypeptide are separated by a self-cleavable peptide.

9. The lentiviral vector of any one of claims 1 to 8, wherein the polypeptide of interest is a fusion polypeptide comprising a first protein and a second protein, and the fusion polypeptide comprises a self-cleavable peptide between the first protein and the second protein.

10. The lentiviral vector of claim 8 or 9, wherein the self-cleavable peptide is a 2A polypeptide, for example, the self-cleavable peptide is selected from P2A, F2A, E2A, or T2A polypeptide, or a functional variant thereof.

11. The lentiviral vector of claim 9 or 10, wherein the first protein is a cancer-associated antigen-specific receptor protein, such as T cell receptor (TCR) or chimeric antigen receptor (CAR).

12. The lentiviral vector of any one of claims 9-11, wherein the second protein is a dominant negative TGF-β type II receptor.

13. The lentiviral vector of claim 12, wherein the dominant negative TGF-β type II receptor lacks the intracellular signaling domain of TGF-β type II receptor, for example, the dominant negative TGF-β type II receptor comprises the amino acid sequence set forth in SEQ ID NO:18.

14. A method for preparing a lentiviral vector particle, comprising:

a) co-transfecting a suitable host cell with the lentiviral vector of any one of claims 1-10, one or more packaging vectors expressing Gag and/or Pol, and an envelope vector expressing an envelope protein such as VSV-G;

b) culturing the transfected host cell to package the lentiviral vector into a lentiviral vector particle; and

c) harvesting the lentiviral vector particle produced in step b).

15. A lentiviral vector particle comprising the lentiviral vector of any one of claims 1-13 or prepared by the method of claim 14.

16. Use of the lentiviral vector particle of claim 15 in the preparation of a therapeutic T cell, wherein the therapeutic T cell expresses a cancer-associated antigen-specific receptor protein, such as T cell receptor (TCR) or chimeric antigen receptor (CAR), and optionally a dominant negative TGF-β type II receptor.

17. A method for preparing a therapeutic T cell, comprising transducing a T cell with the lentiviral vector particle of claim 15.

18. The method of claim 17, wherein the transduction of the lentiviral vector particle causes the therapeutic T cell to express a cancer-associated antigen-specific receptor protein, such as T cell receptor (TCR) or chimeric antigen receptor (CAR), and optionally a dominant negative TGF-β type II receptor.