



(12) **DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION**

(13) **A1**

(86) Date de dépôt PCT/PCT Filing Date: 2019/11/14
(87) Date publication PCT/PCT Publication Date: 2020/05/22
(85) Entrée phase nationale/National Entry: 2021/05/07
(86) N° demande PCT/PCT Application No.: US 2019/061549
(87) N° publication PCT/PCT Publication No.: 2020/102589
(30) Priorité/Priority: 2018/11/14 (US62/767,069)

(51) Cl.Int./Int.Cl. *C07K 16/28* (2006.01),
C12N 15/867 (2006.01)
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(54) Titre : VECTEURS A DEUX GENES POUR LA GENERATION DE CELLULES CAR-T ET UTILISATIONS ASSOCIEES

(54) Title: TWO-GENE VECTORS FOR GENERATING CAR-T CELLS AND USES THEREOF

(57) Abrégé/Abstract:

The present invention provides two-gene retroviral vector compositions comprising polynucleotides encoding an anti-CD7 chimeric activating receptor (CAR) and polynucleotides encoding an anti-CD7 protein expression blocker. Also provided are methods of producing and methods of using such compositions in cancer therapy.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property

Organization

International Bureau

(43) International Publication Date

22 May 2020 (22.05.2020)



(10) International Publication Number

WO 2020/102589 A1

(51) International Patent Classification:

C07K 16/28 (2006.01) C12N 15/867 (2006.01)

(21) International Application Number:

PCT/US2019/061549

(22) International Filing Date:

14 November 2019 (14.11.2019)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/767,069 14 November 2018 (14.11.2018) US

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(81) Designated States (unless otherwise indicated, for every

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every

kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

(54) Title: TWO-GENE VECTORS FOR GENERATING CAR-T CELLS AND USES THEREOF

(57) Abstract: The present invention provides two-gene retroviral vector compositions comprising polynucleotides encoding an anti-CD7 chimeric activating receptor (CAR) and polynucleotides encoding an anti-CD7 protein expression blocker. Also provided are methods of producing and methods of using such compositions in cancer therapy.

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TWO-GENE VECTORS FOR GENERATING CAR-T CELLS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit to U.S. Provisional Application No. 62/767,069 filed November 14, 2018, the disclosure in its entirety is herein incorporated by reference.

REFERENCE TO A SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said Sequence Listing was created on November 14, 2019, and is entitled “119419-5007-WO-Sequence-Listing_ST25.txt” which is 139,264 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Chimeric antigen receptors (CARs) can redirect immune cells to specifically recognize and kill tumor cells. CARs are artificial multi-molecular proteins constituted by a single-chain variable region (scFv) of an antibody linked to a signaling molecule via a transmembrane domain. When the scFv ligates its cognate antigen, signal transduction is triggered, resulting in tumor cell killing by CAR-expressing cytotoxic T lymphocytes (Eshhar Z, Waks T, et al. PNAS USA. 90(2):720-724, 1993; Geiger TL, et al. J Immunol. 162(10):5931-5939, 1999; Brentjens RJ, et al. Nat Med. 9(3):279-286, 2003; Cooper LJ, et al. Blood 101(4):1637-1644, 2003; Imai C, et al. Leukemia. 18:676-684, 2004). Clinical trials with CAR-expressing autologous T lymphocytes have shown positive responses in patients with B-cell refractory leukemia and lymphoma (see, e.g., Till BG, et al. Blood 119(17):3940-3950, 2012; Maude SL, et al. N Engl J Med. 371(16):1507-1517, 2014).

[0004] The development of CAR technology to target T cell malignancies has lagged far behind the progress made for their B-cell counterparts. Novel therapies for T-cell malignancies are needed but progress to date has been slow. In particular, effective immunotherapeutic options are lacking and treatment of T-cell acute lymphocytic leukemia (T-ALL) relies on intensive chemotherapy and hematopoietic stem cell transplant. Despite aggressive treatment regimes associated with significant morbidity, results with these approaches are far from satisfactory.

[0005] CAR-T cells have recently been developed in which the target antigen of the CAR-T is itself expressed in the CAR-T cell (Png et al., Blood, 2017, 1(25):2348-2360, WO 2018/098306). To avoid self-killing (e.g., fratricide), the CAR-T cells also express a PEBL that serves to reduce the expression of the target antigen on the cell surface of the CAR-T. To produce viable CAR-T cells, first a protein expression blocker (PEBL) protein was expressed to bind and sequester the target protein prior to the subsequent expression of the CAR. Due to the pre-existing presence of the target antigen on the cell surface of the resulting engineered T cells, simultaneous expression of the CAR and the PEBL resulted in fratricide. In particular, the pre-existing cell surface target antigens were not susceptible to sequestration by the newly expressed PEBL proteins, and are recognized and targeted by the newly expressed CAR proteins.

[0006] An alternative to simultaneous expression can be sequential expression. However, sequential expression of a PEBL and then a CAR in a T-cell creates several challenges for the clinical implementation of PEBL CAR-T cells. First, sequential engineering of the T cells requires the separate manufacture and administration of distinct viral vectors, one for the PEBL and a second for the CAR. This increases cost and time, as well as the complexity of experimental manipulation to produce the engineered CAR-T cells. In addition, sequential engineering of the T cells results in a complex mix of engineered cells in the final clinical product, creating challenges with product characterization, uniformity and efficacy. Because only a fraction of the T cells integrates the introduced gene at each engineering step, the final product (the engineered T cells) will comprise some cells that only received the PEBL gene, some cells that only received the CAR gene, and some cells that received both genes.

[0007] In sum, there is a significant unmet need for new therapeutic options for patients with T-cell malignancies. Additionally, there is a need for methods for producing an engineered CAR-T cell and eliminating CAR-mediated self-killing or fratricide of the T cell.

SUMMARY OF THE INVENTION

[0008] In some aspects, the present invention provides a bicistronic retroviral vector comprising: (a) a first polynucleotide encoding an anti-CD7 chimeric antigen receptor (CAR) comprising at least 90% sequence identity to the amino acid sequence of any one of SEQ ID NOS:28-31; (b) a second polynucleotide encoding an Internal Ribosome Entry Site (IRES) or a

ribosomal codon skipping site; and (c)) a third polynucleotide encoding an anti-CD7 protein expression blocker (PEBL) comprising at least 90% sequence identity to the amino acid sequence of SEQ ID NOS:24-27, wherein the first polynucleotide is operably linked the second polynucleotide which is operably linked the third polynucleotide.

[0009] In some embodiments, the anti-CD7 CAR comprises the amino acid sequence of any one of SEQ ID NOS:28-31.

[0010] In some embodiments, the anti-CD7 PEBL comprises the amino acid sequence of any one of SEQ ID NOS:24-27.

[0011] In some embodiments, the anti-CD7 CAR comprises the amino acid sequence of SEQ ID NO:29 and the anti-CD7 PEBL comprises the amino acid sequence of SEQ ID NO:25.

[0012] In some embodiments, the anti-CD7 CAR comprises the amino acid sequence of SEQ ID NO:31 and the anti-CD7 PEBL comprises the amino acid sequence of SEQ ID NO:27.

[0013] In some embodiments, the IRES is derived from *Encephalomyocarditis* virus (EMCV) or an *Enterovirus*.

[0014] In some embodiments, the ribosomal codon skipping site comprises a 2A self-cleaving peptide. In some embodiments, the 2A self-cleaving peptide is selected from the group consisting of a F2A peptide (foot-and-mouth disease virus 2A peptide), an E2A peptide (equine rhinitis A virus 2A peptide), a P2A peptide (porcine teschovirus-1 2A peptide), and a T2A peptide (thossea asigna virus 2A).

[0015] In some embodiments, the bicistronic retroviral vector comprises at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO:12.

[0016] In some embodiments, the bicistronic retroviral vector comprises least 90% sequence identity to the nucleic acid sequence of SEQ ID NO:13. some embodiments, the bicistronic retroviral vector comprises the nucleic acid sequence of SEQ ID NO:13.

[0017] In some embodiments, the bicistronic retroviral vector comprises further comprises a promoter element.

[0018] In some embodiments, the promoter element is selected from the group consisting of a CMV promoter, EF1 α promoter, EFS promoter, MSCV promoter, and PGK promoter.

[0019] In some embodiments, the promoter element comprises at least 90% sequence identity to the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:6-10.

[0020] In some embodiments, the promoter element comprises the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:6-10.

[0021] In some embodiments, the bicistronic retroviral vector comprises at least 90% sequence identity to the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:14-16.

[0022] In some embodiments, the bicistronic retroviral vector comprises the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:14-16.

[0023] In some embodiments, the retroviral vector is a lentiviral vector.

[0024] In some aspects, provided herein is an engineered immune cell comprising any one of the bicistronic retroviral vectors outlined herein.

[0025] In some embodiments, the engineered immune cell is an allogeneic T cell. In some embodiments, the engineered immune cell is an autologous T cell.

[0026] In some embodiments, the engineered immune cell has reduced CD7 surface expression compared to a corresponding immune cell and expresses the anti-CD7 CAR.

[0027] In some aspects, provided herein is a pharmaceutical composition comprising any of the engineered immune cells described herein and a pharmaceutically effective carrier.

[0028] In some aspects, provided herein is a method of treating a cancer in a subject comprising administering a therapeutically effective amount of any of the engineered immune cells described herein or a pharmaceutical composition thereof.

[0029] In some aspects, provided herein is a method of producing an engineered immune cell comprising transducing an immune cell with any one of the bicistronic retroviral vectors described herein and recovering the engineered immune cell.

[0030] In some embodiments, the immune cell is selected from the group consisting of a peripheral blood mononuclear cell, an isolated CD4⁺ T cell, an isolated CD8⁺ T cell, and an isolated CD3⁺ T cell.

[0031] In some embodiments, the engineered immune cell has reduced CD7 surface expression compared to a corresponding immune cell and expresses the anti-CD7 CAR.

[0032] In some aspects, provided herein is a recombinant retroviral vector comprising: (a) a first promoter element operably linked to a first polynucleotide encoding an anti-CD7 chimeric antigen receptor (CAR) comprising at least 90% sequence identity to the amino acid sequence of SEQ ID NO:28 or SEQ ID NO:30; and (b) a second promoter element operably linked to a second polynucleotide encoding an anti-CD7 protein expression blocker (PEBL) comprising at least 90% sequence identity to the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:26.

[0033] In some embodiments, the anti-CD7 CAR comprises the amino acid sequence of SEQ ID NO:28 or SEQ ID NO:30.

[0034] In some embodiments, the anti-CD7 PEBL comprises the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:26.

[0035] In some embodiments, the first promoter element and/or the second promoter element are selected from the group consisting of a CMV promoter, EF1 α promoter, EFS promoter, MSCV promoter, and PGK promoter.

[0036] In some embodiments, the first promoter element and/or the second promoter element comprise at least 90% sequence identity to the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:6-10.

[0037] In some embodiments, the first promoter element and/or the second promoter element comprise the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:6-10.

[0038] In some embodiments, the first promoter and the second promoter share less than 95% sequence identity.

[0039] In some embodiments, the first promoter element operably linked to the first polynucleotide is 5' of the second promoter element operably linked to the second polynucleotide.

[0040] In some embodiments, the second promoter element operably linked to the second polynucleotide is 5' of the first promoter element operably linked to the first polynucleotide.

[0041] In some embodiments, the recombinant retroviral vector comprises at least 90% sequence identity to the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:18-23.

[0042] In some embodiments, the retroviral vector is a lentiviral vector.

[0043] Also provided is an engineered immune cell comprising any one of the recombinant retroviral vectors described herein.

[0044] In some embodiments, the engineered immune cell is an allogenic T cell. In some embodiments, the engineered immune cell is an autologous T cell.

[0045] In some embodiments, the engineered immune cell has reduced CD7 surface expression compared to a corresponding immune cell and expresses the anti-CD7 CAR.

[0046] In some aspects, provided herein is a pharmaceutical composition comprising any of the engineered immune cells described herein and a pharmaceutically effective carrier.

[0047] In some aspects, provided herein is a method of treating a cancer in a subject comprising administering a therapeutically effective amount of any of the engineered immune cells described herein or a pharmaceutical composition thereof.

[0048] In some aspects, provided herein is a method of producing an engineered immune cell comprising transducing an immune cell with any one of the recombinant retroviral vectors described herein and recovering the engineered immune cell.

[0049] In some embodiments, the immune cell is selected from the group consisting of a peripheral blood mononuclear cell, an isolated CD4⁺ T cell, an isolated CD8⁺ T cell, and an isolated CD3⁺ T cell.

[0050] In some embodiments, the engineered immune cell has reduced CD7 surface expression compared to a corresponding immune cell and expresses the anti-CD7 CAR.

BRIEF DESCRIPTION OF THE DRAWINGS

[0051] FIG. 1A and FIG. 1B show expression of CAR and PEBL by transduced primary T cells according to flow cytometry (FIG. 1A) and Western blot (FIG. 1B). Primary T cells were

transduced with the indicated retroviruses (e.g., PEBL; CAR; PEBL and CAR sequentially; PEBL-IRES-CAR; and CAR-P2A-PEBL) and analyzed by flow cytometry for CD7 and CAR expression. Cell lysates from primary T cells transduced with the indicated retroviruses were analyzed by Western blot for β -actin, Myc-tagged PEBL, CAR and endogenous CD3 ζ expression.

[0052] FIG. 2A – FIG. 2F provide illustrative schematic diagrams of bicistronic promoter 1-CAR-promoter 2-PEBL lentiviral constructs. FIG. 2A depicts a schematic of an exemplary dual promoter construct comprising a MSCV-promoter-anti-human CD7 (TH69) CAR-EFS promoter-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:19. FIG. 2B depicts a schematic of an exemplary dual promoter construct comprising a MSCV promoter-anti-human CD7 (TH69) CAR-EF1a promoter-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:18. FIG. 2C depicts a schematic of an exemplary dual promoter construct comprising a PGK promoter-anti-human CD7 (TH69) CAR-EFS promoter-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:23. FIG. 2D depicts a schematic of an exemplary dual promoter construct comprising a PGK promoter-anti-human CD7 (TH69) CAR-EF1a promoter-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:22. FIG. 2E depicts a schematic of an exemplary dual promoter construct comprising a PGK promoter-anti-human CD7 (3A1F) CAR-EF1a promoter-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:20. FIG. 2F depicts a schematic of an exemplary dual promoter construct comprising a PGK promoter-anti-human CD7 (TH69) CAR-EF1a promoter-anti-human CD7 (3A1F) PEBL, such as the one of SEQ ID NO:21.

[0053] FIG. 3 shows expression of CAR and CD7 by transduced primary T cells according to flow cytometry. Primary T cells were transduced with the indicated dual-promoter lentiviruses and analyzed by flow cytometry at 5 days and 14 days post transduction.

[0054] FIG. 4A – FIG. 4C provide illustrative schematic diagrams of bicistronic CAR-P2A-PEBL lentiviral constructs. FIG. 4A depicts a schematic of an exemplary bicistronic construct comprising an MSCV promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:14. FIG. 4B depicts a schematic of an exemplary bicistronic construct comprising an EF1a promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:15. FIG. 4C depicts a schematic of an

exemplary bicistronic construct comprising an EFS promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:16.

[0055] FIG. 5 shows expression of CAR and CD7 by primary T cells transduced with MSCV-CD7CAR-P2A-CD7PEBL lentivirus and analyzed by flow cytometry at 3 days, 6 days, and 9 days post transduction.

[0056] FIG. 6 shows expression of CAR and CD7 by transduced primary T cells according to flow cytometry. Primary T cells were transduced with the indicated bicistronic CD7CAR-P2A-CD7PEBL and CD19CAR lentiviruses and analyzed by flow cytometry at 5 days and 14 days post transduction.

[0057] FIG. 7A and FIG. 7B show expression of CAR and PEBL by transduced primary T cells according to flow cytometry (FIG. 7A) and Western blot (FIG. 7B). Primary T cells were transduced with the two independently produced lots of MSCV-CD7CAR-P2A-CD7PEBL lentivirus and analyzed by flow cytometry for CD7 and CAR expression. Cell lysates from transduced cells were analyzed by Western blot for β -actin, Myc-tagged PEBL, CAR and endogenous CD3 ζ expression.

[0058] FIG. 8 shows expression of CAR and CD7 by transduced primary T cells according to flow cytometry. Bulk PBMCs, CD4⁺ and CD8⁺ positively-selected T cells, and CD3⁺ positively-selected T cells were activated with either Dynabeads or TransAct and transduced with MSCV-CD7CAR-P2A-CD7PEBL lentivirus. Cells were analyzed by flow cytometry at 4 days, 7 days, and 10 days post transduction.

[0059] FIG. 9A shows expression of CAR and CD7 by primary T cells transduced with MSCV-CD7CAR-P2A-CD7PEBL lentivirus and cultured in serum-free TexMACS medium, or TexMACS medium supplemented with 3% human AB serum. FIG. 9B shows the total fold expansion of transduced cells at 11 days post activation (mean $\pm$ SEM of biological replicates).

[0060] FIG. 10A and FIG. 10B show percentage of CAR⁺ T cells (FIG. 10A) and total fold expansion (FIG. 10B) of transduced cells at 11 days post activation (mean $\pm$ SEM of biological replicates). Primary T cells were cultured in serum-free TexMACS medium and transduced with MSCV-CD7CAR-P2A-CD7PEBL lentivirus at 1, 2, 3, or 4 days post activation.

[0061] FIG. 11 shows expression of CAR and CD7 by transduced primary T cells according to flow cytometry. CD4⁺ and CD8⁺ positively-selected T cells were activated with TransAct and transduced with MSCV-CD7CAR-P2A-CD7PEBL lentivirus at the indicated multiplicity of infection (MOI). Cells were analyzed by flow cytometry at 3 days and 9 days post transduction.

[0062] FIG. 12A and FIG. 12B show percentage of CAR⁺ T cells (FIG. 12A) and transgene vector copy number (VCN) (FIG. 12B) of transduced cells at 11 days post activation (mean of biological duplicates). Primary T cells were cultured in serum-free TexMACS medium and transduced with MSCV-CD7CAR-P2A-CD7PEBL lentivirus at MOI 3, 5, or 10. T cells were analyzed by flow cytometry for CAR expression. Genomic DNA was extracted from transduced cells to determine transgene VCN by RT-qPCR analysis.

[0063] FIG. 13A – FIG. 13E show expression of various surface markers on primary T cells transduced with MSCV-CD7CAR-P2A-CD7PEBL lentivirus at 11 days post activation. Transduced cells were analyzed by flow cytometry for CAR and CD7 (FIG. 13A), CD3 and CD14/CD19/CD56 (FIG. 13B), CD4 and CD8 (FIG. 13C), CD45RO and CCR7 (FIG. 13D), and PD-1 and Tim-3 (FIG. 13E). The triplicate analyses are of primary T cells from 3 unique donors transduced with MSCV-CD7CAR-P2A-CD7PEBL lentivirus at MOI 10.

[0064] FIG. 14A and FIG. 14B show functional response of PEBL-CAR-T cells, generated with MSCV-CD7CAR-P2A-CD7PEBL lentivirus, to CD7⁺ Jurkat cells and CD7⁻ Nalm6 cells by IFN γ secretion (FIG. 14A) and cytotoxicity (FIG. 14B). IFN γ secretion was measured in culture supernatants of PEBL-CAR-T cells co-cultured with Jurkat or Nalm6 cells at the indicated E:T ratios for 24h (mean $\pm$ SD of technical replicates). Cytolytic activity of PEBL-CAR T cells was measured after a 4h co-culture with Jurkat or Nalm6 cells at the indicated E:T ratios (mean $\pm$ SD of technical replicates).

[0065] FIG. 15 depicts the nucleic acid sequence of CPPT-CMV-MCS-PGK-GFP-WPRE (SEQ ID NO:1). The CPPT is in bold, CMV promoter is single underlined, PGK promoter is double underlined, GFP is bold/single underlined, and the WPRE element is bold/double underlined.

[0066] FIG. 16 depicts the nucleic acid sequence of an exemplary anti-human CD7 PEBL based on the antibody TH69 (SEQ ID NO:2). The CD8 signal peptide starts at position 1, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined,

the anti-CD7 VH domain is in bold/single underlined, the myc-KDEL peptide is double underlined, and a stop codon ends the sequence.

[0067] FIG. 17 depicts the nucleic acid sequence of an exemplary anti-human CD7 PEBL based on the antibody 3A1F (SEQ ID NO:3). The CD8 signal peptide starts at position 1, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined, the anti-CD7 VH domain is in bold/single underlined, the myc-KDEL peptide is double underlined, and a stop codon ends the sequence.

[0068] FIG. 18 depicts the nucleic acid sequence of an exemplary anti-human CD7 CAR based on the antibody TH69 (SEQ ID NO:4). The CD8 signal peptide starts at position 1, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined, the anti-CD7 VH domain is in bold/single underlined, CD8a hinge and transmembrane domain is double underlined, 4-1BB signaling domain is between the CD8a hinge and transmembrane domain and the CD3 ζ signaling domain, CD3 ζ signaling domain is bold/double underlined, and a stop codon ends the sequence.

[0069] FIG. 19 depicts the nucleic acid sequence of an exemplary anti-human CD7 CAR based on the antibody 3A1F (SEQ ID NO:5). The CD8 signal peptide starts at position 1, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined, the anti-CD7 VH domain is in bold/single underlined, CD8a hinge and transmembrane domain is double underlined, 4-1BB signaling domain is between the CD8a hinge and transmembrane domain and the CD3 ζ signaling domain, CD3 ζ signaling domain is bold/double underlined, and a stop codon ends the sequence.

[0070] FIG. 20 depicts the nucleic acid sequence of an exemplary CMV promoter (SEQ ID NO:6).

[0071] FIG. 21 depicts the nucleic acid sequence of an exemplary EF1 α promoter (SEQ ID NO:7).

[0072] FIG. 22 depicts the nucleic acid sequence of an exemplary EFS promoter (SEQ ID NO:8).

[0073] FIG. 23 depicts the nucleic acid sequence of an exemplary MSCV promoter (SEQ ID NO:9).

[0074] FIG. 24 depicts the nucleic acid sequence of an exemplary PGK promoter (SEQ ID NO:10).

[0075] FIG. 25 depicts the nucleic acid sequence of an exemplary bicistronic construct comprising anti-human CD7 (TH69) PEBL-IRES-anti-human CD7 (TH69) CAR (SEQ ID NO:11). Anti-human CD7 (TH69) PEBL is in normal font, IRES is bold, and anti-human CD7 (TH69) CAR is double underlined.

[0076] FIG. 26 depicts the nucleic acid sequence of an exemplary bicistronic construct comprising anti-human CD7 (TH69) CAR-IRES-anti-human CD7 (TH69) PEBL (SEQ ID NO:12). Anti-human CD7 (TH69) CAR is in normal font, IRES is in bold, and anti-human CD7 (TH69) PEBL is single underlined.

[0077] FIG. 27 depicts the nucleic acid sequence of an exemplary bicistronic construct comprising anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL (SEQ ID NO:13). Anti-human CD7 (TH69) CAR is in normal font, P2A is in bold, and anti-human CD7 (TH69) PEBL is single underlined.

[0078] FIG. 28A and FIG. 28B depict the nucleic acid sequence of an exemplary bicistronic construct comprising an MSCV promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL (SEQ ID NO:14). The MSCV promoter is double underlined, a restriction enzyme site and Kozak sequence are between the MSCV promoter and CAR, anti-human CD7 (TH69) CAR is in bold, P2A is in normal font, and anti-human CD7 (TH69) PEBL is single underlined.

[0079] FIG. 29A and FIG. 29B depict the nucleic acid sequence of an exemplary bicistronic construct comprising an EF1 α promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL (SEQ ID NO:15). The EF1 α promoter is double underlined, a restriction enzyme site and Kozak sequence are between the EF1 α promoter and CAR, anti-human CD7 (TH69) CAR is in bold, P2A is in normal font, and anti-human CD7 (TH69) PEBL is single underlined.

[0080] FIG. 30A and FIG. 30B depict the nucleic acid sequence of an exemplary bicistronic construct comprising an EFS promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL (SEQ ID NO:16). The EFS promoter is double underlined, a restriction enzyme

site and Kozak sequence are between the EFS promoter and CAR, anti-human CD7 (TH69) CAR is in bold, P2A is in normal font, and anti-human CD7 (TH69) PEBL is single underlined.

[0081] FIG. 31A and FIG. 31B depict the nucleic acid sequence of an exemplary dual promoter construct comprising a MSCV promoter-anti-human CD7 (TH69) CAR-PGK promoter-anti-human CD7 (TH69) PEBL (SEQ ID NO:17). The MSCV promoter is double underlined, a restriction enzyme site (in italics) and Kozak sequence are between the MSCV promoter and CAR, anti-human CD7 (TH69) CAR is in bold, a restriction enzyme site (in italics) is between CAR and PGK promoter, PGK promoter is single underlined, a restriction enzyme site (italized) and Kozak sequence are between PGK promoter and PEBL, anti-human CD7 (TH69) PEBL is bold/single underlined, and restriction enzyme site (in italics) ends the sequence.

[0082] FIG. 32A and FIG. 32B depict the nucleic acid sequence of an exemplary dual promoter construct comprising a MSCV promoter-anti-human CD7 (TH69) CAR-EF1a promoter-anti-human CD7 (TH69) PEBL (SEQ ID NO:18). The MSCV promoter is double underlined, a restriction enzyme site (in italics) and Kozak sequence are between the MSCV promoter and CAR, anti-human CD7 (TH69) CAR is in bold, a restriction enzyme site (in italics) is between CAR and EF1a promoter, EF1a promoter is single underlined, a restriction enzyme site (in italics) and Kozak sequence are between EF1a promoter and PEBL, and anti-human CD7 (TH69) PEBL is bold/single underlined.

[0083] FIG. 33A and FIG. 33B depict the nucleic acid sequence of an exemplary dual promoter construct comprising a MSCV promoter-anti-human CD7 (TH69) CAR-EFS promoter-anti-human CD7 (TH69) PEBL (SEQ ID NO:19). The MSCV promoter is double underlined, a restriction enzyme site (in italics) and Kozak sequence are between the MSCV promoter and CAR, anti-human CD7 (TH69) CAR is in bold, a restriction enzyme site (in italics) is between CAR and EFS promoter, EFS promoter is single underlined, a restriction enzyme site (in italics) and Kozak sequence are between EFS promoter and PEBL, and anti-human CD7 (TH69) PEBL is bold/single underlined.

[0084] FIG. 34A and FIG. 34B depict the nucleic acid sequence of an exemplary dual promoter construct comprising a PGK promoter-anti-human CD7 (3A1F) CAR-EF1a promoter-anti-human CD7 (TH69) PEBL (SEQ ID NO:20). The PGK promoter is double underlined, a

restriction enzyme site (in italics) and Kozak sequence are between the PGK promoter and CAR, anti-human CD7 (3A1F) CAR is in bold, a restriction enzyme site (in italics) is between CAR and EF1a promoter, EF1a promoter is single underlined, a restriction enzyme site (in italics) and Kozak sequence are between EF1a promoter and PEBL, and anti-human CD7 (TH69) PEBL is bold/single underlined.

[0085] FIG. 35A and FIG. 35B depict the nucleic acid sequence of an exemplary dual promoter construct comprising a PGK promoter-anti-human CD7 (TH69) CAR-EF1a promoter-anti-human CD7 (3A1F) PEBL (SEQ ID NO:21). The PGK promoter is double underlined, a restriction enzyme site (in italics) and Kozak sequence are between the PGK promoter and CAR, anti-human CD7 (TH69) CAR is in bold, a restriction enzyme site (in italics) is between CAR and EF1a promoter, EF1a promoter is single underlined, a restriction enzyme site (in italics) and Kozak sequence are between EF1a promoter and PEBL, and anti-human CD7 (3A1F) PEBL is bold/single underlined.

[0086] FIG. 36A - FIG. 36C depict the nucleic acid sequence of an exemplary dual promoter construct comprising a PGK promoter-anti-human CD7 (TH69) CAR-EF1a promoter-anti-human CD7 (TH69) PEBL (SEQ ID NO:22). The PGK promoter is double underlined, a restriction enzyme site (in italics) and Kozak sequence are between the PGK promoter and CAR, anti-human CD7 (TH69) CAR is in bold, a restriction enzyme site (in italics) is between CAR and EF1a promoter, EF1a promoter is single underlined, a restriction enzyme site (in italics) and Kozak sequence are between EF1a promoter and PEBL, and anti-human CD7 (TH69) PEBL is bold/single underlined.

[0087] FIG. 37A and FIG. 37B depict the nucleic acid sequence of an exemplary dual promoter construct comprising a PGK promoter-anti-human CD7 (TH69) CAR-EFS promoter-anti-human CD7 (TH69) PEBL (SEQ ID NO:23). The PGK promoter is double underlined, a restriction enzyme site (in italics) and Kozak sequence are between the PGK promoter and CAR, anti-human CD7 (TH69) CAR is in bold, a restriction enzyme site (in italics) is between CAR and EFS promoter, EFS promoter is single underlined, a restriction enzyme site (in italics) and Kozak sequence are between EFS promoter and PEBL, and anti-human CD7 (TH69) PEBL is bold/single underlined.

[0088] FIG. 38 depicts the amino acid sequence of an exemplary anti-human CD7 PEBL based on the antibody TH69 (SEQ ID NO:24). The CD8 signal peptide starts at position 1 and is in normal type, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined, the anti-CD7 VH domain is in bold/single underlined, and the myc-KDEL peptide is double underlined.

[0089] FIG. 39 depicts the amino acid sequence of an exemplary anti-human CD7 PEBL variant based on the antibody TH69 (SEQ ID NO:25). The N-terminal proline is in italics, the CD8 signal peptide starts at position 2 and is in normal type, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined, the anti-CD7 VH domain is in bold/single underlined, and the myc-KDEL peptide is double underlined.

[0090] FIG. 40 depicts the amino acid sequence of an exemplary anti-human CD7 PEBL based on the antibody 3A1F (SEQ ID NO:26). The CD8 signal peptide starts at position 1 and is in normal type, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined, the anti-CD7 VH domain is in bold/single underlined, and the myc-KDEL peptide is double underlined.

[0091] FIG. 41 depicts the amino acid sequence of an exemplary anti-human CD7 PEBL variant based on the antibody 3A1F (SEQ ID NO:27). The N-terminal proline is in italics, the CD8 signal peptide starts at position 2 and is in normal type, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined, the anti-CD7 VH domain is in bold/single underlined, and the myc-KDEL peptide is double underlined.

[0092] FIG. 42 depicts the amino acid sequence of an exemplary anti-human CD7 CAR based on the antibody TH69 (SEQ ID NO:28). The CD8 signal peptide starts at position 1, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined, the anti-CD7 VH domain is in bold/single underlined, CD8a hinge and transmembrane domain is double underlined, 4-1BB signaling domain is between the CD8a hinge and transmembrane domain and the CD3 ζ signaling domain and is in normal type, and CD3 ζ signaling domain is bold/double underlined.

[0093] FIG. 43 depicts the amino acid sequence of an exemplary anti-human CD7 CAR variant based on the antibody TH69 (SEQ ID NO:29). The CD8 signal peptide starts at position 1, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single

underlined, the anti-CD7 VH domain is in bold/single underlined, CD8a hinge and transmembrane domain is double underlined, 4-1BB signaling domain is between the CD8a hinge and transmembrane domain and the CD3 ζ signaling domain and is in normal type, CD3 ζ signaling domain is bold/double underlined, and the amino acids at the C-terminus of the CD3 ζ signaling domain arise via ribosome skipping at the P2A site.

[0094] FIG. 44 depicts the amino acid sequence of an exemplary anti-human CD7 CAR based on the antibody 3A1F (SEQ ID NO:30). The CD8 signal peptide starts at position 1, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined, the anti-CD7 VH domain is in bold/single underlined, CD8a hinge and transmembrane domain is double underlined, 4-1BB signaling domain is between the CD8a hinge and transmembrane domain and the CD3 ζ signaling domain and is in normal type, and CD3 ζ signaling domain is bold/double underlined.

[0095] FIG. 45 depicts the amino acid sequence of an exemplary anti-human CD7 CAR variant based on the antibody 3A1F (SEQ ID NO:31). The CD8 signal peptide starts at position 1, the anti-CD7 VL domain is in bold, the linker between the VL and VH domains is single underlined, the anti-CD7 VH domain is in bold/single underlined, CD8a hinge and transmembrane domain is double underlined, 4-1BB signaling domain is between the CD8a hinge and transmembrane domain and the CD3 ζ signaling domain and is in normal type, CD3 ζ signaling domain is bold/double underlined, and the amino acids at the C-terminus of the CD3 ζ signaling domain arise via ribosome skipping at the P2A site.

[0096] FIG. 46 depicts the amino acid sequence of an exemplary anti-human CD7 CAR based on the antibody TH69- P2A- anti-human CD7 PEBL based on the antibody TH69 (SEQ ID NO:95). The CD7 CAR is in normal font, the P2A is double underlined, and the CD7 PEBL is bold/single underlined.

DETAILED DESCRIPTION OF THE INVENTION

Introduction

[0097] The present invention provides methods for simultaneous expression of a fratricide-inducing chimeric antigen receptor (e.g., CAR) and a fratricide-preventing protein (e.g., PEBL)

in T cells that result in viable CAR-expressing cytotoxic T lymphocytes (CAR-T) that target T cell antigens.

[0098] Viral vectors have been produced in which two or more genes can be expressed from a single construct. Typically, these vectors employ either a bicistronic element or a two-promoter configuration. In the case of bicistronic vectors, a sequence element is introduced between two genes that enables the translation of two proteins from a single messenger RNA. Examples include the internal ribosome entry site sequences (IRES) and the virally derived “codon skipping” peptide sequences such as P2A, T2A, F2A, E2A, and the like. In the case of two-promoter designed vectors, separate promoter elements are configured upstream of each gene such that each promoter transcribes the mRNA for its proximally linked gene. In some embodiments, an expression vector (e.g., construct) contains a first promoter operably linked to a CAR and a second promoter operably linked to a PEBL.

[0099] Described herein are methods for producing and testing various bicistronic vectors and two-promoter designed vectors for the expression of two different genes (e.g., a gene encoding a CAR, and a gene encoding a PEBL). It was unexpectedly discovered that certain two gene vectors were able to direct expression of both a PEBL and a CAR protein in T cells in a manner such that the resulting engineered T cells survived, expanded, and were able to kill target cells. The relative timing and level of expression of each gene in the identified two gene vectors enabled the downregulation of the target antigen before the CAR can cause undue fratricide to the engineered T cells.

[00100] Described herein are fratricide-resistant CAR-T cells expressing a CAR directed against CD7 and such CAR-T cell has reduced or no surface expression of CD7. The present invention is based, in part, on co-expression of a chimeric antigen receptor (CAR) directed against CD7 and a protein expression blocker (PEBL) directed against CD7 in immune cells (e.g., T cells) using a bicistronic construct, such as a bicistronic viral vector. In one aspect, the present invention relates to an engineered immune cell (e.g., an engineered T cell) comprising a bicistronic construct comprising a polynucleotide sequence encoding an anti-CD7 CAR and a polynucleotide sequence encoding an anti-CD7 PEBL. In some embodiments, the CAR comprises intracellular signaling domains of 4-1BB and CD3 ζ , and an antibody (e.g., a single chain variable fragment or scFv) that specifically binds CD7. The CD7 CAR of the present

invention is sometimes referred to herein as “anti-CD7-41BB-CD3 ζ ”. In some embodiments, the CAR also includes a CD8 α hinge and transmembrane domain. In some embodiments, the anti-CD7 PEBL comprises an antibody (e.g., a scFv) that specifically binds CD7 and an intracellular localization sequence. In certain embodiments, the anti-CD7 PEBL comprises an antibody (e.g., a scFv) that specifically binds CD7, CD8 α hinge and transmembrane domains, and an intracellular localization sequence.

[00101] CD7 is a 40 kDa type I transmembrane glycoprotein which is the primary marker for T cell malignancies, and which is highly expressed in all cases of T cell ALL, including early T-cell progenitor acute lymphoblastic leukemia (ETP-ALL). An anti-CD7 CAR can induce T cells to exert specific cytotoxicity against T cell malignancies. Further, T cell cytotoxicity has been shown to be markedly increased when an anti-CD7 CAR was used in combination with downregulation of CD7 expression on the effector T cells. Downregulation (e.g., elimination, reduction, and/or relocalization) of CD7 in a T cell via expression of anti-CD7 PEBL prevented the fratricidal effect exerted by the corresponding anti-CD7 CAR. This led to greater T cell recovery after CAR expression as compared to cells that retained the target antigen (e.g., CD7), and a more effective cytotoxicity against T leukemia/lymphoma cells.

Definitions

[00102] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the present invention, the preferred materials and methods are described herein. In describing and claiming the present invention, the following terminology will be used.

[00103] It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[00104] The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[00105] The term "about" and its grammatical equivalents in relation to a reference numerical value and its grammatical equivalents as used herein can include a range of values plus or minus 10% from that value. For example, the amount "about 10" includes amounts from 9 to 11. The term "about" in relation to a reference numerical value can also include a range of values plus or minus 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% from that value.

[00106] As used herein, the term "nucleic acid" refers to a polymer comprising multiple nucleotide monomers (*e.g.*, ribonucleotide monomers or deoxyribonucleotide monomers). "Nucleic acid" includes, for example, genomic DNA, cDNA, RNA, and DNA-RNA hybrid molecules. Nucleic acid molecules can be naturally occurring, recombinant, or synthetic. In addition, nucleic acid molecules can be single-stranded, double-stranded or triple-stranded. In certain embodiments, nucleic acid molecules can be modified. In the case of a double-stranded polymer, "nucleic acid" can refer to either or both strands of the molecule. Nucleic acids and polynucleotides as used herein are interchangeable.

[00107] The term "nucleotide sequence," in reference to a nucleic acid, refers to a contiguous series of nucleotides that are joined by covalent linkages, such as phosphorus linkages (*e.g.*, phosphodiester, alkyl and aryl-phosphonate, phosphorothioate, phosphotriester bonds), and/or non-phosphorus linkages (*e.g.*, peptide and/or sulfamate bonds). In certain embodiments, the nucleotide sequence encoding, *e.g.*, a target-binding molecule linked to a localizing domain is a heterologous sequence (*e.g.*, a gene that is of a different species or cell type origin).

[00108] The terms "nucleotide" and "nucleotide monomer" refer to naturally occurring ribonucleotide or deoxyribonucleotide monomers, as well as non-naturally occurring derivatives and analogs thereof. Accordingly, nucleotides can include, for example, nucleotides comprising naturally occurring bases (*e.g.*, adenosine, thymidine, guanosine, cytidine, uridine, inosine, deoxyadenosine, deoxythymidine, deoxyguanosine, or deoxycytidine) and nucleotides comprising modified bases known in the art.

[00109] The term "operably linked" refers to functional linkage between a regulatory sequence and a heterologous nucleic acid sequence resulting in expression of the latter. For example, a first nucleic acid sequence is operably linked with a second nucleic acid sequence when the first nucleic acid sequence is placed in a functional relationship with the second nucleic acid sequence. For instance, a promoter is operably linked to a coding sequence if the promoter

affects the transcription or expression of the coding sequence. Generally, operably linked DNA sequences are contiguous and, where necessary to join two protein coding regions, in the same reading frame.

[00110] The term “sequence identity” means that two nucleotide sequences or two amino acid sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least, *e.g.*, 70% sequence identity, or at least 80% sequence identity, or at least 85% sequence identity, or at least 90% sequence identity, or at least 95% sequence identity or more. For sequence comparison, typically one sequence acts as a reference sequence (*e.g.*, parent sequence), to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[00111] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel *et al.*, *Current Protocols in Molecular Biology*). One example of algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, *J. Mol. Biol.* 215:403 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (publicly accessible through the National Institutes of Health NCBI internet server). Typically, default program parameters can be used to perform the sequence comparison, although customized parameters can also be used. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

[00112] As will be appreciated by those of skill in the art, in some aspects, the nucleic acid further comprises a plasmid sequence. The plasmid sequence can include, for example, one or more sequences of a promoter sequence, a selection marker sequence, or a locus-targeting sequence.

[00113] The term "promoter" or "promoter element" as used herein is defined as a DNA sequence recognized by the synthetic machinery of the cell, or introduced synthetic machinery, required to initiate the specific transcription of a polynucleotide sequence.

[00114] The term "retroviral vector" can refer to a gammaretroviral vector. A retroviral vector may include, e.g., a promoter, a packaging signal, a primer binding site (PBS), one or more (e.g., two) long terminal repeats (LTR), and polynucleotides of interest, e.g., a polynucleotide encoding a CAR and a polynucleotide encoding a PEBL. A retroviral vector may lack viral structural genes such as gag, pol, and env. Exemplary retroviral (e.g., gammaretroviral) vectors include Murine Embryonic Stem Cell Virus (MESV), Murine Stem Cell Virus (MSCV), Murine Leukemia Virus (MLV), Spleen-Focus Forming Virus (SFFV), and Myeloproliferative Sarcoma Virus (MPSV), and vectors derived therefrom. Other gammaretroviral vectors are described, e.g., in Maetzig et al., *Viruses*, 2011; 3(6): 677-713.

[00115] The term "bicistronic expression" is typically achieved by operably linking the polynucleotides described herein to a promoter, and incorporating the bicistronic construct into an expression vector. The vectors can be suitable for replication and integration in eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence. The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal virus, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors.

[00116] "Expression vector" refers to a vector comprising a recombinant polynucleotide comprising expression control sequences operatively linked to a nucleotide sequence to be expressed. An expression vector comprises sufficient cis-acting elements for expression; other elements for expression can be supplied by the host cell or in an in vitro expression system. Expression vectors include all those known in the art, such as cosmids, plasmids (e.g., naked or

contained in liposomes) and viruses (e.g., lentiviruses, retroviruses, adenoviruses, and adeno-associated viruses) that incorporate the recombinant polynucleotide.

[00117] Further, the expression vector may be provided to a cell in the form of a viral vector. Viral vector technology is well known in the art and is described, for example, in Sambrook et al., 2012, *MOLECULAR CLONING: A LABORATORY MANUAL*, volumes 1-4, Cold Spring Harbor Press, NY), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentiviruses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[00118] Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the start site, although a number of promoters have been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription. Exemplary promoters include the immediate early cytomegalovirus (CMV), EF-1 α , ubiquitin C, or phosphoglycerokinase (PGK) promoters. A strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto can be used. Other constitutive promoter sequences may be used, including, but not limited to the simian virus 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency virus (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the elongation factor-1 Ovis leukemia virus promoter, an Epstein-Barr virus immediate early promoter, a Rous sarcoma virus promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, and the like. In some embodiments, the promoter is an inducible promoter provides a

molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

[00119] As used herein, “antibody” means an intact antibody or antigen-binding fragment of an antibody, including an intact antibody or antigen-binding fragment modified or engineered, or that is a human antibody. Examples of antibodies modified or engineered are chimeric antibodies, humanized antibodies, multiparatopic antibodies (*e.g.*, biparatopic antibodies), and multispecific antibodies (*e.g.*, bispecific antibodies). Examples of antigen-binding fragments include Fab, Fab', F(ab')₂, Fv, single chain antibodies (*e.g.*, scFv), minibodies and diabodies.

[00120] The term “specifically (or selectively) binds” or “specifically (or selectively) immunoreactive with,” when referring to a protein or peptide, refers to a binding reaction that is determinative of the presence of the protein, often in a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein at least two times the background and more typically more than 10 to 100 times background. Specific binding to an antibody under such conditions requires an antibody that is selected for its specificity for a particular protein. For example, polyclonal antibodies can be selected to obtain only those polyclonal antibodies that are specifically immunoreactive with the selected antigen and not with other proteins. This selection may be achieved by subtracting out antibodies that cross-react with other molecules. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (*see, e.g.*, Harlow & Lane, *Using Antibodies, A Laboratory Manual* (1998) for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity).

[00121] In certain embodiments, the antibody that binds CD7 is a single-chain variable fragment antibody (“scFv antibody”). scFv refers to antibody fragments comprising the VH and VL domains of an antibody, wherein these domains are present in a single polypeptide chain. Generally, the Fv polypeptide further comprises a polypeptide linker between the VH and VL

domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Pluckthun (1994) *The Pharmacology Of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315. See also, PCT Publication No. WO 88/01649 and U.S. Pat. Nos. 4,946,778 and 5,260,203. As would be appreciated by those of skill in the art, various suitable linkers can be designed and tested for optimal function, as provided in the art, and as disclosed herein.

[00122] As used herein, an “engineered” immune cell includes an immune cell that has been genetically modified as compared to a naturally-occurring immune cell. For example, an engineered T cell produced according to the present methods carries a nucleic acid comprising a nucleotide sequence that does not naturally occur in a T cell from which it was derived, such as the nucleic acids exemplified herein.

[00123] As used herein, a "substantially purified" cell is a cell that is essentially free of other cell types. A substantially purified cell also refers to a cell which has been separated from other cell types with which it is normally associated in its naturally occurring state. In some instances, a population of substantially purified cells refers to a homogenous population of cells. In other instances, this term refers simply to cell that have been separated from the cells with which they are naturally associated in their natural state. In some embodiments, the cells are cultured in vitro. In other embodiments, the cells are not cultured in vitro.

[00124] As used herein, a “CD7 CAR+/CD7-negative” T cell refers to a T cell expressing a chimeric antigen receptor against human CD7 and having low or no surface expression of endogenous CD7. In some embodiments, the low or no surface expression of endogenous CD7 is due to expression of a PEBL against human CD7 which prevents or hinders endogenous CD7 protein to translocated to the surface of the T cell. In some instances, surface expression of CD7 can be determined using standard methods known to those in the art such as but not limited to immunocytochemistry, flow cytometry, or FACS.

[00125] The term "autologous" and its grammatical equivalents as used herein can refer to as originating from the same being. For example, a sample (e.g., cells) can be removed, processed, and given back to the same subject (e.g., patient) at a later time. An autologous process is distinguished from an allogenic process where the donor and the recipient are different subjects.

[00126] "Allogeneic" refers to a graft derived from a different animal of the same species.

[00127] As used herein, the terms “treat,” “treating,” or “treatment,” refer to counteracting a medical condition (e.g., a condition related to a T cell malignancy) to the extent that the medical condition is improved according to a clinically-acceptable standard.

[00128] As used herein, “subject” refers to a mammal (e.g., human, non-human primate, cow, sheep, goat, horse, dog, cat, rabbit, guinea pig, rat, mouse). In certain embodiments, the subject is a human. A “subject in need thereof” refers to a subject (e.g., patient) who has, or is at risk for developing, a disease or condition that can be treated (e.g., improved, ameliorated, prevented) by inducing T cells to exert specific cytotoxicity against malignant T cells.

[00129] As defined herein, a “therapeutic amount” refers to an amount that, when administered to a subject, is sufficient to achieve a desired therapeutic effect (treats a condition related to a T cell malignancy) in the subject under the conditions of administration. An effective amount of the agent to be administered can be determined by a clinician of ordinary skill using the guidance provided herein and other methods known in the art, and is dependent on several factors including, for example, the particular agent chosen, the subject’s age, sensitivity, tolerance to drugs and overall well-being.

Detailed Description of the Embodiments

[00130] As described in detail below, the anti-CD7 CAR (also referred to as “CD7 CAR”) can comprise an antigen binding domain targeting CD7 based on the TH69 antibody. In some embodiments, the antigen binding domain of the CD7 CAR is based on the 3A1F antibody. In some embodiments, the antigen binding domain of the CD7 CAR is based on the T3-3A1 antibody. In some embodiments, the CD7 CAR of the present invention comprises an amino acid sequence selected from the group consisting of SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:31. In some embodiments, the CD7 CAR comprises an amino acid sequence having at least 90% sequence identity to one selected from the group consisting of SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, and SEQ ID NO:31. In some cases, the engineered immune cell of the present invention comprises the CD7 CAR of SEQ ID NO:28. In some cases, the engineered immune cell comprises the CD7 CAR having at least 90% sequence identity to SEQ ID NO:28. In some cases, the engineered immune cell comprises the CD7 CAR of SEQ ID NO:29. In some cases, the engineered immune cell comprises the CD7 CAR having at least 90%

sequence identity to SEQ ID NO:30. In some cases, the engineered immune cell comprises the CD7 CAR having at least 90% sequence identity to SEQ ID NO:30. In some cases, the engineered immune cell comprises the CD7 CAR having at least 90% sequence identity to SEQ ID NO:31. In some cases, the engineered immune cell comprises the CD7 CAR having at least 90% sequence identity to SEQ ID NO:31.

[00131] In some embodiments, the CD7 PEBL of the present invention comprises an amino acid sequence selected from the group consisting of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27. In some embodiments, the CD7 PEBL of the present invention comprises an amino acid sequence having at least 90% sequence identity to one selected from the group consisting of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, and SEQ ID NO:27. In some instances, the engineered immune cell of the present invention comprises the CD7 PEBL of SEQ ID NO: 24. In some instances, the engineered immune cell of the present invention comprises the CD7 PEBL having at least 90% sequence identity to SEQ ID NO: 25. In some instances, the engineered immune cell comprises the CD7 PEBL of SEQ ID NO: 24. In some instances, the engineered immune cell comprises the CD7 PEBL having at least 90% sequence identity to SEQ ID NO: 25. In some instances, the engineered immune cell comprises the CD7 PEBL of SEQ ID NO: 26. In some instances, the engineered immune cell comprises the CD7 PEBL having at least 90% sequence identity to SEQ ID NO: 26. In some instances, the engineered immune cell comprises the CD7 PEBL of SEQ ID NO: 27. In some instances, the engineered immune cell comprises the CD7 PEBL having at least 90% sequence identity to SEQ ID NO: 27.

[00132] In some embodiments, the engineered immune cell or population of engineered immune cells of the present invention comprises a CD7 PEBL of SEQ ID NO: 24 and a CD7 CAR of SEQ ID NO:28. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL having at least 90% sequence identity to SEQ ID NO: 24 and a CD7 CAR having at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL of SEQ ID NO: 24 and a CD7 CAR of SEQ ID NO:30. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL having at least 90% sequence identity SEQ ID NO: 24 and a CD7 CAR having at least 90% sequence identity SEQ ID NO:30. In some embodiments, the engineered immune cell or

population of engineered immune cells comprises a CD7 PEBL of SEQ ID NO: 26 and a CD7 CAR of SEQ ID NO:28. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL having at least 90% sequence identity SEQ ID NO: 26 and a CD7 CAR having at least 90% sequence identity SEQ ID NO:28. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL of SEQ ID NO: 26 and a CD7 CAR of SEQ ID NO:308. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL having at least 90% sequence identity SEQ ID NO: 26 and a CD7 CAR having at least 90% sequence identity SEQ ID NO:30. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL of SEQ ID NO: 25 and a CD7 CAR of SEQ ID NO:29. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL having at least 90% sequence identity SEQ ID NO: 25 and a CD7 CAR having at least 90% sequence identity SEQ ID NO:29. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL of SEQ ID NO: 25 and a CD7 CAR of SEQ ID NO:31. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL having at least 90% sequence identity SEQ ID NO: 25 and a CD7 CAR having at least 90% sequence identity SEQ ID NO:31. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL of SEQ ID NO: 27 and a CD7 CAR of SEQ ID NO:29. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL having at least 90% sequence identity SEQ ID NO: 27 and a CD7 CAR having at least 90% sequence identity SEQ ID NO:29. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL of SEQ ID NO: 27 and a CD7 CAR of SEQ ID NO:31. In some embodiments, the engineered immune cell or population of engineered immune cells comprises a CD7 PEBL having at least 90% sequence identity SEQ ID NO: 27 and a CD7 CAR having at least 90% sequence identity SEQ ID NO:31.

[00133] In certain embodiments, the engineered immune cell is an engineered T cell. In some embodiments, the engineered immune cell is an engineered CD4⁺ T cell. In some embodiments, the engineered immune cell is an engineered CD8⁺ T cell. In some embodiments, the engineered immune cell harboring the bicistronic construct or dual-promoter construct is

generated from PBMCs. In some embodiments, the engineered immune cell harboring the bicistronic construct or dual-promoter construct is generated from purified CD4⁺ T cells. In some embodiments, the engineered immune cell harboring the bicistronic construct or dual-promoter construct is generated from purified CD8⁺ T cells. In some embodiments, the engineered immune cell harboring the bicistronic construct or dual-promoter construct is generated from a population for cells comprising purified CD4⁺ T cells and purified CD8⁺ T cells. In some embodiments, the engineered immune cell harboring the bicistronic construct or dual-promoter construct is generated from a population for cells comprising purified CD3⁺ T cells.

Bicistronic expression constructs

[00134] Provided herein are recombinant bicistronic viral constructs or vectors that contain a polynucleotide encoding a CAR and a polynucleotide encoding a PEBL, as described herein. In some embodiments, the recombinant bicistronic viral construct includes an internal ribosomal entry site (IRES) sequence between the nucleic acid sequence of the CAR and the nucleic acid sequence of the PEBL. In some embodiments, the recombinant bicistronic viral construct includes a ribosomal codon skipping site sequence (also referred to as a sequence encoding a 2A self-cleaving peptide) between the nucleic acid sequence of the CAR and the nucleic acid sequence of the PEBL. In some embodiments of a bicistronic construct, a polynucleotide encoding a CAR is located upstream (at the 5' end) of an IRES sequence, and a polynucleotide encoding a PEBL is located downstream (at the 3' end) of the IRES. In some cases, a nucleic acid sequence encoding a CAR is operably linked to an IRES sequence and an IRES sequence is operably linked to a nucleic acid sequence encoding a PEBL. In some cases, a nucleic acid sequence encoding a PEBL is operably linked to an IRES sequence and an IRES sequence is operably linked to a nucleic acid sequence encoding a CAR.

[00135] In some embodiments of a bicistronic construct, a polynucleotide encoding a CAR is located upstream (at the 5' end) of a polynucleotide encoding 2A self-cleaving peptide, and a polynucleotide encoding a PEBL is located downstream (at the 3' end) of the polynucleotide encoding 2A self-cleaving peptide. In some cases, a nucleic acid sequence encoding a CAR is operably linked to a nucleic acid sequence encoding a 2A self-cleaving peptide, which is operably linked to a nucleic acid sequence encoding a PEBL. In some cases, a nucleic acid

sequence encoding a PEBL is operably linked to a nucleic acid sequence encoding a 2A self-cleaving peptide, which is operably linked to a nucleic acid sequence encoding a CAR.

[00136] The mechanism of ribosomal codon skipping via a 2A peptide sequence is useful for generating two proteins from one transcript; a normal peptide bond is impaired at the 2A sequence, resulting in two discontinuous protein fragments from one translation event. Self-cleaving 2A peptides (e.g., 2A cleavage sites) are described in Kim et al., PLoS One, 2011, 6(4):e18556.

[00137] In some embodiments, the IRES is from an *Encephalomyocarditis* virus. In some embodiments, the IRES is from an *Enterovirus*. In some embodiments, the nucleic acid sequence of the IRES sequence is set forth in SEQ ID NO:62 (see, e.g., Table 1).

[00138] In some embodiments, the ribosomal codon skipping site is based on a 2A self-cleaving peptide (see, e.g., Table 2). In some embodiments, the 2A self-cleaving peptide is selected from the group consisting of P2A, E2A, F2A, and T2A. In some instances, the amino acid sequence of the P2A peptide comprises the amino acid sequence of SEQ ID NO:67, or an amino acid sequence having at least 90% sequence identity thereto. In some instances, the amino acid sequence of the E2A peptide comprises the amino acid sequence of SEQ ID NO:68, or an amino acid sequence having at least 90% sequence identity thereto. In some instances, the amino acid sequence of the F2A peptide comprises the amino acid sequence of SEQ ID NO:69, or an amino acid sequence having at least 90% sequence identity thereto. In some instances, the amino acid sequence of the T2A peptide comprises the amino acid sequence of SEQ ID NO:70, or an amino acid sequence having at least 90% sequence identity thereto.

[00139] In some embodiments, the viral construct (e.g., retroviral construct) comprises a nucleic acid sequence encoding a 2A self-cleaving peptide (e.g., 2A peptide cleavage site) selected from the group consisting of P2A, E2A, F2A, and T2A, wherein the polynucleotide encoding 2A self-cleaving peptide links the nucleic acid sequence encoding the CAR and the nucleic acid sequence encoding the PEBL. In other words, the polynucleotide encoding 2A self-cleaving peptide is between the nucleic acid sequence encoding the CAR and the nucleic acid sequence encoding the PEBL. As described above, in some embodiments, the construct comprises or consisting of from 5' end to 3' end: a nucleic acid sequence encoding a CAR, a nucleic acid sequence encoding a 2A self-cleaving peptide, and a nucleic acid sequence

encoding a PEBL. In some embodiments, the construct comprises or consisting of from 5' end to 3' end: a nucleic acid sequence encoding any CD7 CAR described herein, a nucleic acid sequence encoding a P2A self-cleaving peptide, and a nucleic acid sequence encoding any CD7 PEBL described herein. In some embodiments, the construct comprises or consisting of from 5' end to 3' end: a nucleic acid sequence encoding any CD7 CAR described herein, a nucleic acid sequence encoding an E2A self-cleaving peptide, and a nucleic acid sequence encoding any CD7 PEBL described herein. In some embodiments, the construct comprises or consisting of from 5' end to 3' end: a nucleic acid sequence encoding any CD7 CAR described herein, a nucleic acid sequence encoding an F2A self-cleaving peptide, and a nucleic acid sequence encoding any CD7 PEBL described herein. In some embodiments, the construct comprises or consisting of from 5' end to 3' end: a nucleic acid sequence encoding any CD7 CAR described herein, a nucleic acid sequence encoding a T2A self-cleaving peptide, and a nucleic acid sequence encoding any CD7 PEBL described herein.

[00140] In some embodiments, the construct comprises or consisting of from 5' end to 3' end: a nucleic acid sequence encoding a PEBL, a nucleic acid sequence encoding a P2A self-cleaving peptide, and a nucleic acid sequence encoding a CAR. In some embodiments, the construct comprises or consisting of from 5' end to 3' end: a nucleic acid sequence encoding a PEBL, a nucleic acid sequence encoding an E2A self-cleaving peptide, and a nucleic acid sequence encoding a CAR. In some embodiments, the construct comprises or consisting of from 5' end to 3' end: a nucleic acid sequence encoding a PEBL, a nucleic acid sequence encoding an F2A self-cleaving peptide, and a nucleic acid sequence encoding a CAR. In some embodiments, the construct comprises or consisting of from 5' end to 3' end: a nucleic acid sequence encoding a PEBL, a nucleic acid sequence encoding a T2A self-cleaving peptide, and a nucleic acid sequence encoding a CAR.

[00141] In some embodiments, the nucleic acid sequence encoding the P2A comprises or consisting of a nucleic acid having at least 90% sequence identity to SEQ ID NO:63. In some embodiments, the nucleic acid sequence encoding the P2A comprises or consisting of a nucleic acid of SEQ ID NO:63. In some embodiments, the nucleic acid sequence encoding the E2A comprises or consisting of a nucleic acid having at least 90% sequence identity to SEQ ID NO:64. In some embodiments, the nucleic acid sequence encoding the E2A comprises or consisting of a nucleic acid of SEQ ID NO:64. In some embodiments, the nucleic acid sequence

encoding the F2A comprises or consisting of a nucleic acid having at least 90% sequence identity to SEQ ID NO:65. In some embodiments, the nucleic acid sequence encoding the F2A comprises or consisting of a nucleic acid of SEQ ID NO:65. In some embodiments, the nucleic acid sequence encoding the T2A comprises or consisting of a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO:66. In some embodiments, the nucleic acid sequence encoding the T2A comprises or consisting of a nucleic acid of SEQ ID NO:66.

[00142] In some embodiments, the nucleic acid sequence encoding the PEBL is disposed (e.g., located) 5' to the nucleic acid sequence encoding the CAR. In some embodiments, the nucleic acid sequence encoding the CAR is disposed 5' to the nucleic acid sequence encoding the PEBL. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: (a1) SEQ ID NO:4, SEQ ID NO:63, and SEQ ID NO:2; (a2) SEQ ID NO:4, SEQ ID NO:63, and SEQ ID NO:3; (a3) SEQ ID NO:5, SEQ ID NO:63, and SEQ ID NO:2; (a4) SEQ ID NO:5, SEQ ID NO:63, and SEQ ID NO:3; (b1) SEQ ID NO:4, SEQ ID NO:64, and SEQ ID NO:2; (b2) SEQ ID NO:4, SEQ ID NO: 64, and SEQ ID NO:3; (b3) SEQ ID NO:5, SEQ ID NO: 64, and SEQ ID NO:2; (b4) SEQ ID NO:5, SEQ ID NO: 64, and SEQ ID NO:3; (c1) SEQ ID NO:4, SEQ ID NO:65, and SEQ ID NO:2; (c2) SEQ ID NO:4, SEQ ID NO: 65, and SEQ ID NO:3; (c3) SEQ ID NO:5, SEQ ID NO: 65, and SEQ ID NO:2; (c4) SEQ ID NO:5, SEQ ID NO: 65, and SEQ ID NO:3; (d1) SEQ ID NO:4, SEQ ID NO:66, and SEQ ID NO:2; (d2) SEQ ID NO:4, SEQ ID NO: 66, and SEQ ID NO:3; (d3) SEQ ID NO:5, SEQ ID NO: 66, and SEQ ID NO:2; (d4) SEQ ID NO:5, SEQ ID NO: 66, and SEQ ID NO:3; (e1) SEQ ID NO:2, SEQ ID NO:63, and SEQ ID NO:4; (e2) SEQ ID NO:3, SEQ ID NO:63, and SEQ ID NO:4; (e3) SEQ ID NO:2, SEQ ID NO:63, and SEQ ID NO:5; (e4) SEQ ID NO:3, SEQ ID NO:63, and SEQ ID NO:5; (f1) SEQ ID NO:2, SEQ ID NO:64, and SEQ ID NO:4; (f2) SEQ ID NO:3, SEQ ID NO:64, and SEQ ID NO:4; (f3) SEQ ID NO:2, SEQ ID NO:63, and SEQ ID NO:5; (f4) SEQ ID NO:3, SEQ ID NO:64, and SEQ ID NO:5; (g1) SEQ ID NO:2, SEQ ID NO:65, and SEQ ID NO:4; (g2) SEQ ID NO:3, SEQ ID NO:65, and SEQ ID NO:4; (g3) SEQ ID NO:2, SEQ ID NO:65, and SEQ ID NO:5; (g4) SEQ ID NO:3, SEQ ID NO:65, and SEQ ID NO:5; (h1) SEQ ID NO:2, SEQ ID NO:66, and SEQ ID NO:4; (h2) SEQ ID NO:3, SEQ ID NO:66, and SEQ ID NO:4; (h3) SEQ ID NO:2, SEQ ID NO:66, and SEQ ID NO:5; or (h4) SEQ ID NO:3, SEQ ID NO:66, and SEQ ID NO:5.

[00143] In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: the sequence of FIG. 16, SEQ ID NO: 63, and the sequence of FIG. 18. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: the sequence of FIG. 16, any one of SEQ ID NOS: 63-66, and the sequence of FIG. 19. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: the sequence of FIG. 17, any one of SEQ ID NOS: 63-66, and the sequence of FIG. 18. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: the sequence of FIG. 17, any one of SEQ ID NOS: 63-66, and the sequence of FIG. 19.

[00144] In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: the sequence of FIG. 18, any one of SEQ ID NOS: 63-66, and the sequence of FIG. 16. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: the sequence of FIG. 19, any one of SEQ ID NOS: 63-66, and the sequence of FIG. 16. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: the sequence of FIG. 18, any one of SEQ ID NOS: 63-66, and the sequence of FIG. 17. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: the sequence of FIG. 19, any one of SEQ ID NOS: 63-66, and the sequence of FIG. 17.

[00145] In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of SEQ ID NO:28, a polynucleotide encoding a P2A peptide of SEQ ID NO:67, and a polynucleotide encoding a CD7 (TH67) PEBL of SEQ ID NO:24. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of FIG. 42, a polynucleotide encoding a P2A peptide of SEQ ID NO:67, and a polynucleotide encoding a CD7 (TH67) PEBL of FIG. 38. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of SEQ ID NO:28, a polynucleotide encoding a P2A peptide of SEQ ID NO:67, and a polynucleotide encoding a CD7 (3A1F) PEBL of SEQ ID NO:26. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of FIG. 42, a polynucleotide encoding a P2A peptide of SEQ ID NO:67, and a polynucleotide encoding a CD7 (3A1F) PEBL of FIG. 40. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of SEQ ID NO:30, a polynucleotide encoding a P2A peptide of SEQ ID NO:67, and a polynucleotide encoding a CD7 (TH67) PEBL

of SEQ ID NO:24. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of FIG. 44, a polynucleotide encoding a P2A peptide of SEQ ID NO:67, and a polynucleotide encoding a CD7 (TH67) PEBL of FIG. 38. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of SEQ ID NO:30, a polynucleotide encoding a P2A peptide of SEQ ID NO:67, and a polynucleotide encoding a CD7 (3A1F) PEBL of SEQ ID NO:26. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of FIG. 44, a polynucleotide encoding a P2A peptide of SEQ ID NO:67, and a polynucleotide encoding a CD7 (3A1F) PEBL of FIG. 40. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of SEQ ID NO:28, a polynucleotide encoding a P2A peptide of SEQ ID NO:68, and a polynucleotide encoding a CD7 (TH67) PEBL of SEQ ID NO:24. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of FIG. 42, a polynucleotide encoding a P2A peptide of SEQ ID NO:68, and a polynucleotide encoding a CD7 (TH67) PEBL of FIG. 38. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of SEQ ID NO:28, a polynucleotide encoding a P2A peptide of SEQ ID NO:68, and a polynucleotide encoding a CD7 (3A1F) PEBL of SEQ ID NO:26. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of FIG. 42, a polynucleotide encoding a P2A peptide of SEQ ID NO:68, and a polynucleotide encoding a CD7 (3A1F) PEBL of FIG. 40. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of SEQ ID NO:30, a polynucleotide encoding a P2A peptide of SEQ ID NO:68, and a polynucleotide encoding a CD7 (TH67) PEBL of SEQ ID NO:24. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of FIG. 44, a polynucleotide encoding a P2A peptide of SEQ ID NO:68, and a polynucleotide encoding a CD7 (TH67) PEBL of FIG. 38. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of SEQ ID NO:30, a polynucleotide encoding a P2A peptide of SEQ ID NO:68, and a polynucleotide encoding a CD7 (3A1F) PEBL of SEQ ID NO:26. In some embodiments, a bicistronic construct comprises or consists of from

5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of FIG. 44, a polynucleotide encoding a P2A peptide of SEQ ID NO:68, and a polynucleotide encoding a CD7 (3A1F) PEBL of FIG. 40. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of SEQ ID NO:28, a polynucleotide encoding a P2A peptide of SEQ ID NO:69, and a polynucleotide encoding a CD7 (TH67) PEBL of SEQ ID NO:24. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of FIG. 42, a polynucleotide encoding a P2A peptide of SEQ ID NO:69, and a polynucleotide encoding a CD7 (TH67) PEBL of FIG. 38. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of SEQ ID NO:28, a polynucleotide encoding a P2A peptide of SEQ ID NO:69, and a polynucleotide encoding a CD7 (3A1F) PEBL of SEQ ID NO:26. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of FIG. 42, a polynucleotide encoding a P2A peptide of SEQ ID NO:69, and a polynucleotide encoding a CD7 (3A1F) PEBL of FIG. 40. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of SEQ ID NO:30, a polynucleotide encoding a P2A peptide of SEQ ID NO:69, and a polynucleotide encoding a CD7 (TH67) PEBL of SEQ ID NO:24. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of FIG. 44, a polynucleotide encoding a P2A peptide of SEQ ID NO:69, and a polynucleotide encoding a CD7 (TH67) PEBL of FIG. 38. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of SEQ ID NO:30, a polynucleotide encoding a P2A peptide of SEQ ID NO:69, and a polynucleotide encoding a CD7 (3A1F) PEBL of SEQ ID NO:26. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of FIG. 44, a polynucleotide encoding a P2A peptide of SEQ ID NO:69, and a polynucleotide encoding a CD7 (3A1F) PEBL of FIG. 40. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of SEQ ID NO:28, a polynucleotide encoding a P2A peptide of SEQ ID NO:70, and a polynucleotide encoding a CD7 (TH67) PEBL of SEQ ID NO:24. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of FIG. 42, a polynucleotide

encoding a P2A peptide of SEQ ID NO:70, and a polynucleotide encoding a CD7 (TH67) PEBL of FIG. 38. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of SEQ ID NO:28, a polynucleotide encoding a P2A peptide of SEQ ID NO:70, and a polynucleotide encoding a CD7 (3A1F) PEBL of SEQ ID NO:26. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (TH67) CAR of FIG. 42, a polynucleotide encoding a P2A peptide of SEQ ID NO:70, and a polynucleotide encoding a CD7 (3A1F) PEBL of FIG. 40. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of SEQ ID NO:30, a polynucleotide encoding a P2A peptide of SEQ ID NO:70, and a polynucleotide encoding a CD7 (TH67) PEBL of SEQ ID NO:24. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of FIG. 44, a polynucleotide encoding a P2A peptide of SEQ ID NO:70, and a polynucleotide encoding a CD7 (TH67) PEBL of FIG. 38. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of SEQ ID NO:30, a polynucleotide encoding a P2A peptide of SEQ ID NO:70, and a polynucleotide encoding a CD7 (3A1F) PEBL of SEQ ID NO:26. In some embodiments, a bicistronic construct comprises or consists of from 5' to 3' end: a polynucleotide encoding a CD7 (3A1F) CAR of FIG. 44, a polynucleotide encoding a P2A peptide of SEQ ID NO:70, and a polynucleotide encoding a CD7 (3A1F) PEBL of FIG. 40.

[00146] In some embodiments, the polynucleotide sequence encoding the PEBL is disposed 5' (upstream) of an IRES site and the IRES site is disposed 5' to the polynucleotide sequence encoding the CAR. In some embodiments, the polynucleotide sequence encoding the CAR is disposed 5' of an IRES site and the IRES site is disposed 5' to the polynucleotide sequence encoding the PEBL.

[00147] In some embodiments, the polynucleotide sequence encoding the PEBL is disposed 5' (upstream) of the ribosomal codon skipping site and the ribosomal codon skipping site is disposed 5' to the polynucleotide sequence encoding the CAR. In some embodiments, the polynucleotide sequence encoding the CAR is disposed 5' of the ribosomal codon skipping site and the ribosomal codon skipping site is disposed 5' to the polynucleotide sequence encoding the PEBL.

[00148] In some aspects, provided herein is a recombinant bicistronic construct comprising at least 90% sequence identity to a nucleic acid sequence of one or more selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, and SEQ ID NO:66. In some embodiments, the recombinant bicistronic construct comprises at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO:63. In some embodiments, the recombinant bicistronic construct comprises at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO:64. In some embodiments, the recombinant bicistronic construct comprises at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO:65. In some embodiments, the recombinant bicistronic construct comprises at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO:66. In some embodiments, the recombinant bicistronic construct comprises a nucleic acid sequence of one selected from the group consisting of SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, and SEQ ID NO:66.

Table 1. Nucleic acid sequences of ribosomal codon skipping peptides and IRES

Name	SEQ ID NO	Nucleic Acid Sequence
IRES	SEQ ID NO:62	CGGGATCAATTCCGCCCCCCCCCTAACGTTACTGGCCGAAGCCGCT TGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCAT ATTGCCGTCTTTTGGCAATGTGAGGGCCCGGAAACCTGGCCCTGTC TTCTTGACGAGCATTCCTAGGGGTCTTTCCCTCTCGCCAAAGGAA TGCAAGGTCTGTTGAATGTCTGAAGGAAGCAGTTCCTCTGGAAGC TTCTTGAAGACAAACAACGTCTGTAGCGACCCCTTTCAGGCAGCGG AACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTG TATAAGATACACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGT GAGTTGGATAGTTGTGGAAGAGTCAAATGGCTCTCCTCAAGCGTA TTCAACAAGGGGCTGAAGGATGCCAGAAGGTACCCATTGTATGG GATCTGATCTGGGGCCTCGGTGCACATGCTTTACATGTGTTTAGTC GAGGTTAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTGGTT TTCCTTTGAAAAACACGATAATACC
P2A	SEQ ID NO:63	GCCACAACTTCTCTCTGCTAAAGCAAGCAGGTGATGTTGAAGAAA ACCCCGGGCCT
E2A	SEQ ID NO:64	CAGTGTAATAATTATGCTCTCTTGAAATTGGCTGGAGATGTTGAGA GCAACGGAGGTCCC
F2A	SEQ ID NO:65	GTGAAACAGACTTTGAATTTTGACCTTCTCAAGTTGGCGGGAGACG TGGAGTCCAACCTGGACCT
T2A	SEQ ID NO:66	GAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATC CCGGCCCA

Table 2. Amino acid sequences of ribosomal codon skipping sites

Name	SEQ ID NO	Amino Acid Sequence
P2A	SEQ ID NO:67	ATNFSLLKQAGDVEENPGP
E2A	SEQ ID NO:68	QCTNYALLKLAGDVESNPGP
F2A	SEQ ID NO:69	VKQTLNFDLLKLAGDVESNPGP
T2A	SEQ ID NO:70	EGRGSLLTCGDVEENPGP

[00149] The present invention provides vectors such as expression vectors in which any of the polynucleotides described herein is inserted. In some embodiments, the vector is derived from retroviruses such as lentiviruses. Such vectors are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of an exogenous polynucleotide (e.g., transgene) and its propagation in daughter cells. Unlike vectors derived from onco-retroviruses such as murine leukemia viruses, lentiviral vectors can transduce non-proliferating cells. Lentiviral vectors also have low immunogenicity. In other embodiments, the vector is an adenoviral vector. In certain embodiments, the vector is a plasmid.

[00150] In some embodiments, the promoter comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to a CMV promoter. In some embodiments, the promoter comprises a CMV promoter. In some embodiments, the CMV promoter comprises the sequence of SEQ ID NO:6. In some embodiments, any of the constructs described herein comprises or consists of a CMV promoter of SEQ ID NO:6.

[00151] In some embodiments, the promoter comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to an EF1 α promoter. In some embodiments, the promoter comprises an EF1 α promoter. In some embodiments, the EF1 α promoter comprises the sequence of SEQ ID NO:7. In some embodiments, the CMV promoter comprises the sequence of SEQ ID NO:6. In some embodiments, any of the constructs described herein comprises or consists of an EF1 α promoter of SEQ ID NO:7.

[00152] In some embodiments, the promoter comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to an EFS promoter. In some embodiments, the promoter comprises an EFS promoter. In some embodiments, the EFS promoter comprises the sequence of SEQ ID NO:8. In some embodiments, the CMV

promoter comprises the sequence of SEQ ID NO:6. In some embodiments, any of the constructs described herein comprises or consists of an EFS promoter of SEQ ID NO:8.

[00153] In some embodiments, the promoter comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to a murine stem cell virus (MSCV) promoter. In some embodiments, the promoter comprises a MSCV promoter. In some embodiments, the MSCV promoter comprises the sequence of SEQ ID NO:9. In some embodiments, any of the constructs described herein comprises or consists of a MSCV promoter of SEQ ID NO:9.

[00154] In some embodiments, the promoter comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to a phosphoglycerate kinase (PGK) promoter. In some embodiments, the promoter comprises a PGK promoter. In some embodiments, the PGK promoter comprises the sequence of SEQ ID NO:10. In some embodiments, any of the constructs described herein comprises or consists of a PGK promoter of SEQ ID NO:10.

[00155] In some embodiments, the bicistronic vector comprises or consists of the nucleic acid sequence of SEQ ID NO:11. An exemplary embodiment of such a sequence is depicted in FIG. 25. In some embodiments, the bicistronic vector comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to the sequence of SEQ ID NO:11. The bicistronic vector comprises a nucleic acid sequence comprising from 5' end to 3' end: a nucleic acid sequence encoding a CD7 PEBL, an IRES sequence, and a nucleic acid sequence encoding a CD7 CAR, and optionally at the 5' end, a promoter selected from the group consisting of a CMV promoter (e.g., SEQ ID NO:6 or FIG. 20), EF1a promoter (e.g., SEQ ID NO:7 or FIG. 21), EFS promoter (e.g., SEQ ID NO:8 or FIG. 22), MSCV promoter (e.g., SEQ ID NO:9 or FIG. 23), and PGK promoter (e.g., SEQ ID NO:10 or FIG. 24).

[00156] In some embodiments, the bicistronic vector comprises the nucleic acid sequence of SEQ ID NO:12. In some embodiments, the bicistronic vector comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to the sequence of SEQ ID NO:12. An exemplary embodiment of such a sequence is depicted in FIG. 26. The bicistronic vector comprises a nucleic acid sequence comprising from 5' end to 3' end: a nucleic acid sequence encoding a CD7 CAR, an IRES sequence, and a nucleic acid sequence

encoding a CD7 PEBL, optionally at the 5' end, a promoter selected from the group consisting of a CMV promoter, EF1a promoter, EFS promoter, MSCV promoter, and PGK promoter.

[00157] In some embodiments, the bicistronic vector comprises the nucleic acid sequence of SEQ ID NO:13. An exemplary embodiment of such a sequence is depicted in FIG. 27. In some embodiments, the bicistronic vector comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to the sequence of SEQ ID NO:13. The bicistronic vector comprises a nucleic acid sequence comprising from 5' end to 3' end: a nucleic acid sequence encoding a CD7 CAR, a nucleic acid sequence encoding a P2A peptide, and a nucleic acid sequence encoding a CD7 PEBL, optionally at the 5' end, a promoter selected from the group consisting of a CMV promoter, EF1a promoter, EFS promoter, MSCV promoter, and PGK promoter.

[00158] In some embodiments, the bicistronic vector comprises the nucleic acid sequence of SEQ ID NO:14. In some embodiments, the bicistronic vector comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to the sequence of SEQ ID NO:14. The bicistronic vector comprises a nucleic acid sequence comprising from 5' end to 3' end: a promoter, a nucleic acid sequence encoding a CD7 CAR, a P2A sequence, and a nucleic acid sequence encoding a CD7 PEBL. In some instances the bicistronic vector comprises a nucleic acid sequence comprising from 5' end to 3' end: a MSCV promoter, a nucleic acid sequence encoding a CD7 CAR, a nucleic acid sequence encoding a P2A peptide, and a nucleic acid sequence encoding a CD7 PEBL. An exemplary embodiment of such a sequence is depicted in FIG. 28A-FIG. 28B.

[00159] In some embodiments, the bicistronic vector comprises the nucleic acid sequence of SEQ ID NO:15. In some embodiments, the bicistronic vector comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to the sequence of SEQ ID NO:15. In some instances the bicistronic vector comprises a nucleic acid sequence comprising from 5' end to 3' end: an EF1a promoter, a nucleic acid sequence encoding a CD7 CAR, a nucleic acid sequence encoding a P2A peptide, and a nucleic acid sequence encoding a CD7 PEBL. An exemplary embodiment of such a sequence is depicted in FIG. 29A-FIG. 29B.

[00160] In some embodiments, the bicistronic vector comprises the nucleic acid sequence of SEQ ID NO:16. In some embodiments, the bicistronic vector comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to the sequence of SEQ ID NO:16. In some instances the bicistronic vector comprises a nucleic acid sequence comprising from 5' end to 3' end: an EFS promoter, a nucleic acid sequence encoding a CD7 CAR, a nucleic acid sequence encoding a P2A peptide, and a nucleic acid sequence encoding a CD7 PEBL. An exemplary embodiment of such a sequence is depicted in FIG. 30A-FIG. 30B.

Dual promoter retroviral constructs

[00161] Provided herein are recombinant retroviral constructs (or vectors) for simultaneous expression of a CAR and a PEBL in a cell such as a T cell. In some embodiments, the retroviral constructs include a promoter operably linked to a polynucleotide encoding any of the CARs described herein and a promoter operably linked to a polynucleotide encoding any of the PEBLs described herein. In some embodiments, the promoter for the CAR and the promoter for the PEBL share less than 90% sequence identity, e.g., less than 90% identity, less than 80% identity, less than 75% sequence identity, less than 70% sequence identity, less than 65% sequence identity, less than 60% sequence identity, less than 55% sequence identity, and the like. In some embodiments, the promoter for the CAR and the promoter for the PEBL share 80% sequence identity or less, e.g., 80% identity, 75% sequence identity, 70% sequence identity, 65% sequence identity, 60% sequence identity, 55% sequence identity, and the like. In some embodiments, the promoter for the CAR and the promoter for the PEBL share at least 50% sequence identity, e.g., 50% sequence identity, 55% sequence identity, 60% sequence identity, 65% sequence identity, 70% sequence identity, 75% sequence identity, 80% sequence identity, 85% sequence identity, 90% sequence identity, 95% sequence identity, or more sequence identity.

[00162] In some embodiments, the promoter for the CAR (referred to as the first promoter) is different than the promoter for the PEBL (referred to as the second promoter). The first promoter and the second promoter can have the same sequence. In other instances, the first promoter and the second promoter have different sequences.

[00163] In some embodiments, the first promoter and/or second promoter comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to a CMV promoter. In some embodiments, the first promoter and/or second promoter comprises a CMV promoter. In some embodiments, the CMV promoter comprises the sequence of SEQ ID NO:6.

[00164] In some embodiments, the first promoter and/or second promoter comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to an EF1 α promoter. In some embodiments, the first promoter and/or second promoter comprises an EF1 α promoter. In some embodiments, the EF1 α promoter comprises the sequence of SEQ ID NO:7.

[00165] In some embodiments, the first promoter and/or second promoter comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to an EFS promoter. In some embodiments, the first promoter and/or second promoter comprises an EFS promoter. In some embodiments, the EFS promoter comprises the sequence of SEQ ID NO:8.

[00166] In some embodiments, the first promoter and/or second promoter comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to a murine stem cell virus (MSCV) promoter. In some embodiments, the first promoter and/or second promoter comprises a MSCV promoter. In some embodiments, the MSCV promoter comprises the sequence of SEQ ID NO:9.

[00167] In some embodiments, the first promoter and/or second promoter comprises at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to a phosphoglycerate kinase (PGK) promoter. In some embodiments, the first promoter and/or second promoter comprises a PGK promoter. In some embodiments, the PGK promoter comprises the sequence of SEQ ID NO:10.

[00168] In some embodiments, the retroviral constructs from 5' to 3' include the first promoter operably linked to the polynucleotide encoding the CAR and the second promoter operably linked to the polynucleotide encoding the PEBL. In various embodiments, the retroviral constructs from 5' to 3' include the second promoter operably linked to the polynucleotide

encoding the PEBL and the first promoter operably linked to the polynucleotide encoding the CAR.

[00169] In some embodiments, the first promoter is located upstream of the second promoter. In some embodiments, the first promoter is a CMV promoter and the second promoter is an EFS promoter. In some embodiments, the first promoter is a CMV promoter and the second promoter is an EF1 α promoter. In some embodiments, the first promoter is a CMV promoter and the second promoter is a PGK promoter. In some embodiments, the first promoter is a CMV promoter and the second promoter is a MSCV promoter. In some embodiments, the first promoter is a CMV promoter and the second promoter is a CMV promoter. In some embodiments, the first promoter is a MSCV promoter and the second promoter is an EFS promoter. In some embodiments, the first promoter is a MSCV promoter and the second promoter is an EF1 α promoter. In some embodiments, the first promoter is a MSCV promoter and the second promoter is a PGK promoter. In some embodiments, the first promoter is a MSCV promoter and the second promoter is a CMV promoter. In some embodiments, the first promoter is a MSCV promoter and the second promoter is a MSCV promoter. In some embodiments, the first promoter is a PGK promoter and the second promoter is an EFS promoter. In some embodiments, the first promoter is a PGK promoter and the second promoter is an EF1 α promoter. In some embodiments, the first promoter is a PGK promoter and the second promoter is a MSCV promoter. In some embodiments, the first promoter is a PGK promoter and the second promoter is a CMV promoter. In some embodiments, the first promoter is a PGK promoter and the second promoter is a PGK promoter. In some embodiments, the first promoter is an EF1 α promoter and the second promoter is an MSCV promoter. In some embodiments, the first promoter is an EF1 α promoter and the second promoter is an PGK promoter. In some embodiments, the first promoter is an EF1 α promoter and the second promoter is an EFS promoter. In some embodiments, the first promoter is an EF1 α promoter and the second promoter is a CMV promoter. In some embodiments, the first promoter is an EF1 α promoter and the second promoter is an EF1 α promoter. In some embodiments, the first promoter is an EFS promoter and the second promoter is an MSCV promoter. In some embodiments, the first promoter is an EFS promoter and the second promoter is an EF1 α promoter. In some embodiments, the first promoter is an EFS promoter and the second promoter is an PGK promoter. In some embodiments, the first promoter is an EFS promoter and the

second promoter is a CMV promoter. In some embodiments, the first promoter is an EFS promoter and the second promoter is an EFS promoter.

[00170] In some embodiments, the retroviral construct of the present invention comprises a nucleic acid sequence having at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to SEQ ID NO:17. In some embodiments, the retroviral construct of the present invention comprises the nucleic acid sequence of SEQ ID NO:17. In some embodiments, the retroviral construct of the present invention comprises a nucleic acid sequence having at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to SEQ ID NO:18. In some embodiments, the retroviral construct of the present invention comprises the nucleic acid sequence of SEQ ID NO:18. In some embodiments, the retroviral construct of the present invention comprises a nucleic acid sequence having at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to SEQ ID NO:19. In some embodiments, the retroviral construct of the present invention comprises the nucleic acid sequence of SEQ ID NO:19. In some embodiments, the retroviral construct of the present invention comprises a nucleic acid sequence having at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to SEQ ID NO:20. In some embodiments, the retroviral construct of the present invention comprises the nucleic acid sequence of SEQ ID NO:20. In some embodiments, the retroviral construct of the present invention comprises a nucleic acid sequence having at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to SEQ ID NO:21. In some embodiments, the retroviral construct of the present invention comprises the nucleic acid sequence of SEQ ID NO:21. In some embodiments, the retroviral construct of the present invention comprises a nucleic acid sequence having at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to SEQ ID NO:22. In some embodiments, the retroviral construct of the present invention comprises the nucleic acid sequence of SEQ ID NO:22. In some embodiments, the retroviral construct of the present invention comprises a nucleic acid sequence having at least 90%, e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.9%, or more sequence identity to SEQ ID NO:23. In some embodiments, the retroviral construct of the present invention comprises the nucleic acid sequence of SEQ ID NO:23.

Antibodies that bind CD7

[00171] In certain embodiments, the anti-CD7 scFv based on the TH69 antibody comprises a variable heavy chain (heavy chain variable region or VH) and a variable light chain (light chain variable region or VL) having an amino acid sequence that each have at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the VH and VL sequences set forth in SEQ ID NOS:32 and 33, respectively. The heavy chain variable region can comprise at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the VH sequence of SEQ ID NO:32. The light chain variable region can comprise at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the VL sequence of SEQ ID NO:33. In some instances, the heavy chain variable region comprises at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) amino acid substitution in the sequence set forth in SEQ ID NO:32. In certain instances, the heavy chain variable region comprises 10 or fewer amino acid (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) substitutions in the sequence set forth in SEQ ID NO:32. In some instances, the light chain variable region comprises at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) amino acid substitution in the sequence set forth in SEQ ID NO:33. In certain instances, the light chain variable region comprises 10 or fewer amino acid (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) substitutions in the sequence set forth in SEQ ID NO:33. Any of the amino acid substitutions described herein can be conservative or non-conservative substitutions.

[00172] In some embodiments, the anti-CD7 scFv comprises a VL CDR1 of SEQ ID NO:44 (SASQGISNYLN), a VL CDR2 of SEQ ID NO:45 (YTSSLHS), and a VL CDR3 of SEQ ID NO:46 (QQYSKLPYT). In some embodiments, the anti-CD7 scFv comprises a VH CDR1 of

SEQ ID NO:47 (SYAMS), a VH CDR2 of SEQ ID NO:48 (SISSGGFTYYPDSVKG), and a VH CDR3 of SEQ ID NO:49 (DEVRGYLDV). In some embodiments, the anti-CD7 scFv comprises a VL CDR1 of SEQ ID NO:44, a VL CDR2 of SEQ ID NO:45, a VL CDR3 of SEQ ID NO:46, a VH CDR1 of SEQ ID NO:47, a VH CDR2 of SEQ ID NO:48, and a VH CDR3 of SEQ ID NO:49.

[00173] In some embodiments, the nucleic acid sequence encoding the VH comprises at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the nucleic acid sequence set forth in SEQ ID NO:38. In other embodiments, the nucleic acid sequence encoding the VL comprises at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the nucleic acid sequence set forth in SEQ ID NO:39.

[00174] In certain embodiments, the anti-CD7 scFv based on the 3A1F antibody comprises a variable heavy chain (heavy chain variable region or VH) and a variable light chain (light chain variable region or VL) having a sequence that each have at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the VH and VL sequences set forth in SEQ ID NOS:34 and 35, respectively. The heavy chain variable region can comprise at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the VH sequence of SEQ ID NO:34. The light chain variable region can comprise at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence

identity, at least 99% sequence identity, or 100% sequence identity to the VL sequence of SEQ ID NO:35. In some instances, the heavy chain variable region comprises at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) amino acid substitution in the sequence set forth in SEQ ID NO:34. In certain instances, the heavy chain variable region comprises 10 or fewer amino acid (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) substitutions in the sequence set forth in SEQ ID NO:34. In some cases, the light chain variable region comprises at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or more) amino acid substitution in the sequence set forth in SEQ ID NO:35. In certain cases, the heavy chain variable region comprises 10 or fewer amino acid (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10) substitutions in the sequence set forth in SEQ ID NO:35. Any of the amino acid substitutions described herein can be conservative or non-conservative substitutions.

[00175] In some embodiments, the anti-CD7 scFv comprises a VL CDR1 of SEQ ID NO:50 (RASQISNNLH), a VL CDR2 of SEQ ID NO:51 (SASQSIS), and a VL CDR3 of SEQ ID NO:52 (QQSNSWPYT). In some embodiments, the anti-CD7 scFv comprises a VH CDR1 of SEQ ID NO:53 (SYWMH), a VH CDR2 of SEQ ID NO:54 (KINPSNGRTNYNEKFKS), and a VH CDR3 of SEQ ID NO:55 (GGVYYDLYYYALDY). In various embodiments, the anti-CD7 scFv comprises a VL CDR1 of SEQ ID NO:50, a VL CDR2 of SEQ ID NO:51, a VL CDR3 of SEQ ID NO:52, a VH CDR1 of SEQ ID NO:53, a VH CDR2 of SEQ ID NO:54, and a VH CDR3 of SEQ ID NO:55.

[00176] In some embodiments, the nucleic acid sequence encoding the VH comprises at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the nucleic acid sequence set forth in SEQ ID NO:40. In other embodiments, the nucleic acid sequence encoding a VL comprises at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the nucleic acid sequence set forth in SEQ ID NO:41.

[00177] In certain embodiments, the anti-CD7 scFv based on the T3-3A1 antibody comprises a variable heavy chain (heavy chain variable region or VH) and a variable light chain (light chain variable region or VL) having a sequence that each have at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the VH and VL sequences set forth in SEQ ID NOS:36 and 37, respectively. The heavy chain variable region can comprise at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the VH sequence of SEQ ID NO:36. The light chain variable region can comprise at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the VL sequence of SEQ ID NO:37. In some instances, the heavy chain variable region comprises at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) amino acid substitution in the sequence set forth in SEQ ID NO:36. In certain instances, the heavy chain variable region comprises 13 or fewer amino acid (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13) substitutions in the sequence set forth in SEQ ID NO:36. In some cases, the light chain variable region comprises at least one (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more) amino acid substitution in the sequence set forth in SEQ ID NO:37. In certain cases, the heavy chain variable region comprises 5 or fewer amino acid (e.g., 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11) substitutions in the sequence set forth in SEQ ID NO:37. Any of the amino acid substitutions described herein can be conservative or non-conservative substitutions.

[00178] In some embodiments, the anti-CD7 scFv comprises a VL CDR1 of SEQ ID NO:56 (RASKSVSASGYSYMH), a VL CDR2 of SEQ ID NO:57 (LASNLES), and a VL CDR3 of SEQ ID NO:58 (QHSRELPTY). In some embodiments, the anti-CD7 scFv comprises a VH CDR1 of SEQ ID NO:59 (SFGMH), a VH CDR2 of SEQ ID NO:60(YISSGSSTLHYADTVKG), and a VH CDR3 of SEQ ID NO:61 (WGNYPHYAMDY). In various embodiments, the anti-CD7 scFv comprises a VL CDR1 of SEQ ID NO:56, a VL

CDR2 of SEQ ID NO:57, a VL CDR3 of SEQ ID NO:58, a VH CDR1 of SEQ ID NO:59, a VH CDR2 of SEQ ID NO:60, and a VH CDR3 of SEQ ID NO:61.

[00179] In some embodiments, the nucleic acid sequence encoding the VH comprises at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the nucleic acid sequence set forth in SEQ ID NO:42. In other embodiments, the nucleic acid sequence encoding the VL comprises at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the nucleic acid sequence set forth in SEQ ID NO:43.

[00180] In some embodiments, the scFv of the present invention comprises a variable heavy chain sequence having at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to a variable heavy chain sequence of an anti-CD7 antibody. In some embodiments, the scFv of the present invention comprises a variable light chain sequence having at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to a variable light chain sequence of an anti-CD7 antibody. For instance, the anti-CD7 antibody can be any such recognized by one skilled in the art.

Table 3. Amino acid sequences of VH regions and VL regions of anti-CD7 scFvs

Antibody	Component	Amino Acid Sequence
TH69	VH	EVQLVESGGGLVVKPGGSLKLSCAASGLTFSSYAMSWVRQTPEKRLE WVASISSGGFTYYPD SVKGRFTISRDNARNILYLQMSSLRSED TAM YYCARDEV RGYLDVWGAGTTVT VSS (SEQ ID NO:32)
	VL	AAYKDIQMTQTSSLSASLGDRVTISCSASQGISNYLNWYQQKPDG TVKLLIYYTSSLHSGVPSRFSGSGSGTDYSLTISNLEPEDIATYYC QQYSKLPYTFGGGTKLEIKR (SEQ ID NO:33)
3A1F	VH	QVQLQESGAELVKPGASVKLSCKASGYTFSTSYWMHWVKQRPGQGLE WIGKINPSNGRTNYNEKF KSKATLTVDKSSSTAYMQLSSLTSEDSA VYYCARGGVYYDLYYYALDYWGQGTTVT VSS (SEQ ID NO:34)
	VL	DIELTQSPATLSVTPGDSVSLSCRASQSI SNNLHWYQQKSHES PRL LIK SASQSI SGIPSRFSGSGSGTDFTLSINSVETEDFGMYFCQQSN SWPYTFGGGTKLEIKR (SEQ ID NO:35)
T3-3A1	VH	DVQLVESGGGLVQPGGSRKLSCAASGFTFSSFGMHWVRQAPEKGLE WVAYISSGSSTLHYADTVKGRFTISRDNPKNTLFLQMTSLRSED TA MYYCARWGNYPHYAMDYWGQGTSVTVSS (SEQ ID NO:36)
	VL	DIVMTQSPASLAVSLGQRATISCRASKSVSASGYSYMHWYQQKPGQ PPKLLIYLASNLES GVPARFSGSGSGTDFTLNHPVEEEDAVTYYC QHSRELPTYTFGGGTKLEIK (SEQ ID NO:37)

Table 4. Nucleic acid sequences of VH regions and VL regions of anti-CD7 scFvs

Antibody	Component	Nucleic Acid Sequence
TH69	VH	GAGGTGCAGCTGGT CGAATCTGGAGGAGGACTGGTGAAGCCAGGAG GATCTCTGAAACTGAGTTGTGCCGCTTCAGGCCTGACCTTCTCAAG CTACGCCATGAGCTGGGTGCGACAGACACCTGAGAAGCGGCTGGAA TGGGTCGCTAGCATCTCCTCTGGCGGGTTCACATACTATCCAGACT CCGTGAAAGGCAGATTTACTATCTCTCGGGATAACGCAAGAAATAT TCTGTACCTGCAGATGAGTTCAGT GAGGAGCGAGGACACCGCAATG TACTATTGTGCCAGGGACGAAGTGC GCGGCTATCTGGATGTCTGGG GAGCTGGCACTACCGTCACCGTCTCCAGC (SEQ ID NO:38)
	VL	GCCGCATACAAGGATATT CAGATGACTCAGACCACAAGCTCCCTGA GCGCCTCCCTGGGAGACCGAGTGACAATCTCTTGCA GTGCATCACA GGGAATTAGCAACTACCTGAATTGGTATCAGCAGAAGCCAGATGGC ACTGTGAAACTGCTGATCTACTATACCTCTAGTCTGCACAGTGGGG TCCCCTCACGATT CAGCGGATCCGGCTCTGGGACAGACTACAGCCT GACTATCTCCAACCTGGAGCCCGAAGATATTGCCACCTACTATTGC CAGCAGTACTCCAAGCTGCCTTATACCTTTGGCGGGGGAACAAAGC TGGAGATTA AAAG (SEQ ID NO:39)
3A1F	VH	CAGGTCCAGCTGCAGGAGTCAGGAGCTGAGCTGGTGAAGCCAGGGG CAAGCGTCAAACCTGTCCTGCAAGGCCTCTGGATATACATTCAGTAG CTACTGGATGCACTGGGTGAAACAGAGACCCGGACAGGGCCTGGAG TGGATCGGAAAGATTAACCTAGCAATGGCAGGACCAACTACAACG

Antibody	Component	Nucleic Acid Sequence
		AAAAGTTTAAATCCAAGGCAACCCTGACAGTGGACAAGAGCTCCTC TACAGCCTACATGCAGCTGAGTTCAGTTCAGAGGATAGCGCA GTGTACTATTGCGCCAGAGGCGGGTCTACTATGACCTGTACTATT ACGCCCTGGATTATTGGGGGCAGGGAACCACAGTGAAGTGTGAGCTC C (SEQ ID NO:40)
	VL	GACATCGAGCTGACCCAGAGTCCTGCTACACTGAGCGTGAAGTCCAG GCGATTCTGTGAGTCTGTCTGTCGCGGCAAGCCAGTCCATCTCTAA CAATCTGCACTGGTACCAGCAGAAATCCCATGAATCTCCACGACTG CTGATTAAGAGTGCCTCACAGAGCATCTCCGGCATTCCTCCCGGT TCTCTGGCAGTGGGTCAGGAACTGACTTTACCCTGAGTATTAAGTC AGTGGAGACAGAAAGATTTCCGGCATGTATTTTGGCAGCAGAGCAAT TCCTGGCCCTACACTTTCCGGAGGCGGGACCAAAGTGGAGATCAAGC GG (SEQ ID NO:41)
T3-3A1	VH	GATGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAG GGTCCCGGAAACTCTCCTGTGCAGCCTCTGGATTCACTTTCAAGTAG CTTTGGAATGCACTGGGTTTCGTGAGGCTCCAGAGAAGGGGCTGGAG TGGGTCGCATACATTAGTAGTGGCAGTAGTACCCTCCACTATGCAG ACACAGTGAAGGGCCGATTACCATCTCCAGAGACAATCCCAAGAA CACCTGTTCTGCAATGACCAGTCTAAGGTCTGAGGACACGGCC ATGTATTACTGTGCAAGATGGGGTAACTACCCTCACTATGCTATGG ACTACTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCA SEQ ID NO:42)
	VL	GACATTGTGATGACCCAGTCTCCTGCTTCCTTAGCTGTATCTCTGG GGCAGAGGGCCACCATCTCATGCAGGGCCAGCAAAAGTGTGAGTGC ATCTGGCTATAGTTATATGCACTGGTACCAACAGAAACCAGGACAG CCACCCAACTCCTCATCTATCTTGCATCCAACCTAGAATCTGGGG TCCCTGCCAGGTTCAAGTGGCAGTGGGTCTGGGACAGACTTCACCT CAACATCCATCCTGTGGAGGAGGAGGATGCTGTAACCTATTACTGT CAGCACAGTAGGGAGCTTCCGTACACGTTCCGGAGGGGGGACCAAGC TGGAAATAAAA (SEQ ID NO:43)

Downregulation of intracellular CD7 via CD7 PEBL

[00181] As described herein, T cell cytotoxicity was shown to be markedly increased when anti-CD7 CAR was used in combination with downregulation of CD7 expression on the effector T cells. As demonstrated herein, downregulation (e.g., elimination, reduction, and/or relocalization) of CD7 prevented the fratricidal effect exerted by the corresponding anti-CD7 CAR, allowing greater T cell recovery after CAR expression as compared to cells that retained the target antigen (e.g., CD7), and a more effective cytotoxicity against T leukemia/lymphoma cells. As those of skill in the art would appreciate, downregulation of CD7 expression on the

effector T cells can be achieved according to a variety of known methods including, for example, protein expression blockers (PEBLs) against CD7 (as described in WO2016/126213), RNAi against CD7, or gene editing methods such as, e.g., meganucleases, TALEN, CRISPR/Cas9, and zinc finger nucleases. The present invention describes PEBLs that bind target antigens and sequester the target antigens to the cytoplasm of a cell. The target antigens are synthesized and bind to the PEBLs intracellularly.

[00182] In certain embodiments, provided herein is a polynucleotide comprising a nucleic acid sequence encoding a PEBL comprising a target-binding molecule (e.g., a CD7 antigen binding domain) linked to a localizing domain. In some instances, the PEBL comprises from the N-terminus to the C-terminus: a CD7 antigen binding domain, an optional domain linker, and a cellular localizing domain. In some embodiments, the PEBL further comprises a signal peptide fused N-terminal to the CD7 antigen binding domain. In some embodiments, the CD7 antigen binding domain comprises a VL domain, a domain linker, and a VH domain. Exemplary embodiments of a PEBL are shown in FIG. 3E and FIG. 17 of US 2018/0179280, which is herein incorporated by reference.

[00183] As used herein, “linked” in the context of the protein expression blocker refers to a gene encoding a target-binding molecule directly in frame (e.g., without a linker) adjacent to one or more genes encoding one or more localizing domains. Alternatively, the gene encoding a target-binding molecule may be connected to one or more gene encoding one or more localizing domains through a linker sequence, e.g., as described in WO2016/126213. As would be appreciated by those of skill in the art, such linker sequences as well as variants of such linker sequences are known in the art. Methods of designing constructs that incorporate linker sequences as well as methods of assessing functionality are readily available to those of skill in the art.

[00184] In some embodiments, the localizing domain of the PEBL comprises an endoplasmic reticulum (ER) or Golgi retention sequence; or a proteasome localizing sequence. In certain embodiments, the localizing domain comprises an endoplasmic reticulum (ER) retention peptide of Table 5. In certain embodiments, the localizing domain comprises a proteasome localizing sequence set forth in Table 5. The localizing domain can direct the PEBL to a specific cellular

compartment, such as the Golgi or endoplasmic reticulum, the proteasome, or the cell membrane, depending on the application.

[00185] In some embodiments, proteasome localization is achieved by linking the scFv sequence to a tripartite motif containing 21 (TRIM21) targeting domain sequence and coexpressing the sequence encoding the human TRIM21 E3 ubiquitin ligase protein. TRIM21 binds with high affinity to the Fc domains of antibodies and can recruit the ubiquitin-proteasome complex to degrade molecules (e.g., proteins and peptides) bound to the antibodies. The TRIM21 targeting domain sequence encodes amino acid sequences selected from the group of human immunoglobulin G (IgG) constant regions (Fc) genes such as IgG1, IgG2, or IgG4 and is used to form a fusion protein comprising scFv and Fc domains. In this embodiment, the exogenously expressed TRIM21 protein binds the scFv-Fc fusion protein bound to the target protein (e.g., CD7) and directs the complex to the proteasome for degradation.

[00186] Details of the amino acid sequence of the human TRIM21 E3 ligase protein can be found, for example, in NCBI Protein database under NCBI Ref. Seq. No. NP_003132.2. Details of the nucleic acid sequence encoding the human TRIM21 E3 ligase protein can be found, for example, in NCBI Protein database under NCBI Ref. Seq. No. NM_003141.3.

[00187] In some embodiments, the PEBL also includes a hinge domain and transmembrane domain sequence derived from CD8 α , CD8 β , 4-1BB, CD28, CD34, CD4, Fc ϵ RI γ , CD16, OX40, CD3 ζ , CD3 ϵ , CD3 γ , CD3 δ , TCR α , CD32, CD64, VEGFR2, FAS, or FGFR2B. In some embodiments, the PEBL comprises a hinge and transmembrane domain selected from the group consisting of a hinge and transmembrane domain of CD8 α , a hinge and transmembrane domain of CD8 β , a hinge and transmembrane domain of 4-1BB, a hinge and transmembrane domain of CD28, a hinge and transmembrane domain of CD34, a hinge and transmembrane domain of CD4, a hinge and transmembrane domain of Fc ϵ RI γ , a hinge domain and transmembrane domain of CD16, a hinge and transmembrane domain of OX40, a hinge and transmembrane domain of CD3 ζ , a hinge and transmembrane domain of CD3 ϵ , a hinge and transmembrane domain of CD3 γ , a hinge and transmembrane domain of CD3 δ , a hinge and transmembrane domain of TCR α , a hinge and transmembrane domain of CD32, a hinge and transmembrane domain of CD64, a hinge and transmembrane domain of VEGFR2, a hinge and transmembrane domain of FAS, and a hinge and transmembrane domain of FGFR2B.

[00188] In certain embodiments, the PEBL comprises one or more of the components set forth in Table 5.

Table 5. Amino acid sequence information for select components of a CD7 PEBL

Component	SEQ ID NO	Amino Acid Sequence
ER localization domain KDEL tethered to scFv with myc (“myc KDEL”)	SEQ ID NO:71	EQKLISEEDLKDEL
Localization domain “link(20)AEKDEL”	SEQ ID NO:72	GGGGSGGGSGGGSGGGGSAEKDEL
KDEL domain	SEQ ID NO:73	KDEL
KKXX domain	SEQ ID NO:74	KKXX where X is any amino acid
KXD/E domain		KXD or KXE where X is any amino acid
YQRL domain	SEQ ID NO:75	YQRL
PEST motif	SEQ ID NO:76	PEST
Localization domain “mb DEKKMP” domain	SEQ ID NO:77	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLYKYKSRR SFIDEKKMP
CD8 α hinge and transmembrane domain	SEQ ID NO:78	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHT RGLDFACDIYIWAPLAGTCGVLLLSLVITLY
VH-VL linker	SEQ ID NO:79	GGGGSGGGSGGGSGGGGS
CD8 α signal peptide	SEQ ID NO:80	MALPVTALLLPLALLLHAARP

[00189] In some embodiments, the CD7 PEBL contains CD7 antigen binding domain comprising an amino acid sequence of SEQ ID NO:32, an amino acid sequence of SEQ ID NO:33, and a VH-VL linker. The VH-VL linker can be a (G₄S)_n linker where n can range from 1 to 6, e.g., 1, 2, 3, 4, 5, or 6. In one embodiment, the CD7 PEBL comprises an amino acid sequence of SEQ ID NO:32, an amino acid sequence of SEQ ID NO:33, and an amino acid sequence of SEQ ID NO:79. In some embodiments, the CD7 PEBL comprises an amino acid sequence having at least 90% sequence identity or at least 95% sequence identity to SEQ ID NO:32, the amino acid sequence of SEQ ID NO:33, and the amino acid sequence of SEQ ID NO:79. In certain embodiments, the CD7 PEBL comprises an amino acid sequence of SEQ ID NO:32, an amino acid sequence having at least 90% sequence identity or at least 95% sequence

identity to SEQ ID NO: 33, and an amino acid sequence of SEQ ID NO:79. In other embodiments, the anti-CD7 protein expression blocker comprises an amino acid sequence having at least 90% sequence identity or at least 95% sequence identity to SEQ ID NO:32, an amino acid sequence having at least 90% sequence identity or at least 95% sequence identity to SEQ ID NO:33, and an amino acid sequence of SEQ ID NO:79.

[00190] In some embodiments, the CD7 PEBL contains CD7 antigen binding domain comprising an amino acid sequence of SEQ ID NO:34, an amino acid sequence of SEQ ID NO:35, and a VH-VL linker. The VH-VL linker can be a (G₄S)_n linker where n can range from 1 to 6, e.g., 1, 2, 3, 4, 5, or 6. In one embodiment, the CD7 PEBL comprises an amino acid sequence of SEQ ID NO:34, an amino acid sequence of SEQ ID NO:35, and an amino acid sequence of SEQ ID NO:79. In some embodiments, the CD7 PEBL comprises an amino acid sequence having at least 95% sequence identity to SEQ ID NO:34, the amino acid sequence of SEQ ID NO:35, and the amino acid sequence of SEQ ID NO:79. In certain embodiments, the CD7 PEBL comprises an amino acid sequence of SEQ ID NO:34, an amino acid sequence having at least 95% sequence identity to SEQ ID NO:35, and an amino acid sequence of SEQ ID NO:79. In other embodiments, the CD7 PEBL comprises an amino acid sequence having at least 90% sequence identity or at least 95% sequence identity to SEQ ID NO:34, an amino acid sequence having at least 90% sequence identity or at least 95% sequence identity to SEQ ID NO:35, and an amino acid sequence of SEQ ID NO:79.

[00191] In some embodiments, the CD7 PEBL contains CD7 antigen binding domain comprising an amino acid sequence of SEQ ID NO:36, an amino acid sequence of SEQ ID NO:37, and a VH-VL linker. The VH-VL linker can be a (G₄S)_n linker where n can range from 1 to 5, e.g., 1, 2, 3, 4, 5, or 6. In one embodiment, the CD7 PEBL comprises an amino acid sequence of SEQ ID NO:36, an amino acid sequence of SEQ ID NO:37, and an amino acid sequence of SEQ ID NO:79. In some embodiments, the CD7 PEBL comprises an amino acid sequence having at least 90% sequence identity or at least 95% sequence identity to SEQ ID NO:36, the amino acid sequence of SEQ ID NO:37, and the amino acid sequence of SEQ ID NO:79. In certain embodiments, the CD7 PEBL comprises an amino acid sequence of SEQ ID NO:36, an amino acid sequence having at least 90% sequence identity or at least 95% sequence identity to SEQ ID NO:37, and an amino acid sequence of SEQ ID NO:79. In other embodiments, the CD7 PEBL comprises an amino acid sequence having at least 90% sequence

identity or at least 95% sequence identity to SEQ ID NO:36, an amino acid sequence having at least 90% sequence identity or at least 95% sequence identity to SEQ ID NO:37, and an amino acid sequence of SEQ ID NO:79.

[00192] In some instance, CD7 PEBL also comprises a localization domain selected from any one sequence set forth in SEQ ID NOS:72-77. In some cases, the CD7 PEBL also comprises a CD8 α signal peptide such as but not limited to the CD8 α signal peptide set forth in SEQ ID NO:80. In other cases, the anti-CD7 protein expression blocker also comprises CD8 α hinge and transmembrane domains such as but not limited to the CD8 α hinge and transmembrane domains set forth in SEQ ID NO:78.

[00193] In one embodiment, the CD7 PEBL encoded by the bicistronic vector described herein comprises the sequence of SEQ ID NO:24 and a proline at the N-terminus. In some embodiments, the CD7 PEBL comprises the sequence of SEQ ID NO:25. The N-terminal proline residue arises from the 2A cleavage. In some embodiments, the CD7 PEBL encoded by the bicistronic vector described herein comprises the sequence of SEQ ID NO:26 and a proline at the N-terminus. In some embodiments, the CD7 PEBL comprises the sequence of SEQ ID NO:27.

[00194] In some embodiments, an engineered immune cell of the present invention comprises a CD7 PEBL encoded by a bicistronic vector such that the CD7 PEBL comprises the sequence of SEQ ID NO:24 and a proline at the N-terminus or the sequence of SEQ ID NO:25. In some embodiments, the engineered immune cell is a CD4⁺ T cell comprising a CD7 PEBL encoded by a bicistronic vector such that the CD7 PEBL comprises the sequence of SEQ ID NO:24 and a proline at the N-terminus or the sequence of SEQ ID NO:25. In some embodiments, the engineered immune cell is a CD8⁺ T cell comprising a CD7 PEBL encoded by the bicistronic vector wherein the CD7 PEBL comprises the sequence of SEQ ID NO:24 and a proline at the N-terminus or the sequence of SEQ ID NO:25. In some embodiments, the engineered immune cell is a CD3⁺ T cell comprising a CD7 PEBL encoded by the bicistronic vector wherein the CD7 PEBL comprises the sequence of SEQ ID NO:24 and a proline at the N-terminus or the sequence of SEQ ID NO:25.

[00195] In some embodiments, an engineered immune cell of the present invention comprises a CD7 PEBL encoded by a bicistronic vector such that the CD7 PEBL comprises the sequence of

SEQ ID NO:26 and a proline at the N-terminus or the sequence of SEQ ID NO:27. In some embodiments, the engineered immune cell is a CD4⁺ T cell comprising a CD7 PEBL encoded by the bicistronic vector wherein the CD7 PEBL comprises the sequence of SEQ ID NO:26 and a proline at the N-terminus or the sequence of SEQ ID NO:27. In some embodiments, the engineered immune cell is a CD8⁺ T cell comprising a CD7 PEBL encoded by the bicistronic vector wherein the CD7 PEBL comprises the sequence of SEQ ID NO:26 and a proline at the N-terminus or the sequence of SEQ ID NO:27. In some embodiments, the engineered immune cell is a CD3⁺ T cell comprising a CD7 PEBL encoded by the bicistronic vector wherein the CD7 PEBL comprises the sequence of SEQ ID NO:26 and a proline at the N-terminus or the sequence of SEQ ID NO:27.

[00196] In some embodiments, the CD7 PEBL encoded by the dual promoter vector described herein comprises the sequence of SEQ ID NO:24. In some embodiments, the CD7 PEBL encoded by the dual promoter vector described herein binds to CD7 and comprises at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the CD7 PEBL encoded by the dual promoter vector described herein comprises the sequence of SEQ ID NO:26. In some embodiments, the CD7 PEBL encoded by the dual promoter vector described herein binds to CD7 and comprises at least 90% sequence identity to SEQ ID NO:26.

[00197] In some embodiments, the polynucleotide encoding the CD7 PEBL comprises one or more nucleic acid sequences set forth in Table 6.

[00198] In some embodiments, the VH domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence of SEQ ID NO:38 and the VL domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence of SEQ ID NO:39. In certain embodiments, the VH domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:38 and the VL domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:39. In some embodiments, the VH domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:38 and the VL domain of the anti-

CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:39, or a codon optimized variant thereof.

[00199] In some embodiments, the VH domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence of SEQ ID NO:40 and the VL domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence of SEQ ID NO:41. In certain embodiments, the VH domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:40 and the VL domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:41. In some embodiments, the VH domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:40 and the VL domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:41, or a codon optimized variant thereof.

[00200] In some embodiments, the VH domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence of SEQ ID NO:42 and the VL domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence of SEQ ID NO:43. In certain embodiments, the VH domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:42 and the VL domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:43. In some embodiments, the VH domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:42 and the VL domain of the anti-CD7 scFv of the PEBL comprises the nucleotide sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to SEQ ID NO:43, or a codon optimized variant thereof.

Table 6. Nucleic acid sequence information for select components of a CD7 PEBL

Component	SEQ ID NO	Sequence
CD8 α signal peptide	SEQ ID NO:81	ATGGCTCTGCCTGTGACCGCACTGCTGCTGCCC CTGGCTCTGCTGCTGCACGCCGCAAGACCT
VH-VL Linker	SEQ ID NO:82	GGAGGAGGAGGAAGCGGAGGAGGAGGATCCGGA GGCGGGGGATCTGGAGGAGGAGGAAGT
ER localization domain KDEL tethered to scFv with myc ("myc KDEL")	SEQ ID NO:83	GAGCAGAACTGATTAGCGAAGAGGACCTGAAA GATGAACTG

[00201] In certain aspects of the present invention, the PEBL can bind to a molecule that is expressed on the surface of a cell including, but not limited to members of the CD1 family of glycoproteins, CD2, CD3, CD4, CD5, CD7, CD8, CD25, CD28, CD30, CD38, CD45, CD45RA, CD45RO, CD52, CD56, CD57, CD99, CD127, and CD137.

[00202] In some embodiments, the CD7 PEBL comprises a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, or more) sequence identity to SEQ ID NO:2 and binds to CD7. In some embodiments, the CD7 PEBL comprises a nucleic acid sequence having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:2 and binds to CD7. In some embodiments, the CD7 PEBL comprises a nucleic acid sequence having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:2.

[00203] In some embodiments, the CD7 PEBL comprises a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, or more) sequence identity to SEQ ID NO:3 and binds to CD7. In some embodiments, the CD7 PEBL comprises a nucleic acid sequence having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:3 and binds to CD7. In some embodiments, the CD7 PEBL comprises a nucleic acid sequence having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:3.

[00204] In some embodiments, an engineered immune cell of the present invention comprises a CD7 PEBL encoded by a bicistronic construct comprising a nucleic acid sequence of the CD7

PEBL having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:2. In some embodiments, the engineered immune cell is a CD4⁺ T cell comprising a CD7 PEBL encoded by the bicistronic vector construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:2. In some embodiments, the engineered immune cell is a CD8⁺ T cell comprising a CD7 PEBL encoded by the bicistronic construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:2. In some embodiments, the engineered immune cell is a CD3⁺ T cell comprising a CD7 PEBL encoded by the bicistronic construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:2. Also, provided herein is a population comprising such cells.

[00205] In some embodiments, an engineered immune cell of the present invention comprises a CD7 PEBL encoded by a bicistronic construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:3. In some embodiments, the engineered immune cell is a CD4⁺ T cell comprising a CD7 PEBL encoded by the bicistronic vector construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:3. In some embodiments, the engineered immune cell is a CD8⁺ T cell comprising a CD7 PEBL encoded by the bicistronic construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:3. In some embodiments, the engineered immune cell is a CD3⁺ T cell comprising a CD7 PEBL encoded by the bicistronic construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:3. Also, provided herein is a population comprising such cells.

Chimeric antigen receptors that bind CD7

[00206] In some embodiments, the CAR of the present invention comprises intracellular signaling domains of 4-1BB and CD3 ζ , and an antigen binding domain (e.g., a single chain variable fragment or scFv) that specifically binds CD7. The CD7 CAR of the present invention is sometimes referred to herein as “anti-CD7-41BB-CD3 ζ ”. In some embodiments, the CAR also includes a CD8 α hinge domain and transmembrane domain, such as but not limited the amino acid sequence of SEQ ID NO:84.

[00207] As those skilled in the art would appreciate, in certain embodiments, any of the amino acid sequences of the various components disclosed herein (e.g., scFv, intracellular signaling domain, linker, and combinations thereof) can have at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the specific corresponding sequences disclosed herein. For example, in certain embodiments, the intracellular signaling domain 4-1BB can have at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to SEQ ID NO:85, as long as it possesses the desired function. In certain embodiments, the intracellular signaling domain of 4-1BB comprises the amino acid sequence set forth in SEQ ID NO:85.

[00208] As another example, in certain embodiments, the intracellular signaling domain 4-1BB can be replaced by another intracellular signaling domain from a co-stimulatory molecule such as CD28, OX40, ICOS, CD27, GITR, HVEM, TIM1, LFA1, or CD2. In some embodiments, the intracellular signaling domain of the CAR can have at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the intracellular signaling domain of CD28, OX40, ICOS, CD27, GITR, HVEM, TIM1, LFA1, or CD2.

[00209] As another example, in certain instances, the intracellular signaling domain of 4-1BB can also include another intracellular signaling domain (or a portion thereof) from a co-stimulatory molecule such as CD28, OX40, ICOS, CD27, GITR, HVEM, TIM1, LFA1, or CD2. In some embodiments, the additional intracellular signaling domain can have at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the intracellular signaling domain of CD28,

OX40, ICOS, CD27, GITR, HVEM, TIM1, LFA1, or CD2. In other embodiments, the additional intracellular signaling domain comprises at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to one or more intracellular signaling domain fragment(s) of CD28, OX40, ICOS, CD27, GITR, HVEM, TIM1, LFA1, or CD2.

[00210] As another example, in certain embodiments, the intracellular signaling domain CD3 ζ can have at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to SEQ ID NO:86, as long as it possesses the desired function. In certain embodiments, the intracellular signaling domain of CD3 ζ comprises the amino acid sequence set forth in SEQ ID NO:86.

[00211] In some instances, the intracellular signaling domain comprises an immunoreceptor tyrosine-based activation motif (ITAM) or a portion thereof, as long as it possesses the desired function. The intracellular signaling domain of the CAR can include a sequence having at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to an ITAM. In certain embodiments, the intracellular signaling domain can have at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to Fc ϵ RI γ , CD4, CD7, CD8, CD28, OX40 or H2-Kb, as long as it possesses the desired function.

[00212] In certain embodiments, the anti-CD7 CAR further comprises a hinge domain and/or a transmembrane domain. Hinge and transmembrane domains suitable for use in the present invention are known in the art, and provided in, e.g., publication WO2016/126213, incorporated by reference in its entirety. In some embodiments, the hinge and transmembrane domains of the anti-CD7 CAR includes a signaling domain (e.g., hinge and transmembrane domains) from

CD8 β , 4-1BB, CD28, CD34, CD4, Fc ϵ RI γ , CD16, OX40, CD3 ζ , CD3 ϵ , CD3 γ , CD3 δ , TCR α , CD32, CD64, VEGFR2, FAS, FGFR2B, or another transmembrane protein.

[00213] In certain embodiments, the anti-CD7 CAR further comprises a CD8 α signal peptide. A schematic of the anti-CD7 CAR comprising the embodiments described herein is shown in FIG. 17 of US 2018/0179280.

[00214] In some embodiments, the chimeric antigen receptor (CAR) can bind to a molecule that is expressed on the surface of a cell including, but not limited to members of the CD1 family of glycoproteins, CD2, CD3, CD4, CD5, CD7, CD8, CD25, CD28, CD30, CD38, CD45, CD45RA, CD45RO, CD52, CD56, CD57, CD99, CD127, and CD137.

[0215] In certain embodiments, an isolated polynucleotide of the present invention comprises a nucleic acid sequence that encodes a CAR according to Table 7. In some embodiments, the polynucleotide comprises a nucleic acid sequence that encodes a component of the CAR according to Table 7.

Table 7. Amino acid sequence information for select components of a CD7 CAR

Component	SEQ ID NO	Amino Acid Sequence
CD8 α hinge and transmembrane domain	SEQ ID NO:84	TTTPAPRPPTPAPTIASQPLSLRPEACRPAA GGAVHTRGLDFACDIYIWAPLAGTCGVLLLS LVITLY
Intracellular signaling domain of 4-1BB	SEQ ID NO:85	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCR FPEEEEGGCEL
Intracellular signaling domain CD3 ζ	SEQ ID NO:86	RVKFSSRSADAPAYQQGQNQLYNEINLGRREE YDVLDKRRGRDPFEMGGKPRRKNPQEGLYNEL QKDKMAEAYSEIGMKGERRRGKGHGGLYQGL STATKDTYDALHMQALPPR

[0216] In some embodiments, the CD7 CAR comprises a CD7 antigen binding domain, a 4-1BB intracellular signaling domain, a CD3 ζ intracellular signaling domain, and CD8 hinge and transmembrane domain. In some embodiments, the CD7 antigen binding domain comprises a VH domain and a VL domain, and a VH-VL linker, such as but not limited to a (G₄S)_n linker where n can range from 1 to 6, e.g., 1, 2, 3, 4, 5, or 6. In some embodiments, the CD7 CAR comprises from N-terminus to C-terminus: a CD8 signal peptide, a CD7 antigen binding domain,

aCD8 hinge and transmembrane domain, a 4-1BB intracellular signaling domain, and a CD3 ζ intracellular signaling domain.

[0217] In some embodiments, the CD7 CAR encoded by the bicistronic vector described herein comprises the amino acid sequence of SEQ ID NO:28. In some embodiments, the CD7 CAR encoded by the bicistronic vector described herein comprises the amino acid sequence of SEQ ID NO:29. In some embodiments, the CD7 CAR encoded by the bicistronic vector described herein comprises the amino acid sequence of SEQ ID NO:30. In some embodiments, the CD7 CAR encoded by the bicistronic vector described herein comprises the amino acid sequence of SEQ ID NO:31. Exemplary embodiments of CD7 CARs of the present invention are depicted in FIGS. 42-45.

[0218] In some embodiments, an engineered immune cell of the present invention comprises a CD7 CAR encoded by a bicistronic vector such that the CD7 CAR comprises the sequence of SEQ ID NO:28 and additional amino acid residues at the N-terminus produced by cleavage of the 2A self-cleaving peptide, or the CD7 CAR comprises the sequence of SEQ ID NO:29. In some embodiments, the engineered immune cell is a CD4⁺ T cell comprising a CD7 CAR encoded by a bicistronic vector such that the CD7 CAR comprises the sequence of SEQ ID NO:28 and additional amino acid residues at the N-terminus produced by cleavage of the 2A self-cleaving peptide, or the CD7 CAR comprises the sequence of SEQ ID NO:29. In some embodiments, the engineered immune cell is a CD8⁺ T cell comprising a CD7 CAR encoded by a bicistronic vector such that the CD7 CAR comprises the sequence of SEQ ID NO:28 and additional amino acid residues at the N-terminus produced by cleavage of the 2A self-cleaving peptide, or the CD7 CAR comprises the sequence of SEQ ID NO:29. In some embodiments, the engineered immune cell is a CD3⁺ T cell comprising a CD7 CAR encoded by a bicistronic vector such that the CD7 CAR comprises the sequence of SEQ ID NO:28 and additional amino acid residues at the N-terminus produced by cleavage of the 2A self-cleaving peptide, or the CD7 CAR comprises the sequence of SEQ ID NO:29. Also, provided herein are populations comprising such cells.

[0219] In some embodiments, an engineered immune cell of the present invention comprises a CD7 CAR encoded by a bicistronic vector such that the CD7 CAR comprises the sequence of SEQ ID NO:30 and additional amino acid residues at the N-terminus produced by cleavage of

the 2A self-cleaving peptide, or the CD7 CAR comprises the sequence of SEQ ID NO:31. In some embodiments, the engineered immune cell is a CD4⁺ T cell comprising a CD7 CAR encoded by a bicistronic vector such that the CD7 CAR comprises the sequence of SEQ ID NO:30 and additional amino acid residues at the N-terminus produced by cleavage of the 2A self-cleaving peptide, or the CD7 CAR comprises the sequence of SEQ ID NO:31. In some embodiments, the engineered immune cell is a CD8⁺ T cell comprising a CD7 CAR encoded by a bicistronic vector such that the CD7 CAR comprises the sequence of SEQ ID NO:30 and additional amino acid residues at the N-terminus produced by cleavage of the 2A self-cleaving peptide, or the CD7 CAR comprises the sequence of SEQ ID NO:31. In some embodiments, the engineered immune cell is a CD3⁺ T cell comprising a CD7 CAR encoded by a bicistronic vector such that the CD7 CAR comprises the sequence of SEQ ID NO:30 and additional amino acid residues at the N-terminus produced by cleavage of the 2A self-cleaving peptide, or the CD7 CAR comprises the sequence of SEQ ID NO:31. Also, provided herein are populations of such cells.

[0220] In some embodiments, the CD7 CAR encoded by the dual promoter vector described herein comprises the amino acid sequence of SEQ ID NO:28. In some embodiments, an engineered immune cell of the present invention comprises a CD7 CAR encoded by a dual promoter vector such that the CD7 CAR comprises the sequence of SEQ ID NO:28. In some embodiments, the engineered immune cell is a CD4⁺ T cell comprising a CD7 CAR encoded by a dual promoter vector such that the CD7 CAR comprises the sequence of SEQ ID NO:28. In some embodiments, the engineered immune cell is a CD8⁺ T cell comprising a CD7 CAR encoded by a dual promoter vector such that the CD7 CAR comprises the sequence of SEQ ID NO:28. In some embodiments, the engineered immune cell is a CD3⁺ T cell comprising a CD7 CAR encoded by a dual promoter vector such that the CD7 CAR comprises the sequence of SEQ ID NO:28. In some embodiments, the CD7 CAR encoded by the bicistronic vector described herein comprises the amino acid sequence of SEQ ID NO:30. In some embodiments, an engineered immune cell of the present invention comprises a CD7 CAR encoded by a dual promoter vector such that the CD7 CAR comprises the sequence of SEQ ID NO:30. In some embodiments, the engineered immune cell is a CD4⁺ T cell comprising a CD7 CAR encoded by a dual promoter vector such that the CD7 CAR comprises the sequence of SEQ ID NO:30. In some embodiments, the engineered immune cell is a CD8⁺ T cell comprising a CD7 CAR

encoded by a dual promoter vector such that the CD7 CAR comprises the sequence of SEQ ID NO:30. In some embodiments, the engineered immune cell is a CD3⁺ T cell comprising a CD7 CAR encoded by a dual promoter vector such that the CD7 CAR comprises the sequence of SEQ ID NO:30. Also, provided herein are populations of such cells.

[0221] In certain embodiments, an isolated polynucleotide of a CD7 CAR of the present invention comprises one or more nucleic acid sequences of Table 8. In some embodiments, the nucleic acid sequence comprises a sequence encoding one or more components of the CAR as set forth in Table 8.

Table 8. Nucleic acid sequence information for select components of a CD7 CAR

Component	SEQ ID NO	Nucleic Acid Sequence
CD8 α hinge and transmembrane domain	SEQ ID NO:96	ACCACTACACCTGCACCAAGGCCTCCCACACCCGCT CCCCTATCGCTTCCCAGCCACTGTCCTGAGGCC GAGGCTGCAGGCCAGCAGCTGGCGGAGCCGTGCAT ACTAGGGGGCTGGACTTCGCTTGCACATCTACATC TGGGCCCCACTGGCAGGGACATGCGGAGTCCTGCTG CTGTCCCTGGTCATCACACTGTACTGC
Intracellular signaling domain of 4-1BB	SEQ ID NO:97	AAGCGGGGGCGCAAAAACTGCTGTATATCTTTAAG CAGCCTTTCATGAGACCAGTGCAGACAACCCAGGAG GAAGATGGGTGCTCATGCCGTTTCCCAGGAGGAG GAAGGCGGCTGCGAGCTG
Intracellular signaling domain of CD3 ζ	SEQ ID NO:98	GGGTGAAGTTTTCCCGCTCAGCAGATGCTCCTGCCT ACCAGCAGGGCCAGAACCAGCTGTATAATGAGCTGA ACCTGGGCAGACGCGAAGAGTATGATGTGCTGGACA AAAGGCGGGGAAGAGACCCCGAAATGGGAGGGGAAGC CAAGGCGGAAAAACCCCGAGGAGGCGCTGTACAATG AGCTGCAGAAGGACAAAATGGCAGAGGCTTACAGTG AGATTGGGATGAAGGAGAGAGACGGAGGGGAAAAG GGCACGATGGCCTGTACCAGGGGCTGAGCACAGCAA CCAAAGATACTTATGACGCACTGCACATGCAGGCAC TGCCACCCAGA

[0222] In some embodiments, the polynucleotide encoding the CD7 CAR comprises a nucleic acid sequence for an antigen binding domain that binds CD7, a nucleic acid sequence for a CD8 α hinge and transmembrane domain, a nucleic acid sequence for an intracellular signaling domain of 4-1BB, and intracellular signaling domain of CD3 ζ . In certain embodiments, the polynucleotide also includes a nucleic acid sequence for a CD8 signal peptide.

[0223] In certain embodiments, the antigen binding domain is a anti-CD7 scFv. In some embodiments, the VH sequence of the scFv comprises a nucleic acid sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to the sequence of SEQ ID NO:38 and the VL sequence comprises a nucleic acid sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to the sequence of SEQ ID NO:39.

[0224] In some embodiments, the VH sequence of the scFv comprises a nucleic acid sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to the sequence of SEQ ID NO:40 and the VL sequence comprises a nucleic acid sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to the sequence of SEQ ID NO:41. In some embodiments, the VH sequence of the scFv comprises a nucleic acid sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to the sequence of SEQ ID NO:42 and the VL sequence comprises a nucleic acid sequence having at least 90% sequence identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity) to the sequence of SEQ ID NO:43.

[0225] In some embodiments, the polynucleotide encoding the CD7 CAR comprises from the 5' end to the 3' end: a nucleic acid sequence for an antigen binding domain that binds CD7, SEQ ID NO:96, SEQ ID NO:97, and SEQ ID NO:98. In some embodiments, the polynucleotide encoding the CD7 CAR comprises from the 5' end to the 3' end: SEQ ID NO:81, a nucleic acid sequence for an antigen binding domain that binds CD7, SEQ ID NO:96, SEQ ID NO:97, and SEQ ID NO:98.

[0226] In some embodiments, the CD7 CAR comprises a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, or more) sequence identity to SEQ ID NO:4 and binds to CD7. In some embodiments, the CD7 CAR comprises a nucleic acid sequence having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:3 and binds to CD7. In some embodiments, the CD7 PEBL comprises a nucleic acid sequence having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:4.

[0227] In some embodiments, the CD7 CAR comprises a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, or more) sequence identity to SEQ ID NO:5 and binds to CD7. In some embodiments, the CD7 CAR comprises a nucleic acid sequence having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:3 and binds to CD7. In some embodiments, the CD7 PEBL comprises a nucleic acid sequence having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:5.

[0228] In some embodiments, an engineered immune cell of the present invention comprises a CD7 CAR encoded by a bicistronic construct or a dual promoter construct comprising a nucleic acid sequence of the CD7 CAR having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:4. In some embodiments, the engineered immune cell is a CD4⁺ T cell comprising a CD7 CAR encoded by the bicistronic vector construct or a dual promoter construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:4. In some embodiments, the engineered immune cell is a CD8⁺ T cell comprising a CD7 PEBL encoded by the bicistronic construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:4. In some embodiments, the engineered immune cell is a CD3⁺ T cell comprising a CD7 PEBL encoded by the bicistronic construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:4. Also, provided herein is a population comprising such cells.

[0229] In some embodiments, an engineered immune cell of the present invention comprises a CD7 CAR encoded by a bicistronic construct or a dual promoter construct comprising a nucleic acid sequence of the CD7 CAR having at least 90% (e.g., 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:5. In some embodiments, the engineered immune cell is a CD4⁺ T cell comprising a CD7 CAR encoded by the bicistronic vector construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:5. In some embodiments, the engineered immune cell is a CD8⁺ T cell comprising a CD7 PEBL encoded by the bicistronic construct comprising a nucleic acid sequence of the CD7 PEBL having at least 90% sequence identity to SEQ ID NO:5. In some embodiments, the engineered immune cell is a CD3⁺ T cell comprising a CD7 PEBL encoded by the bicistronic construct comprising a nucleic acid sequence of the CD7 PEBL having at least

90% sequence identity to SEQ ID NO:5. Also, provided herein is a population comprising such cells.

Engineered immune cells expressing bicistronic vectors

[0230] In certain embodiments, provided is an engineered immune cell comprising a bicistronic construct comprising: (i) a polynucleotide encoding a chimeric antigen receptor (CAR), wherein the CAR comprises intracellular signaling domains of 4-1BB and CD3 ζ , and an antigen binding domain that specifically binds CD7; (ii) a polynucleotide encoding a target-binding molecule linked to a localizing domain, wherein the target-binding molecule is an antigen binding domain that binds CD7, and the localizing domain comprises an endoplasmic reticulum retention sequence; and (iii) a nucleic acid sequence encoding a 2A self-cleaving peptide or an IRES sequence, as exemplified herein.

[0231] In certain embodiments, the antigen binding domain that binds CD7 in the context of the CAR, as well as in the context of the antigen binding domain against CD7 comprises: a VH sequence set forth in SEQ ID NO:32 and a VL sequence set forth in SEQ ID NO:33; a VH sequence set forth in SEQ ID NO:34 and a VL sequence set forth in SEQ ID NO:35; or a VH sequence set forth in SEQ ID NO:36 and a VL sequence set forth in SEQ ID NO:37. As described herein, in certain embodiments, the antigen binding domain comprises a VH and a VL having sequence that each comprise at least 90% sequence identity, at least 91% sequence identity, at least 92% sequence identity, at least 93% sequence identity, at least 94% sequence identity, at least 95% sequence identity, at least 96% sequence identity, at least 97% sequence identity, at least 98% sequence identity, at least 99% sequence identity, or 100% sequence identity to the VH and VL sequences set forth in SEQ ID NOS:32 and 33, respectively; SEQ ID NOS:34 and 35, respectively; or SEQ ID NOS:36 and 37, respectively. In certain embodiments, the antigen binding domain that binds CD7 in the context of the CAR can be different from the antibody that binds CD7 in the context of the target-binding molecule (the protein expression blocker or PEBL), as described herein.

[0232] In some embodiments, the engineered immune cell comprising a bicistronic construct comprising a nucleic acid construct comprising from the 5' end to 3' end: a polynucleotide encoding a target-binding molecule linked to a localizing domain wherein the target-binding

molecule binds CD7 (e.g., a CD7 PEBL), an IRES sequence, and a polynucleotide encoding a chimeric antigen receptor against CD7 (e.g., a CD7 CAR). In some instances, the engineered immune cell comprises a nucleic acid construct comprising SEQ ID NO:11. In some embodiments, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:11. In other embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:11. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:11.

[0233] In some embodiments, the engineered immune cell comprising a bicistronic construct comprising a nucleic acid construct comprising from the 5' end to 3' end: a polynucleotide encoding a chimeric antigen receptor against CD7, a IRES sequence, and a polynucleotide encoding a target-binding molecule linked to a localizing domain wherein the target-binding molecule binds CD7 (e.g., a PEBL against CD7). In some instances, the engineered immune cell comprises a nucleic acid construct comprising SEQ ID NO:12 or the sequence depicted in FIG. 26. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:12 or the sequence depicted in FIG. 26. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:12 or the sequence depicted in FIG. 26. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:12 or the sequence depicted in FIG. 26.

[0234] In some embodiments, the engineered immune cell comprising a bicistronic construct comprising a nucleic acid construct comprising from the 5' end to 3' end: a polynucleotide encoding a chimeric antigen receptor against CD7 (e.g., a CD7 CAR), a nucleic acid sequence encoding a 2A self-cleaving peptide, and a polynucleotide encoding a target-binding molecule linked to a localizing domain wherein the target-binding molecule binds CD7 (e.g., a CD7 PEBL). In some instances, the engineered immune cell comprises a nucleic acid construct comprising SEQ ID NO:13 or the sequence depicted in FIG. 27. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:13 or the sequence depicted in FIG. 27. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid

construct comprising SEQ ID NO:13 or the sequence depicted in FIG. 27. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:13 or the sequence depicted in FIG. 27.

[0235] In some embodiments, the engineered immune cell comprising a bicistronic construct comprising a nucleic acid construct comprising from the 5' end to 3' end: a polynucleotide encoding a target-binding molecule linked to a localizing domain wherein the target-binding molecule binds CD7, a nucleic acid sequence encoding a 2A self-cleaving peptide, and a polynucleotide encoding a chimeric antigen receptor against CD7.

[0236] In some embodiments, the engineered immune cell comprising a bicistronic construct comprising a nucleic acid construct comprising from the 5' end to 3' end: a promoter, a polynucleotide encoding a chimeric antigen receptor against CD7 (e.g., a CD7 CAR), a nucleic acid sequence encoding a 2A self-cleaving peptide, and a polynucleotide encoding a target-binding molecule linked to a localizing domain wherein the target-binding molecule binds CD7 (e.g., a CD7 PEBL). In some instances, the engineered immune cell comprises a nucleic acid construct comprising at least 85% sequence identity to any one of the nucleic acid sequences of SEQ ID NOS:14-16. In some instances, the engineered immune cell comprises a nucleic acid construct comprising any one of the nucleic acid sequences of SEQ ID NOS:14-16. In some embodiments, the engineered immune cell comprises a nucleic acid construct comprising at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:14. In some embodiments, the engineered immune cell comprises a nucleic acid construct comprising at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:15. In some embodiments, the engineered immune cell comprises a nucleic acid construct comprising at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:16.

[0237] In some embodiments, the engineered immune cell comprising a bicistronic construct comprising a nucleic acid construct comprising from the 5' end to 3' end: a promoter, a polynucleotide encoding a chimeric antigen receptor against CD7, a nucleic acid sequence encoding a 2A self-cleaving peptide, and a polynucleotide encoding a target-binding molecule linked to a localizing domain wherein the target-binding molecule binds CD7. In some instances,

the promoter is selected from a MSCV promoter, PGK promoter, EF1 α promoter, and EFS promoter. In some instances, the engineered immune cell comprises a polynucleotide comprising SEQ ID NO:14 or the sequence as depicted in FIGS. 28A-28B. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:14 or the sequence depicted in FIGS. 28A-B. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:14 or the sequence depicted in FIGS. 28A-B. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:14 or the sequence depicted in FIGS. 28A-B.

[0238] In some instances, the engineered immune cell comprises a polynucleotide comprising SEQ ID NO:15. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:15 or the sequence depicted in FIGS. 29A-B. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:15 or the sequence depicted in FIGS. 29A-B. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:15 or the sequence depicted in FIGS. 29A-B. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:15 or the sequence depicted in FIGS. 29A-B.

[0239] In some instances, the engineered immune cell comprises a polynucleotide comprising SEQ ID NO:16 or the sequence depicted in FIGS. 30A-B. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:16 or the sequence depicted in FIGS. 30A-B. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:16 or the sequence depicted in FIGS. 30A-B. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:16 or the sequence depicted in FIGS. 30A-B.

[0240] In some embodiments, the engineered immune cells described herein or a population thereof comprise at least 10% CD7 CAR⁺/CD7-negative T cells, at least 15% CD7 CAR⁺/CD7-

negative T cells, at least 20% CD7 CAR+/CD7-negative T cells, at least 25% CD7 CAR+/CD7-negative T cells, at least 30% CD7 CAR+/CD7-negative T cells, at least 35% CD7 CAR+/CD7-negative T cells, at least 40% CD7 CAR+/CD7-negative T cells, at least 45% CD7 CAR+/CD7-negative T cells, at least 50% CD7 CAR+/CD7-negative T cells, at least 55% CD7 CAR+/CD7-negative T cells, at least 60% CD7 CAR+/CD7-negative T cells, at least 65% CD7 CAR+/CD7-negative T cells, at least 70% CD7 CAR+/CD7-negative T cells, at least 75% CD7 CAR+/CD7-negative T cells, at least 80% CD7 CAR+/CD7-negative T cells, at least 85% CD7 CAR+/CD7-negative T cells, at least 90% CD7 CAR+/CD7-negative T cells, at least 95% CD7 CAR+/CD7-negative T cells, at least 96% CD7 CAR+/CD7-negative T cells, at least 97% CD7 CAR+/CD7-negative T cells, at least 98% CD7 CAR+/CD7-negative T cells, at least 99% CD7 CAR+/CD7-negative T cells, or 100% CD7 CAR+/CD7-negative T cells. In some embodiments, the engineered immune cells outlined herein include a population of substantially purified CD7 CAR/CD7-negative T cells wherein such cells express any one of the bicistronic constructs described.

Engineered immune cells expressing dual promoter vectors

[0241] In some embodiments, provided is an engineered immune cell comprising a recombinant retroviral vector comprising (a) a first promoter operably linked to a first polynucleotide encoding any of the CARs described herein, and (b) a second promoter operably linked to a second polynucleotide encoding any of the PEBLs described herein. In some embodiments, the engineered immune cell comprises any of the recombinant retroviral vectors described herein containing a promoter driving CAR expression and another promoter driving PEBL expression.

[0242] In some embodiments, the engineered immune cell comprises a recombinant retroviral vector comprising a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:17. In some embodiments, the engineered immune cell comprises a recombinant retroviral vector comprising a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:18. In some embodiments, the engineered immune cell comprises a recombinant retroviral vector comprising a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%,

90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:19. In some embodiments, the engineered immune cell comprises a recombinant retroviral vector comprising a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:20. In some embodiments, the engineered immune cell comprises a recombinant retroviral vector comprising a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:21. In some embodiments, the engineered immune cell comprises a recombinant retroviral vector comprising a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:22. In some embodiments, the engineered immune cell comprises a recombinant retroviral vector comprising a nucleic acid sequence having at least 85% (e.g., 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO:23. In some instances, the engineered immune cell is an engineered CD4⁺ T cell or a population thereof or a population comprising such. In some instances, the engineered immune cell is an engineered CD8⁺ T cell or a population thereof or a population comprising such.

[0243] In some instances, the engineered immune cell comprises a polynucleotide comprising SEQ ID NO:17 or the sequence depicted in FIGS. 31A-B. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:17 or the sequence depicted in FIGS. 31A-B. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:17 or the sequence depicted in FIGS. 31A-B. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:17 or the sequence depicted in FIGS. 31A-B.

[0244] In some instances, the engineered immune cell comprises a polynucleotide comprising SEQ ID NO:18. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:18 or the sequence depicted in FIGS. 32A-B. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:18 or the sequence depicted in FIGS. 32A-B. In some embodiments, provided herein is an

engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:18 or the sequence depicted in FIGS. 32A-B.

[0245] In some instances, the engineered immune cell comprises a polynucleotide comprising SEQ ID NO:19 or the sequence depicted in FIGS. 33A-B. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:19 or the sequence depicted in FIGS. 33A-B. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:19 or the sequence depicted in FIGS. 33A-B. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:19 or the sequence depicted in FIGS. 33A-B.

[0246] In some instances, the engineered immune cell comprises a polynucleotide comprising SEQ ID NO:20 or the sequence depicted in FIGS. 34A-B. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:20 or the sequence depicted in FIGS. 34A-B. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:20 or the sequence depicted in FIGS. 34A-B. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:20 or the sequence depicted in FIGS. 34A-B.

[0247] In some instances, the engineered immune cell comprises a polynucleotide comprising SEQ ID NO:21 or the sequence depicted in FIGS. 35A-B. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:21 or the sequence depicted in FIGS. 35A-B. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:21 or the sequence depicted in FIGS. 35A-B. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:21 or the sequence depicted in FIGS. 35A-B.

[0248] In some instances, the engineered immune cell comprises a polynucleotide comprising SEQ ID NO:22 or the sequence depicted in FIGS. 36A-B. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:22 or the sequence depicted in FIGS. 36A-B. In some embodiments,

provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:22 or the sequence depicted in FIGS. 36A-B. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:22 or the sequence depicted in FIGS. 36A-B.

[0249] In some instances, the engineered immune cell comprises a polynucleotide comprising SEQ ID NO:23 or the sequence depicted in FIGS. 37A-B. In one embodiment, provided herein is an engineered CD4⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:23 or the sequence depicted in FIGS. 37A-B. In some embodiments, provided herein is an engineered CD8⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:23 or the sequence depicted in FIGS. 37A-B. In some embodiments, provided herein is an engineered CD3⁺ T cell or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:23 or the sequence depicted in FIGS. 37A-B.

[0250] In some embodiments, the engineered immune cells described herein or a population thereof comprise at least 10% CD7 CAR⁺/CD7-negative T cells, at least 15% CD7 CAR⁺/CD7-negative T cells, at least 20% CD7 CAR⁺/CD7-negative T cells, at least 25% CD7 CAR⁺/CD7-negative T cells, at least 30% CD7 CAR⁺/CD7-negative T cells, at least 35% CD7 CAR⁺/CD7-negative T cells, at least 40% CD7 CAR⁺/CD7-negative T cells, at least 45% CD7 CAR⁺/CD7-negative T cells, at least 50% CD7 CAR⁺/CD7-negative T cells, at least 55% CD7 CAR⁺/CD7-negative T cells, at least 60% CD7 CAR⁺/CD7-negative T cells, at least 65% CD7 CAR⁺/CD7-negative T cells, at least 70% CD7 CAR⁺/CD7-negative T cells, at least 75% CD7 CAR⁺/CD7-negative T cells, at least 80% CD7 CAR⁺/CD7-negative T cells, at least 85% CD7 CAR⁺/CD7-negative T cells, at least 90% CD7 CAR⁺/CD7-negative T cells, at least 95% CD7 CAR⁺/CD7-negative T cells, at least 96% CD7 CAR⁺/CD7-negative T cells, at least 97% CD7 CAR⁺/CD7-negative T cells, at least 98% CD7 CAR⁺/CD7-negative T cells, at least 99% CD7 CAR⁺/CD7-negative T cells, or 100% CD7 CAR⁺/CD7-negative T cells. In some embodiments, the engineered immune cells outlined herein include a population of substantially purified CD7 CAR/CD7-negative T cells wherein such cells express any one of the dual promoter constructs described.

CD7 CAR⁺ engineered immune cells with reduced expression of endogenous CD7

[0251] In some embodiments, the engineered immune cells described herein express a CD7 CAR and have reduced or no endogenous CD7 expression compared to a non-engineered immune cell. Such engineered immune cells express a CD7 PEBL that minimizes or eliminates endogenous expression of CD7 on the surface of the immune cell. In some embodiments, reduced expression of CD7 refers to a downregulation or partial downregulation of surface CD7 by the cell. In some cases, reduced expression includes an at least 5% (e.g., at least 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 24%, 25%, 28%, 40%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) reduction in expression level compared to the expression level of a comparable wild-type or non-engineered cell. In some embodiments, engineered immune cells outlined herein include a population of substantially purified CD7 CAR/CD7-negative T cells.

[0252] In some embodiments, the engineered immune cells described herein express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:24. In some embodiments, the engineered immune cells express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:25. In some embodiments, the engineered immune cells express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:26. In some embodiments, the engineered immune cells express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:27.

[0253] In some embodiments, the engineered immune cells described herein express a CD7 CAR having at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the engineered immune cells express a CD7 CAR having at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the engineered immune cells express a CD7 CAR having at least 90% sequence identity to SEQ ID NO:30. In some embodiments, the engineered immune cells express a CD7 CAR having at least 90% sequence identity to SEQ ID NO:31.

[0254] In some embodiments, the engineered immune cells described herein express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:24 and a CD7 CAR having at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the engineered immune cells described herein express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:24 and a CD7 CAR having at least 90% sequence identity to SEQ ID NO:30.

[0255] In some embodiments, the engineered immune cells express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:25 and express a CD7 CAR having at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the engineered immune cells express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:25 and express a CD7 CAR having at least 90% sequence identity to SEQ ID NO:31.

[0256] In some embodiments, the engineered immune cells express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:26 and a CD7 CAR having at least 90% sequence identity to SEQ ID NO:28. In some embodiments, the engineered immune cells express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:26 and a CD7 CAR having at least 90% sequence identity to SEQ ID NO:30.

[0257] In some embodiments, the engineered immune cells express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:27 and express a CD7 CAR having at least 90% sequence identity to SEQ ID NO:29. In some embodiments, the engineered immune cells express a CD7 PEBL having at least 90% sequence identity to SEQ ID NO:27 and express a CD7 CAR having at least 90% sequence identity to SEQ ID NO:31.

[0258] In certain embodiments, the engineered immune cell is an engineered T cell, an engineered natural killer (NK) cell, an engineered NK/T cell, an engineered monocyte, an engineered macrophage, or an engineered dendritic cell. In some embodiments, the engineered immune cell is an engineered CD4⁺ T cell. In some embodiments, the engineered immune cell is an engineered CD8⁺ T cell. In some embodiments, the engineered immune cell is an engineered CD3⁺ T cell. Also provided is a population of any one of the engineered cells described herein.

[0259] In some embodiments, provided herein is a population of engineered immune cells (e.g., CD3⁺ T cells, CD4⁺ T cells, or CD8⁺ T cells) comprising at least about 50% (e.g., about 50%, 55%, 58%, 60%, 62%, 64%, 66%, 68%, 70%, 71%, 73%, 75%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) CD7 CAR-positive, endogenous CD7-negative cells. In some embodiments, provided herein is a population of engineered immune cells comprising at least about 50% (e.g., about 50%, 55%, 58%, 60%, 62%, 64%, 66%, 68%, 70%, 71%, 73%, 75%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) CD7 CAR-positive, endogenous CD7-negative

CD4⁺ T cells. In some embodiments, provided herein is a population of engineered immune cells comprising at least about 50% (e.g., about 50%, 55%, 58%, 60%, 62%, 64%, 66%, 68%, 70%, 71%, 73%, 75%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) CD7 CAR-positive, endogenous CD7-negative CD8⁺ T cells. Such a population of cells can be produced from peripheral blood mononuclear cells (PBMC), purified CD4⁺ T cells, purified CD8⁺ T cells, or a population comprising purified CD4⁺ T cells and purified CD8⁺ T cells.

[0260] In some embodiments, the engineered immune cells described herein are cultured to generate a highly pure population of CD7 CAR-T cells that have reduced expression of endogenous CD7. The level of purity can be at least about 75% (e.g., about 75%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 90%, 91%, 92%, 94%, 94%, 95%, 96%, 97%, 98%, 99%, or more) CD7 CAR-T cells with no surface expression of CD7. The expression level CD7 can be determined according to standard methods known to those in the art including, but not limited to immunocytochemistry, flow cytometry, and FACS analysis.

[0261] In some embodiments, the engineered immune cells of the present invention include CD45RO⁺ cells. In some embodiments, the engineered immune cells include CCR7-negative cells. In some embodiments, the engineered immune cells include central memory T cells. In some embodiments, the engineered immune cells include effector memory T cells. In some embodiments, the engineered immune cells include effector T cells. In some embodiments, the engineered immune cells include naive T cells.

[0262] In some instances, a population of engineered immune cells comprises effector memory T cells, central memory T cells, effector T cells, and naive T cells. In some embodiments, the population of engineered immune cells comprises a higher percentage of effector memory T cells and central memory T cells than effector T cells and naive T cells. In some embodiments, the population of engineered immune cells comprises about 40% or more effector memory T cells.

[0263] In some instances, a population of engineered immune cells comprises PD1-negative cells. In some instances, a population of engineered immune cells comprises TIM-1-negative cells. In some embodiments, the population comprises about 60% or more PD1-negative, TIM-1-

negative cells. In some embodiments, the population comprises about 4% to about 20% PD1 positive, TIM-1 positive cells.

[0264] In some embodiments, the engineered immune cells generate an immune response and secrete interferon- γ . The engineered immune cells induce T-cell mediated cytotoxicity in response to a cancer cell such as a CD7 expressing cancer cell.

[0265] In some embodiments, cells described herein comprising a bicistronic expression vector can be used to generate a population of CD7 CAR+/CD7-neg T cells. The CD7 CAR+/CD7-neg T cells can be expanded and enriched over time. The CD7 CAR+/CD7-neg T cells can be generated from cells including, but not limited to, bulk PBMCs, purified T cells comprising CD4+ and CD8+ T cells, and purified CD3+ T cells. The CD7 CAR+/CD7-neg T cells can be used to produce different subsets of T cells including T_{EM} cells, T_{CM} cells, Teff cells, and naïve T cells.

[0266] In another aspect, also provided is a method for producing the engineered immune cell (e.g., engineered CD3+ T cell, engineered CD4+ T cell, and engineered CD8+ T cell) having any of the embodiments described herein, the method comprising introducing into an immune cell any of the bicistronic constructs or dual promoter constructs of the present invention. In some embodiments, the engineered immune cells are derived from immune cells obtained from a subject that will receive the engineered immune cells as a therapy. In some embodiments, the engineered immune cells are derived from immune cells obtained from a donor and the resulting engineered immune cells are administered to a subject as a therapy.

[0267] In various aspects, also provided is a kit for producing an engineered immune cell described herein. The present kit can be used to produce, e.g., allogeneic or autologous T cells having anti-CD7 CAR-mediated cytotoxic activity. In some embodiments, the kit is useful for producing allogeneic effector T cells having anti-CD7 CAR-mediated cytotoxic activity. In certain embodiments, the kit is useful for producing autologous effector T cells having anti-CD7 CAR-mediated cytotoxic activity.

[0268] Accordingly, provided herein is a kit comprising any one of the bicistronic constructs or dual promoter constructs described herein.

[0269] In certain embodiments, the bicistronic construct further comprise sequences (e.g., plasmid or vector sequences) that allow, e.g., cloning and/or expression. For example, the nucleotide sequence can be provided as part of a plasmid for ease of cloning into other plasmids and/or vectors (expression vectors or viral expression vectors) for, e.g., transfection, transduction, or electroporation into a cell (e.g., an immune cell).

[0270] Typically, the kits are compartmentalized for ease of use and can include one or more containers with reagents. In certain embodiments, all of the kit components are packaged together. Alternatively, one or more individual components of the kit can be provided in a separate package from the other kits components. The kits can also include instructions for using the kit components.

Administering engineered immune cells

[0271] In other aspects, also provided is a method of treating cancer in a subject in need thereof, comprising administering a therapeutic amount of an engineered immune cell having any of the embodiments described herein to the subject, thereby treating cancer in a subject in need thereof.

[0272] In certain embodiments, the method comprises administering a therapeutic amount of an engineered immune cell comprising a bicistronic viral construct comprising a polynucleotide comprising a nucleic acid sequence encoding a CAR and a polynucleotide comprising a nucleic acid sequence encoding a PEBL. In various embodiments, the method comprises administering a therapeutic amount of any one of the engineered immune cells described herein comprising a recombinant retroviral vector comprising: (a) a first promoter operably linked to a first polynucleotide encoding a CD7 chimeric antigen receptor (CD7 CAR) as outlined herein; and (b) a second promoter operably linked to a second polynucleotide encoding a CD7 protein expression blocker (CD7 PEBL) as outlined herein.

[0273] In some embodiments, a therapeutic amount of an engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:11 or the sequence depicted in FIG. 25 is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell,

engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:12 or the sequence depicted in FIG. 26 is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:13 or the sequence depicted in FIG. 27 is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:14 or the sequence depicted in FIGS. 28A-28B is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:15 or the sequence depicted in FIGS. 29A-29B is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:16 or the sequence depicted in FIGS. 30A-30B is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:17 or the sequence depicted in FIGS. 31A-31B is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:18 or the sequence depicted in FIGS. 32A-B is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:19 or the sequence depicted in FIGS. 33A-B is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:20 or the sequence depicted in FIGS. 34A-B is administered to a subject having cancer. In some embodiments, a therapeutic amount of an

engineered immune cell or a population thereof (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) comprising a nucleic acid construct comprising SEQ ID NO:21 or the sequence depicted in FIGS. 35A-B is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:22 or the sequence depicted in FIGS. 36A-B is administered to a subject having cancer. In some embodiments, a therapeutic amount of an engineered immune cell (e.g., engineered CD3⁺ T cell, engineered CD4⁺ T cell, or engineered CD8⁺ T cell) or a population thereof comprising a nucleic acid construct comprising SEQ ID NO:23 or the sequence depicted in FIGS. 37A-B is administered to a subject having cancer.

[0274] In some embodiments, a therapeutic amount of a population of engineered immune cells (e.g., engineered CD3⁺ T cells, engineered CD4⁺ T cells, or engineered CD8⁺ T cells) comprising a CD7 PEBL of SEQ ID NO:25 is administered to a subject with cancer. In some embodiments, a therapeutic amount of a population of engineered immune cells (e.g., engineered CD3⁺ T cells, engineered CD4⁺ T cells, or engineered CD8⁺ T cells) comprising a CD7 PEBL of SEQ ID NO:27 is administered to a subject with cancer.

[0275] In some embodiments, a therapeutic amount of a population of engineered immune cells (e.g., engineered CD3⁺ T cells, engineered CD4⁺ T cells, or engineered CD8⁺ T cells) comprising a CD7 CAR of SEQ ID NO:29 is administered to a subject with cancer. In some embodiments, a therapeutic amount of a population of engineered immune cells (e.g., engineered CD3⁺ T cells, engineered CD4⁺ T cells, or engineered CD8⁺ T cells) comprising a CD7 CAR of SEQ ID NO:31 is administered to a subject with cancer.

[0276] In some embodiments, a therapeutic amount of a population of engineered immune cells (e.g., engineered CD3⁺ T cells, engineered CD4⁺ T cells, or engineered CD8⁺ T cells) comprising a CD7 PEBL of SEQ ID NO:25 and a CD7 CAR of SEQ ID NO:29 is administered to a subject with cancer. In some embodiments, a therapeutic amount of a population of engineered immune cells (e.g., engineered CD3⁺ T cells, engineered CD4⁺ T cells, or engineered CD8⁺ T cells) comprising a CD7 PEBL of SEQ ID NO:27 and a CD7 CAR of SEQ ID NO:29 is administered to a subject with cancer.

[0277] In some embodiments, a therapeutic amount of a population of engineered immune cells (e.g., engineered CD3+ T cells, engineered CD4+ T cells, or engineered CD8+ T cells) comprising a CD7 PEBL of SEQ ID NO:25 and a CD7 CAR of SEQ ID NO:31 is administered to a subject with cancer. In some embodiments, a therapeutic amount of a population of engineered immune cells (e.g., engineered CD3+ T cells, engineered CD4+ T cells, or engineered CD8+ T cells) comprising a CD7 PEBL of SEQ ID NO:27 and a CD7 CAR of SEQ ID NO:31 is administered to a subject with cancer.

[0278] In some embodiments, a therapeutic amount of a population of engineered immune cells (e.g., engineered CD3+ T cells, engineered CD4+ T cells, or engineered CD8+ T cells) is administered to a subject with cancer, wherein the engineered immune cells comprise SEQ ID NO:95.

[0279] In certain embodiments, the cancer is a T cell malignancy, e.g., T cell leukemia or T cell lymphoma, such a T-cell acute lymphoblastic leukemia, T-cell prolymphocytic leukemia, T-cell large granular lymphocytic leukemia, enteropathy-associated T-cell lymphoma, hepatosplenic T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, mycosis fungoides, Sézary syndrome, primary cutaneous gamma-delta T-cell lymphoma, peripheral T-cell lymphoma not otherwise specified, angioimmunoblastic T-cell lymphoma, anaplastic large cell lymphoma. In certain embodiments, the T cell malignancy is early T-cell progenitor acute lymphoblastic leukemia (ETP-ALL).

[0280] In some embodiments, the engineered immune cell is autologous to the subject in need of treatment, e.g., cancer treatment. In other embodiments, the engineered immune cell is allogenic to the subject in need of treatment.

[0281] In certain embodiments, the engineered immune cell is administered into the subject by intravenous infusion, intra-arterial infusion, direct injection into tumor and/or perfusion of tumor bed after surgery, implantation at a tumor site in an artificial scaffold, intrathecal administration, and intraocular administration.

[0282] In certain embodiments, the engineered immune cell is administered by infusion into the subject. Methods of infusing immune cells (e.g., allogeneic or autologous immune cells) are known in the art. A sufficient number of cells are administered to the recipient in order to ameliorate the symptoms of the disease. Typically, dosages of 10^7 to 10^{10} cells are infused in a

single setting, *e.g.*, dosages of 10^9 cells. Infusions are administered either as a single 10^9 cell dose or divided into several 10^9 cell dosages. The frequency of infusions can be daily, every 2 to 30 days or even longer intervals if desired or indicated. The quantity of infusions is generally at least 1 infusion per subject and preferably at least 3 infusions, as tolerated, or until the disease symptoms have been ameliorated. The cells can be infused intravenously at a rate of 50-250 ml/hr. Other suitable modes of administration include intra-arterial infusion, intraperitoneal infusion, direct injection into tumor and/or perfusion of tumor bed after surgery, implantation at the tumor site in an artificial scaffold, intrathecal administration. Methods of adapting the present invention to such modes of delivery are readily available to one skilled in the art.

[0283] In certain embodiments, the method of treating cancer according to the present invention is combined with at least one other known cancer therapy, *e.g.*, radiotherapy, chemotherapy, or other immunotherapy.

[0284] In other aspects, also provided is use of an engineered immune cell having any of the embodiments described herein for treating cancer, comprising administering a therapeutic amount of the engineered immune cell to a subject in need thereof. In certain embodiments, the cancer is a T cell malignancy. In certain embodiments, the T cell malignancy is early T-cell progenitor acute lymphoblastic leukemia (ETP-ALL).

[0285] In certain embodiments, the engineered immune cell is administered into the subject by intravenous infusion, intra-arterial infusion, intraperitoneal infusion, direct injection into tumor and/or perfusion of tumor bed after surgery, implantation at a tumor site in an artificial scaffold, and intrathecal administration.

EXAMPLES

Example 1: USING BICISTRONIC EXPRESSION VECTORS FOR BLOCKADE OF CD7 EXPRESSION IN CHIMERIC ANTIGEN-RECEPTOR T-CELLS

[0286] This example illustrates blockade of CD7 expression in anti-CD7 CAR-T cells using bicistronic expression constructs.

METHODS

Cell culture

[0287] 293T cells (ATCC CRL-3216) were maintained in DMEM (Gibco) with 10% FBS (Hyclone), 100U/mL penicillin and 100ug/mL streptomycin (Gibco). Jurkat clone E6-1 cells (ATCC TIB-152) and NALM6 clone G5 cells (CRL-3273) were maintained in RPMI1640 (Gibco) with 10% FBS (Hyclone), 100U/mL penicillin, 100ug/mL streptomycin (Gibco) and 1x GlutaMAX (Gibco).

Lentivirus production

[0288] 293T cells were cotransfected with lentiviral transfer vectors and Virapower packaging plasmids mix (Invitrogen) at a ratio of 1:3 using Lipofectamine 2000 (Invitrogen). Transfection medium was replaced with fresh DMEM (Gibco) with 10% FBS (Hyclone) 6 hours post transfection. 48h later, the virus supernatant was collected, passed through a 0.45μM filter and then concentrated 100x using Lenti-X Concentrator (Clontech). Concentrated lentivirus stock was stored at -150°C until use.

Retrovirus production

[0289] 293T cells were cotransfected with retroviral transfer vectors and pEQ and pRDF packaging plasmids using X-tremeGENE 9 DNA transfection reagent (Roche). Transfection medium was replaced with fresh DMEM (Gibco) with 10% FBS (Hyclone) 6 hours post transfection. 24h and 48h later, the virus supernatant was collected and passed through a 0.45μM filter. Retrovirus was used fresh or stored at -150°C until use.

Lentivirus titration on 293T cells

[0290] 293T cells were transduced with varying volumes of lentiviruses in the presence of 5μg/mL polybrene (Sigma). After 15h overnight culture, transduction medium was removed and cells were treated with 10U/mL DNaseI (New England Biolabs) in fresh culture media for 15min at 37°C. The media was then replaced with fresh DMEM with 10% FBS for further culture. Transduced cells were harvested for analysis at ≥ 72h post transduction. Virus titers were determined using flow cytometry and RT-qPCR.

[0291] Transducing unit (TU) titers were calculated from flow cytometry data using the equation: $[TU/mL = (\text{Number of 293T cells per sample} \times \%CAR+ \text{ cells}) \div \text{Virus volume in mL}]$. TU titers were calculated using samples within the linear range of %CAR+ cells and virus volume.

[0292] Integration unit (IU) titers were calculated from RT-qPCR data on genomic DNA using the equation: $[IU/ml = (\text{Number of 293T cells per sample} \times \text{number of proviral gene copies per genome}) \div \text{Virus volume in mL}]$. IU titers were calculated using samples within the linear range of proviral gene copy number and virus volume.

Lentiviral transduction of Jurkat cells

[0293] Lentiviruses were directly added to Jurkat cells with or without 8 μ g/mL polybrene (Sigma). A complete media change was performed two days later to remove lentiviruses from cultures. Transduced cells were harvested for analysis at ≥ 2 days post transduction.

Primary T cell culture

[0294] Frozen human primary peripheral blood mononuclear cells (PBMCs) (ATCC Cat# PCS-800-011 and Stemcell Technologies Cat# 70025) were thawed, recovered overnight, and maintained at 1million cells/mL in either RPMI1640 (Gibco) with 10% FBS (Hyclone), 100U/mL penicillin, 100ug/mL streptomycin (Gibco) and 1x GlutaMAX (Gibco), TexMACS medium (Miltenyi Biotec) supplemented with 3% human AB serum (Sigma), or serum-free TexMACS medium. Culture media was supplemented with 120IU/mL Interleukin-2 (Miltenyi Biotec) every 2 to 3 days.

[0295] PBMCs were either cultured in bulk without further selection or purified for T cells after overnight recovery. CD4⁺ and CD8⁺ T cells were isolated using CD4 Microbeads (Miltenyi Biotec) and CD8 Microbeads (Miltenyi Biotec). CD3⁺ T cells were isolated using CD3 Microbeads (Miltenyi Biotec).

[0296] T cells were activated with either T Cell TransAct (Miltenyi Biotec) or Dynabeads Human T-Activator CD3/CD28 for T Cell Expansion and Activation (Gibco) according to manufacturers' recommendations. Dynabeads were added at a bead to cell ratio of 1:1. Bead depletion was performed after 4 days.

[0297] Lentiviral transduction of primary T cells

[0298] Primary T cells were transduced at 1 to 4 days post activation. Static transduction was performed where lentiviruses were directly added to T cells. A complete media change was performed two days later to remove lentiviruses from cultures. Transduced cells were analysed by flow cytometry at ≥ 3 days post transduction.

Retroviral transduction of primary T cells

[0299] RetroNectin-based retroviral transduction was performed on primary T cells. Non-treated tissue culture plates were coated with 2.5µg/cm² of RetroNectin Recombinant Human Fibronectin Fragment (Takara) according to manufacturer's recommendations. Retrovirus supernatants were added to retronectin-coated plates and centrifuged at 1000xg for 2h at 32°C. Virus supernatants were then removed from the wells. Wells were rinsed with culture media before adding T cells. Transduced cells were analysed by flow cytometry at ≥ 3 days post transduction.

Flow cytometry

[0300] Antibody staining and washes are performed with staining buffer (1xPBS pH 7.4, 0.2%BSA, 0.02% sodium azide). Cells were incubated with antibodies on ice for 15 min and washed 3 times. The following antibodies were used for staining: anti-mouse F(ab')₂-biotin (Jackson ImmunoResearch 115-066-072), streptavidin-APC (Jackson ImmunoResearch 016-130-084), anti-human CD7-PE (BD 555361), anti-human CD3 eFluor780 (eBioscience 47-0037-42). Cells stained with anti-mouse F(ab')₂-biotin were blocked with 3µg of mouse IgG1 isotype control antibody (BioXCell) for 5min before adding the remaining antibodies. DAPI was used at 1µg/mL for live/dead discrimination. Stained cells were collected on an Invitrogen Attune NxT flow cytometer and analyzed with FlowJo v10 software.

Real-Time quantitative PCR (RT-qPCR)

[0301] Genomic DNA was extracted from cells using the DNeasy Blood and Tissue Kit (Qiagen) and RNase A (Qiagen). Total RNA was extracted from cells using the MN NucleoSpin RNA Kit (Macherey-Nagel), and cDNA was synthesized using the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific). All kits were used according to manufacturers' recommendations. RT-qPCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad) on a CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad).

[0302] **Primers used were:**

[0303] RPPH1-F: 5'-GAGGGAAGCTCATCAGTGGG-3' (SEQ ID NO:87)

[0304] RPPH1-R: 5'-CATCTCCTGCCCAGTCTGAC-3' (SEQ ID NO:88)

[0305] WPRE-F: 5'-CCTTTCCGGGACTTTCGCTTT-3' (SEQ ID NO:89)

- [0306] WPRE-R: 5'-GCAGAATCCAGGTGGCAACA-3' (SEQ ID NO:90)
- [0307] TH69CD7CAR-F: 5'-GCAGCCTTTCATGAGACCAG-3' (SEQ ID NO:91)
- [0308] TH69CD7CAR-R: 5'-TGCCCAGGTTCAGCTCATTA-3' (SEQ ID NO:92)
- [0309] TH69CD7PEBL-F: 5'-ACCTGCCGCATACAAGGATA-3' (SEQ ID NO:93)
- [0310] TH69CD7PEBL-R: 5'-CCACTGTGCAGACTAGAGGT-3' (SEQ ID NO:94)

[0311] All assay primers had primer efficiencies between 90% and 110%.

[0312] Fold changes of all genes were normalized to a housekeeping gene using the equation: $[\text{Fold change} = 2^{-(\text{Ct}(\text{target gene}) - \text{Ct}(\text{housekeeping gene}))}]$. Copy number of target genes was normalized to the genomic copy number of RNaseP in 293T cells.

Western blot

[0313] Cells were lysed with RIPA buffer (Pierce) and protease inhibitor (Pierce). Protein quantitation of cell lysates was performed using a Bradford Coomassie protein assay kit (Thermo Scientific), according to manufacturer's recommendations. Western blots were performed using an automated western blot system, Simple Western Wes (ProteinSimple), with a 12-230kDa Wes Separation Module. The following primary antibodies were used: anti- β -Actin, clone 13E5 (Cell Signaling Technology); anti-Myc-Tag, clone 71D10 (Cell Signaling Technology); and anti-CD3 ζ polyclonal antibody, Cat# ab226475 (Abcam). Secondary antibodies from Wes Anti-Rabbit Detection Module were used. The data was analysed with Compass for Simple Western software.

IFN γ secretion

[0314] Effector CAR-T cells were resuspended at a cell density of 10^6 cells/mL, and 100,000 CAR-T cells were plated per well in a 96-well round-bottom plate. Target cells were cocultured with effector CAR-T cells at various effector:target (E:T) ratios for 24h. After 24h, the cells were spun down and supernatants were collected and stored at -150°C . The supernatants were evaluated for IFN γ secretion using the ELISA MAX Standard Set Human IFN- γ kit (Biolegend) according to manufacturer's recommendations.

Cytotoxicity assay

[0315] Target cells were resuspended at a cell density of 10^6 cells/mL and loaded with $0.4\mu\text{g/ml}$ of calcein red-orange AM (Invitrogen) for 10min. Loaded cells were then washed

thrice to remove excess calcein. 100,000 target cells were plated per well in a 96-well round-bottom plate. Target cells were cocultured with effector CAR-T cells at various effector:target (E:T) ratios for 4h. After 4h, DAPI was added to all wells, and cells were collected on the flow cytometer. The number of remaining live target cells was counted in all wells. The percentage cytotoxicity was calculated with the following equation:

[0316] Percentage cytotoxicity = $[(S-E)/S] * 100\%$

[0317] S = Remaining live target cells in target cell only control wells

[0318] E = Remaining live target cells after coculture with effector T cells in experimental wells

RESULTS

[0319] Primary T cells were transduced with the different retroviruses expressing (1) PEBL; (2) CAR; (3) PEBL and CAR sequentially; (4) PEBL-IRES-CAR; or (5) CAR-P2A-PEBL. The transduced cells were analyzed by flow cytometry for CD7 and CAR expression (FIG. 1A). Cell lysates from primary T cells transduced with the indicated retroviruses were analyzed by Western blot for β -actin, Myc-tagged PEBL, CAR and endogenous CD3 ζ expression (FIG. 1B).

[0320] Dual promoter lentiviral constructs were prepared to express an anti-CD7 CAR and an anti-CD7 PEBL from a single vector. As shown in FIG. 2A-FIG. 2F, the general format of the dual promoter construct from 5' end to 3' end included a first promoter – an anti-CD7 CAR – a second promoter – an anti-CD7 PEBL. The promoters tested include a MSCV promoter, an EFS promoter, a PGK promoter, and an EF1a promoter. Nucleic acid sequences of exemplary dual promoter constructs are provided as SEQ ID NOS:19-23 and shown in FIGS. 33A-33B, FIGS. 34A-34B, FIGS. 35A-35B, FIGS. 36A-36B, and FIGS. 37A-37B. Such constructs encoded anti-CD7 CARs including an anti-CD7 CAR based on the TH69 antibody and an anti-CD7 CAR based on the 3A1F antibody, as well as anti-CD7 PEBLs including an anti-CD7 PEBL based on the TH69 antibody and an anti-CD7 PEBL based on the 3A1F antibody.

[0321] The dual promoter lentiviral vectors were transduced into cells to produce cells with partial downregulation of surface CD7 expression and low expression of the anti-CD7 CAR. The percentage of cells expressing the CAR -remained low after an extended time in culture. FIG. 3 shows the expression of the CAR (y-axis) and the expression of CD7 (x-axis) in the cells

at 5 days after transduction and at 14 days after transduction. The figure shows expression of cells (e.g., healthy donor cells including healthy donor lymphocytes) transduced with the exemplary dual promoter constructs are provided as SEQ ID NOS:18-23 and shown in FIGS. 32A-32B, 33A-33B, FIGS. 34A-34B, FIGS. 35A-35B, FIGS. 36A-36B, and FIGS. 37A-37B. As an example, the cells transduced with the dual promoter lentiviral vector comprising the MSCV promoter-CD7 (TH69) CAR-EF1a promoter-CD7 (TH69) PEBL produced a population of cells comprising CD7 CAR-neg /CD7-neg cells (52.8%), CD7 CAR+/CD7-neg cells (2.98%), CD7 CAR-neg /CD7+ cells (40.4%), and CD7 CAR+/CD7+ cells (3.84%) at 5 days post transduction. At 14 days post transduction the MSCV promoter-CD7 (TH69) CAR-EF1a promoter-CD7 (TH69) PEBL transduced cells included a population of cells comprising CD7 CAR-neg /CD7-neg cells (42.6%), CD7 CAR+/CD7-neg cells (0.14%), CD7 CAR-neg /CD7+ cells (54.9%), and CD7 CAR+/CD7+ cells (2.37%).

[0322] In an effort to optimize expression of the CD7 CAR and CD7 PEBL single promoter bicistronic lentiviral constructs were generated. Exemplary schematic diagrams of such constructs are provided in FIG. 4A, FIG. 4B, and FIG. 4C. FIG. 4A depicts a schematic of an exemplary bicistronic construct comprising an MSCV promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:14. FIG. 4B depicts a schematic of an exemplary bicistronic construct comprising an EF1a promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:15. FIG. 4C depicts a schematic of an exemplary bicistronic construct comprising an EFS promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL, such as the one of SEQ ID NO:16.

[0323] Cells transduced with an MSCV promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL lentivirus generated a population of CD7 CAR+/CD7-neg T cells. FIG. 5 shows an expansion and enrichment of the CD7 CAR+/CD7-neg T cells from day 0 to day 9 post transduction. For example at day 0, 10.9% of the cells were CD7 CAR-neg /CD7-neg cells, 0.016% were CD7 CAR+/CD7-neg cells, 87.9% were CD7 CAR-neg /CD7+ cells, and 1.21% were CD7 CAR+/CD7+ cells. At day 3, 24.1% of the cells were CD7 CAR-neg /CD7-neg cells, 17.7% were CD7 CAR+/CD7-neg cells, 53.8% were CD7 CAR-neg /CD7+ cells, and 4.33% were CD7 CAR+/CD7+ cells. At day 6, 27.5% of the cells were CD7 CAR-neg /CD7-neg cells, 63.7% were CD7 CAR+/CD7-neg cells, 6.25% were CD7 CAR-neg /CD7+ cells, and

2.57% were CD7 CAR+/CD7+ cells. At day 9, 16.1% of the cells were CD7 CAR-neg /CD7-neg cells, 83.7% were CD7 CAR+/CD7- cells, 0.012% were CD7 CAR-neg /CD7+ cells, and 0.095% were CD7 CAR+/CD7+ cells.

[0324] Cells transduced with an anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL bicistronic lentivirus vectors with different promoters produced CD7 CAR+/CD7-neg cells that were enriched in culture over time. FIG. 6 shows an increase in the percentage of CD7 CAR+/CD7-neg cells at 5 days post transduction and 14 days post transduction. For example, cell transduced with an EFS promoter anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL lentiviral vector produced 67.3% CD7 CAR-neg /CD7- cells, 31.2% were CD7 CAR+/CD7-neg cells, 0.44% were CD7 CAR-neg /CD7+ cells, and 1.06% were CD7 CAR+/CD7+ cells at 5 days post transduction. By 14 days post transduction 25.0% CD7 CAR-neg /CD7- cells, 73.8% were CD7 CAR+/CD7-neg cells, 0.80% were CD7 CAR-neg /CD7+ cells, and 0.46% were CD7 CAR+/CD7+ cells. As a control, cells were transduced with a lentiviral vector containing a EF1a promoter upstream of an anti-CD19 CAR at 5 days post transduction 34.7% of the cells were CD19 CAR+/CD7+, 60.1% were CD19 CAR-neg /CD7+, 4.58% were double negative, and 0.68% were CD19 CAR+/CD7-neg. And at 14 days post transduction, 47.5% of the cells were CD19 CAR+/CD7+, 50.4% were CD19 CAR-neg /CD7+, 1.01% were double negative, and 1.06% were CD19 CAR+/CD7-neg. Thus, almost no enrichment of CD19 CAR expressing cells was detected over time.

[0325] To evaluate consistency and reproducibility of the single promoter bicistronic vectors, cells were transduced with two independent lots of an MSCV promoter-anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL lentivirus. The CD7 PEBL was myc tagged and detected by Western blot. The CD7 CAR was also detected (FIG. 7B). FIG. 7A shows flow cytometry analysis of the CD7 CAR and CD7 expression in the transduced cells. The first lot generated a population of transduced cells comprising 53.8% CD7 CAR+/CD7-neg cells and 46.1% CD7-neg /CD7-neg cells. The second lot produced a population of transduced cells comprising 65.5% CD7 CAR+/CD7-neg cells and 34.5% CD7-neg /CD7-neg cells. It was noted that the untransduced cells included 98.6% CAR-neg /CD7+ cells.

[0326] It should be noted that the single promoter bicistronic vectors described herein were successfully used to produce CD7 PEBL-CAR-T cells from different starting cells including bulk

PBMCs, purified T cells comprising CD4⁺ and CD8⁺ T cells, and purified CD3⁺ T cells. In addition, different T cell activation reagents including Dynabeads® Human T-Activator CD3/CD28 for T Cell Expansion and Activation (Gibco) and T Cell TransAct™ (Miltenyi Biotec) were used. FIG. 8 shows an increasing percentage of CD7 CAR⁺/CD7-neg T cells when the anti-human CD7 (TH69) CAR-P2A-anti-human CD7 (TH69) PEBL transduced cells were cultured over time. A comparable expansion of the CD7 CAR⁺/CD7-neg T cells was detected among the different starting cell types and between the two activation reagents.

[0327] The cells described herein (e.g., CD7 CAR⁺/CD7-neg T cells) were generated from purified CD4⁺ positively selected and CD8⁺ positively selected T cells cultured in either serum-free TexMACS media or TexMACS media supplemented with 3% human AB serum. T cells were transduced with CD7CAR-P2A-CD7PEBL lentivirus at MOI 10 to generate CD7-CAR⁺ T cells. Total fold change of transduced cells at 11 days post cell activation was higher with serum-supplemented media (FIG. 9A and FIG. 9B).

[0328] Purified CD4⁺ and CD8⁺ selected T cells transduced with CD7CAR-P2A-CD7PEBL lentivirus at different days post activation (day 1 to day 4) generated a highly pure population of CAR⁺ T cells (FIG. 10A and FIG. 10B). Cells transduced on different days expanded and proliferated during the manufacturing process. FIG. 10B shows that the transduced T cells exhibited about an average of 5-fold to 10-fold expansion when the cells were transduced at 1, 2, 3, or 4 days after activation.

[0329] Expression of the CAR and endogenous CD7 in T cells transduced with different MOIs of the CD7CAR-P2A-CD7PEBL lentivirus was measured by FACS. T cells transduced at lower MOIs had a lower percentage of CD7 CAR⁺/CD7-neg T cells early in the transduction process, but increased to match the percentage of CD7 CAR⁺/CD7-neg T cells obtained at higher MOIs of transduction (see, e.g., FIG. 11). For instance, T cells from Donor 1 that were transduced with the lentivirus at an MOI of 3 were 9.76% CD7 CAR⁺/CD7-neg T cells at 3 days post transduction and 69.9% CD7 CAR⁺/CD7-neg T cells at 9 days post transduction. T cells from Donor 1 that were transduced with the lentivirus at an MOI of 10 were 17.7% CD7 CAR⁺/CD7-neg at 3 days post transduction and 83.7% CD7 CAR⁺/CD7-neg at 9 days post transduction.

[0330] Purified CD4⁺ and CD8⁺ T cells from three unique donors were transduced with CD7CAR-P2A-CD7PEBL lentivirus at the indicated MOI in two individual wells. The percentage of CAR⁺ cells were analysed by flow cytometry. Cell pellets were collected and genomic DNA was extracted to determine vector copy number (VCN) by RT-qPCR analysis. Higher MOI correlated with higher VCN (FIG. 12B), however the percentage of CD7 CAR⁺ T cells was similar at MOI 5 and 10 (FIG. 12A).

[0331] Expression of various surface markers was measured in the primary T cells transduced with MSCV-CD7CAR-P2A-CD7PEBL lentivirus at 11 days post activation. FIG. 13A shows CD7 CAR and endogenous CD7 expression in transduced cells from three different donors. Expression of CD3 compared to CD14/CD19/CD56 is shown in FIG. 13B. CD4 and CD8 expression is shown in FIG. 13C. FIG. 13D shows that transduced cells generated different subsets of T cells including T_{EM} cells, T_{CM} cells, T_{eff} cells, and naïve T cells as determined by CD45RO and CCR7 expression. FIG. 13D shows PD-1 and TIM-3 expression in the transduced T cells.

[0332] The response of the transduced PEBL-CAR T cells to CD7⁺ Jurkat cells and CD7-negative Nalm6 cells was determined by IFN γ secretion (FIG. 14A) and cytotoxicity (FIG. 14B). IFN-g secretion measured in culture supernatant of PEBL-CAR T cells co-cultured with Jurkat or Nalm6 cells at the indicated E:T ratios for 24h (mean $\pm$ SD of technical replicates). PEBL-CAR-T cells showed target-specific functional responses, as IFN γ was secreted by the PEBL-CAR-T cells when cultured with CD7⁺ Jurkat cells, and not with Nalm6 cells. In addition, PEBL-CAR-T cells killed CD7⁺ Jurkat cells but not CD7-negative Nalm6 cells in a cytotoxicity assay.

[0333] This example demonstrates the generation and expansion of PEBL-CAR-T cells produced using a CD7 CAR-P2A-CD7 PEBL bicistronic lentiviral vector. Such cells showed antigen-specific T cell functional responses such as IFN γ secretion and specific toxicity against CD7⁺ target cell lines. The PEBL-CAR-T cells exhibited high percentage purity of CD7 negative, CAR⁺ T cells.

[0334] The teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety.

[0335] While this invention has been particularly shown and described with references to example embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

WHAT IS CLAIMED IS:

1. A bicistronic retroviral vector comprising:
 - (a) a first polynucleotide encoding an anti-CD7 chimeric antigen receptor (CAR) comprising at least 90% sequence identity to the amino acid sequence of any one of SEQ ID NOS:28-31;
 - (b) a second polynucleotide encoding an Internal Ribosome Entry Site (IRES) or a ribosomal codon skipping site; and
 - (c) a third polynucleotide encoding an anti-CD7 protein expression blocker (PEBL) comprising at least 90% sequence identity to the amino acid sequence of SEQ ID NOS:24-27;wherein the first polynucleotide is operably linked the second polynucleotide which is operably linked the third polynucleotide.
2. The bicistronic retroviral vector of claim 1, wherein the anti-CD7 CAR comprises the amino acid sequence of any one of SEQ ID NOS:28-31.
3. The bicistronic retroviral vector composition of claim 1 or 2, wherein the anti-CD7 PEBL comprises the amino acid sequence of any one of SEQ ID NOS:24-27.
4. The bicistronic retroviral vector composition of any one of claims 1 to 3, wherein the anti-CD7 CAR comprises the amino acid sequence of SEQ ID NO:29 and the anti-CD7 PEBL comprises the amino acid sequence of SEQ ID NO:25.
5. The bicistronic retroviral vector composition of any one of claims 1 to 3, wherein the anti-CD7 CAR comprises the amino acid sequence of SEQ ID NO:31 and the anti-CD7 PEBL comprises the amino acid sequence of SEQ ID NO:27.
6. The bicistronic retroviral vector of any one of claims 1 to 5, wherein the IRES is derived from *Encephalomyocarditis* virus (EMCV) or an *Enterovirus*.
7. The bicistronic retroviral vector of any one of claims 1 to 5, wherein the ribosomal codon skipping site comprises a 2A self-cleaving peptide.

8. The bicistronic retroviral vector of claim 7, wherein the 2A self-cleaving peptide is selected from the group consisting of a F2A peptide (foot-and-mouth disease virus 2A peptide), an E2A peptide (equine rhinitis A virus 2A peptide), a P2A peptide (porcine teschovirus-1 2A peptide), and a T2A peptide (thosea asigna virus 2A).

9. The bicistronic retroviral vector of any one of claims 1 to 6, comprising at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO:12.

10. The bicistronic retroviral vector of any one of claims 1 to 5 and 6, comprising at least 90% sequence identity to the nucleic acid sequence of SEQ ID NO:13.

11. The bicistronic retroviral vector of claim 10, comprising the nucleic acid sequence of SEQ ID NO:13.

12. The bicistronic retroviral vector of any one of claims 1 to 11, further comprises a promoter element.

13. The bicistronic retroviral vector of claim 12, wherein the promoter element is selected from the group consisting of a CMV promoter, EF1 α promoter, EFS promoter, MSCV promoter, and PGK promoter.

14. The bicistronic retroviral vector of claim 13, wherein the promoter element comprises at least 90% sequence identity to the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:6-10.

15. The bicistronic retroviral vector of claim 13 or 14, wherein the promoter element comprises the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:6-10.

16. The bicistronic retroviral vector of any one of claims 12 to 15, comprising at least 90% sequence identity to the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:14-16.

17. The bicistronic retroviral vector of any one of claims 12 to 16, comprising the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:14-16.

18. The bicistronic retroviral vector of any one of claims 1 to 18, wherein the retroviral vector is a lentiviral vector.

19. An engineered immune cell comprising the bicistronic retroviral vector of any one of claims 1 to 18.

20. The engineered immune cell of claim 19, wherein the engineered immune cell is an allogeneic T cell.

21. The engineered immune cell of claim 20, wherein the engineered immune cell is an autologous T cell.

22. The engineered immune cell of claim 20 or 21, wherein the engineered immune cell has reduced CD7 surface expression compared to a corresponding immune cell and expresses the anti-CD7 CAR.

23. A pharmaceutical composition comprising the engineered immune cell of claim 19 and a pharmaceutically effective carrier.

24. A method of treating a cancer in a subject comprising administering a therapeutically effective amount of the engineered immune cell of any one of claims 19 to 21, or the pharmaceutical composition of claim 23.

25. A method of producing an engineered immune cell comprising transducing an immune cell with the bicistronic retroviral vector of any one of claims 1 to 18, and recovering the engineered immune cell.

26. The method of claim 25, wherein the immune cell is selected from the group consisting of a peripheral blood mononuclear cell, an isolated CD4⁺ T cell, an isolated CD8⁺ T cell, and an isolated CD3⁺ T cell.

27. The method of claim 25 or 26, wherein the engineered immune cell has reduced CD7 surface expression compared to a corresponding immune cell and expresses the anti-CD7 CAR.

28. A recombinant retroviral vector comprising:
- (a) a first promoter element operably linked to a first polynucleotide encoding an anti-CD7 chimeric antigen receptor (CAR) comprising at least 90% sequence identity to the amino acid sequence of SEQ ID NO:28 or SEQ ID NO:30; and
 - (b) a second promoter element operably linked to a second polynucleotide encoding an anti-CD7 protein expression blocker (PEBL) comprising at least 90% sequence identity to the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:26.
29. The recombinant retroviral vector of claim 28, wherein the anti-CD7 CAR comprises the amino acid sequence of SEQ ID NO:28 or SEQ ID NO:30.
30. The recombinant retroviral vector of claim 28 or 29, wherein the anti-CD7 PEBL comprises the amino acid sequence of SEQ ID NO:24 or SEQ ID NO:26.
31. The recombinant retroviral vector of any one of claims 28 to 30, wherein the first promoter element and/or the second promoter element are selected from the group consisting of a CMV promoter, EF1 α promoter, EFS promoter, MSCV promoter, and PGK promoter.
32. The recombinant retroviral vector of any one of claims 28 to 31, wherein the first promoter element and/or the second promoter element comprise at least 90% sequence identity to the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:6-10.
33. The recombinant retroviral vector of any one of claims 28 to 32, wherein the first promoter element and/or the second promoter element comprise the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:6-10.
34. The recombinant retroviral vector of any one of claims 28 to 33, wherein the first promoter and the second promoter share less than 95% sequence identity.
35. The recombinant retroviral vector of any one of claims 28 to 34, wherein the first promoter element operably linked to the first polynucleotide is 5' of the second promoter element operably linked to the second polynucleotide.

36. The recombinant retroviral vector of any one of claims 28 to 34, wherein the second promoter element operably linked to the second polynucleotide is 5' of the first promoter element operably linked to the first polynucleotide.

37. The recombinant retroviral vector of any one of claims 28 to 35, comprising at least 90% sequence identity to the nucleic acid sequence of any one selected from the group consisting of SEQ ID NOS:18-23.

38. The recombinant retroviral vector of any one of claims 28 to 37, wherein the retroviral vector is a lentiviral vector.

39. An engineered immune cell comprising the recombinant retroviral vector of any one of claims 28 to 38.

40. The engineered immune cell of claim 39, wherein the engineered immune cell is an allogenic T cell.

41. The engineered immune cell of claim 40, wherein the engineered immune cell is an autologous T cell.

42. The engineered immune cell of claim 40, wherein the engineered immune cell has reduced CD7 surface expression compared to a corresponding immune cell and expresses the anti-CD7 CAR.

43. A pharmaceutical composition comprising the engineered immune cell of any one of claim 39 to 42 and a pharmaceutically effective carrier.

44. A method of treating a cancer in a subject comprising administering therapeutically effective amount of the engineered immune cell of any one of claims 39 to 41, or the pharmaceutical composition of claim 43.

45. A method of producing an engineered immune cell comprising transducing an immune cell with the recombinant retroviral vector of any one of claims 28 to 38, and recovering the engineered immune cell.

46. The method of claim 45, wherein the immune cell is selected from the group consisting of a peripheral blood mononuclear cell, an isolated CD4⁺ T cell, an isolated CD8⁺ T cell, and an isolated CD3⁺ T cell.

47. The method of claim 45 or 46, wherein the engineered immune cell has reduced CD7 surface expression compared to a corresponding immune cell and expresses the anti-CD7 CAR.

FIG. 1A

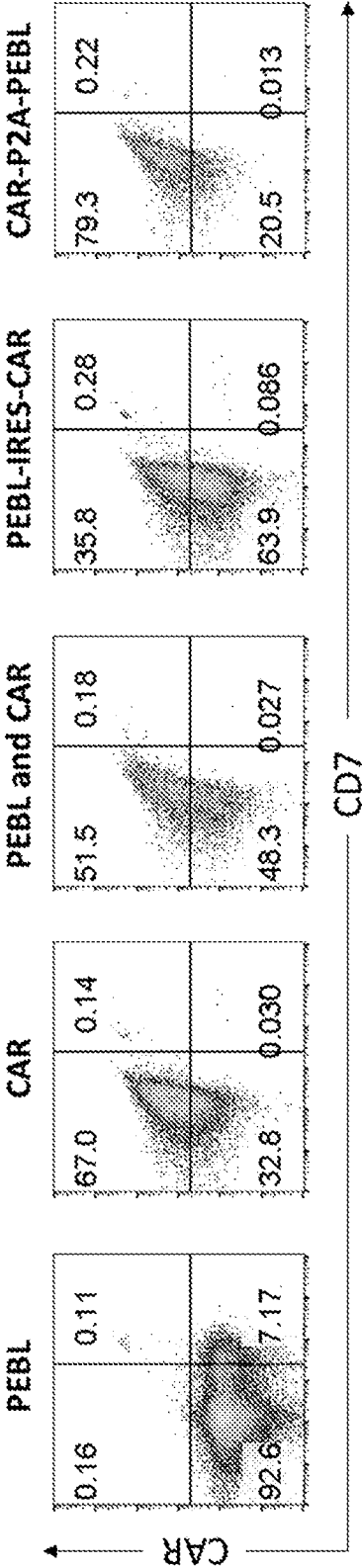
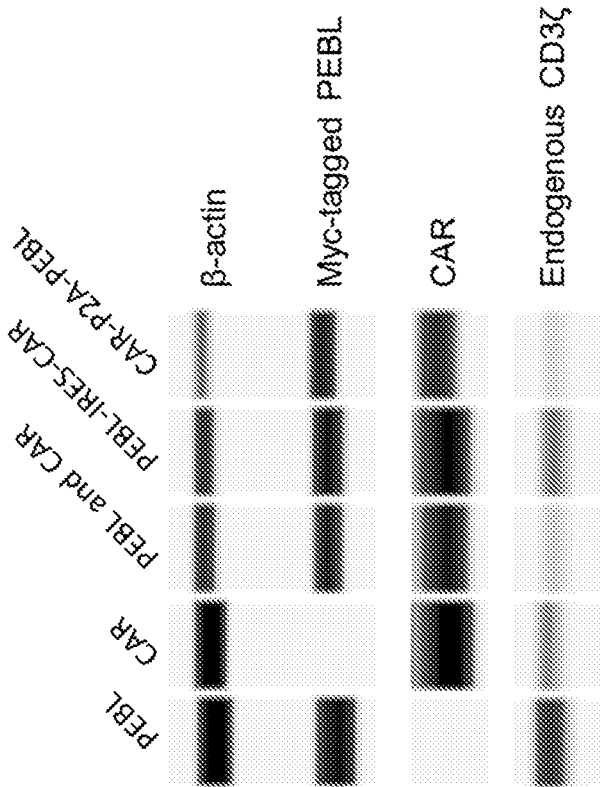


FIG. 1B



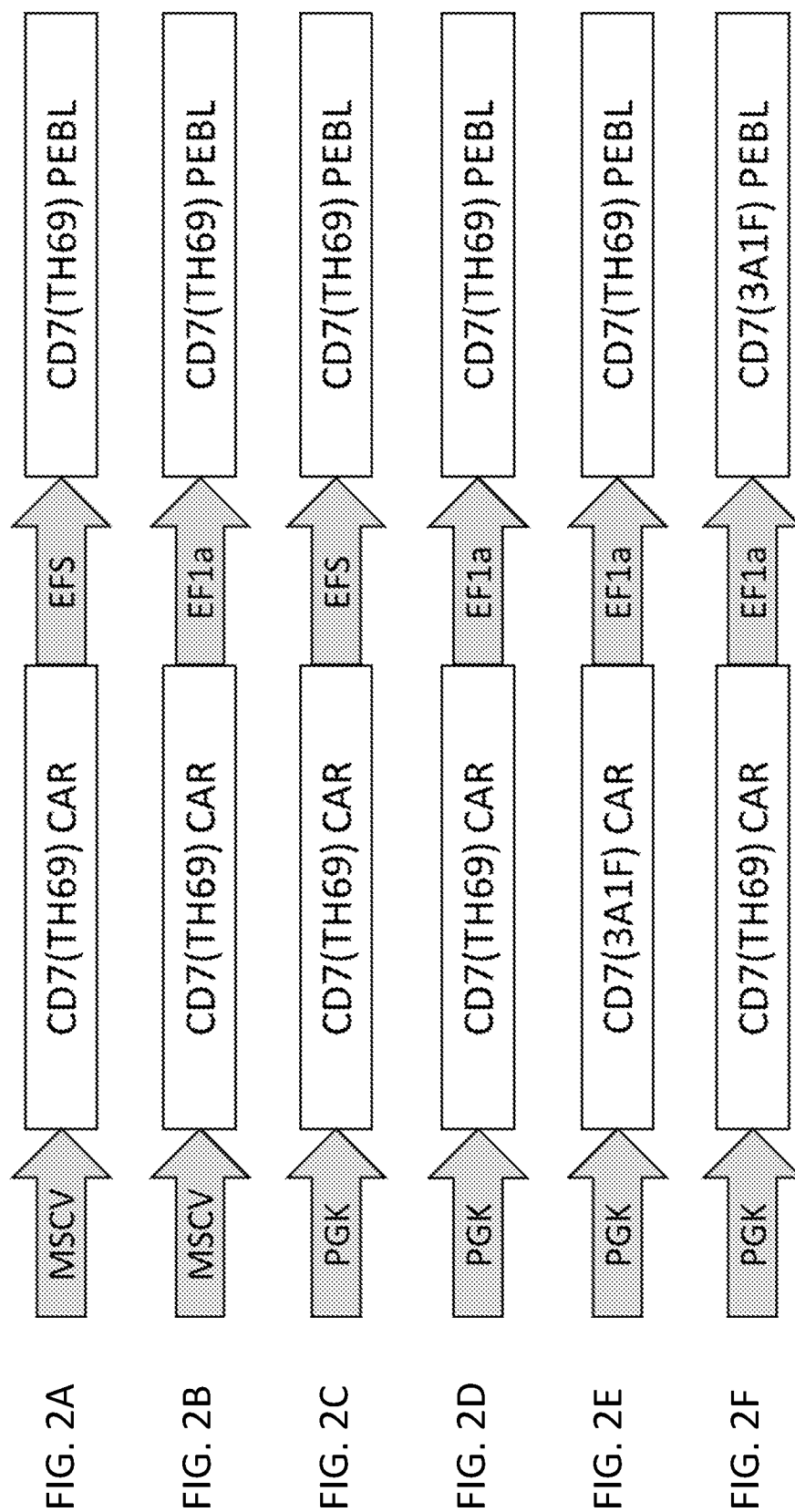
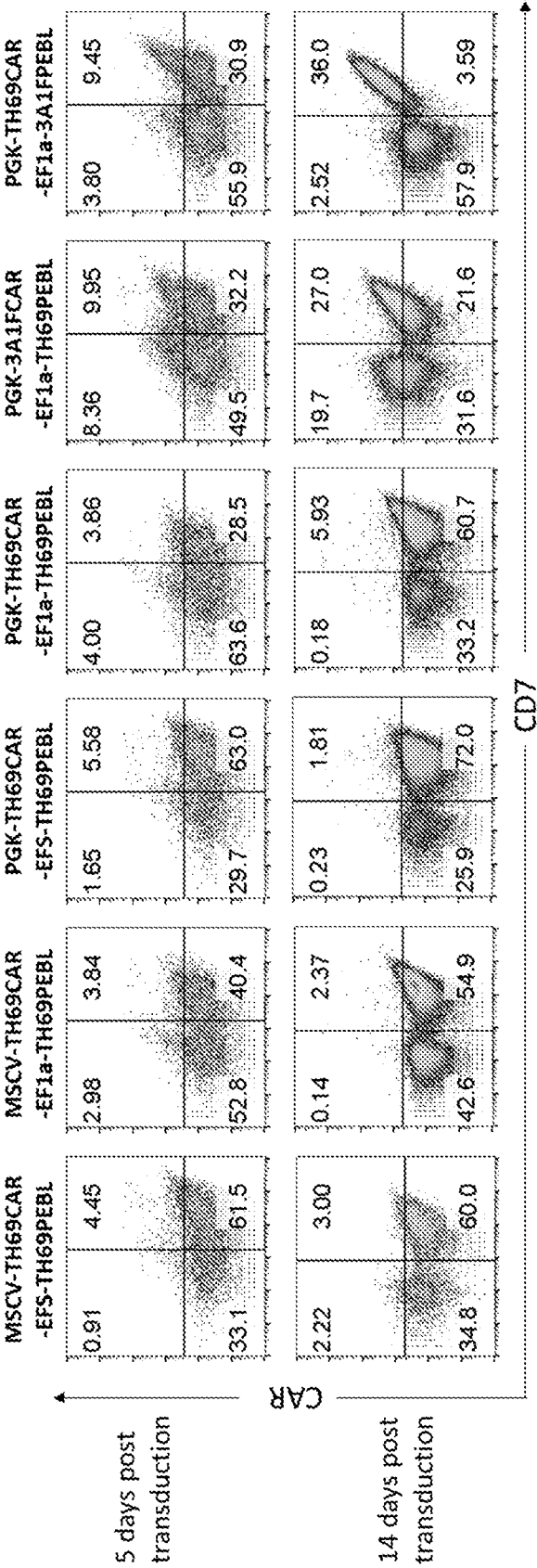


FIG. 3



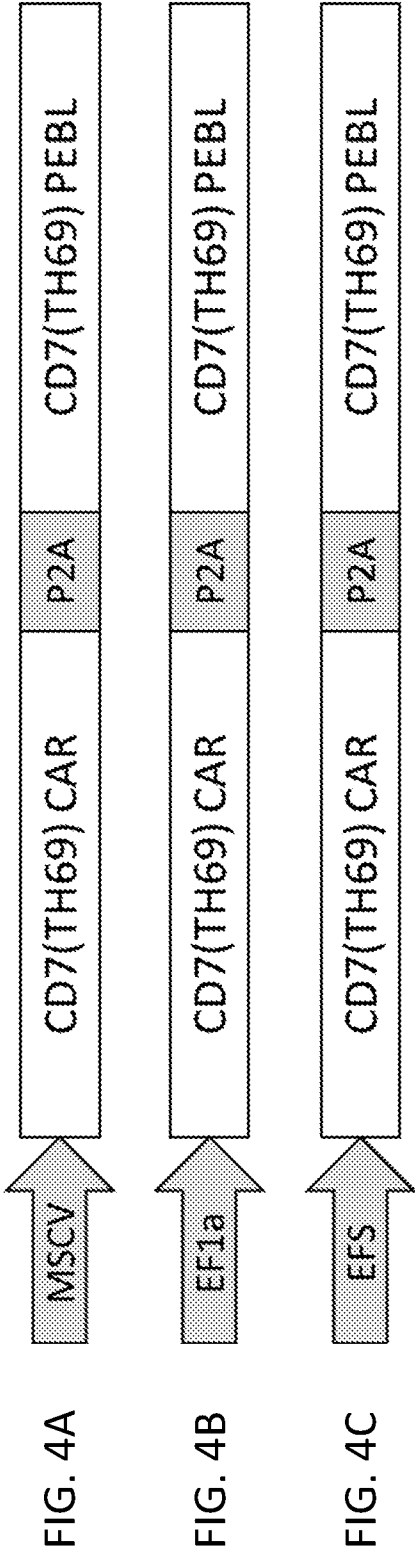


FIG. 5

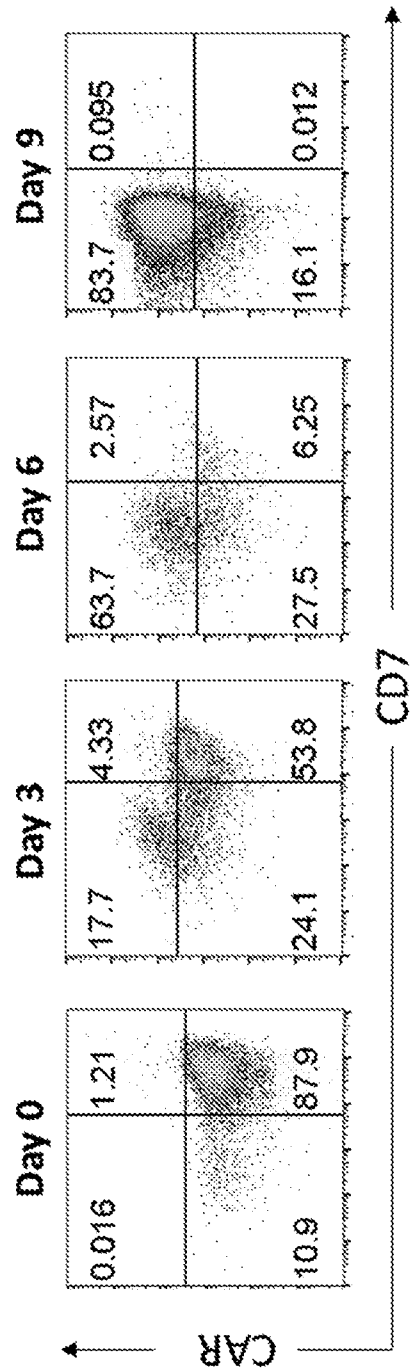


FIG. 6

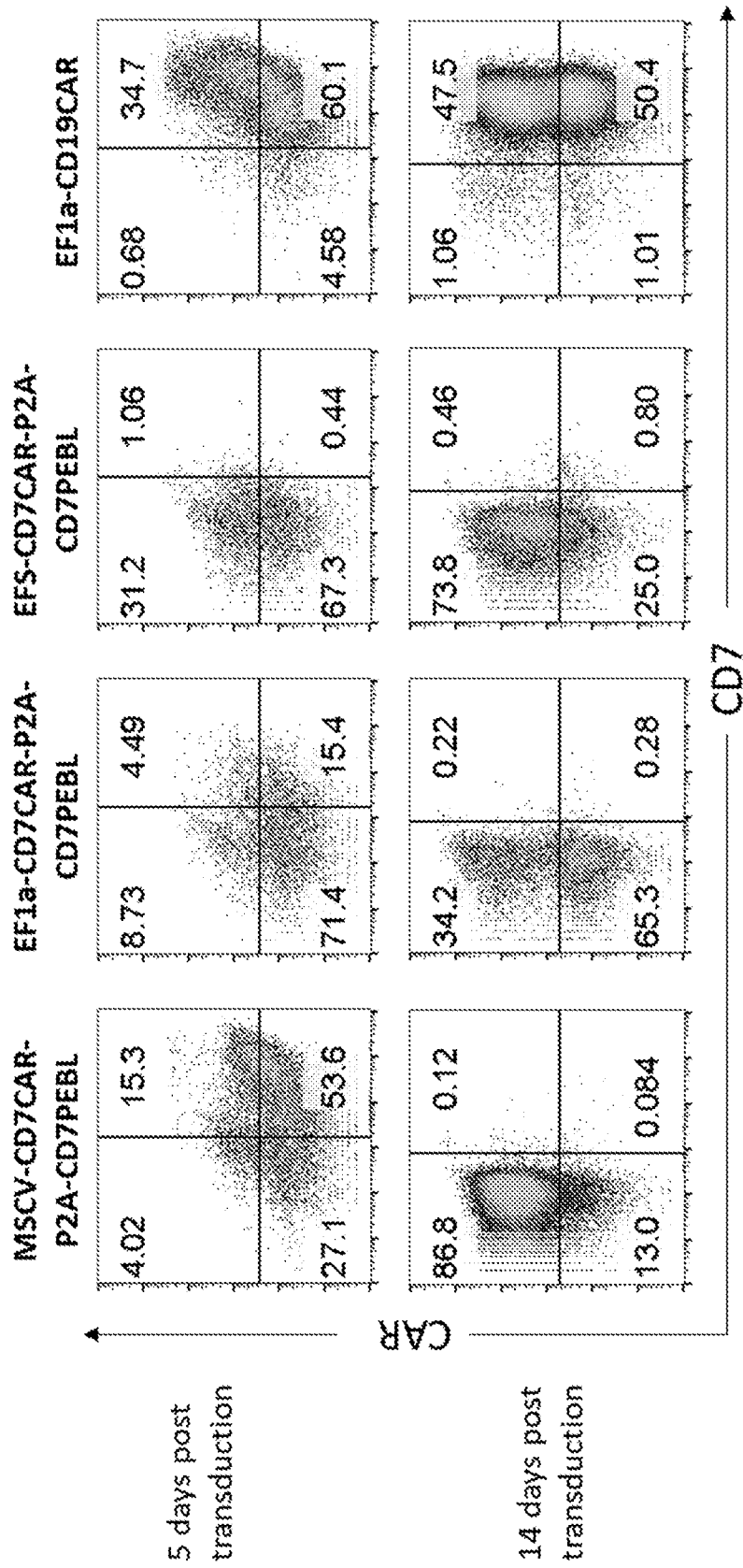


FIG. 7A

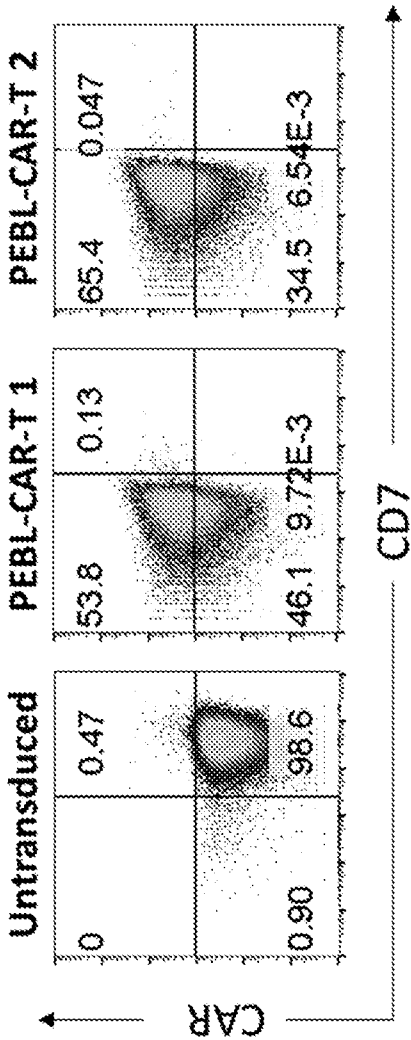


FIG. 7B

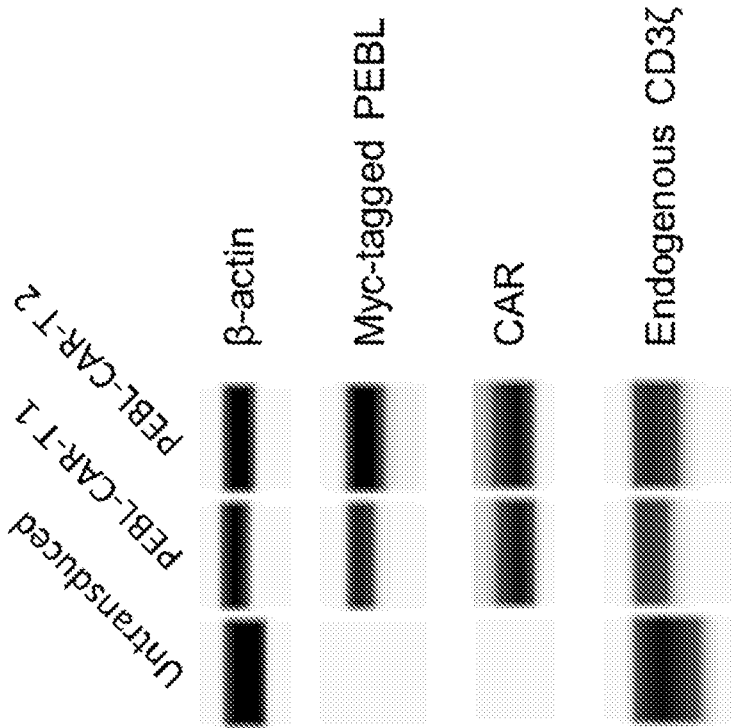


FIG. 8

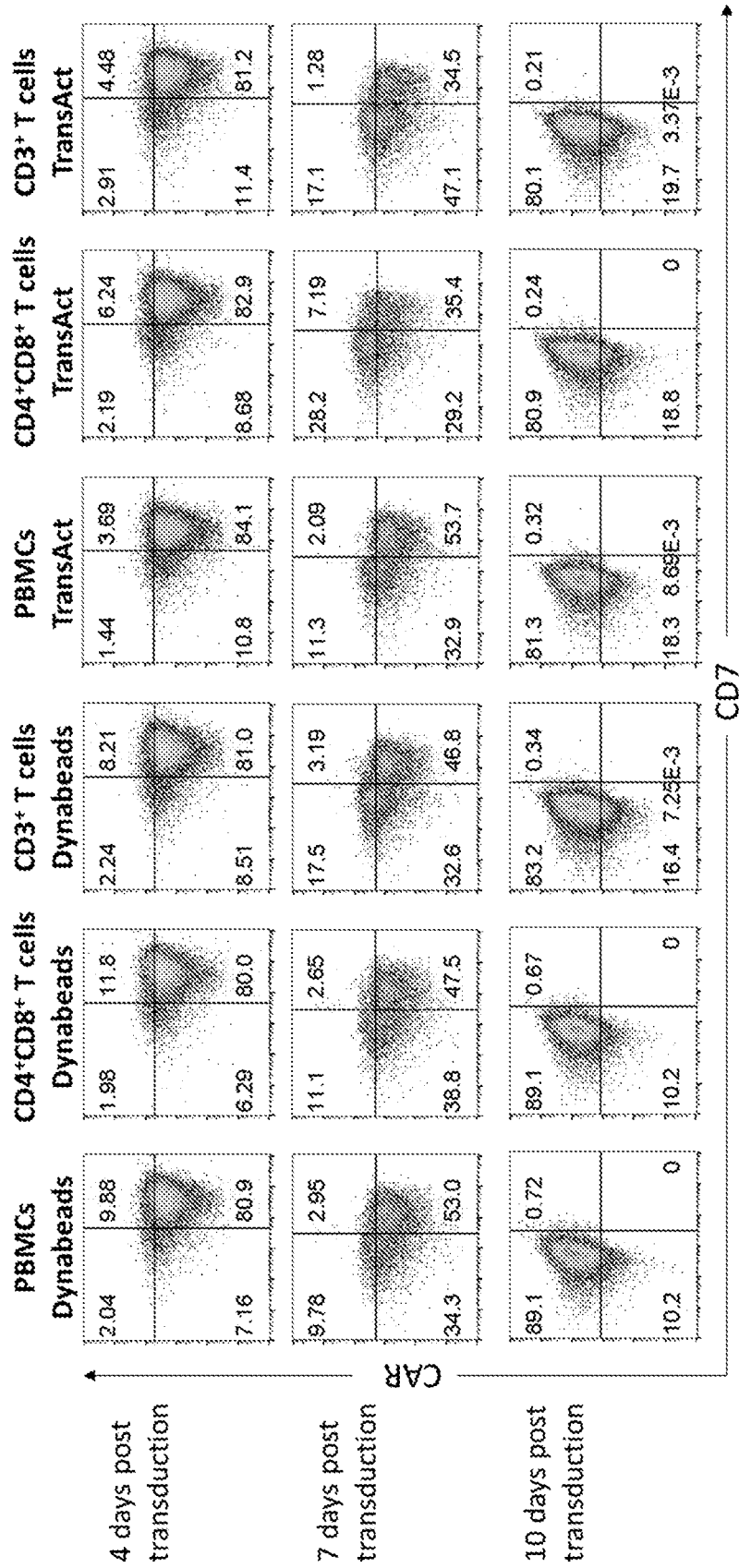


FIG. 9A

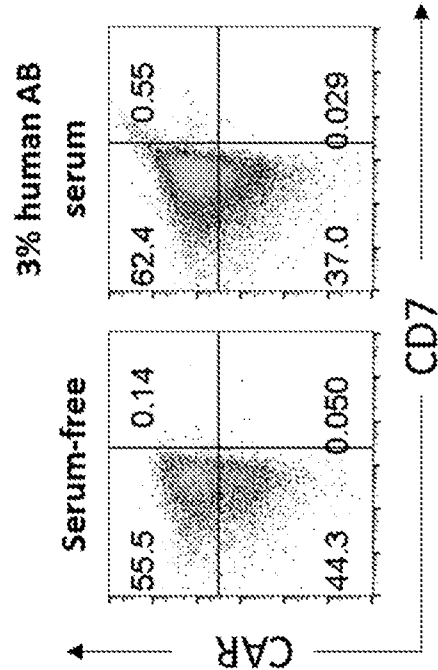


FIG. 9B

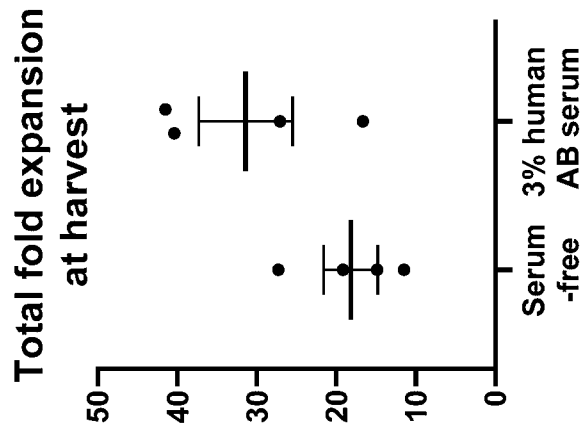


FIG. 10A

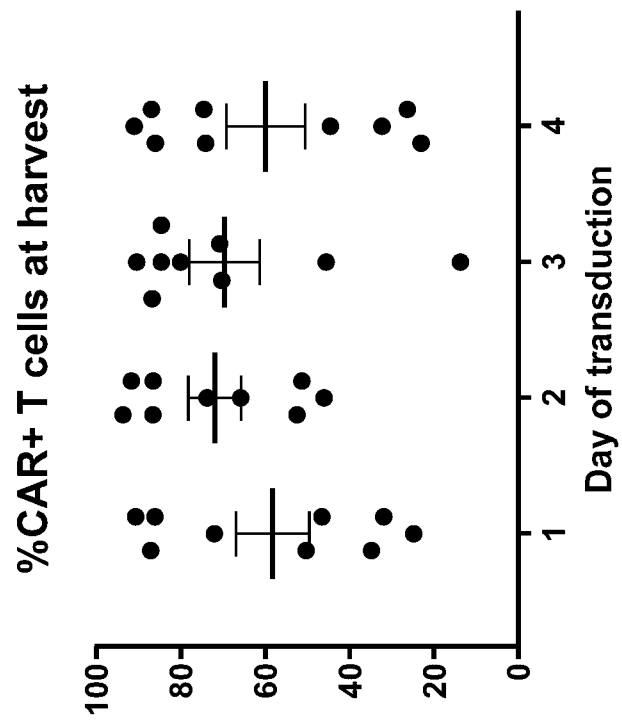


FIG. 10B

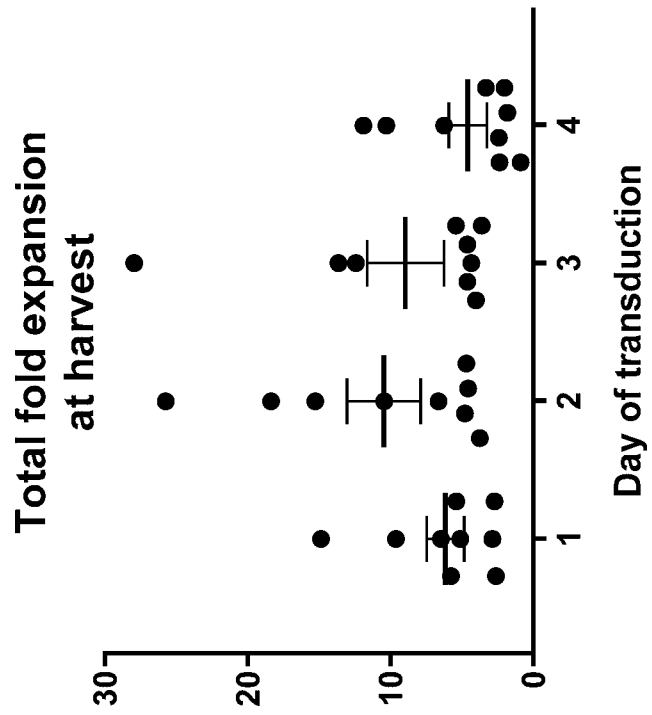
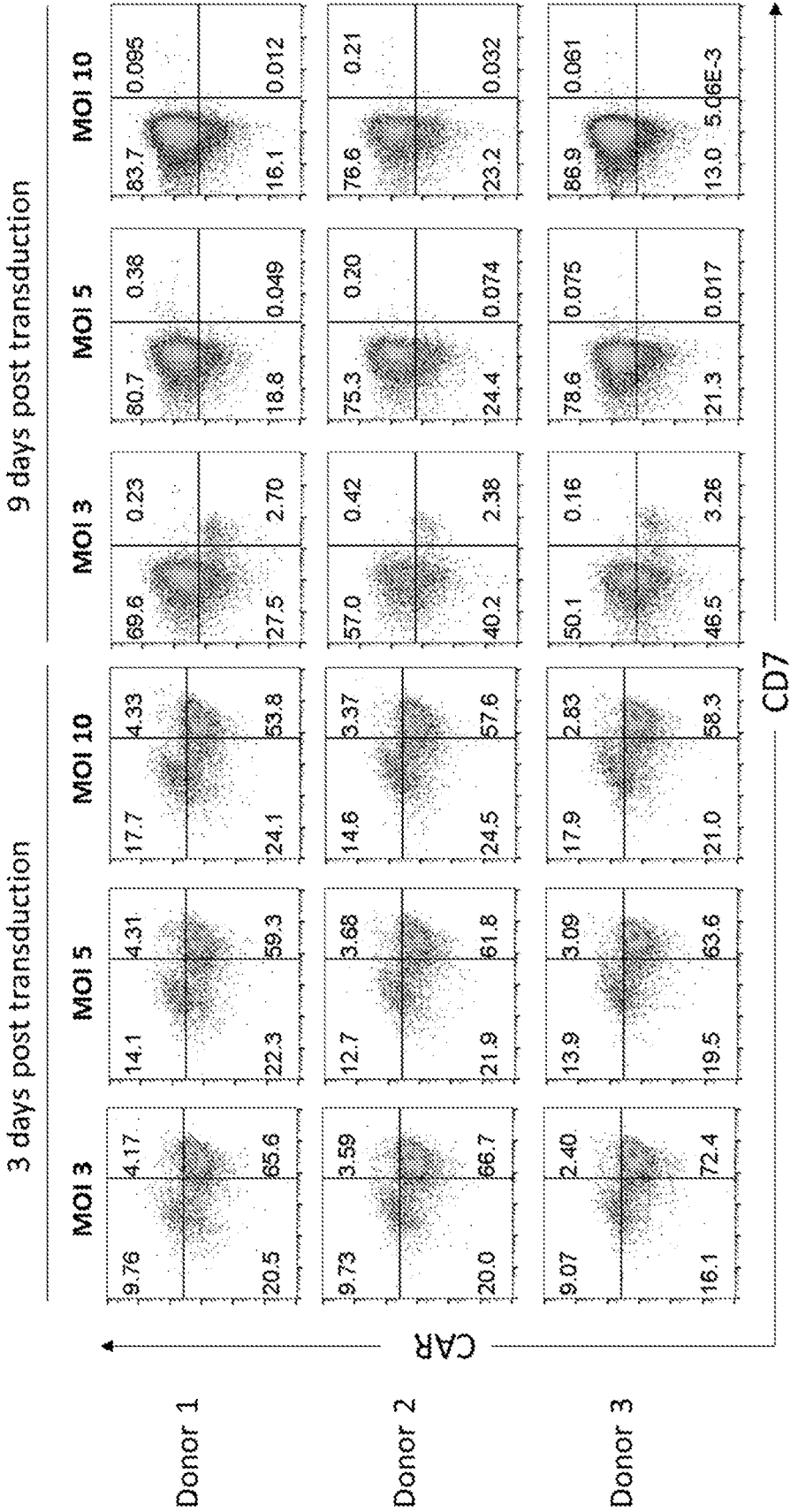


FIG. 11



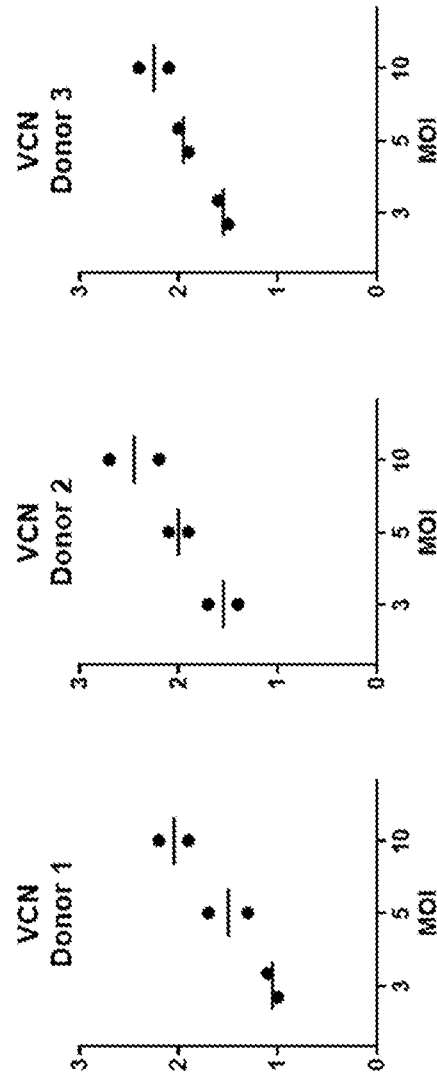
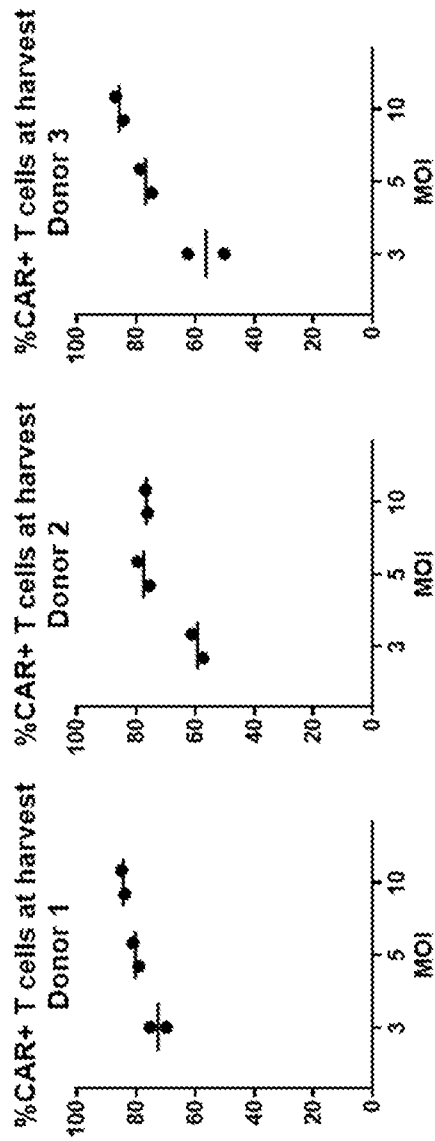


FIG. 13A

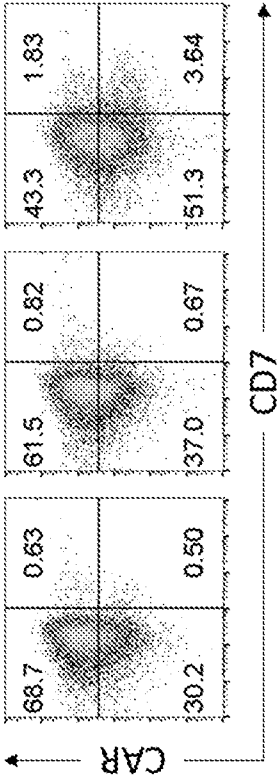


FIG. 13B

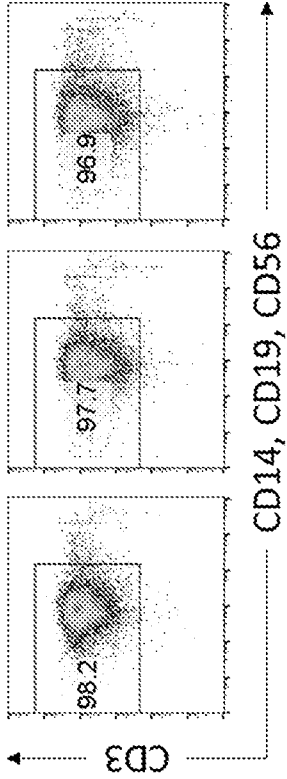


FIG. 13C

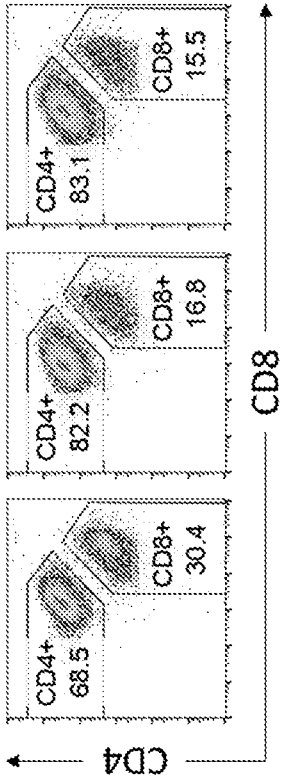


FIG. 13D

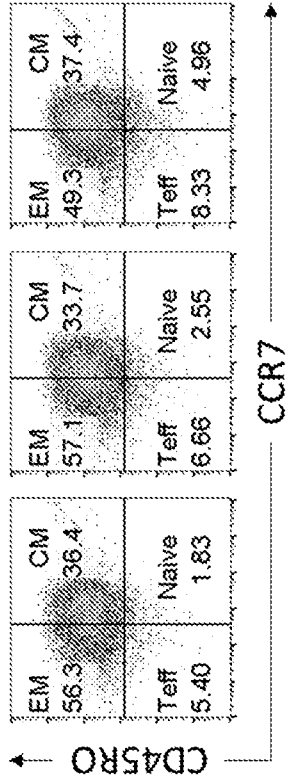


FIG. 13E

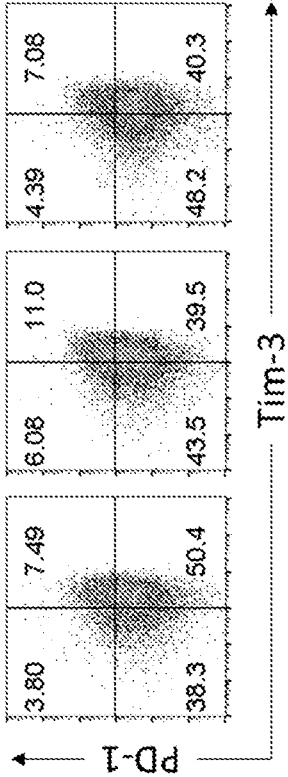


FIG. 14A

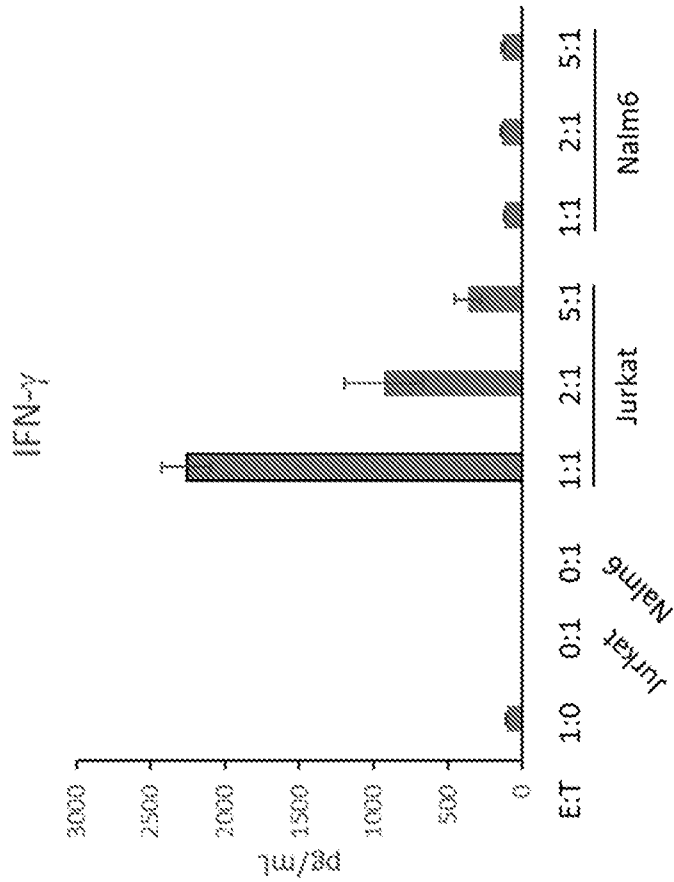
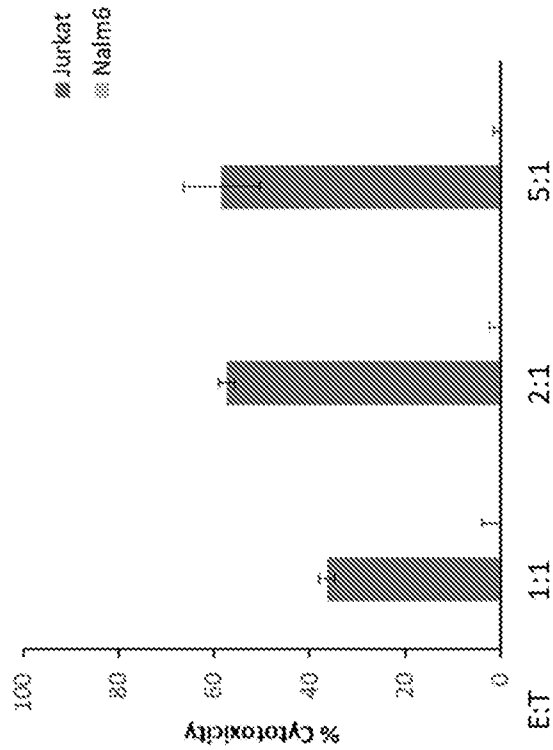


FIG. 14B



Jurkat: CD7+ T-ALL target cell-line
Nalm6: CD7- non-target cell-line

FIG. 15

CPPT-CMV-MCS-PGK-GFP-WPRE (SEQ ID NO:1)

1 ATCGATTTTA AAAGAAAGG GGGGATTGGG GGGTACAGTG CAGGGAAAG AATAGTAGAC ATAATAGCAA CAGACATACA
 81 AACTAAGAA TTACAAAAC AAATTACAAA AATTCAAAAT TTTCGGGTTT ATTAATTAAC AGGACATGC AGAGATCCAG
 161 TTTAAGCTTG GGAGTTCGC GTTACATAAC TTACGGTAA TGGCCCGCTT TGGCCCGCTT TGGCCCGCTT TGGCCCGCTT
 241 ACCTGATTA TGGAGTATGT TCCCATATGT TCCCATATGT TCCCATATGT TCCCATATGT TCCCATATGT TCCCATATGT
 321 AGTGGCCAC TTGGCAGTAC ATCAAGTGA TCAATATGCA AGTACGCCCT CTATTGACGT CAATGACGGT AAATGGCCCC
 401 CCTGGCATTA TGCCAGTAC ATGACCTTAT GGGAGTTTCC TACTTGGCAG TACATCTACG TATTAGTCAAT CGCTATTACC
 481 ATGGTATGC GGTTTTGGCA GTACATCAAT GGGCGTGGAT AGCGTTTGA CTCACGGGA TTTCCAAATC TCCACCCCAT
 561 TGACGTCAAT GGGAGTTTGT TTTGGCACCA AAATCAACGG GACTTTCCAA AATGTCGTA CAATCCGCC CCATTGACGC
 641 AATGGGGGG TAGGCGTGA CCGTGGGAGG TCTATATAAG CAGAGTCTGT TTAGTGAACC GTACATCGC CTGGAGACGC
 721 CATCCACGCT GTTTTGACCT CCATAGAAGA CACCGACTCT ACTAGAGGAT CGTAGCGCT ACCGGACTCA GATCGAATTC
 801 CTCGAAGCTC CTCGAGTGCA GTCGACGGG GCGCGGATC CCGGACTCT AGATAATCTT ACCGGTAGG GAGGGCGCTT
 881 TTCCCAAGG AGTCTGGAG ATGCGCTTTA GCAGCCCGC TGGGCACTTG GCGTACACA AGTGGCTCT GGCCTCGCAC
 961 ACATTCCACA TCCACCGGTA GCGCCCAACC GGTCCGTTT TTTGGTGGC CTTTCGCGC ACCTTCTACT CCTCCCTAG
 1041 TCAGGAAGTT CCCCCCGGC CCGAGCTCG GTCGTGGAG GACGTGACA ATGGAAGTAG CAGTCTCAC TAGTCTCGTG
 1121 CAGATGACA GCACCGCTGA GCAATGGAAG CCGGTAGGCC TTTGGGGCAG CGGCCAATAG CAGCTTGTCT CTTCCGCTTT
 1201 CTGGGCTCAG AGGCTGGGA GGGTGGGTC CCGGGGGGG CTAAGGGGG GGTCAAGGG CCGGGGGGG GCGCGAAGGT
 1281 CCTCCGAG CCGGCATTG TGCACGCTT AAAAGGCAC GTCTGCGCG CTGTTCTCT CTTCTCATC TCCGGGCTT
 1361 TCGACTGGCC ACCGGCTTC ACCATGGTA GCAAGGCGA GGAGTGTTC ACCGGGTGG TCCCATCTT GGTGAGCTG
 1441 GACGCGACG TAAACGGCCA CAAGTTTACG GTGTCGGCG AGGCGAGGG CGATGCCACC TACGGCAAGC TGACCCCTGAA
 1521 GTTCATCTGC ACCACCGCA AGCTGCCCGT GCCCTGGCC ACCCTCGTGA CCACCTGAC CTACGGCGTG CAGTGCITCA
 1601 GCCGCTACCC CGACCACATG AAGCAGCAG ACTTCTTCAA GTCCGCCATG CCCGAAGGT ACCTCCAGGA GCGCACCATC
 1681 TTCTTCAAGG ACGACGGCA CTACAAGACC CCGCCGAGG TGAAGTTTGA GGGCGACACC CTGGTGAACC GCATCGAGCT
 1761 GAAGGCATC GACTTCAAG AGGACGGCAA CATCTGGGG CACAAGCTGG AGTACAATA CAACAGCCAC AACGTCTATA
 1841 TCATGGCCGA CAAGCAGAAG AACGGCATCA AGGTGACTT CAAGATCCGC CACAACATCG AGGACGGCAG CGTGACGCTC
 1921 GCCGACCACT ACCAGCAGAA CACCCCCATC GCGGACGGCC CCGTGTCTGT GCGCGACAAC CACTACCTGA GCACCCAGTC
 2001 CGCCCTGAGC AAAGACCCCA ACGAGAAGCG CGATCACATG GTCTGTCTGT AGTTCTGTAC CGCCGCGGG ATCACTCTCG
 2081 GCATGGACGA GCTGTACAAG TAAAGCGTCT GGAACAATCA ACCTCTGGAT TACAAAATTT GTGAAGATT GACTGGTATT
 2161 CTTAAGTATG TTGCTCCTTT TAGCTATGT GATACGCTG CTTTAAATGCC TTTGTATCAT GCTATGTCTT CCCGTATGGC
 2241 TTTTCATTTT TCCTCCTTGT ATAAATCCTG GTTGTGTCT CTTTATGAGG AGTTGTGGC CGTTGTACAG CAACGTGGCG
 2321 TGCTGTGCAC TGTGTCTTGT GACGCAACCC CCACTGTTG GGCATTTGC CCGTGTCTG ACAGGGCTC GGCTGTGGG
 2401 GCTTTCCCC TCCCTATTG CACGGCGGAA CTATGCGCG CTTGCCATGC CCGTGTCTG ACAGGGCTC GGCTGTGGG
 2481 CACTGCAAT TCCGTGTGT TGTGGGGGAA GCTGAGTCC TTTCCATGG TGTCTGCTG TGTGTCCACC TGGATTCTGC
 2561 GCGGACGCTC CTTCTGTCTC GTCCCTTCCG CCCTCAATCC AGCGACCTT CTTTCCCGG GCTGTCTGCC GGTCTGTGGG
 2641 CCTCTTCCGC GTCTTCCGCT TCGCCCTCAG ACGAGTCGGA TCTCCCTTTG GCGCGCCTCC CCGCCTGG

FIG. 16

Anti-human CD7 (TH69) PEBL (SEQ ID NO:2)

```

1  ATGGCTCTGC  CTGTGACCGC  ACTGCTGCTG  CCCCTGGCTC  TGCTGCTGCA  CGCCGCAAGA  CCTGCCGCAT  ACAAGGATAT
81  TCAGATGACT  CAGACCACAA  GCTCCCTGAG  CGCCTCCCTG  GGAGACCGAG  TGACAATCTC  TTGCAGTGCA  TCACAGGGA
161  TTAGCAACTA  CCTGAATTGG  TATCAGCAGA  AGCCAGATGG  CACTGTGAAA  CTGCTGATCT  ACTATACCTC  TAGTCTGCAC
241  AGTGGGTCC  CCTCACGATT  CAGCGGATCC  GGCTCTGGGA  CAGACTACAG  CCTGACTATC  TCCAACCTGG  AGCCGGAAGA
321  TATTGCCACC  TACTATTGCC  TACTATTGCC  CAAGCTGCCT  TATACCTTTG  GCGGGGGAAC  AAAGCTGGAG  ATTAAAAAGG
401  GAGGAGGAGG  AAGCGGAGGA  GGAGGATCCG  GAGGCGGGGG  ATCTGGAGGA  GGAGGAAGTG  AGGTGCAGCT  GGTGGAATCT
481  GGAGGAGGAC  TGGTGAAGCC  AGGAGGATCT  CTGAAACTGA  GTTGTGCCGC  TTCAGGCCCTG  ACCTTCTCAA  GCTACGCCAT
561  GAGCTGGGTG  CGACAGACAC  CTGAGAAGCG  GCTGGAATGG  GTCGCTAGCA  TCTCCTCTGG  CGGGTTTACA  TACTATCCAG
641  ACTCCGTGAA  AGCAGATTT  ACTATCTCT  GGGATAACGC  AAGAAATATT  CTGTACCTGC  AGATGAGTTC  ACTGAGGAGC
721  GAGGACACCG  CAATGTACTA  TTGTGCCAGG  GACGAAGTGC  GCGGCTATCT  GGAATGCTGG  GGAGCTGGCA  CTACCGTCAC
801  CGTCTCCAGC  GAGCAGAAAC  TGATTAGCGA  AGAGGACCTG  AAAGATGAAC  TGTA

```

FIG. 17

Anti-human CD7 (3A1F) PEBL (SEQ ID NO:3)

```

1  ATGGCTCTGC  CCGTCACCGC  TCTGCTGCTG  CCTCTGGCTC  TGCTGCTGCA  CGCCGCAAGA  CCCCAGGTCC  AGCTGCAGGA
81  GTCAGGGGCA  GAGCTGGTGA  AACCCGGAGC  CAGTGTCAA  CTGTCTCTGTA  AGGCCAGCGG  CTATACTTTC  ACCAGCTACT
161  GGATGCACCTG  GGTGAAACAG  AGGCCAGGAC  AGGCCCTGGA  GTGGATCGGC  AAGATTAAAC  CCAGCAATGG  GCGCACCAAC
241  TACAACGAAA  AGTTTAAATC  CAAGGCTACA  CTGACTGTGG  ACAAGAGCTC  CTCTACCGCA  TACATGCAGC  TGAGTTCAC
321  GACATCTGAA  GATAGTGCCG  TGTAATAATTG  TGTACTAGCT  GGGTCTACT  ATGACCTGTA  CTATTACGCA  CTGGATTATT
401  GGGGGCAGGG  AACACAGTG  ACTGTACGCT  CCGGAGGAGG  AGGATCCGGC  GGAGGAGGCT  CTGGGGGAGG  CCGGAGTGAC
481  ATCAGACTGA  CACAGTCTCC  AGCCACTCTG  AGCGTGACCC  CTGGCGATTTC  TGTCAGTCTG  TCATGTAGAG  CTAGCCAGTC
561  CATCTCTAAC  AATCTGCACT  GGTACCAGCA  GAAATCACAT  GAAAGCCCTC  GGCTGCTGAT  TAAGAGTGCT  TCACAGAGCA
641  TCTCCGGGAT  TCCAAAGCAG  TTCTCTGGCA  GTGGGTGAGG  AACCGACTTT  ACACGTGTCC  TTAACCTCTG  GGAGACCGAA
721  GATTTCGGCA  TGATATTTTG  CCAGCAGAGC  AATTCTGGC  CTTACACATT  CGGAGGCGGG  ACTAAACTGG  AGATTAAAG
801  GGAGCAGAAA  CTGATTAGCG  AAGAGGACCT  GAAGGACGAA  CTGTGA

```

FIG. 18

Anti-human CD7 (TH69) CAR (SEQ ID NO:4)

1	ATGGCTCTGC	CTGTGACTGC	CCTGCTGCTG	CCCCCTGGCCC	TGCTGCTGCA	CGCTGCTAGA	CCCCCGGCAT	ACAAGGACAT
81	TCAGATGACT	CAGACTACAT	CCAGCCTGTC	AGCTTCCCTG	GGCGATCGAG	TGACTATCAG	CTGTTACGCA	AGCCAGGGGA
161	TCAGCAATTA	CCTGAACCTGG	TACACGACGA	AGCCCGACGG	AACTGTCAAG	CTGCTGATCT	ACTATACTTC	CAGTCTGCAT
241	AGCGGGGTCC	CTAGCCGATT	CTCTGGGAGT	GGAAGCGGCA	CCGACTATAG	CCTGACCATT	TCCAACCTGG	AACCAGAGGA
321	CATTGCCACC	TATTACTGTC	AGCAGTATTC	AAAGCTGCCC	TATACCTTTG	GCGGAGGGAC	TAAAGCTGGAG	ATCAAGCGAG
401	GAGGGGAGG	ATCAGGGGGA	GGGGGGAGCG	GAGGCGGGGG	GAGTGGCGGC	GGAGGGTCAG	AAGTCCAGCT	GGTCGAGAGT
481	GGGGGCCGGAC	TGGTGAAACC	TGGGGGCTCT	CTGAAACTGA	GCTGTGCAGC	CAGTGGCCTG	ACATTCTCCT	CATATGCTAT
561	GTCTTGGGTG	CGGCAGACCC	CAGAAAGAG	ACTGGAGTGG	GTGGCTTCAA	TCTCTTCCGG	GGGCTTCACT	TACTATCCTG
641	ACAGCGTGAA	AGCAGATTTC	ACTATCTCCA	GGGATAACGC	ACGGAATATC	CTGTATCTGC	AGATGAGCTC	ACTGAGATCC
721	GAGGACACCG	CAATGTACTA	CTGCGCAAGA	GACGAAGTGC	GGGGGTATCT	GGACGTGTGG	GGAGCAGGCA	CTACCCGTAC
801	CGTGTCTAGC	ACCACTACAC	CTGCACCAAG	GCCTCCACAC	CCCCGTCCCA	CTATCGCTTC	CCAGCCACTG	TCCCTGAGGC
881	CCGAGGCCTG	CAGGCCAGCA	GCTGGCGGAG	CCGTGCATAC	TAGGGGGCTG	GACTTCGCTT	GGACATCTA	CATCTGGGCC
961	CCACTGGCAG	GGACATGCGG	AGTCCCTGCTG	CTGTCCCTGG	TCATCACACT	GTA CTGCAAG	CGGGGGCGCA	AAAAACTGCT
1041	GTATATCTTT	AAGCAGCCTT	TCATGAGACC	AGTGCAGACA	ACCCAGGAGG	AAGATGGGTG	CTCATGCCGG	TTTCCCGAGG
1121	AGGAGGAAGG	CGGCTGCGAG	CTGAGGGTGA	AGTTTTCCTG	CTCAGCAGAT	GCTCCTGCCT	ACCAGCAGGG	CCAGAACCCAG
1201	CTGTATAATG	AGCTGAACCT	GGGCAGACGC	GAAGAGTATG	ATGTGCTGGA	CAAAAGGCCG	GGAGAGAGCC	CCGAAATGGG
1281	AGGGAAGCCA	AGGCGGAAA	ACCCCCAGGA	GGGCCTGTAC	AATGAGCTGC	AGAAGGACAA	AATGGCAGAG	GCTTACAGTG
1361	AGATTGGGAT	GAAGGAGAG	AGACGGAGGG	GAAAAGGGCA	CGATGGCCTG	TACCAGGGGG	TGAGCACAGC	AACCAAGAT
1441	ACTTATGACG	CACTGCACAT	GCAGGCACCTG	CCACCCAGAT	GA			

FIG. 19

Anti-human CD7 (3A1F) CAR (SEQ ID NO:5)

```

1  ATGGCTCTGC  CCGTCACCGC  TCTGTGCTGTG  CCTCTGGCTC  TGCTGCTGGA  CGCTGCTCGA  CCACAGGTCC  AGCTGCAGGA
81  GTCAGGAGCT  GAGCTGGTGA  AGCCAGGGGC  AAGCGTCAAA  CTGTCTCTGCA  AGGCCTCTGG  ATATACATTC  ACTAGCTACT
161  GGATGCAC TG  GGTGAAACAG  AGACCCGGAC  AGGCCCTGGA  GTGGATCGGA  AAGATTAACC  CTAGCAATGG  CAGGACCAAC
241  TACAACGAAA  AGTTTAAATC  CAAGGCAACC  CTGACAGTGG  ACAAGAGCTC  CTCTACAGCC  TACATGCAGC  TGAGTTCACT
321  GACTTCAGAG  GATAGCGCAG  AATCTGCACT  TGTACTATTG  CGCCAGAGGC  ATGACCTGTA  CTATTACGCC  CTGGATTATT
401  GGGGGCAGGG  AACACACAGT  CCCAGAGTCC  ACTGTCACTG  CCGGAGGAGG  GGAGGAGGAT  CCGGAGGAGG  AGGATCTGAC
481  ATCAGCTGA  ATCTGCACT  GGTACCACTG  TGTACACTG  AGCGTGACTC  GAGGCGATTC  TCATGTCGGG  CAAGCCAGTC
561  CATCTCTAAC  TCCCTCCCGG  TTCTCTGGCA  GTGGGTCAAG  AACTGACTTT  GACTGCTGAT  TAAGAGTGCC  TCACAGAGCA
641  TCTCCGGCAT  TGTATTTTGG  CCAGCAGAGC  AATTCCTGGC  CCTACACTTT  ACCCTGAGTA  TTAACCTCAGT  GGAGACAGAA
721  GATTTCGGCA  CCAGCTCCAA  GACTACCACT  GCTGTCTCTG  GCTGTCTCTG  GCGTGTCTCT  TGCATATATCT  ACATTTGGGC  TCCTCTGGCA
801  GACTACCACT  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG
881  GTAGGCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG
961  GGAACCTGTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG  GCTGTCTCTG
1041  CAAGCAGCCC  TTTATGAGGC  CTGTGAGGC  CTGTGAGGC  CTGTGAGGC  CTGTGAGGC  CTGTGAGGC  CTGTGAGGC
1121  GGGGATGTA  GCTGCGCGTC  GCTGCGCGTC  GCTGCGCGTC  GCTGCGCGTC  GCTGCGCGTC  GCTGCGCGTC  GCTGCGCGTC
1201  GAGCTGAATC  TGGGGCGGAG  TGGGGCGGAG  TGGGGCGGAG  TGGGGCGGAG  TGGGGCGGAG  TGGGGCGGAG  TGGGGCGGAG
1281  ACGACGGAAG  AACCCCGAGG  AACCCCGAGG  AACCCCGAGG  AACCCCGAGG  AACCCCGAGG  AACCCCGAGG  AACCCCGAGG
1361  TGAAGGAGAG  GAGAAAGGCG  GAGAAAGGCG  GAGAAAGGCG  GAGAAAGGCG  GAGAAAGGCG  GAGAAAGGCG  GAGAAAGGCG
1441  GCACTGCACA  TGCAGGCACT  TGCAGGCACT  TGCAGGCACT  TGCAGGCACT  TGCAGGCACT  TGCAGGCACT  TGCAGGCACT

```

FIG. 20

CMV promoter (SEQ ID NO:6)

```

1  AAGCTTGGGA  GTTCCGCGGTT  ACATAACTTA  CGGTAAATGG  CCCGCCCTGGC  TGACCGCCCCA  ACGACCCCCCG  CCCATTGACG
81  TCAATAATGA  CGTATGTTCC  CATAGTAACG  CCAATAGGGA  CTTTCCATTG  ACGTCAATGG  GTGGAGTATT  TACGGTAAAC
161  TGCCCACTTG  GCAGTACATC  AAGTGATATCA  TATGCCAAGT  ACGCCCCCTTA  TTGACGTCAA  TGACGGTAAA  TGGCCCCCCT
241  GGCATTATGC  CCAGT

```

FIG. 21

EF1 α promoter (SEQ ID NO:7)

```

1  GGCTCCGGTG  CCCGTCAGTG  GGCAGAGCGC  ACATCGCCCA  CAGTCCCCCA  GAAGTTGGG  GGAGGGGTCTG  GCAATTGAAC
81  CGGTGCCTAG  AGAAGGTGGC  GCGGGTAAA  CTGGGAAAGT  GATGTCGTGT  ACTGGCTCCG  CCTTTTCCC  GAGGGTGGG
161  GAGAACCGTA  TATAAGTGCA  GTAGTCGCC  TGAACGTTCT  TTTTCGCAAC  GGGTTTGCCG  CCAGAACACA  GGTAAAGTGC
241  GTGTGTGGTT  CCCGCGGGCC  TCGCGGTGG  TGGCCTCTTT  GCCCTTGCCT  GCCTTGAATT  ACTTCCACCT  GGCTGCAGTA
321  CGTGATTCTT  GATCCCGAGC  TTCGGGTGG  TCGGGGTGG  GAGAGTTCGA  GGCCTTGCG  TTAAGGAGCC  CCTTCGCCTC
401  GTGCTTGAGT  TGAGGCCTGG  CCTGGCGCT  CCGCGGTGG  GGGCCGCCG  CGTGCGAATC  TCGCGCCTG  TCTCGCTGCT
481  TTCGATAAGT  CTCTAGCCAT  CTGACACTG  GTATTTCGGT  TGATGACCTG  CTGCGACGCT  TTTTCTCTGG  CAAGATAGTC  TTGTAAATGC
561  GGGCCAAGAT  CTGCACACTG  GGGCCTGCCA  GCGCGGTGAT  CGCCCGGCC  TTTTGGGGC  ACGGGCCCG  TGCGTCCCAG  CGCACATGTT
641  CGGCGAGGCG  GCGCTCGCT  GCGCGGTGAT  CAGGAGCTC  CAGGAGCTC  TCGCTTCCG  TCGCTTCCG  GCGGGCTGC  TCTGGTGCCT
721  GCGCTCGCT  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC
801  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC
881  AAAGGAAAAG  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC
961  TAGTTCTCGA  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC
1041  GGTGGAGACT  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC
1121  TCATTCTCAA  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC  GCGCTTCCC

```

FIG. 22

EFS promoter (SEQ ID NO:8)

```

1  ATTCTACCGG  GTAGGGGAGG  CGCTTTTCCC  AAGGCAGTCT  GGAGCATGCG  CTTTAGCAGC  CCCGCTGGGC  ACTTGGCGCT
81  ACACAAGTGG  CCTCTGGCCT  CGCACACATT  CCACATCCAC  CCGTAGGCGC  CAACCGGCTC  CGTCTTTGG  TGGCCCCCTC
161  GCGCCACCTT  CTAATCCTCC  CTAATCCTCC  CTAATCCTCC  CTAATCCTCC  CTAATCCTCC  CTAATCCTCC  CTAATCCTCC  CTAATCCTCC
241  AGTAGCACGT  CTCACATAGT  CTCACATAGT  CTCACATAGT  CTCACATAGT  CTCACATAGT  CTCACATAGT  CTCACATAGT  CTCACATAGT
321  AATAGCAGCT  TTGCTCCTTC  TTGCTCCTTC  TTGCTCCTTC  TTGCTCCTTC  TTGCTCCTTC  TTGCTCCTTC  TTGCTCCTTC  TTGCTCCTTC
401  AGGGGCGGGG  CGGGCGGGGG  CGGGCGGGGG  CGGGCGGGGG  CGGGCGGGGG  CGGGCGGGGG  CGGGCGGGGG  CGGGCGGGGG  CGGGCGGGGG
481  CTCCTCTTCC  TCATCTCCGG  TCATCTCCGG  TCATCTCCGG  TCATCTCCGG  TCATCTCCGG  TCATCTCCGG  TCATCTCCGG  TCATCTCCGG

```

FIG. 23

MSCV promoter (SEQ ID NO:9)

```

1  GGAATGAAAG  ACCCCACCTG  TAGGTTTGGC  AAGCTAGCTT  AAGTAACGCC  ATTTTGCAAG  GCATGGAAAA  TACATAAAGT
81  AGAATAGAGA  AGTTTCAATC  AAGGTTAGGA  ACAGAGAGAC  AGCAGAATAT  GGGCCAAACA  GGATATCTGT  GGTAAAGCAGT
161  TCCTGCCCCG  GCTCAGGGCC  AAGAACAGAT  GGTCCCCAGA  TGCGGTCCCG  CCCTCAGCAG  TTTCTAGAGA  ACCATCAGAT
241  GTTTCAGGG  TGCCCCAAGG  ACCTGAAATG  ACCCTGTGCC  TTATTTGAAC  TAACCAATCA  GTTCGCTTCT  CGCTTCTGTT
321  CGCGCGCTTC  TGCTCCCCGA  GCTCAATAAA  AGAGCCACAC  ACCCTCACT  CGGCGCGCCA  GTCC

```

FIG. 24

PGK promoter (SEQ ID NO:10)

```

1  ATTTCTACCG  GTAGGGGAGG  CGCTTTTCCC  AAGGCAGTCT  GGAGCATGG  CTTTAGCAGC  CCCGCTGGGC  ACTTGGCGCT
81  ACACAAAGTG  CCTCTGGCCT  CGCACACATT  CCACATCCAC  CGGTAGGGC  CAACCGGCTC  CGTTCTTTGG  TGGCCCCCTC
161  GCGCCACCTT  CTAATCCTCC  CTAATCCTCC  AAGTTCCCCC  CCGCCCCGCA  GCTCGCGTCG  TGCAGGACGT  GACAAATGGA
241  AGTAGCACGT  CTCACCTAGT  TCGTGCAGAT  GGACAGCAC  GCTGAGCAAT  GGAAGCGGGT  AGGCCTTTGG  GGCAGCGGCC
321  AATAGCAGT  TTGCTCCTTC  GCTTCTGGG  CTCAGAGGCT  GGAAGGGGT  GGTCCGGGG  GCGGGCTCAG  GGGCGGGCTC
401  AGGGCGGGG  CGGGCGGGG  AAGTCTCTCC  GGAGGCCCGG  CATTCTGCAC  GCTTCAAAAG  CGCACGTCTG  CCGCGCTGTT
481  CTCCTCTTCC  TCATCTCCGG  GCCTTTCGA

```

FIG. 25

Anti-human CD7 (TH69) PEBL-IREs-Anti-human CD7 (TH69) CAR (SEQ ID NO:11)

1 ATGGCTCTGC CTGTGACCGC ACTGCTGCTG CCCCTGGCTC TGCTGTGCA CGCCGCAAGA CCTGCGCGAT ACAAGGATAT TCAGATGACT
 91 CAGACACAA GCTCCCTGAG CGCCTCCCTG GGAGACCGAG TGACAATCTC TTGACAGTGA TCACAGGGA TTAGCAACTA CCTGAATTGG
 181 TATCAGCAGA AGCCAGATGG CACTGTGAAA CTGTGATCT ACTATACCTC TAGTCTGCAC AGTGGGTCC CTTACGATT CAGCGGATCC
 271 GGCTCTGGGA CAGACTACAG CCTGACTATC TCCAACTGG AGCCGAAGA TATTGCCACC TACTATTGCC AGCAGTACTC CAAGCTGCCT
 361 TATACCTTTG GCGGGGAAC AAAGCTGGAG ATTAAAGGG GAGGAGGAGG AAGCGGAGG GAGGCGGGG ATCTGGAGGA ATCTGGAGGA
 451 GGAGGAAGTG AGGTGCAGCT GGTGCAATCT GGAGGAGGAC TGGTGAAGCC AGGAGGATCT CTGAATGA GTTGTGCCG TTCAGGCCTG
 541 ACCTTCTCAA GCTACGCCAT GAGCTGGTG CGACAGACAC CTGAGAAGCG GCTGGAATGG GTCCCTCTGG TCTCCTCTGG CCGGTTTACA
 631 TACTATCCAG ACTCCGTGAA AGGCAGATTT ACTATCTCTC GGGATAACGC AAGAAATATT CTGTACCTGC AGATGATTC ACTGAGGAGC
 721 GAGGACACCG CAATGTACTA TTGTGCCAGG GACGAAGTGC GCGGCTATCT GGATGTCTGG GGAGCTGGCA CTACCGTCAC CGTCTCCAGC
 811 GAGCAGAAAC TGATTAGCGA AGAGGACCTG AAAGATGAAC TGTAAACGGG TCAATTCCGC CCCCCCTA ACCTTACTGG CCGAAGCCGC
 901 TTGGAATAAG GCCGGTGGC GTTGTCTCTAT ATGTTATTTT CCACATATTT GCCAATGTGA GGGCCCGGAA ACCTGGCCCT
 991 GTCTTCTTGA CGAGCATTTCC TAGGGGTCTT TCCCCTCTCG CCAAGGAAT GCAAGGTCTG TTGAATGTCTG TGAAGGAAGC AGTTCCCTCTG
 1081 GAAGCTTCTT GAAGACAAAC AACGTCTGTA GCGACCTTTT CAGGCGAGCG GAACCCCCCA CCGTGGCGACA GGTGCCCTCTG CCGCCAAAAG
 1171 CCACGTGTAT AAGATACACC TGCAAAAGCG GCACAACCCC AGTGCCACGT TGTGAGTTGG ATAGTTGTGG AAAGAGTCAA ATGGCTCTCC
 1261 TCAAGCGTAT TCAACAAGGG GCTGAAGGAT GCCCAAGAGG TACCCCATTTG TATGGGATCT GATCTGGGGC CTCGGTGCAC ATGCTTTTACA
 1351 TGTGTTTAGT CGAGGTTAA AAAACGTCTA GGGCCCGCGA ACCACGGGGA CGTGGTTTTC CTTTGAAGAA CACGATAATA CCATGGCTCT
 1441 GCCTGTGACT GCCCTGTCTG TGGCCCTGGC CCTGCTGCTG CAGCTGCTA GACCCGCGC ATACAAGGAC ATTACAGATGA CTCAGACTAC
 1531 ATCCAGCCTG TCAGCTTCCC TGGCGATCG AGTGACTATC AGCTGTTTCA CAAGCCAGGG GATCAGCAAT TACCTGAACT GGTACCAGCA
 1621 GAAGCCGAC GGAACGTGTA AGTGTCTGAT CTACTATACT TCCAGTCTGC ATAGCGGGT CCTTAGCGA TTCTCTGGG GTGGAAGCGG
 1711 CACCGACTAT AGCCTGACCA TTTCACACCT GGAACACAG GACATTGCGA CCTATTACTG TCAGCAGTAT TCAAGCTGC CCTATACCTT
 1801 TGGCGAGGG ACTAAGCTG AGATCAAGCG AGGAGGGGGA GGATCAGGG GAGGGGGAG GGGAGTGGG GGGAGTGGG GCGGAGGGT
 1891 AGAATCCAG CTGGTCGAGA GTGGGGGCG ACTGTTGAAA CCTGGGGCT CTCTGAACT GAGCTGTGA GCCAGTGGC TGACATTCTC
 1981 CTCATATGCT ATGTCTTGGG TCGGGCAGC CCCAGAAAAG AGACTGGAGT GGGTGGCTTC AATCTCTTC GGGGGCTTCA CTACTATCC
 2071 TGACAGGGTG AAAGGCAGAT TCACTATCTC CAGGGATAAC GCACGGAATA TCCGTATCT GCAGATGAGC TCACTGAGT CCGAGGACAC
 2161 CGCAATGTAC TACTGCGCA GAGACGAAGT GCGGGGTAT CTGGACGTGT GGGGAGCAGG CACTACCGTC ACCGTGTCTA GCACCACTAC
 2251 ACCTGCACA AGGCCTCCCA CACCCGCTCC CACTATCGCT TCCCAGCCAC TGTCCCTGAG GCCGAGGCC TGCAGGCCAG CAGCTGGCGG
 2341 AGCCGTGCAT ACTAGGGGC TGGACTTCG TTGCGACATC TACATCTGG CCCACTGGC AGGCATGTC GGAGTCTGC TGCTGTCCCT
 2431 GGTCAATACA CTGTACTGCA AGCGGGGCG CAAAAAATG CTGTATATCT TTAAGCAGCC TTTCATGAGA CCAGTGCAGA CAACCCAGGA
 2521 GGAAGATGGG TGCTCATGCC GGTTCCTCGA GGAGGAGGAA GCGGGCTGC AGCTGAGGT GAAGTTTTC CGCTCAGCAG ATGCTCTCTG
 2611 CTACAGCAG GGCAGAAC AGCTGTATAA TGAGCTGAAC CTGGCAGAC GCGAAGAGTA TGATGTGCTG GACAAAAGGC GGGGAAGAGA
 2701 CCGCAATG GGAGGGAAGC CAAGCGGGA AAACCCCGA GAGGGCTGT ACAATGAGCT GCAGAAAGAC AAAATGGCAG AGGCTTACAG
 2791 TCCAGTTGG ATGAAGGAG AGAGACGGG GGGAAAAGG CACCATGGC TGTACCGAG GCTGAGCACA GCAACCAAAG ATACTTATGA
 2881 CGCACTGC ATGACGGCAC TGCCACCCAG ATGACACCCA GGGGATTTCA CCACTCAAAG GCCAGACCTG CAGACGCCCA GATTATGAGA
 2971 CACA

FIG. 26

Anti-human CD7 (TH69) CAR-IRES-Anti-human CD7 (TH69) PEBL (SEQ ID NO:12)

1	ATGGCTCTGC	CTGTGACTGC	CCTGCTGCTG	CCCCTGGGCC	TGCTGTGCTGCA	CGTGTGCTAGA	CCCGCCGCAT	ACAAAGGACAT	TCAGATGACT
91	CAGACTACAT	CCAGCCTGTC	AGCTTCCCTG	GGCGATCGAG	TGACTATACAG	CTGTTTCAGCA	AGCCAGGGGA	TCAGCAATTGA	CCTGAACTGG
181	TACCAACAGA	AGCCCGACGG	AACGTGTCAAG	CTGTGTATCT	ACTATATCTTC	CAGTCTGCAT	AGCGGGGTCC	CTAGCCGATT	CTCTGGGAGT
271	GGAAGCGGCA	CCGACTATAG	CCTGACCATT	TCCAACTGG	AACCAGAGGA	CATTGCCACC	TATTACTGTC	AGCAGTATTC	AAAGCTGCC
361	TATACCTTTG	GCGGAGGGAC	TAAAGCTGGAG	ATCAAGCGAG	GAGGGGGAGG	ATCAGGGGGA	GGGGGAGCG	GAGGCGGGG	GAGTGGCGGC
451	GGAGGTCAG	AAGTCCAGCT	GGTCCGAGAGT	GGGGCGGAC	TGTTGAAACC	TGGGGGCTCT	CTGAAACTGA	GCTGTGCAG	CAGTGGCCTG
541	ACATTCTCCT	CATATGCTAT	GTCCTTGGGTG	CGGCAGACCC	CAGAAAAGAG	ACTGGAGTGG	GTGGCTTCAA	TCTCTTCCGG	GGGCTTCACT
631	TACTATCCTG	ACAGCGTGAA	AGGCAGATTTC	ACTATCTCCA	GGGATAACGC	ACGGAATATC	CTGTATCTGC	AGATGAGCTC	ACTGAGATCC
721	GAGGACACCG	CAATGTACTA	CTGCGCAAGA	GACGAAGTGC	GGGGTATCT	GGACGTGTGG	GGAGCAGGCA	CTACCGTCAC	CGTGTCTAGC
811	ACCAGTACAC	CTGCACCAAG	GCCTCCACA	CCCGCTCCCA	CTATCGCTTC	CCAGCCACTG	TCCCTGAGGC	CCGAGGCCCTG	CAGGCCAGCA
901	CTGCGCGGAG	CCGTGCATAC	TAGGGGGCTG	GACTTCGCTT	CGACATCTA	CATCTGGGC	CCACTGGCAG	GGACATGCCG	AGTCTGTCTG
991	GCTTCCCTGG	TCAATCACAT	GTAATGCAAG	CGGGGGCGG	AAAACTGCT	GTATATCTTT	AAGCAGCCTT	TCAAGAGACC	AGTGCAGACA
1081	ACCCAGGAGG	AAGATGGGTG	CTCATGCCGG	TTTCCCGAGG	AGGAGGAAG	CGGCTGCGAG	CTGAGGGTGA	AGTTTCCCG	CTCAGCAGAT
1171	GCTCCTGCCT	ACCAGCAGGG	CCAGAACCAG	CTGTATAATG	AGCTGAACCT	GGGCAGACGC	GAAGACTATG	ATGTGCTGGA	CAAAAAGCGG
1261	GGAAGAGACC	CCGAAATGGG	AGGGAAGCCA	AGGCGGAAA	ACCCCCAGGA	GGGCTGTAC	AATGAGCTGC	AGAAGGACAA	AATGGCAGAG
1351	GCTTACAGTG	AGATTGGGAT	GAAAGGAGAG	AGACGGAGGG	GAAAAGGGCA	CGATGGCCTG	TACCAAGGGC	TGAGCACAG	AACCAAAAGAT
1441	ACTTATGACG	CACATGCACAT	GCAGGCACTG	CCACCCAGAT	GACAGCCAGG	GGATTTCACC	ACTCAAAAGC	CAGACCTGCA	GACGCCCAGA
1531	TTATGAGACA	CACGGGATCA	ATTCCGCCCC	CCCCCTAACG	TTACTGGCCG	AAGCCGCTTG	GAATAAGGCC	GGTGTGCGTT	TGCTCTATATG
1621	TTATTTTCCA	CCATATTGCC	GTCCTTTTGGC	AATGTGAGGG	CCCGGAAACC	TGGCCCTGTC	TTCTTTGACGA	GCATTCCTAG	GGGTCTTTCC
1711	CCTCTCGCCA	AAGGAATGCA	AGGTCTGTTG	AATGTCTGTA	AGGAAGCAGT	TCCTCTGGAA	GCTTCTTGAA	GACAAACAAAC	GTCCTGTAGCG
1801	ACCTTTTGCA	GGCAGCGGAA	CCCCCACCT	GGCGACAGGT	GCCTCTGCGG	CCAAAGCCCA	CGTGTATAAG	ATACACCTGC	AAAGCGCGCA
1891	CAACCCAGT	GCCACGTTGT	GAGTTGGATA	GTTTGGGAAA	GAGTCAAAATG	GCTCTCCTCA	AGCCTATTCA	ACAAGGGGCT	GAAGGATGCC
1981	CAGAAAGTAC	CCCATTTGTAT	GGGATCTGAT	CTGGGGCCTC	GGTGACATG	CTTTACATGT	GTTTACTCGA	GGTTAAAAAA	ACGTCTAGGC
2071	CCCCCGAACC	ACGGGGACGT	GGTTTTCCTT	TGAAAAACAC	GATAATACCA	TGGCTCTGCC	TGTGACCGCA	CTGCTGCTGC	CCCTGGCTCT
2161	GCTGTGTCAC	GCCGCAAGAC	CTGCCGCATA	CAAGGATATT	CAGATGACTC	AGACCACAAAG	CTCCCTGAGC	GCCTCCCTGG	GAGACCGAGT
2251	GACAACTCT	TGCAGTGCAT	CACAGGGAAT	TAGCAACTAC	CTGAATTGGT	ATCAGCAGAA	GCCAGATGGC	ACTGTGAAAC	TGCTGATCTA
2341	CTATACCTCT	AGTCTGCACA	GTGGGGTCCC	CTCACGATTC	AGCGGATCCG	GCTCTGGAC	AGACTACAGC	CTGACTATCT	CCAACTTGA
2431	GCCCGAAGAT	ATTGCCACCT	ACTATTGCCA	GCAGTACTCC	AAGCTGCCCT	ATACCTTTGG	CGGGGGAACA	AAGCTGGAGA	TTAAAAGGGG
2521	AGGAGGAGGA	AGCGGAGGAG	GAGGATCCGG	AGGCGGGGGA	TCTGGAGGAG	GAGGAAGTGA	GGTGCACTG	GTCGAATCTG	GAGGAGGACT
2611	GGTGAAGCCA	GGAGGATCTC	TGAAACTGAG	TTGTGCCGCT	TCAGGCCCTGA	CCTTCTCAAG	CTACGCCATG	AGCTGGGTGC	GACAGACACC
2701	GTGAAAGCGG	CTGGAATGGG	TCGCTAGCAT	CTCCTCTGGC	GGGTTACAT	ACTATCCAGA	CTCCGTGAAA	GGCAGATTGA	CTATCTCTCG
2791	GGATAACGCA	AGAAATATTC	TGTACCTGCA	GATGATGTCA	CTGAGGAGCG	AGGACACCCG	AATGTACTAT	TGTGCCAGGG	ACGAAGTCCG
2881	CGGCTATCTG	GATGTCTGGG	GAGCTGGCAC	TACCGTCACC	GTCTCCAGCG	AGCAGAAACT	GATTAGCGAA	GAGGACCTGA	AAGATGAAC
2971	GTAA								

FIG. 27
Anti-human CD7 (TH69) CAR-P2A-Anti-human CD7 (TH69) PEBL (SEQ ID NO:13)

1	ATGGCTCTGC	CTGTGACTGC	CCTGCTGCTG	CCCCTGGGCC	TGCTGTGCTA	CGTGTGCTAGA	CCCGCCGCAT	ACAAAGACAT	TCAGATGACT
91	CAGACTACAT	CCAGCCTGTC	AGCTTCCCTG	GGCGATCGAG	TGACTATACG	CTGTTTCAGCA	AGCCAGGGGA	TCAGCAATTG	CCTGAACTGG
181	TACCAAGCAGA	AGCCCGACGG	AACGTGTCAAG	CTGCTGATTC	ACTATACTTC	CAGTCTGCAT	AGCGGGGTCC	CTAGCCGATT	CTCTGGGAGT
271	GGAAGCGGCA	CCGACTATAG	CCTGACCATT	TCCAACTGG	AACCAGAGGA	CATTGCCACC	TATTACTGTC	AGCAGTATTC	AAAGCTGCCC
361	TATACCTTTG	GCGGAGGGAC	TAAAGCTGGAG	ATCAAGCGAG	GAGGGGGAGG	ATCAGGGGGA	GGGGGAGCG	GAGGCGGGG	GAGTGGCGGC
451	GGAGGTCAG	AAGTCCAGCT	GGTCCAGAGT	GGGGGCGGAC	TGTTGAAACC	TGGGGGCTCT	CTGAAACTGA	GCTGTGCAG	CAGTGGCCTG
541	ACATTCTCCT	CATATGCTAT	GTCTTGGGTG	CGGACAGCCC	CAGAAAAGAG	ACTGGAGTGG	GTGGCTTCAA	TCTCTTCCGG	GGGCTTCACT
631	TACTATCCTG	ACAGCGTGAA	AGGCAGATTG	ACTATCTCCA	GGATAAACGC	ACGGAATATC	CTGTATCTGC	AGATGAGCTC	ACTGAGATCC
721	GAGGACACCG	CAATGTACTA	CTGCGCAAGA	GACGAAGTGC	GGGGTATCT	GGACGTGTGG	GGAGCAGGCA	CTACCGTCAC	CGTGTCTAGC
811	ACCACCTACAC	CTGCACCAAG	GCCTCCACA	CCCGCTCCCA	CTATCGCTTC	CCAGCCACTG	TCCCTGAGGC	CCGAGGCCTG	CAGGCCAGCA
901	GCTGGCGGAG	CCGTGCATAC	TAGGGGGCTG	GACTTCGCTT	GCGACATCTA	CCACTGGGCC	CCACTGGCAG	GGACATGCCG	AGTCTGTCTG
991	CTGTCCCTGG	TCATCACACT	GTACTGCAAG	CGGGGGCGCA	AAAACTGCT	GTATATCTTT	AAGCAGCCTT	TCAAGAGACC	AGTGCAGACA
1081	ACCCAGGAGG	AAGATGGGTG	CTCATGCCCG	TTTCCCGAGG	AGGAGGAAGG	CGGCTGCGAG	CTGAGGGTGA	AGTTTCCCG	CTCAGCAGAT
1171	GCTCCTGCCT	ACCAGCAGGG	CCAGAACCAG	CTGTATAATG	AGCTGAACCT	GGGCAGACGC	GAAGACTATG	ATGTGCTGGA	CAAAAAGGCGG
1261	GGAAGAGACC	CCGAAATGGG	AGGGAAGCCA	AGGCGGAAAA	ACCCCCAGGA	GGGCCTGTAC	AATGAGCTGC	AGAAGGACAA	AATGGCAGAG
1351	GCTTACAGTG	AGATTGGGAT	GAAAGGAGAG	AGACGGAGGG	GAAAAGGGCA	CGATGGCCTG	TACCAAGGGC	TGAGCACAGC	AACCAAAAGAT
1441	ACTTATGACG	CACATGCACAT	GCAGGCACTG	CCACCCAGAG	GCAGCGGCGC	CACAACTTC	TCTCTGCTAA	AGCAAGCAGG	TGATGTTGAA
1531	GAAACCCCG	GGCCTATGGC	TCTGCTGTG	ACCGCACTGC	TGCTGCCCT	GGCTCTGCTG	CTGCAAGCCG	CAAGACCTGC	CGCATACAAG
1621	GATATTTCAGA	TGACTCAGAC	CACAAGCTCC	CTGAGCGCT	CCCTGGGAGA	CCGAGTGACA	ATCTCTTGA	GTGCATCACA	GGGAATTAGC
1711	AACATCCTGA	ATTGGTATCA	GCAGAAAGCCA	GATGGCACTG	TGAAACTGCT	GATCTACTAT	ACCTCTAGTC	TGCACAGTGG	GGTCCCTCA
1801	CGATTACGG	GATCCGGCTC	TGGGACAGAC	TACAGCCTGA	CTATCTCCA	CCTGGAGCCC	GAAGATATTG	CCACCTACTA	TTGCCAGCAG
1891	TACTCCAAGC	TGCCTTATAC	CTTTGGCGGG	GGAAACAAGC	TGGAGATTAA	AAGGGGAGGA	GGAGGAAGCG	GAGGAGGAGG	ATCCGGAGGC
1981	GGGGGATCTG	GAGGAGGAGG	AAGTGAGGTG	CAGCTGGTCG	AATCTGGAGG	AGGACTGGTG	AAGCCAGGAG	GATCTCTGAA	ACTGAGTTGT
2071	GCCGCTTCAG	GCCTGACCTT	CTCAAGCTAC	GCCATGAGCT	GGGTGCGACA	GACACCTGAG	AAGCGGCTGG	AATGGGTGCG	TAGCATCTCC
2161	TCTGGCGGGT	TCACATACTA	TCCAGACTCC	GTGAAAAGGCA	GATTTACTAT	CTCTCGGGAT	AACGCAAGAA	ATATTCTGTA	CCTGCAGATG
2251	AGTTCACTGA	GGAGCGAGGA	CACCGCAATG	TACTATTGTG	CCAGGGAGCA	AGTGGCGGGC	TATCTGGATG	TCTGGGGAGC	TGGCACTACC
2341	GTCAACCTCT	CCAGCGAGCA	GAACCTGATT	AGCGAAGAGG	ACCTGAAAAG	TGAACTGTAA			

FIG. 28A

MSCV promoter -Anti-human CD7 (TH69) CAR-p2A-Anti-human CD7 (TH69) PEBL (SEQ ID NO:14)

1 GGAATGAAAG ACCCCACCTG TAGTTTGGC AAGCTAGCTT AAGTAACGCC ATTTTGCAG GCATGGAAAA TACATAACTG
 81 AGAATAGAGA AGTTCAGATC AAGTTTAGG ACAGAGAGAC AGCAGAAATAT GGGCCAAACA GGATATCTGT GGTAAAGCAGT
 161 TCCTGCCCCG GCTCAGGGCC AAGAACAGAT GGTCGCCAGA TGGGTCCTCG CCTCAGCAG TTTCTAGAGA ACCATCAGAT
 241 GTTTCAGGG TGCCCCAAGG ACCTGAAATG ACCCTGTGCC TTATTGAA TAACCAATCA GTTCGCTTCT CGTTTCTGTT
 321 CGCGCGCTTC TGCTCCCCGA GCTCAATAAA AGAGCCCA CAACCCCTCACT CGGCGCGCCA GTCCGAATTC GGCTTCCACC
 401 **ATGGCTCTGC CTGTGACTGC CCTGCTGCTG CCCCTGGCCC TGCTGCTGCA CGCTGCTAGA CCGCCGCGAT ACAAGGACAT**
 481 **TCAGATGACT CAGACTACAT CCAGCCTGTC AGCTTCCCTG GCGATCGAG TGAATATCAG CTGTTTCAGCA AGCCAGGGGA**
 561 **TCAGCAATTA CCTGAACCTGG TACCAGCAGA AGCCCGACGG AACTGTCAAG CTGCTGATCT ACTATACTTC CAGTCTGCAT**
 641 **AGCGGGGTCC CTAGCCGATT CTCTGGGAGT GGAAGCGGCA CCGACTATAG CCTGACCATT TCCAACCTGG AACCAAGGGA**
 721 **CATTGCCACC TATTACTGTC AGCAGTATTC AAAGCTGCCC TATACCTTTG GCGAGGGGAC TAAGCTGGAG ATCAAGCGAG**
 801 **GAGGGGGAGG ATCAGGGGGA GGGGGGAGCG GAGGCGGGG GAGTGGCGGC GGAGGCTCAG AAGTCCAGCT GGTGAGAGT**
 881 **GGGGCGGAC TGGTGAAACC TGGGGGCTCT CTGAAACTGA GCTGTGCAGC CAGTGGCCTG ACATTCTCCT CATATGCTAT**
 961 **GTCTTGGGTG CGGCAGACCC CAGAAAAGAG ACTGGAGTGG GTGGCTTCAA TCTCTTCCGG GGGCTTCACT TACTATCCTG**
 1041 **ACAGCGTGAA AGGCAGATTC ACTATCTCCA GGGATAACGC ACGGAATATC CTGTATCTGC AGATGAGCTC ACTGAGATCC**
 1121 **GAGGACACCG CAATGTACTA CTGCGCAAGA GACGAAGTGC GGGGGTATCT GGACGTGTGG GGAGCAGGCA CTACCGTAC**
 1201 **CGTGTCTAGC ACCACTACAC CTGCACCAAG GCCTCCACA CCGCTCCCA CTATCGCTTC CCAGCCACTG TCCCTGAGGC**
 1281 **CCGAGGCCCTG CAGGCCAGCA GCTGGCGGAG AGTCCCTGCTG TAGGGGGCTG GACTTCGCTT GCGACATCTA CATCTGGGCC**
 1361 **CCACTGGCAG GGACATGCGG AGTCCCTGCTG CTGTCCCTGG TCATCACACT GTACTGCAAG CGGGGGCGCA AAAAAGTGT**
 1441 **GTATATCTTT AAGCAGCCTT TCATGAGACC AGTGCAGACA ACCCAGGAGG AAGATGGGTG CTGATGCCGG TTTCCCGAGG**
 1521 **AGGAGGAAGG CGGCTGCGAG CTGAGGGTGA AGTTTCCCG CTCAGCAGAT GCTCCTGCCT ACCAGAGGG CCAGAACCCAG**
 1601 **CTGTATAATG AGCTGAACCT GGGCAGACGC GAAGAGTATG ATGTGCTGGA CAAAAGGCGG GGAAGAGACC CCGAAATGGG**
 1681 **AGGAAGCCA AGCGGAAAA ACCCCAGGA GGCCTGTAC AATGAGCTGC AGAAGGACAA AATGGCAGAG GCTTACAGTG**
 1761 **AGATTGGGAT GAAGGGAGAG AGACGGAGG GAAAGGGCA CGATGGCCTG TACCAGGGG TGAGCACAGC AACCAAAGAT**
 1841 **ACTTATGACG CACTGCACAT GCAGGGACTG CCACCCAGAT GAGGCAGCGG CGCCACAAAC TTCTCTCTGC TAAAGCAAGC**
 1921 **AGGTGATGTT GAAGAAAAAC CCGGGCCTAT GGCTCTGCCT GTGACCGCAC TGCTGTGCC CCTGGCTCTG CTGCTGCACG**
 2001 **CCGCAAGACC TGCCGCATAC AAGGATATTC AGATGACTCA GACCACAAGC TCCCTGAGCG CTTCCCTGGG AGACCGAGTG**
 2081 **ACAATCTCTT GCAGTGATC ACAGGGAATT AGCAACTACC TGAATTGTTA TCAGCAGAAG CCAGATGGCA CTGTGAAACT**
 2161 **GCTGATCTAC TATACCTCTA GTCTGCACAG TGGGTCCCC TCAGGATTCA GCGGATCCGG CTCTGGGACA GACTACAGCC**
 2241 **TGACTATCTC CAACCTGGAG CCCGAAGATA TTGCCACCTA CTATTGCCAG CAGTACTCCA AGCTGCCCTTA TACCTTTGGC**
 2321 **GGGGGAACAA AGCTGGAGAT TAAAGGGGA GGAGAGGAA CCGGAGGAGG AGGATCCGGA GCGGGGGAT CTGGAGGAGG**
 2401 **AGGAAGTGAG GTGCAGCTGG TCGAATCTGG AGGAGGACTG GTGAAGCCAG GAGGATCTCT GAAACTGAGT TGTCCCGCTT**
 2481 **CAGGCCGTGAC CTTCTCAAG TACGCCATGA GCTGGGTGCG ACAGACACCT GGAATGGGT CGCTAGCATC**

FIG. 28B
SEQ ID NO:14 continued

2561 TCCTCTGGCG GGTTCACATA CTATCCAGAC TCCGTGAAAG GCAGATTATC TATCTCTCGG GATAACGCAA GAAATATTCT
 2641 GTACCTGCAG ATGAGTTTCA TGAAGAGCGA GGACACCGCA ATGTACTATT GTGCCAGGGA CGAAGTGCGC GGCTATCTGG
 2721 ATGTCTGGG AGCTGGCACT ACCGTACCG TCTCCAGCGA GCAGAACTG ATTAGCGAAG AGGACCTGAA AGATGAACATG
 2801 TAA

FIG. 29A
EF1a promoter -Anti-human CD7 (TH69) CAR-P2A-Anti-human CD7 (TH69) PEBL (SEQ ID NO:15)

1 GGCTCCGGTG CCCGTCAGTG GGCAGAGCGC ACATCGCCCA CAGTCCCCA GAAGTTGGG GGAGGGGTG GCAATTGAAC
 81 CGGTGCCTAG AGAAGGTGGC GCGGGGTAAA CTGGGAAAGT GATGTCGTGT ACTGGCTCCG CCTTTTCCC GAGGTGGGG
 161 GAGAACCGTA TATAAGTGCA GTAGTCGCCG TGAACGTTCT TTTTCGCAAC GGGTTTGGC CCAGAACACA GGTAAGTGCC
 241 GTGTGTGGTT CCCGGGGCC TGGCCTCTTT ACGGTTATG GCCCTTGGT GCCTTGAATT ACTTCCACCT GGTGACAGTA
 321 CGTGATTCTT GATCCCCGAG TTCGGGTTGG AAGTGGTGG GAGAGTTCA GGCCTTGGC TTAAGGAGCC CCTTCGCCCTC
 401 GTGCTTGAGT TGAGGCCCTG CTTGGCGCTT GGGCCGCCG CGTGCGAATC TGGTGGCACC TTCGCGCCTG TCTCGCTGCT
 481 TTCCGATAAGT CTCTAGCCAT TTAATAATTT TGATGACCTG CTGCGACGCT TTTTCTCTGG CAAGATAGTC TTGTAATGTC
 561 GGGCCAAGAT CTGCACACTG GTATTTCGGT TTTTGGGGC GCGGGCGG ACGGGGCCG TGCCTCCAG CGCACATGTT
 641 CGCGGAGGCG GGGCTGCGA GCGCGGCCAC CGAGAAATCGG ACGGGGTAG TCTCAAGCTG GCCGGCCTG TCTGGTGCCT
 721 GGCTCGCGC CGCGGTGTAT CGCCCCGCC TGGCGGCAA GGCTGGCCG GTCGGCACA GTTCGTGAG CGGAAAGATG
 801 CGCGCTTCCC GCGCTGCTG CAGGGAGCTC AAAATGGAG ACGCGCGCT CGGGAGAGCG GCGGGGTGAG TCACCCACAC
 881 AAAGGAAAAG GGCCTTTCCG TCCTCAGCCG TCGCTTCATG TGAATCCAG GAGTACCGG CGCCGTCCAG GCACCTCGAT
 961 TAGTTCTCGA GCTTTTGGAG TACGTCGTCT TTAGGTTGG GGGAGGGGT TTAGCGATG GAGTTTCCC ACACGTAGTG
 1041 GGTGGAGACT GAAGTTAGG CAGCTTGGCA CTTGATGTAA TTCTCCTGG AATTGGCCCT TTTTGAGTTT GGATCTTGGT
 1121 TCATTCTCAA GCCTCAGACA GTGGTTCAA GTTTTTTCT TCCATTTCAG GTGTCGTGAG AATTGGGCTT CCACCATGGC
 1201 TCTGCCTGTG ACTGCCCTGC TGCTGCCCTT GGCCTGTCTG CTGCACGCTG CTAGACCCCG CGCATACAAG GACATTTCAGA
 1281 TGACTCAGAC TACATCCAGC CTGTCACTT CCCTGGGCGA TCGAGTGACT ATCAGCTGTT CAGCAAGCCA GGGGATCAGC
 1361 AATTACCTGA ACTGGTACCA GCAGGAAGCCC GACGGAACCTG TCAAGCTGCT GATCTACTAT ACTTCCAGTC TGATAGCGG
 1441 GGTCCCTAGC CGATTCTCTG GGAAGTGAAG CGGCACCGAC TATAGCCTGA CCATTTCCAA CCTGGAACCA GAGGACATTG
 1521 CCACCTATTA CTGTCAGCAG TATTCAAAGC TGCCCTATAC CTTTGGCGGA GGGACTAAGC TGGAGATCAA GCGAGGAGG
 1601 GGAGGATCAG GGGGAGGGG GAGCGGAGG GGGGGAGTG GCGCGGAGG GTCAGAAATC CAGCTGGTGG AGAGTGGGG
 1681 CGGACTGGTG AAACCTGGG GCTCTCTGAA ACTGAGCTGT GCAGCCAGTG GCCTGACATT CTCCTCATAT GCTATGTCTT
 1761 GGGTGGCGCA GACCCAGAA AAGAGACTGG AGTGGGTGGC TTCAATCTCT TCCGGGGGCT TCACCTACTA TCCTGACAGC

FIG. 29B

SEQ ID NO:15 continued

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1841 GTGAAAGGCA GATTCACTAT CTCAGGGAT AACGCACGGA ATATCTCTGTA TCTGCAGATG AGCTCACTGA GATCCGAGGA
1921 CACCGCAATG TACTACTGCG CAAGAGACGA AGTGGGGGG TATCTGGACG TGTGGGGAGC AGGCACCTACC GTCACCGTGT
2001 CTAGCACCAC TACACCTGCA CCAAGGCCCTC CCACACCCGC TCCCACCTATC TCCCTCCAGC CACTGTCCCT GAGGCCCGAG
2081 GCCTGCAGGC CAGCAGCTGG CGGAGCCGTG CATACTAGGG GGCTGGACTT CGCTTGCAGC ATCTACATCT GGGCCCCACT
2161 GGCAGGGACA TGGCGAGTCC TGCTGCTGTC CCTGGTCATC ACACGTACT GCAAGCGGGG GCGCAAAAAA CTGCTGTATA
2241 TCTTTAAGCA GCCTTTTCATG AGACCAAGTC AGACAACCCA GGAGGAAGAT GGTGCTCAT GCGGTTTCC CGAGGAGGAG
2321 GAAGCGGCT GCGAGCTGAG GGTGAAGTTT TCCCGCTCAG CAGATGCTCC TGCCTACCAG CAGGGCCAGA ACCAGCTGTA
2401 TAATGAGCTG AACCTGGGCA GACGCGAAGA GTATGATGTG CTGGACAAAA GCGGGGGAAG AGACCCCGAA ATGGGAGGGA
2481 AGCCAAGGCG GAAAAACCCC CAGGAGGGCC TGTACAATGA GCTGCAGAA GACAAAAATGG CAGAGGCTTA CAGTGAGATT
2561 GGGATGAAGG GAGAGAGACG GAGGGGAAA GGGCACGATG GCCTGTACCA GGGGCTGAGC ACAGCAACCA AAGATACTTA
2641 TGACGCACTG CACATGCAGG CACTGCCACC CAGATGAGGC AGCGGCGCCA CAAACTTCTC TCTGCTAAAG CAAGCAGGTG
2721 ATGTTGAAGA AAACCCCGGG CCTATGGCTC TGCTGTGAC CGCACTGCTG CTGCCCTGG CTCTGCTGCT GCACGCCGCA
2801 AGACCTGCCG CATACAAGGA TATTCAGATG ACTCAGACCA CAAAGCTCCTT GAGCGCCTCC CTGGGAGACC GAGTGACAAAT
2881 CTCTTGCACT GCATCACAGG GAAATTAGCAA CTACCTGAAT TGGTATCAGC AGAAGCCAGA TGGCACTGTG AAACCTGCTGA
2961 TCTACTATAC CTCTAGTCTG CACAGTGGG GAAATTAGCAA CTACCTGAAT TGGTATCAGC AGAAGCCAGA TGGCACTGTG AAACCTGCTGA
3041 ATCTCCAACC TGGAGCCCGA AGATATTGCC ACCTACTATT GCCAGCAGTA CTCCAAGCTG CCTTATACCT TTGGCGGGG
3121 AACAAAGCTG GAGATTAAAA GGGAGGAGG AGGAAAGCGGA GGAGGAGGAT CCGGAGGCGG GGGATCTGGA GGAGGAGGAA
3201 GTGAGGTGCA GCTGGTCGAA TCTGGAGGAG GACTGGTGAA GCCAGGAGGA TCTCTGAAAC TGAGTTGTGC CGCTTCAGGC
3281 CTGACCTTCT CAAGCTACGC CATGAGCTGG GTGCGACAGA CACCTGAGAA GCGGCTGGAA TGGGTGCTA GCATCTCCTC
3361 TGGCGGGTTC ACATACTATC CAGACTCCGT GAAAGGCAGA TTTACTATCT CTCGGGATAA CGCAAGAAAT ATTCTGTACC
3441 TGCAGATGAG TTCACTGAGG AGCGAGGACA CCGCAATGTA CTATTGTGCC AGGACGGAAG TCGCGGGCTA TCTGGATGTC
3521 TGGGAGCTG GCACTACCGT CACCGTCTCC AGCGAGCAGA AACTGATTAG CGAAGAGGAC CTGAAAGATG AACTGTAA

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FIG. 30A

EFS promoter -Anti-human CD7 (TH69) CAR-P2A-Anti-human CD7 (TH69) PEBL (SEQ ID NO:16)

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1 GGGCAGAGCG CACATCGCCC ACAGTCCCCG AGAAGTTGGG GGGAGGGGTC GGCAATTGAA CCGGTGCCTA GAGAAAGGTGG
81 CGCGGGGTAA ACTGGGAAAG TGATGTCGTG TACTGGCTCC GCCTTTTCC CGAGGGTGGG GGAGAACCGT ATATAAGTGC
161 AGTAGTCGCC GTGAACGTTT TTTTCGCAA CGGGTTTCC GCCAGAACAC AGGTGTCGTG ACCGCGGTC TAGAGGATCC
241 GGTACTCGAG GAACTGAAAA ACCAGAAAGT TAACTGGTAA GTTTAGTCTT TTTGTCTTTT ATTTCAGGTC CCGGATCCGG

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FIG. 30B

SEQ ID NO:16 continued

321	TGGTGGTGCA	AATCAAAGAA	CTGCTCCTCA	GTGGATGTTG	CCTTTACTTC	TAGGAATTGC	GCTTCCACCA	TGGTCTTGCC
401	TGTGACTGCC	CTGCTGCTGC	CCCTGGCCCT	GCTGCTGCAC	GCTGCTAGAC	CCGCCGCATA	CAAGGACATT	CAGATGACTC
481	AGACTACATC	CAGCCTGTCA	GCTTCCCTGG	GCGATCGAGT	GACTATCAGC	TGTTTCAGCAA	GCCAGGGGAT	CAGCAATTAC
561	CTGAACCTGGT	ACCAGCAGAA	GCCCGACGGA	ACTGTCAAGC	TGCTGATCTA	CTATACTTCC	AGTCTGCATA	GCGGGTCCC
641	TAGCCGATTTC	TCTGGGAGTG	GAAGCGGCAC	CGACTATAGC	CTGACCAATT	CCAACCTGGA	ACCAGAGGAC	ATTGCCACCT
721	ATTACTGTCA	GCAGTATTCA	AAGCTGCCCT	ATACCTTTGG	CGGAGGGACT	AAGCTGGAGA	TCAAAGCGAGG	AGGGGAGGA
801	TCAGGGGGAG	GGGGAGCGG	AGGCGGGGG	AGTGCGGGG	GAGGGTCAGA	AGTCCAGCTG	GTGAGAGTG	GGGGCGGACT
881	GGTGAACCT	GGGGCTCTC	TGAAACTGAG	CTGTGCAGCC	AGTGGCCTGA	CATTCTCCTC	ATATGCTATG	TCTTGGGTGC
961	GGCAGACCCC	AGAAAAGAGA	CTGGAGTGGG	TGGCTTCAAT	CTCTTCCGGG	GGCTTCACTT	ACTATCCTGA	CAGCGTGAAA
1041	GGCAGATTCA	CTATCTCCAG	GGATAACGCA	CGGAATATCC	TGTATCTGCA	GATGAGCTCA	CTGAGATCCG	AGGACACCGC
1121	AATGTACTAC	TGCGCAAGAG	ACGAAGTGCG	GGGGTATCTG	GACGTGTGGG	GAGCAGGCAC	TACCGTCACC	GTGTCTAGCA
1201	CCACTACACC	TGCACCAAGG	CCTCCACAC	CCGCTCCAC	TATCGCTTCC	CAGCCACTGT	CCCTGAGGCC	CGAGGCCCTGC
1281	AGGCCAGCAG	CTGGCGGAGC	CGTGCATACT	AGGGGGCTGG	ACTTCGCTTG	CGACATCTAC	ATCTGGGCC	CAC'TGGCAGG
1361	GACATGCGGA	GTCTGTCTGC	TGTCCCTGGT	CATCACACTG	TACTGCAAGC	GGGGCGGCAA	AAAACTGCTG	TATATCTTTA
1441	AGCAGCCTTT	CATGAGACCA	GTGCAGACAA	CCCAGGAGGA	AGATGGGTGC	TCATGCCGGT	TTCCCGAGGA	GGAGGAAAGC
1521	GGCTGCGAGC	TGAGGGTGAA	GTTTTCCTCG	TCAGCAGATG	CTCCTGCCTA	CCAGCAGGGC	CAGAACCAGC	TGTATAATGA
1601	GCTGAACCTG	GGCAGACGCG	AAGAGTATGA	TGTGCTGGAC	AAAAGGCGGG	GAAGAGACCC	CGAAATGGGA	GGGAAAGCCAA
1681	GGCGGAAAAA	CCCCCAGGAG	GGCCTGTACA	ATGAGCTGCA	GAAGGACAAA	ATGGCAGAGG	CTTACAGTGA	GATTGGGATG
1761	AAGGAGAGA	GACGGAGGGG	AAAAGGGCAC	GATGGCCTGT	ACCAGGGGCT	GAGCACAGCA	ACCAAAGATA	CTTATGACGC
1841	ACTGCACATG	CAGGCACCTGC	CACCCAGATG	AGGCAGCGGC	GCCACAAAAT	TCTCTCTGCT	AAAGCAAAGCA	GGTGATGTTG
1921	AAGAAAACCC	CGGGCCCTATG	GCTCTGCCTG	TGACCGCACT	GCTGCTGCCC	CTGGCTCTGC	TGCTGCACGC	CGCAAAGACCT
2001	GCCGCATACA	AGGATATTCA	GATGACTCAG	ACCACAAGCT	CCCTGAGCGC	CTCCCTGGGA	GACCGAGTGA	CAATCTCTTG
2081	CAGTGCATCA	CAGGGAATTA	GCAACTACCT	GAATTGGTAT	CAGCAGAAAGC	CAGATGGCAC	TGTGAAACTG	CTGATCTACT
2161	ATACCTCTAG	TCTGCACAGT	GGGTCCCCT	CACGATTCAG	CGGATCCGGC	TCTGGGACAG	ACTATGACCT	GACTATCTCC
2241	AACCTGGAGC	CCGAAGATAT	TGCCACCTAC	TATTGCCAGC	AGTACTCCAA	GCTGCCCTTAT	ACCTTTGGCG	GGGAAACAAA
2321	GCTGGAGATT	AAAAGGGGAG	GAGGAGGAAG	CGGAGGAGGA	GGATCCGAG	GCGGGGGATC	TGGAGGAGGA	GGAAGTGAGG
2401	TGCAGCTGGT	CGAATCTGGA	GGAGGACTGG	TGAAGCCAGG	AGGATCTCTG	AAACTGAGTT	GTGCCGCTTC	AGGCCTGACC
2481	TTCTCAAGCT	ACGCCATGAG	CTGGGTGCGA	CAGACACCTG	AGAAAGCGGT	GGAATGGGTC	GCTAGCATCT	CCTCTGGCGG
2561	TTTCACATAC	TATCCAGACT	CCGTGAAAGG	CAGATTACT	ATCTCTCGGG	ATAACGCAAG	AAATATTCTG	TACCTGCGAG
2641	TGAGTTCACT	GAGGAGCGAG	GACACCGCAA	TGTACTATTG	TGCCAGGGAC	GAAGTGGCGC	GCTATCTGGA	TGTCTGGGGA
2721	GCTGGCACTA	CCGTACCCGT	CTCCAGCGAG	CAGAAACTGA	TTAGCGAAGA	GGACCTGAAA	GATGAACTGT	AA

FIG. 31A

MSCV promoter -Anti-human CD7 (TH69) CAR-PGK promoter-Anti-human CD7 (TH69) PEBL (SEQ ID NO:17)

1 TTAATTAATT TATTAGTCT CCAGAAAAAG GGGGGAATGA AAGACCCAC CTGTAGGTTT GGCAAGCTAG CTTAAGTAAAC
 81 GCCATTTTGC AAGGCATGGA AAATACATAA CTGAGAATAG AGAAGTTTCAG ATCAAGGTTA GGAACAGAGA GACAGCAGAA
 161 TATGGGCCAA ACAGGATATC TGTGGTAAGC AGTTCCTGCC CCGGCTCAGG GCCAAGAACA GATGGTCCCC AGATCGGCTC
 241 CCGCCCTCAG CAGTTTCTAG AGAACCATCA GATGTTTCCA GGGTGCCCCA AGGACCTGAA ATGACCTGT GCCTTATTG
 321 AACTAACCAA TCAGTTCGCT TCTCGCTTCT GTTCGGCGCG TTCTGCTCC CGAGCTCAAT AAAAGAGCCC ACAACCCCTC
 401 ACTCGGCGCG CCAGTCCGAA TTCCGGCTTC ACCATGGCTC TGCCTGTGAC TGCCTGTGCT GTGCCCCCTGG CCCTGCTGCT
 481 GCACGCTGCT AGACCCGCGG CATACAAGGA CATTCAGATG ACTCAGACTA CATCCAGCCT GTCAGCTTCC CTGGCGGATC
 561 GAGTGACTAT CAGCTGTTCA GCAAGCCAGG GGATCAGCAA TTACCTGAAC TGGTACCAGC AGAAGCCCCG CGGAACCTGC
 641 AAGCTGCTGA TCTACTATAC TTCCAGTCTG CATAGCGGG TCCCTAGCCG ATTCTCTGG AGTGGAAGCG GCACCGACTA
 721 TAGCCTGACC ATTTCCAACC TGGAAACCAGA GGACATTGCC ACCTATTACT GTCAGCAGTA TTCAAAGCTG CCTATACCT
 801 TTGGCGGAGG GACTAAGCTG GAGATCAAGC GAGGAGGGG AGGATCAGGG GGAGGGGGG TCTCTGAAAC TGAGCTGTGC
 881 GCGGAGGGT CAGAAGTCCA GCTGGTCGAG AGTGGGGCG GACTGGTGAA ACCTGGGGC TCTCTGAAAC TGAGCTGTGC
 961 AGCCAGTGGC CTGACATTCT CCTCATATGC TATGTCTTGG GTGCGGCAGA CCCAGAAAA GAGACTGGAG TGGGTGGCTT
 1041 CAATCTCTTC CCGGGGCTTC ACTTACTATC CTGACAGCGT GAAAGGCAGA TTTACTATCT CCAGGGATAA CGCACGGAAAT
 1121 ATCCTGTATC TGCAGATGAG CTCACTGAGA TCCGAGGACA CCGCAATGTA CTACTGCGCA AGAGACGAAG TCGGGGGGTA
 1201 TCTGGACGTG TGGGAGCAG GCACATACCGT CACCGTGTCT AGCACCACTA CACCTGCACC AAGGCCCTCC ACACCCGCTC
 1281 CCACATATCG TTCCAGCCA CTGTCCCTGA GCGCCGAGGC CTGCAGGCCA GCAGCTGGCG GAGCCGTGCA TACTAGGGG
 1361 CTGGACTTCG CTTGCGACAT CTACATCTGG GCGCCACTGG CAGGGACATG CCGAGTCTCT GTGCTGTCCC TGGTCATCAC
 1441 ACTGTACTGC AAGCGGGGGC GCAAAAACCT GCTGTATATC TTTAAGCAGC CTTTTCATGAG ACCAGTGCAG ACAACCCAGG
 1521 AGGAAGATGG GTGCTCATGC CCGTTTCCCG AGGAGGAGGA AGCGGCTGC GAGCTGAGGG TGAAGTTTTC CCGCTCAGCA
 1601 GATGCTCCTG CTACCCAGCA GGGCCGAAC CAGCTGTATA ATGAGCTGAA CTTGGGCAGA CGCGAAGAGT ATGATGTGCT
 1681 GGACAAAAGG CCGGGAAGAG ACCCCGAAT GGGAGGGAAG CCAAGGCGGA AAAACCCCA GAGGGCCTG TACAATGAGC
 1761 TGCAGAAGGA CAAAATGGCA GAGGCTTACA GTGAGATTGG GATGAAGGGA GAGAGACGGA GGGGAAAAGG GCACGATGGC
 1841 CTGTACCAGG GGCTGAGCAC AGCAACCAA GATACTTATG ACGCACTGCA CATGCACGCA CTGCCACCCA GATGACTCGA
 1921 GTGCAGTCGA CGCGGGCCCC GGATCCCGG ACTCTAGATA ATTCTACCG GTAGGGGAGG CGCTTTTCCC AAGGCAGTCT
 2001 GGAGCATGCG CTTTAGCAG CCGGCTGGG ACTTGGCGT ACACAAGTGG CCTCTGGCT CGCACACATT CCACATCCAC
 2081 CGGTAGGCG CAACCGGCTC CGTCTTTTGG TGGCCCTTTC GCGCCACCTT CTACTCTCTC CTAAGTCAGG AAGTTCCCC
 2161 CCGCCCGCA GTCGCGTGC TGCAGGACGT GACAAATGGA AGTAGACAGT CTCACTAGTC TCGTGCAGAT GGACAGCACC
 2241 CGTGAGCAAT GGAAGCGGGT AGGCTTTTGG GGCACGGGC AATAGCAGT TTGCTCTTTC GCTTCTTGG CTGAGAGGCT
 2321 GGAAGGGGT GGTCCGGG GCGGGCTCAG GCGCGGCTC AGGGCGGG CCGGCGCTCC AAGTCTCTCC TGAGGCCGG
 2401 CATTCTGCAC GCTTCAAAAG CGACGCTCT CCGCGCTGT TCTCTCTTCC TCATCTCCG GCCTTTCGAC TGGCCAGGCT
 2481 TCCACCATGG CTCTGCCTGT GACCGCACTG CTGCTGCCCC TGGCTCTGCT GCTGCACGCC GCAAGACCTG CCGCATACAA

FIG. 31B

SEQ ID NO:17 continued

2561 GGATATTTCAG ATGACTTCAGA CCACAAGCTC CTTGAGCGCC TCCCTGGGAG ACCGAGTGAC AATCTCTTGC AGTGCATCAC
 2641 AGGGAATTAG CAACTACCTG AATTGGTATC AGCAGAAGCC AGATGGCACT GTGAAACTGC TGATCTACTA TACCTCTAGT
 2721 CTGCACAGTG GGGTCCCTC ACGATTTCAGC GGATCCGGCT CTGGGACAGA CTACAGCCTG ACTATCTCCA ACCTGGAGCC
 2801 CGAAGATATT GCCACCTACT ATTGCCAGCA GTACTCCAAG CTGCCTTATA CCTTTGGCGG GGGAACAAAG CTGGAGATTA
 2881 AAAGGGGAGG AGGAGGAAGC GGAGGAGGAG GATCCGGAGG CGGGGGATCT GGAGGAGGAG GAAGTGAGGT GCAGCTGGTC
 2961 GAATCTGGAG GAGGACTGGT GAAGCCAGGA GGATCTCTGA AACTGAGTTG TGCCGCTTCA GGCCTGACCT TCTCAAGCTA
 3041 CGCCATGAGC TGGGTGCGAC AGACACCTGA GAAGCGGCTG GAATGGGTCG CTAGCATCTC CTCTGGCGGG TTCACATACT
 3121 ATCCAGACTC CGTGAAAGGC AGATTTACTA TCTCTCGGGA TAACGCAAGA AATATTCTGT ACCTGCAGAT GAGTTCACATG
 3201 AGGAGCGAGG ACACCGCAAT GTACTATTGT GCCAGGGAGC AAGTGCGCGG CTATCTGGAT GTCTGGGGAG CTGGCACTAC
 3281 CGTACCCGTC TCCAGCGAGC AGAAACTGAT TAGCGAAGAG GACCTGAAAAG ATGAACTGTA *ACGGGT*

FIG. 32A

MSCV promoter - Anti-human CD7 (TH69) CAR-EF1 α promoter-Anti-human CD7 (TH69) PEBL (SEQ ID NO:18)

1 GGAATGAAAG ACCCACCTG TAGGTTTGGC AAGTAGCTT AAGTAACGCC ATTTTGCAAG GCATGGAAAA TACATAACTG
 81 AGAATAGAGA AGTTCAGATC AAGTTAGGA ACAGAGAGAC AGCAGAAATAT GGGCCAAACA GGATATCTGT GGTAAAGCAGT
 161 TCCTGCCCCG GCTCAGGGCC AAGAACAGAT GGTCCTCCAG TGCGGTCCCG CCTCAGCAG TTTCTAGAGA ACCATCAGAT
 241 GTTTCCAGGG TGCCCCAAGG ACCTGAAATG ACCCTGTGCC TTATTGAACTAACCAATCA GTTCGCTTCT CGCTTCTGTT
 321 CGCGCGCTTC TGCTCCCCGA GCTCAATAAA AGAGCCCAACA ACCCTCACT CGGCGCGCCA GTCCGAATTC GGCTTCCACC
 401 ATGGCTCTGC CTGTGACTGC CCTGTGCTG CCCCTGGCCC TGCTGTGCA CGCTGCCGAT CCCGCCGAT ACAAGGACAT
 481 TCAGATGACT CAGACTACAT CCAGCCTGTC AGCTTCCCTG GCGATCGAG TGACTATCAG CTGTTTCAGCA AGCCAGGGGA
 561 TCAGCAATTA CCTGAACCTG TACCAGCAGA AGCCCGACGG AACTGTCAAG CTGCTGATCT ACTATACTTC CAGTCTGCAT
 641 AGCGGGGTCC CTAGCCGATT CTCTGGGAGT GGAAGCGGCA CCGACTATAG CTTGACCATTT TCCAACCTGG AACCAAGGGA
 721 CATTTGCCACC TATTACTGTC AGCAGTATTC AAGCTGCCC TATACCTTTG GCGGAGGGAC TAAGCTGGAG ATCAAGCGAG
 801 GAGGGGAGG ATCAGGGGGA GGGGGGAGCG GAGGCGGGGG GAGTGGCGGC GGAGGGTCAG AAGTCCAGCT GGTGAGAGT
 881 GGGGGCGGAC TGGTGAAACC TGGGGGCTCT CTGAAACTGA GCTGTGCAGC CAGTGGCCTG ACATTCTCCT CATATGCTAT
 961 GTCTTGGGTG CGGCAGACCC CAGAAAAGAG ACTGGAGTGG GTGGCTTCAA TCTCTTCCGG GGGCTTCACT TACTATCCTG
 1041 ACAGCGTGAA AGGCAGATTC ACTATCTCCA GGGATAACGC ACGGAATATC CTGTATCTGC AGATGAGCTC ACTGAGATCC
 1121 GAGGACACCG CAATGTACTA CTGCGCAAGA GACGAAGTGC GGGGGTATCT GGACGTGTGG GGAGCAGGCA CTACCGTCAC
 1201 CGTGTCTAGC ACCACTACAC CTGCACCAAG GCCTCCCAACA CCCGCTCCCA CTATCGCTTC CCAGCCACTG TCCCTGAGGC
 1281 CCGAGGCGCTG CAGGCCAGCA GCTGGCGGAG CCGTGCATAC TAGGGGGCTG GACTTCGCTT GCGACATCTA CATCTGGGCC
 1361 CCACTGGCAG GGACATGCGG AGTCCCTGCTG CTGTCCCTGG TCATCACACT GTACTGCAAG CGGGGGCGCA AAAAACTGCT

FIG. 32B

SEQ ID NO:18 continued

1441 GTATATCTTT AAGCAGCCTT TCATGAGACC AGTGCAGACA ACCCAGGAGG AAGATGGGTG CTCATGCCGG TTTCCTGGAG
 1521 AGGAGGAAGG CCGCTGCGAG CTGAGGGTGA AGTTTTCCTG CTCAGCAGAT GCTCCTGCCT ACCAGCAGGG CCAGAACCAG
 1601 CTGTATAATG AGCTGAACCT GGGCAGACGC GAAGAGTATG ATGTGCTGGA CAAAAGGCGG GGAAGAGACC CCGAAATGGG
 1681 AGGGAAGCCA AGCGGAAAA ACCCCAGGA GGGCTGTAC AATGAGCTGC AGAAGGACAA AATGGCAGAG GCTTACAGTG
 1761 AGATTGGGAT GAAGGGAGAG AGACGGAGG GAAAAGGCA CGATGGCCTG TACCAGGGG TGAGCACAGC AACCAAGAT
 1841 ACTTATGACG CACTGCACAT GCAGGCACTG CCACCCAGAT GAGTCGACGG CTCGGTGCC CGTCAGTGG CAGAGCGCAC
 1921 ATCGCCACA GTCCCGGAGA AGTTGGGGG AGGGGTGGC AATTGAACCG GTGCCTAGAG AAGGTGGCG GGGTAAACT
 2001 GGGAAAGTGA TGTCGTGTAC TGGCTCCGC TTTTTCCTG GGTGGGGA GAACCGTATA TAAGTGCAGT AGTCGCCGTG
 2081 AACGTTCTTT TTGCGCAACGG GTTGGCCGC AGAACACAGG TAAAGTCCGT GTGTGTTCC CGCGGGCCTG GCCTCTTAC
 2161 GGGTTATGCG CCTTGCCTGC CTTGAATTAC TTCCACCTGG CTGCAGTACG TGATTCTTGA TCCCGAGCTT CCGGTTGGAA
 2241 GTGGGTGGGA GAGTTCGAGG CTTGCGCTT AAGGAGCCCC TTCGCCCTCGT GCTTGAGTTG AGGCCTGGCC TGGCGCTGG
 2321 GGCCGCCGCG TGCGAATCTG GTGGCACCTT CGCGCCTGTC TCGCTGCTT CGATAAGTCT CTAGCCATTT AAAATTTTG
 2401 ATGACCTGCT GCGACGCTTT TTTTCTGGCA AGATAGTCTT GTAAATGGG GCCAAGATCT GCACACTGGT ATTTCGGTTT
 2481 TTGGGGCCGC GGGCGGCGAC GGGGCTAGTC TCAAGCTGGC CGGCTGCTC TGGTGCTGG CCTCGGCCG CCCTGTATCG CCCCGCCCTG
 2561 AGAATCGGAC GGGGGTGGT CTGGCCCGGT CGGCACCATG TGCGTGAGCG GAAAGATGG CGCTTCCCG CCCTGTGCA GGGAGCTCAA
 2641 GGCGGCAAGG CTGGCCCGGT GAGAGCGGG GAGAGCGGG CCGTGAGTC ACCCACAAA AGGAAAAGG CCTTCCGTC CTCAGCCGTC
 2721 AATGGAGGAC GCGGCGCTCG GTACCCGGA GTACCGGGG ATCCGATGGA GTTCTCGAG TGGAGACTGA AGTTAGGCCA GCTTGGCACT
 2801 GCTTCATGTG ACTCCACGGA GTCCGCTTTT ATCCGATGGA GTTCCCCAC ACTGAGTGG ATCTGTTG ATCTCAAG CTCAGACAGT GGTCAAAGT
 2881 AGTTTGGGG CTCTTGGAA TTTGCCCTTT TTGAGTTTGG ATCTGTTG ACCATGGCTC TGCCCTGAC CGCAGTCTG CTGCCCCCTG
 2961 TGATGTAATT CTCTTGGAA TTTGCCCTTT TTGAGTTTGG ATCTGTTG ACCATGGCTC TGCCCTGAC CGCAGTCTG CTGCCCCCTG
 3041 TTTTCTCTTC CATTTACGGT GTCGTGACCC GGGGGCTTCC ACCATGGCTC TGCCCTGAC CGCAGTCTG CTGCCCCCTG
 3121 CTCTGCTGCT GCACGCCGCA AGACCTGCC CATACAAGGA TATTCAGATG ACTCAGACCA CAAGTCCCT GAGCGCTCC
 3201 TGGGAGACC GAGTGACAA CTCTTGCACT GCATCACAGG GAATTAGCAA TATTCAGATG TGGTATCAGC AGAAGCCAGA
 3281 TGGCACTGTG AAACCTGCTA TCTACTATC CTCTAGTCTG CACAGTGGG TCCCTCAGC ATTCAGCGGA TCCGGCTCTG
 3361 GGACAGACTA CAGCCTGACT ATCTCCACC TGGAGCCCGA AGATATTGCC ACCTACTATT GCCAGCAGTA CTCCAAGCTG
 3441 CTTTATACCT TTGGCGGGG AACAAAGCTG GAGATTAAA GGGGAGGAG AGGAAGCGGA GGAGGAGGAT CCGGAGCGG
 3521 GGGATCTGGA GGAGGAGGAA GTGAGGTGCA GCTGGTCGAA TCTGGAGGAG GACTGGTGA GCCAGGAGGA TCTCTGAAC
 3601 TGAGTTGTGC CGCTTCAGC CTGACCTTCT CAAGTACGC CATGAGCTGG GTGCGACGA CACCTGAGAA CCGGCTGGA
 3681 TGGGTCGCTA GCATCTCCTC TGGCGGTTT ACATACTATC CAGACTCCGT GAAAGGCAGA TTTACTATCT CTCGGGATAA
 3761 CGCAAGAAAT ATTCTGTACC TGCAGATGAG TTCACTGAGG AGCGAGGACA CCGCAATGTA CTATTGTGCC AGGACCGAAG
 3841 TGCGCGGCTA TCTGGATGTC TGGGAGCTG GCACTACCGT CACCGTCTCC AGCGAGCAGA AACTGATTAG CGAAGAGGAC
 3921 CTGAAAGATG AACTGTAA

FIG. 33A

MSCV promoter-anti-human CD7 (TH69) CAR-EFS promoter- Anti-human CD7 (TH69) PEBL (SEQ ID NO:19)

1 GGAATGAAAG ACCCCACCTG TAGTTTGGC AAGCTAGCTT AAGTAACGCC ATTTTGCAG GCATGGAAAA TACATAACTG
 81 AGAATAGAGA AGTTCAGATC AAGTTAGGA ACAGAGAGAC AGCAGAAATAT GGGCCAAACA GGATATCTGT GGTAAAGCAGT
 161 TCCTGCCCCG GCTCAGGGCC AAGAACAGAT GGTCGCCAGA TCGGGTCCCG CCTCAGCAG TTTCTAGAGA ACCATCAGAT
 241 GTTTCAGGG TGCCCCAAGG ACCTGAAATG ACCCTGTGCC TTATTTGAAC TAACCAATCA GTTCGCTTCT CGCTTCTGTT
 321 CGCGCGCTTC TGCTCCCCGA GCTCAATAAA AGAGCCACACA ACCCTCACT CGGGCGGCCA GTCC/GAATTC GGCTTCCACC/
 401 ATGGCTCTGC CTGTGACTGC CCTGTGCTG CCCCTGGCCC TGCTGTGCA CGCTGCTAGA CCCGCCGCAT ACAAGGACAT
 481 TCAGATGACT CAGACTACAT CCAGCCTGTC AGCTTCCCTG GCGGATCGAG TGACTATCAG CTGTTTCAGCA AGCCAGGGGA
 561 TCAGCAATTA CCTGAACTGG TACCAGCAGA AGCCCGACGG AACTGTCAAAG CTGCTGATCT ACTATACTTC CAGTCTGCAT
 641 AGCGGGGTCC CTAGCCGATT CTCTGGGAGT GGAAGCGGCA CCGACTATAG CCTGACCATT TCCAACCTGG AACCCAGAGGA
 721 CATTGCCACC TATTACTGTC AGCAGTATTC AAAGCTGCCC TATACCTTTG GCGGAGGGAC TAAGCTGGAG ATCAAGCGAG
 801 GAGGGGGAGG ATCAGGGGGA GGGGGGAGCG GAGGCGGGGG GAGTGGCGGC GAGGGGTGAG AAGTCCAGCT GGTGAGAGT
 881 GGGGGCGGAC TGGTGAACCC TGGGGGCTCT CTGAAACTGA GCTGTGCAGC CAGTGGCCTG ACATTCTCCT CATATGCTAT
 961 GTCTTGGGTG CGGCAGACCC CAGAAAGAG ACTGGAGTGG GTGGCTTCAA TCTCTTCCGG GGGCTTCACT TACTATCCTG
 1041 ACAGCGTGAA AGGCAGATTC ACTATCTCCA GGGATAACGC ACGGAATATC CTGTATCTGC AGATGAGCTC ACTGAGATCC
 1121 GAGGACACCG CAATGTACTA CTGCGCAAGA GACGAAAGTC GGGGGTATCT GGACGTGTGG GGAGCAGGCA CTACCGTCAC
 1201 CGTGTCTAGC ACCACTACAC CTGACCCAAAG GCCTCCACAC CCCGCTCCCA CTATCGCTTC CCAGCCACTG TCCCTGAGGC
 1281 CCGAGGCCCTG CAGGCCAGCA GCTGGCGGAG AGTCCTGCTG CTGTCCCTGG TCATCACACT TAGGGGGCTG GACTTCGCTT GCACATCTA CATCTGGGCC
 1361 CCACTGGCAG GGACATGCGG AGTCCTGCTG CTGTCCCTGG ACCCAGGAGG AAGATGGGTG CTGATGCCGG TTTCCCGAGG
 1441 GTATATCTTT AAGCAGCCTT TCATGAGACC AGTGCAGACA ACCCAGGAGG ATGTGCTGGA CAAAGGGCGG GGAAGAGACC CCGAAATGGG
 1521 AGGAGGAAGG CGGCTGCGAG CTGAGGGTGA AGTTTCCCG CTCAGCAGAT GCTCCTGCTT ACCAGCAGGG CCAGAACCCAG
 1601 CTGTATAATG AGCTGAACCT GGGCAGACGC GAAGAGTATG ATGTGCTGGA CAAAGGGCGG GGAAGAGACC CCGAAATGGG
 1681 AGGGAAGCCA AGCGGAAAA ACCCCAGGA AGACCGAGG GAAAGGGCA CGATGGCCTG AATGAGCTGC AGAAGGACAA AATGGCAGAG GCTTACAGTG
 1761 AGATTGGGAT GAAGGGAGAG AGACGGAGG GAAAGGGCA CGATGGCCTG TACCAGGGGC TGAGCACAGC AACCAAAGAT
 1841 ACTTATGACG CACTGCACAT GCAGGGCACTG CCACCCAGAT GAGTCGACGG GCAGAGCGCA CATCGCCAC AGTCCCCGAG
 1921 AAGTTGGGG GAGGGTCCG CAATTGAACC GGTGCTTGA GAAGGTGGG CGGGGTAAC TGGGAAAGTG ATGTCGTGTA
 2001 CTGGCTCCGC CTTTTTCCG AGGGTGGGG AGAACCGTAT ATAAGTGCAG TAGTCGCCGT GAACGTTCTT TTTGCGAACG
 2081 GGTTCGCCGC CAGAACACAG GTGTCGTGAC CGCGGGTCTA GAGGATCCGG TACTCGAGGA ACTGAAAAAC CAGAAAGTTA
 2161 ACTGGTAAGT TTAGTCTTTT TGTCTTTTAT TTCAGGTCCC GGATCCGGTG GTGGTGCAA TCAAAGAACT GCTCCTCAGT
 2241 GGATGTTGCC TTACTTCTA GCCCGGGGGG TTCCACCATG GCTCTGCTG TGACCCGCAT GCTGTGCCCC CTGGCTCTGC
 2321 TGCTGCACGC CGCAAGACCT GCCGCATACA AGGATATTCA GATGACTCAG ACCACAAGCT CCCTGAGCGC CTCCTTGGGA
 2401 GACCGAGTGA CAATCTCTTG CAGTGCAATCA CAGGGAATTA GCAACTACCT GAATTGGTAT CAGCAGAAAGC CAGATGGCAC

FIG. 33B

SEQ ID NO:19 continued

2481 TGTGAAACTG CTGATCTACT ATACCTCTAG TCTGCACAGT GGGGTCCCT CACGATTTCAG CGGATCCGGC TCTGGGACAG
 2561 ACTACAGCCT GACTATCTCC AACCTGGAGC CCGAAGATAT TGCCACCTAC TATTGCCAGC AGTACTCCAA GCTGCCTTAT
 2641 ACCTTTGGCG GGGGAACAAA GCTGGAGATT AAAAGGGAG GAGGAGGAAG CGGAGGAGGA GGATCCGGAG CGGGGGATC
 2721 TGGAGGAGGA GGAAGTGAGG TGCAGCTGGT CGAATCTGGA GGAGGACTGG TGAAGCCAGG AGGATCTCTG AAATGAGTT
 2801 GTGCCGCTTC AGCCTGACC TTCTCAAGCT ACGCCATGAG CTGGGTGCGA CAGACACCTG AGAAGCGGCT GGAATGGGTC
 2881 GCTAGCATCT CCTCTGGCGG GTTCACATAC TATCCAGACT CCGTGAAAGG CAGATTACT ATCTCTCGG ATAACGCAAG
 2961 AAATATTCTG TACCTGCAGA TGAGTTCACT GAGGAGCGAG GACACCGCAA TGTACTATTG TGCCAGGGAC GAAGTGCGCG
 3041 GCTATCTGGA TGTCTGGGA GCTGGCACTA CCGTCACCGT CTCCAGCGAG CAGAAACTGA TTAGCGAAGA GGACCTGAAA
 3121 GATGAACGTG AA

FIG. 34A

PGK promoter-anti-human CD7 (3A1F) CAR-EF1a promoter- Anti-human CD7 (TH69) PEBL (SEQ ID NO:20)

1 ATTTCTACCG GTAGGGGAGG CGCTTTTCCC AAGCAGTCT GGAGCATGGC CTTTAGCAGC CCCGCTGGG ACTTGGCGCT
 81 ACACAAGTGG CCTCTGGCCT CGACACATT CCACATCCAC CGGTAGGGC CAACCGGCTC CGTTCTTTGG TGGCCCTTC
 161 GGGCCACCTT CTACTCCTCC CTAAGTCAGG AGTTCCCCC CGGCCCGGA GCTCGGCTCG TGCAGGACGT GACAAATGGA
 241 AGTAGCACGT CTCACTAGTC TCGTGACAT GGACAGCAC GCTGAGCAAT GGAAGCGGT AGGCCTTTGG GGCAGCGGCC
 321 AATAGCAGCT TTGCTCCTC GCTTCTGGG CTCAGAGGT GGAAGGGGT GGTCCCGGG CGGGCTCAG GGGCGGCTC
 401 AGGGCGGGG CGGGCGGGG AAGTCTCC GAGGCCCGG CATCTGCAC GCTTCAAAAG CGCACGCTC CCGCGCTGT
 481 TCCTCTTCC TCATCTCCG GCCTTTCGAG AATTCGGCT CCACCATGGC TCTGCCCCG ACCGCTCTGC TGCTGCCCTCT
 561 GGCTCTGCTG CTGCACGCTG CTCGACCACA GGTCAGCTG CAGGAGTCAG GAGCTGAGCT GGTGAAGCCA GGGCAAGCG
 641 TCAAACTGTC CTGCAAGGCC TCTGGATATA CATTCACCTAG CTAAGTGGATG CACTGGGTGA AACAGAGACC CGGACAGGGC
 721 CTGGAGTGA TCGGAAAGAT TAACCTTAGC AATGGCAGGA CCAACTACAA CGAAAAGTTT AAATCCAAG CAACCTGAC
 801 AGTGGACAAG AGCTCCTCTA CAGCCTACAT GCAGCTGAGT TCACTGACTT CAGAGGATAG CGCAGTGAT TATTGCGCCA
 881 GAGCGGGGT CTACTATGAC CTGTACTATT ACGCCCTGGA TTATTGGGG CAGGGAACCA CAGTGAAGT CAGCTCCGGA
 961 GGAGGAGGAA GCGGAGGAG AGGATCCGGA GGAGGAGGAT CTGACATCGA GCTGACCCAG AGTCTGCTA CACTGAGCGT
 1041 GACTCCAGGC GATCTGTCA GTCTGTCTG TCGGCAAGC CAGTCCATCT CTAACAATCT GCACCTGGTAC CAGCAGAAAT
 1121 CCCATGAATC TCCACGACTG CTGATTAAGA GTGCCTCACA GAGCATCTCC GGCATTCCTT CCGGTTCTC TGGCAGTGGG
 1201 TCAGGAACCTG ACTTTACCTT GAGTATTAA CAGAGGATTT CCGCATGTAT TTTTGGCAGC AGAGCAATTC
 1281 CTGGCCCTAC ACTTTGGGAG GCGGGACCAA ACTGGAGATC AAGCGGACTA CCACACCCAGC TCCAAGACCA CCTACCCCTG
 1361 CACCAACAAT TGCTAGTCAG CCACTGTCTC TGAGACCAGA AGCATGTAGG CCTGCAGCTG GAGGAGCTGT GCACACCAGA
 1441 GGCCTGGACT TTGCCTGCGA TATCTACATT TGGGCTCCTC TGGCAGGAAC CTGTGGCGTG CTGCTGCTGT CTCTGGTCA
 1521 CACACTGTAT TGCAAAACGAG GCGGGAAGAA ACTGCTGTAC ATTTTCAAGC AGCCCTTTAT GAGGCCTGTG CAGACTACCC

FIG. 34B

SEQ ID NO:20 continued

1601 **AGGAGGAAGA** CGGCTGCAGC TGTCGCTTCC CTGAGGAAGA GGAAGGGGGA TGTGAGCTGC GCGTCAAATT TTCTCGAAGT
 1681 **GCAGATGCCC** CAGCTTATCA GCAGGGCCAG AACAGCTGT ACAACGAGCT GAATCTGGG CGGAGAGAGG AATATGACGT
 1761 **GCTGGATAAG** AGCGAGGAA GGGACCCAGA AATGGGAGG AAACACGAC GGAAGAACCC CCAGGAGGGC CTGTATAATG
 1841 **AACATGCAGAA** AGACAAGATG GCAGAGGCTT ACTCTGAAAT CGGGATGAAA GGAGAGAGAA GGCGCGGCAA GGGGCATGAT
 1921 **GGCCTGTACC** AGGGCTGAG CACAGCAACC AAAGACACCT ATGACGCACT GCACATGCAG GCACGTGCCAC CACGGTAATG
 2001 **AGTCGACGGC** TCCGGTGCCC GTCAGTGGG AGAGCGACA TCGCCACAG TCCCGAGAA GTTGGGGGA GGGTCCGGCA
 2081 **ATTGAACCGG** TGCCTAGAGA AGGTGGCGG GGTAAACTG GAAAGTGAT GTCGTGTACT GGCTCCGCTT TTTTCCCGAG
 2161 **GGTGGGGGAG** AACCGTATAT AAGTGCACTA GTCGCCGTGA ACGTTCTTTT TCGCAACGGG TTTGCCGCCA GAACACAGGT
 2241 **AAGTGCCGTG** TGTGGTTCCC GCGGCCCTGG CCTCTTACG GGTATGGCC CTTGCGTGCC TTGAATTACT TCCACCTGGC
 2321 **TGCAGTACGT** GATTCTTGAT CCCGAGCTTC GGGCTGGCT GGGCTGGAG AGTTGAGGC CTTGCGCTTA AGGAGCCCT
 2401 **TCGCCCTCGT** CTTGAGTTGA GGCCTGGCT GGGCTGGG GCGCCGCGT GCGAATCTGG TGGACCTTC GCGCTGTCT
 2481 **CGCTGCTTTC** GATAAGTCTC TAGCCATTGA AAATTTTGA TGACCTGCTG CGACGCTTTT TTTCTGGCAA GATAGTCTG
 2561 **TAAATGCGGG** CCAAGATCTG CACACTGGTA TTTGCGTTT TGGGGCCGG GCGCGGACG GGGCCCGTGC GTCCAGCGC
 2641 **ACATGTTCCG** CGAGGCGGGG CCGTGGAGCG CGGCCACCGA GAATCGGAC GGGGTAGTCT CAAGCTGGCC GGCCTGCTCT
 2721 **GGTGCTGGC** CTCGCGCCGC CGTGATCGC CCGCCCTGG GCGCAAGGC TGGCCCGTGC GGCACCACTT GCGTGAGCGG
 2801 **AAAGATGGCC** GCTTCCCGC CCGTGTGAG GAGCTCAAA ATGAGGACG CGCGCTCGG GAGAGCGGC GGTGAGTCA
 2881 **CCCACACAAA** GGAAGAGGGC CTTCCCGTCC TCAGCCGTGC CTTATGTGA CTCACGGAG TACCAGGCGC CGTCCAGGCA
 2961 **CCTCGATTAG** TTCTCGAGCT TTTGGAGTAC GTCGTCTTTA GGTGGGGG AGGGTTTGA TCGGATGGAG TTTCCCCACA
 3041 **CTGAGTGGGT** GGAGACTGAA GTTAGGCCAG CTTGGCACTT GATGTAATC TCCTTGGAA TTGCCCTTTT TGAGTTTGA
 3121 **TCTTGGTTCA** TTCTCAAGCC TCAGACAGTG GTTCAAAAGT TTTTCTTCC ATTTCAAGTG TCGTGACCCG GGGCTTCCA
 3201 **CCATGGCTCT** GCCTGTGACC GCACTGCTGC TGCCCTTGGC TCTGTGCTG CACGCCGCAA GACCTGCCGC ATACAAGGAT
 3281 **ATTCAGATGA** CTCAGACCAC AAGTCCCTG AGCCCTCCC TGGGAGACCG AGTGACAATC TCTTGCACTG CATCACAGGG
 3361 **AATTAGCAAC** TACCTGAAT GGTATCAGCA GAAGCCAGAT GGCACGTGTA AACTGCTGAT CTACTATACC TCTAGTCTGC
 3441 **ACAGTGGGGT** CCCCTCACGA TTCAGCGGAT CCGGCTCTGG GACAGACTAC AGCCTGACTA TCTCCAACCT GGAGCCCGAA
 3521 **GATATTGCCA** CCTACTATTG CCAGCAGTAC TCCAAGCTGC CTTATACCTT TGGCGGGGGA ACAAGCTGG AGATTAAAG
 3601 **GGGAGGAGGA** GGAAGCGGAG GAGGAGGATC CGGAGGCGG GATCTGGAG GAGGAGGAAG TGAGGTGCAG CTGGTGGAT
 3681 **CTGGAGGAGG** ACTGGTGAAG CCAGGAGGAT CTCTGAACT GAGTTGTGCC GCTTCAGGCC TGACCTTCTC AAGCTACGCC
 3761 **ATGAGCTGGG** TGGACAGAC ACCTGAGAAG CGGCTGGAAT GGGTCGCTAG CATCTCCTCT GCGGGTTCA CATACTATCC
 3841 **AGACTCCGTG** AAAGGCAGAT TTAATACTC TCGGATAAC GCAAGAAATA TTCTGTACCT GCAGATGAGT TCACTGAGGA
 3921 **CGGAGGACAC** CGCAATGTAC TATTGTCCA GGGACGAAGT GCGGGCTAT CTGGATGTCT GGGGAGCTGG CACTACCGTC
 4001 **ACCGTCTCCA** GCGAGCAGAA ACTGATTAGC GAAGAGGACC TGAAGATGA ACTGTAA

FIG. 35A

PGK promoter-anti-human CD7 (TH69) CAR -EF1a promoter- Anti-human CD7 (3A1F) PEBL (SEQ ID NO:21)

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1  ATTTCTACCGG GTAGGGGAGG CGCTTTTCCC AAGCAGTCT GGAGCATGGG CTTTAGCAGC CCCGCTGGGC ACTTGGCGCT
81  ACACAAAGTGG CCTCTGGCCT CGACACATT CCACATCCAC CGGTAGGCGC CAACCGGCTC CGTCTTTTGG TGGCCCTTTC
161  GCGCCACCTT CTACTCCTCC CTAAGTCCAG AAGTTCCCC CGCCCCGCA GCTCGCGTCG TGCAGGACGT GACAAATGGA
241  AGTAGCACGT CTCACATAGT TCGTGCAGT GGACAGCACC GCTGAGCAAT GGAAGCGGGT AGGCCCTTGG GGCAGCGGCC
321  AATAGCAGCT TTGCTCCTTC GCTTCTGGG CTCAGAGGCT GGAAGGGGT GGGTCCGGG GCGGGCTCAG GCGCGGGCTC
401  AGGGGCGGG GCGGGCGCGG AAGTCCCTCC GGAGGCCCGG CATTCGAC GCTTCAAAAG CGCACGTCTG CCGCGCTGTT
481  CTCCTCTTCC TCATCTCCGG GCCTTTCGAG AATTGGGCTT CCACCATGGC TCTGCTGTG ACTGCCCTGC TGCTGCCCTT
561  GGCCCTGCTG CTGCACGCTG CTAGACCCCG CGCATACAAG GACATTGAGA TGACTCAGC TACATCCAGC CTGTGAGCTT
641  CCCTGGGCGA TCGAGTGACT ATCAGCTGTT CAGCAAGCCA GGGGATCAGC AATTACCTGA ACTGGTACCA GCAGAAGCCC
721  GACGGAACGT TCAAGCTGCT GATCTACTAT ACTTCCAGTC TGCATAGCGG GGTCCCTAGC CGATTCTCTG GGAGTGAAG
801  CGGCACCGAC TATAGCCTGA CCATTTCGA CCTGGAACCA GAGGACATTG CCACCTATTA CTGTGAGCAG TATTCAAAAGC
881  TGCCCTATAC CTTTGGCGGA GGGACTAAGC TGGAGATCAA GCGAGGAGGG GGAGGATCAG GGGGAGGGGG GAGCGGAGGC
961  GGGGGGAGTG GCGGCGGAGG GTCAGAAATC CAGCTGGTGC AGAGTGGGG CGGACTGGTG AAACCTGGGG GCTCTCTGAA
1041  ACTGAGCTGT GCAGCCAGTG GCCTGACATT CTCCTCATAT GCTATGTCTT GGGTGGGCA GACCCAGAA AAGAGACTGG
1121  AGTGGGTGGC TTCAATCTCT TCCGGGGCT TCACCTACTA TCCTGACAGC GTGAAAGGCA GATTCACTAT CTCAGGGAT
1201  AACGCACGGA ATATCTGTGA TCTGCAGAT AGCTCACTGA GATCCGAGGA CACCGCAATG TACTACTGCG CAAGAGACGA
1281  AGTGGGGGG TATCTGGACG TGTGGGAGC AGGCATACC GTACCCGCTGT CTAGCACCACT TACACCTGCA CCAAGGCCCTC
1361  CCACACCCGC TCCACTATC GCTTCCAGC CACTGTCCCT GAGGCCCGAG GCCTGCAGGC CAGCAGCTGG CGGAGCCGTG
1441  CATACTAGG GGTGGACTT CGCTTGCAGC ATCTACATCT GGGCCCCACT CTGCTGTATA TCTTTAAGCA GCCTTTTCATG AGACCAGTGC
1521  CCTGGTCATC ACCTGTACT GCAAGCGGG GGTGCTCAT GCGGTTTCC CGAGGAGGAG GAAGCGGGCT GCGAGCTGAG GGTGAAGTTT
1601  AGACAACCCA GGAGGAAGAT GGTGCTCAT TGCCTACCAG CAGGCCAGA ACCAGCTGTA TAATGAGCTG AACCTGGGCA GACGCGAAGA
1681  TCCCGCTCAG CAGATGCTCC TGCCTACCAG GCGGGGGAAG AGACCCCGAA ATGGGAGGGA AGCCAAGGCG GAAAACCCC CAGGAGGGCC
1761  GTATGATGTG CTGGACAAA GCTGCAGAAG GACAAATGG CAGAGGCTTA CAGTGAGATT GGGATGAAGG GAGAGAGACG GAGGGGAAA
1841  TGTACAATGA GCTGCAGAAG GCGGCTGAGC ACAGCAACCA AAGATACTTA TGACGCACTG CACATGACAG CACTGCCACC
1921  GGGCAGGATG GCCTGTACCA GCGGCTCCG GTGCCCCGCA GTGGGCAGAG CGCATATCGC CCACAGTCCC CGAGAAAGTTG GGGGAGGGG
2001  CAGATGAGTC GACGGCTCCG GTGCCCCGCA GTGGGCAGAG CGCATATCGC CCACAGTCCC CGAGAAAGTTG GGGGAGGGG
2081  TCGGCAATTG AACCGGTGCC TAGAAGGT GCGCGGGGT AAACCTGGGA AGTGATGTCG TGTAATGGCT CCGCTTTT
2161  CCGAGGGTG GGGGAGAAC GTATATAAGT GCAGTAGTGC CCGTGAACGT TCTTTTTCG AACGGTTTG CCGCAGAAC
2241  ACAGGTAAGT GCCGTGTGTG GTTCCCGCG GCTTGGCTC TTTACGGGTT ATGGCCCTTG CGTGCTTGA ATTACTTCA
2321  CCTGGCTGCA GTACGTGATT CTTGATCCG AGCTTCGGT TGGAAAGTGG TGGAGAGATT CGAGGCCCTG CGCTTAAGGA
2401  GCGCCTTCGC CTCGTGCTTG AGTTGAGGCC TGGCCTGGG GCTGGGGCG CCGCTGGCG ATCTGGTGGC ACCTTCGCG
2481  CTGTCTCGCT GCTTTCGATA AGTCTCTAGC CATTAAAAA TTTTGATGAC CTGCTGGCAG GCTTTTTTTC TGGCAAGATA

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FIG. 35B

SEQ ID NO:21 continued

2561 GTCTTGTAAT TCGGGGCCAA GATCTGCACA CTGGTATTTC GGTTTTITGG GCCCGGGGGC GCGACGGGGC CCGTGGCTCC
 2641 CAGCGCACAT GTTCGGCGAG GCGGGGCTG CGAGCGCGG CACCGAGAAAT CCGACGGGG TAGTCTCAAG CTGGCCGGCC
 2721 TGCTCTGGTG CCGTGGCTCG GCGCGCCCTG TATCGCCCTG CCGTGGCGG CAAGGCTGGC CCGTCCGCA CCAGTTGCGT
 2801 GAGCGGAAAG ATGGCCGCTT CCGGGCCCTG CTGACGGGAG CTCAAAATGG AGGACGCGG GCTCGGAGA GCGGGCGGGT
 2881 GAGTCAACCA CACAAAGGAA AAGGGCCCTT CCGTCCCTCAG CCGTCCCTC ATGTGACTCC ACGGAGTACC GGGCGCCGTC
 2961 CAGGCACTC GATTAGTTCT CGAGCTTTTG GAGTACGTCG TCTTTAGGT GGGGGGAGG GTTTTATGCG ATGAGATTTC
 3041 CCCACACTGA GTGGGTGGAG ACTGAAGTTA GGCCAGCTTG GCACTTGATG TAATTCCTCT TGAATTTTGC CTTTTTTGAG
 3121 TTTGGATCTT GGTTCATTCT CAAGCTCTCAG ACAGTGGTTC AAAGTTTTTT TCTTCCATTT CAGGTGTCGT GACCGGGGGG
 3201 CTTCCACCAT GGCTCTGCCC GTACCCGCTC TGCTGCTGCC TCTGGCTCTG CTGCTGCACG CCGCAAGACC CCAGGTTCCAG
 3281 CTGCAGGAGT CAGGGGCAGA GCTGGTGAAA CCCGGAGCCA GTGTCAAACT GTCTGTGAAG GCCAGCGGT ATACTTTAC
 3361 CAGCTACTGG ATGCACTGGG TGAACACAGAG GCCAGGACAG GGCTGGAGT GGATCGGCAA GATTAACCCC AGCAATGGGC
 3441 GCACCAACTA CAACGAAAAG TTTAAATCCA AGGTACACT GACTGTGGAC AAGAGCTCCT CTACCGCATA CATGCAGCTG
 3521 AGTTCACTGA CATCTGAAGA TAGTGCCGTG TACTATTGCG CCAGAGGGCG GGTCTACTAT GACCTGTACT ATTACGCACT
 3601 GGATTATTGG GGGCAGGGAA CCACAGTGAC TGTCAGCTCC GGAGGAGGAG GATCCGGCGG AGGAGGCTCT GGGGGAGGCG
 3681 GGAGTGACAT CGAGCTGACA CAGTCTCCAG CCACTCTGAG CGTGACCCCT GGCGATTCTG TCAGTCTGTC ATGTAGAGCT
 3761 AGCCAGTCCA TCTCTAAACA TCTGCACTGG TACCAGCAGA AATCACATGA AAGCCCTCGG CTGCTGATTA AGAGTGCTTC
 3841 ACAGAGCATC TCCGGGATTC CAAGCAGATT CTCTGGCAGT GGGTCAAGAA CCGACTTTAC ACTGTCCATT AACTCTGTGG
 3921 AGACCGAAGA TTTCGGCATG TATTTTGGC AGCAGAGCAA TTCTTGGCCT TACACATTCTG GAGGCGGGAC TAAACTGGAG
 4001 ATTAAGAGGG AGCAGAAAAT GATTAGCGAA GAGGACCTGA AGGACGAACT GTGA

FIG. 36A

PGK promoter-anti-human CD7 (TH69) CAR -EF1a promoter- Anti-human CD7 (TH69) PEBL (SEQ ID NO:22)

1 ATTCTACCGG GTAGGGGAGG CGCTTTTCCC AAGCAGTCT GGAGCATGCG CTTTAGCAGC CCCGCTGGGC ACTTGGCGCT
 81 ACACAAGTGG CCTCTGGCCT CGCACACATT CCACATCCAC CGGTAGGCG CAACCGGCTC CGTTCTTTGG TGGCCCTTC
 161 GCGCCACCTT CTACTCCTCC CCTAGTCAGG AAGTTCCCC CCGCCCCGCA GCTCGCGTCG TGCAGGACGT GACAAATGGA
 241 AGTAGCACGT CTCACTAGTC TCGTGCAGAT GGACAGCACC GCTGAGCAAT GGAAGCGGGT AGGCCCTTGG GGCAGCGGCC
 321 AATAGCAGCT TTGCTCCTTC GCTTCTGGG CTCAGAGGCT GGGAAGGGGT GGGTCCGGGG GCGGGCTCAG GGGCGGGCTC
 401 AGGGCGGGG GCGGGCCCCG AAGGTCCCTCC GGAGGCCCGG CATTCTGCAC GCTTCAAAAG CGCACGTCTG CCGCGCTGTT
 481 CTCCTCTTCC TCATCTCCGG GCCTTCCGAG AATTCGGCTT CCACCATGGC TCTGCCTGTG ACTGCCCTGC TGCTGCCCCCT
 561 GGCCCTGCTG CTGCACGCTG CTAGACCCCG CGCATACAAG GACATTACAG TGACTCAGAC TACATCCAGC CTGTACAGCTT
 641 CCCTGGGCGA TCGAGTGACT ATCAGCTGTT CAGCAAGCCA GGGGATCAGC AATTACCTGA ACTGGTACCA GCAGAAGCCC

FIG. 36B

SEQ ID NO:22 continued

721 GACGGAACCTG TCAAGCTGCT GATCTACTAT ACTTCCAGTC TGCATAGCGG GGTCCCTAGC CGATTCTCTG GGAGTGAAG
 801 CGGCACCGAC TATAGCCTGA CCATTTCAC CTTGGAACCA GAGGACATTG CCACCTATTA CTGTACAGCAG TATTCAAAGC
 881 TGCCCTATAC CTTTGGCGGA GGGACTAAGC TGGAGATCAA GCGAGGAGGG GGAGGATCAG GGGAGGGGG GAGCGGAGGC
 961 GGGGGGAGTG GCGGCGGAGG GTCAGAAATC CAGCTGGTCG AGAGTGGGG CGGACTGGTG AAACCTGGGG GCTCTCTGAA
 1041 ACTGAGCTGT GCAGCCAGTG GCCTGACATT TCCGGGGCT TCACTTACTA TCCTGACAGC GTGAAAGGCA GATTAATAT CTCAGGGAT
 1121 AGTGGGTGGC TTCAATCTCT TCTGCGGAGC TGTGGGAGC AGGCACTACC GTACCCGAGG CACCGCAATG TACTACTGCG CAAGAGACGA
 1201 AACGCACGGA ATATCTGTGA TCTGCGGAGC TGTGGGAGC AGGCACTACC GTACCCGAGG CACCGCAATG TACTACTGCG CAAGAGACGA
 1281 AGTGGGGGG TATCTGGACG TGTGGGAGC AGGCACTACC GTACCCGAGG CACCGCAATG TACTACTGCG CAAGAGACGA
 1361 CCACACCCGC TCCCACTATC GCTTCCGAGC CACTGTCCCT GAGGCCCGAG GCGTGCAGGC CAGCAGCTGG CGGAGCCCTG
 1441 CATACTAGGG GGCTGGACTT CGCTTGCAGC ATCTACATCT GGGCCCCACT GGCAGGGACA TGCGGAGTCC TGCTGCTGTC
 1521 CCTGGTCATC ACACGTGACT GCAAGCGGGG GCGCAAAAAA CTGCTGTATA TCTTTAAGCA GCCTTTTCATG AGACCACTGC
 1601 AGACAACCCA GGAGGAAGAT GGGTGCTCAT GCGGTTTCC CGAGGAGGAG GAAGGCGGCT GCGAGCTGAG GGTGAAGTTT
 1681 TCCCGCTCAG CAGATGCTCC TGCCTACCAG CAGGGCCAGA ACCAGCTGTA TAATGAGCTG AACCTGGGCA GACGCGAAGA
 1761 GTATGATGTG CTGGACAAAA GCGGGGGAAG AGACCCCGAA ATGGGAGGGA AGCCAAAGCG AGGCAAGGCG CAGGAGGGCC
 1841 TGTACAATGA GCTGCAGAA GACAAATGG CAGAGGCTTA CAGTGAGATT GGGATGAAGG GAGAGAGACG GAGGGGAAAA
 1921 GGGCACGATG GCCTGTACCA GGGGCTGAGC ACAGCAACCA AAGATACTTA TGACGCACTG CACATGCAGG CACTGCCACC
 2001 CAGATGAGTC GACGGCTCCG GTGCCCGTCA GTGGCAGAG CGCACATCGC CCACAGTCCC CGAGAAAGTTG GGGGAGGGG
 2081 TCGGCAATTG AACCGGTGCC TAGAGAAAGT GCGCGGGGT AAACCTGGGA AGTGATGTCG TGTACTGGCT CCGCTTTT
 2161 CCCGAGGGTG GGGGAGAAC GTATATAAGT GCAGTAGTCG CCGTGAACGT TCTTTTTCGC AACGGGTTTG CCGCCAGAAC
 2241 ACAGGTAAGT GCCGTGTGTG GTTCCCGCGG GCCTGGCCTC TTTACGGGTT ATGGCCCTTG CGTGCCTTGA ATTACTTCCA
 2321 CCTGGCTGCA GTACGTGATT CTTGATCCCG AGCTTCGGGT TGGAGTGGG TGGGAGAGTT CGAGGCCCTG ACCTTAAGGA
 2401 GCGCCTTCGC CTCGTGCTTG AGTTGAGGCC TGGCCTGGGC GCTGGGGCCG CCGCGTGGCA ATCTGGTGGC ACCTTCGCGC
 2481 CTGTCTCGCT GCTTTCGATA AGTCTCTAGC CATTTAAAAT TTTTGATGAC CTGCTGCGAC GCTTTTTCGAC TGGCAAGATA
 2561 GTCTTGTAAT TCGGGGCCAA GATCTGCACA CTGGTATTTT GGTTTTGGG GCGCGGGGCG GCGACGGGG TAGTCTCAAG CTGGCCGGC
 2641 CAGGCACAT GTTCGGCGAG GCGGGGCGCT GAGCGCGGC CACCGAGAA TCGGAGGGGG CAGGCTGGC CCGTCTGGC
 2721 TGCTCTGGTG CCTGGCCTCG CGCGCCCTG TATCGCCCGG CCGTGGCGG CAGGCTGGC CCGTCTGGC CAGTTCGGT
 2801 GAGGGAAAG ATGGCCGCTT CCGGGCCCTG CTGACGGAG CTCAAAATGG AGGACGGGG GCTCGGGAGA GCGGGCGGT
 2881 GAGTCAACCA CACAAAGGAA AAGGGCTTT CCGTCTCAG CCGTGGCTT ATGTGACTCC ACGAGTACC GGGCGCGTC
 2961 CAGGCACCTC GATTAGTTCT CGAGCTTTT GAGTACGTCG TCTTTAGGT GGGGGGAGG GTTTATGCG ATGAGTTTC
 3041 CCCACACTGA GTGGGTGGAG ACTGAAGTTA GGCAGCTTG GCACCTGATG TAATCTCCT TGGAAATTCG CCTTTTGAG
 3121 TTTGGATCTT GGTTCATTCT CAAGCCTCAG ACAGTGGTTT AAAGTTTTT TCTTCCATTT CAGGTGCTGT GACCCGGGG
 3201 CTTCCACCAT GGCTCTGCCT GTGACCGCAC TGCTGCTGCT CCTGGCTCTG CTGCTGCACG CCGCAAGACC TGCCGCATAC

FIG. 36C

SEQ ID NO:22 continued

3281 AAGGATATTC AGATGACTCA GACCACAAGC TCCCTGAGCG CCTCCCTGGG AGACCGAGTG ACAATCTCTT GCAGTGCATC
 3361 ACAGGGAATT AGCAACTACC TGAATTGGTA TCAGCAGAAG CCAGATGGCA CTGTGAAACT GCTGATCTAC TATACCTCTA
 3441 GTCTGCACAG TGGGGTCCCC TCACGATTCA GCGGATCCGG CTCTGGGACA GACTACAGCC TGACTATCTC CAACCTGGAG
 3521 CCCGAAGATA TTGCCACCTA CTATTGCCAG CAGTACTCCA AGTGCCTTAA TACCTTTGGC GGGGGAACAA AGCTGGAGAT
 3601 TAAAGGGGA GGAGGAGGAA GCGGAGGAGG AGGATCCGGA GCGGGGGGAT CTGGAGGAGG AGGAAGTGAG GTGCAGCTGG
 3681 TCGAATCTGG AGGAGGACTG GTGAAGCCAG GAGGATCTCT GAAACTGAGT TGTGCCGCTT CAGGCCCTGAC CTTCTCAAGC
 3761 TACGCCATGA GCTGGGTGCG ACAGACACCT GAGAAGCGGC TGGAAATGGT CGCTAGCATC TCCTCTGGCG GGTTCACATA
 3841 CTATCCAGAC TCCGTGAAAG GCAGATTAC TATCTCTCGG GATAACGCAA GAAATATTTCT GTACCTGCAG ATGAGTTAC
 3921 TGAGGAGCGA GGACACCGCA ATGTACTATT GTGCCAGGA CGAAGTGCGC GGCTATCTGG ATGTCTGGG AGCTGGCACT
 4001 ACCGTCACCG TCTCCAGCGA GCAGAAACTG ATTAGCGAAG AGACCTGAA AGATGAACTG TAA

FIG. 37A

PGK promoter-anti-human CD7 (TH69) CAR-EFS promoter- Anti-human CD7 (TH69) PEBL (SEQ ID NO:23)

1 ATTCTACCGG GTAGGGGAGG CGCTTTTCCC AAGGCAGTCT GGAGCATGGG CTTTAGCAGC CCCGCTGGGC ACTTGGCGCT
 81 ACACAAGTGG CCTCTGGCCT CGACACATT CCACATCCAC CGTAGGGCGC CAACCGGCTC CGTTCTTTGG TGGCCCCCTC
 161 GCGCCACCTT CTACTCCTCC CTAAGTCAGG AAGTTCCCC CGCCCCGCA GCTCGCGTGC TGACGGACGT GACAAATGGA
 241 AGTAGCACGT CTCACTAGTC TCGTGAGAT TCGAGAGCACC GCTGAGCAAT GGAAGCGGGT AGGCCTTTGG GGCAGCGGCC
 321 AATAGCAGCT TTGCTCCTTC GCTTCTGGG CTCAGAGGCT GGAAGGGGT GGGTCCGGG GCGGGCTCAG GGGCGGGCTC
 401 AGGGCGGGG CGGGCGCCG AAGTCTCTCC GGAGGCCCGG CATCTGCAC GCTTCAAAAG CGCACGCTCTG CCGCGCTGTT
 481 CTCTCTTCC TCATCTCCG GCTTCTCGA AATTCGGCTT CCACCATGGC TCTGCCCTGTG ACTGCCCTGC TGCTGCCCCCT
 561 GGCCCTGCTG CTGACGCTG CTAGACCCG CGCATAACA GACATTCAGA TGACTCAGC TACATCCAGC CTGTCAGCTT
 641 CCTTGGCGA TCGAGTGACT ATCAGCTGTT CAGCAAGCCA GGGGATCAGC AATTACCTGA ACTGGTACCA GCAGAAGCCC
 721 GACGGAAGT TCAAGCTGCT GATCTACTAT ACTTCCAGTC TGCAATAGCG GGTCCCTAGC CGATTCTCTG GGAGTGAAG
 801 CGGCACCGAC TATAGCCTGA CCATTTCGA CTTGGAACCA GAGGACATTG CCACCTATTA CTGTACGAG TATTCAAAGC
 881 TGGCCCTATC CTTTGGCGA GGGACTAAGC TGGAGATCAA GCGAGGAGG GGAGGATCAG GGGGAGGGG GAGCGGAGGC
 961 GGGGGGAGT GCGGCGGAGG GTCAGAAAGT CAGCTGGTGC AGAGTGGGG CGGACTGGTG AAACCTGGG GCTCTCTGAA
 1041 ACTGAGCTGT GCAGCCAGTG GCCTGACATT CTCTCATAT TCTTGTCTT GGTGCGGCA GACCCAGAA AAGAGACTGG
 1121 AGTGGGTGGC TTCAATCTCT TCCGGGGGCT TCACTTACTA TCCGTGACAG GTGAAAGGCA GATTCATAT CTCCAGGAT
 1201 AACGCACGGA ATATCTGTA TCTGCAGATG AGCTCACTGA GATCCGAGGA CACCGCAATG TACTACTGCG CAAGAGACGA
 1281 AGTGGGGGG TATCTGGACG TGTGGGAGC AGGCACCTACC GTCACCGTGT CTAGCACCCAC TACACCTGCA CCAAGGCCCTC
 1361 CCACACCCCG TCCCACTATC GCTTCCCAGC CACTGTCCCT GAGGCCCGAG GCCTGCAGGC CAGCAGCTGG CGGAGCCCTG

FIG. 37B

SEQ ID NO:23 continued

1441 CATACTAGGG GGCTGGACTTT CGCTTGGCAG ATCTACATCT GGGCCCCCACT GGCAGGGACA TGCGGAGTCC TGCTGCTGTC
 1521 CCTGGTCATC ACACGTGACTT GCAAGCGGGG GCGCAAAAAA CTGCTGTATA TCCTTTAAGCA GCCTTTTCATG AGACCAGTGC
 1601 AGACAACCCA GGAGGAAGAT GGGTGTCTAT GCGGGTTTCC CGAGGAGGAG GAAGGCGGCT GCGAGCTGAG GGTGAAGTTT
 1681 TCCCGCTCAG CAGATGCTCC TGCCTACCA GAGGGCCAGA ACCAGCTGTA TAATGAGCTG AACCTGGCA GACGCGAAGA
 1761 GTATGATGTG CTGGACAAA GCGGGGGAAG AGACCCCGAA ATGGGAGGGA AGCCAAGGCG GAAAAACCCC CAGGAGGGCC
 1841 TGTACAATGA GCTGCAGAAG GACAAAATGG CAGAGGCTTA CAGTGAGATT GGGATGAAGG GAGAGAGACG GAGGGGAAAA
 1921 GGGCACGATG GCCTGTACCA GGGGCTGAGC ACAGCAACCA AAGATACTTA TGACGCACTG CACATGCAGG CACTGCCACC
 2001 CAGATGAGTC GACGGGCAGA GCGCACATCG CCCACAGTCC CCGAGAAATT GGGGGGAGGG GTCGGCAATT GAACCGGTGC
 2081 CTAGAGAAAG TGGCGCGGGG TAAACTGGGA AAGTGATGTC GTGTACTGGC TCCGCCCTTTT TCCCGAGGGT GGGGAGAAAC
 2161 CGTATATAAG TGCAGTAGTC GCGGTGAACG TTCTTTTTCG CAACGGGTTT GCGGCCAGAA CACAGGTGTC GTGACCGCGG
 2241 GTCTAGAGGA TCCGGTACTC GAGGAACTGA AAAACCCAGAA AGTTAACTGG TAAGTTTAGT CTTTTTGTCT TTTATTTTCA
 2321 GTCCCGGATC CGGTGGTGGT GCAAAATCAA GAACTGCTCC TCAGTGGATG TTGCCCTTAC TTCTAGCCCG GGGCTTCCA
 2401 CCATGGCTCT GCCTGTGACC GCACTGCTGC TGCCCTGGC TCTGCTGCTG CACGCCGCAA GACCTGCCCG ATACAAGGAT
 2481 ATTCAGATGA CTCAGACCAC AAGCTCCCTG AGCGCCTCCC TGGGAGACCG AGTGACAATC TCTTGCAGTG CATCACAGGG
 2561 AATTAGCAAC TACCTGAATT GGTATCAGCA GAAGCCAGAT GGCACGTGTA AACTGCTGAT CTACTATACC TCTAGTCTGC
 2641 ACAGTGGGGT CCCCTCACGA TTCAGCGGAT CCGGCTCTGG GACAGACTAC AGCCTGACTA TCTCCAACCT GGAGCCCGAA
 2721 GATATTGCCA CCTACTATTG CCAGCAGTAC TCCAAGCTGC CTTATACCTT TGGCGGGGGA ACAAAGCTGG AGATTAAAAAG
 2801 GGGAGGAGGA GGAAGCGGAG GAGGAGGATC CCGAGGCGGG GGATCTGGAG GAGGAGGAAG TGAGGTGCAG CTGGTCGAAT
 2881 CTGGAGGAGG ACTGGTGAAG CCAGGAGGAT CTCTGAAACT GAGTTGTGCC GCTTCAGGCC TGACCTTCTC AAGCTAGGCC
 2961 ATGAGCTGGG TGCACACAGC ACCTGAGAAG CCGCTGGAAT GGGTCGCTAG CATCTCCTCT GCGGGGTTCA CATACTATCC
 3041 AGACTCCGTG AAAGGCAGAT TTAATATCTC TCGGGATAAC GCAAGAAATA TTCTGTACCT GCAGATGAGT TCACTGAGGA
 3121 GCGAGGACAC CGCAATGTAC TATTGTGCCA GGGACGAAAT GCGCGGCTAT CTGGATGTCT GGGGAGCTGG CACTACCGTC
 3201 ACCGTCTCCA GCGAGCAGAA ACTGATTAGC GAAGAGGACC TGAAGATGA ACTGTAA

FIG. 38

Anti CD7 (TH69) PEBL (SEQ ID NO:24)

1 MALPVTALLL PLALLLHAAR PAAYKDIQMT QTSSLSASL GDRVITSCSA SQGISNYLNW YQKQPDGTVK LLIYYTSSLH
 81 SGVPSRFSGS GSGTDYSLTI SNLEPEDIAI TYCQYQSKLP YTFGGGTKLE IKR//GGGGSG GSGGGGGSGG GGSSEVQLVES
 161 GGGLVKPGGS LKLSCAASGL TFSSYAMSW RQTPKRIEW VASISSGGFT YYPDSVKGRF TISRDNARNI IYLQSSLSR
 241 EDTAMYICAR DEVRGYLDVW GAGTTVTVSS EQKLISEEDL KDEL

FIG. 39

Anti CD7 (TH69) PEBL variant (SEQ ID NO:25)

1 FMALPVTALL LPLALLHAA RPAAYKDIQM TQTTSSLSAS LGDRVITSCS ASQGISNYLN WYQKQPDGTV KLLIYYTSSL
 81 HSGVPSRFSG SSGTDYSLT ISNLEPEDIA TYCQYQSKL PYTFGGGTKL EIKRGGGGSG GSGGGGGSGG GGSSEVQLVE
 161 SGGGLVKPGG SLKLSCAASG LTFSSYAMSW VRQTPKRIE WVASISSGGF TYYPDSVKGR FTISRDNARN IYLQSSLSR
 241 SEDTAMYICA RDEVRGYLDV WGAGTTVTVS SEQKLISEED LKDEL

FIG. 40

Anti CD7 (3A1F) PEBL (SEQ ID NO:26)

1 MALPVTALLL PLALLLHAAR PQVQLQESG ELVKPGASVK LSCKASGYTF TSYMMHWVKQ RPKQGLEWIG KINPSNGRTN
 81 YNEKFKSKAT LTVDKSSSTA YMQLSLTSE DSAVYICARG GVYDLYYYA LDYWGQGTIV TVSSGGGGSGG GSGGGGGSD
 161 IELTQSPATL SVTPGDSVSL SCRASQSIS NLHWYQKSH ESPRLIKSA SQSISGIPSR FSGSGSGTDF TLSINSVETE
 241 DFGMYFCQQS NSWPYTFGGG TKLEIKREQ LISEEDLKDE L

FIG. 41

Anti CD7 (3A1F) PEBL variant (SEQ ID NO:27)

1 FMALPVTALL LPLALLHAA RPQVQLQESG AELVKPGASV KLSCKASGYT FTSYMMHWVK QRPQGLEWI GKINPSNGRT
 81 NYNEKFKSKA TLTVDKSSST AYMQLSLT S EDSAVYICAR GGVYDLYYY ALDYWGQGTIT VTVSSGGGGSG GSGGGGGSGG
 161 DIELTQSPAT LSVTPGDSVS LSCRASQSIS NNLHWYQKSH HESPRLLIKS ASQISGIPS RFSGSGSGTD FTLSINSVET
 241 EDFGMYFCQQ SNSWPYTFGG GTKLEIKREQ KLISEEDLKDE EL

FIG. 42

Anti CD7 (TH69) CAR (SEQ ID NO:28)

1 MALPVTALLL PLALLLHAAR PAAYKDIQMT QTTSSLSASL GDRVTISCSA SQGISNYLW YQKPDGTVK LLIYTTSSLH
 81 SGVPSRFSGS GSGTDYSLTI SNLEPEDIAT YYCQYSKLP YTFGGGTKLE IKRGGGGSGG GSGGGGGSGG GGSEVQLVES
 161 GGGLVKPGGS LKLSCAASGL TFSSYAMSW RQTPKRLW VASISSGGFT YPDSVKGRF TISRDNARNI LYLQMSILRS
 241 EDTAMYCAR DEVGYLDVW GAGTTVTVSS TTTTAPRRPPT PAPTIASQPL SLRPEACRPA AGGAVHTRGL DFACDIYIWA
 321 PLAGTCGVLL LSLVITLYCK RGRKKLLYIF KOPFMRPVOT TOEEDGCSCR FPEEEGGCE LRVKFSRSAD APAYQOQGNQ
 401 LYNELNLGRR EEYDVLDKRR GRDPEMGGKP RRKNPOEGLY NELQDKMAE AYSEIGMKGE RRRGKHDGL YQGLSTATKD
 481 TYDALHMOAL PPR

FIG. 43

Anti CD7 (TH69) CAR variant (SEQ ID NO:29)

1 MALPVTALLL PLALLLHAAR PAAYKDIQMT QTTSSLSASL GDRVTISCSA SQGISNYLW YQKPDGTVK LLIYTTSSLH
 81 SGVPSRFSGS GSGTDYSLTI SNLEPEDIAT YYCQYSKLP YTFGGGTKLE IKRGGGGSGG GSGGGGGSGG GGSEVQLVES
 161 GGGLVKPGGS LKLSCAASGL TFSSYAMSW RQTPKRLW VASISSGGFT YPDSVKGRF TISRDNARNI LYLQMSILRS
 241 EDTAMYCAR DEVGYLDVW GAGTTVTVSS TTTTAPRRPPT PAPTIASQPL SLRPEACRPA AGGAVHTRGL DFACDIYIWA
 321 PLAGTCGVLL LSLVITLYCK RGRKKLLYIF KOPFMRPVOT TOEEDGCSCR FPEEEGGCE LRVKFSRSAD APAYQOQGNQ
 401 LYNELNLGRR EEYDVLDKRR GRDPEMGGKP RRKNPOEGLY NELQDKMAE AYSEIGMKGE RRRGKHDGL YQGLSTATKD
 481 TYDALHMOAL PPR GSGATNF SLLKQAGDVE ENPG

FIG. 44

Anti CD7 (3A1F) CAR (SEQ ID NO:30)

1 MALPVTALLL PLALLLHAAR PQVQLQESGA ELVKPGASVK LSCKASGYTF TSYMHVWKQ RPKQGLEWIG KINPSNGRTN
 81 YNEKFKSKAT LTVDKSSSTA YMQLSLTSE DSAVYICARG GVYDLYYYA LDYWGQGTIV TVSSGGGGSGG GGGGGGGSD
 161 IELTQSPATL SVTPGDSVSL SCRASQISN NLHWYQKSH EPRLLIKSA SQSISGIPSR FSGSGGTDF TLSINSVETE
 241 DFGMFCQQS NSWPYTFGGG TKLEIKRTTT PAPRPPTAP TIASQPLSLR PEACRPAAG AVHTRGLDFA CDIYIWAFLA
 321 GTCGVLLLSL VITLYCKRGR KKLLYIFKQP FMRPVQTTQE EDGCSRFPE EEEGGCELRV KFSRSADAPA YQOQONOLYN
 401 ELNLGRREY DVLDKRRGRD PEMGGKPRK NPQEGLYNEL QDKMAEAYS EIGMKGERR GKGDGLYQG LSTATKDTYD
 481 ALHMOALPPR

FIG. 45

Anti CD7 (3A1F) CAR variant (SEQ ID NO:31)

1 MALPVTALLL PLALLHAAR PQVQLQESGA ELVKPGASVK LSCKASGYTF TSYMHVWKQ RPKQGLEWIG KINPSNGRTN
 81 YNEKFSKAT LTVDKSSSTA YMQLSLTSE DSAVYCARG GYYDLYYYA LDYWGQGTIV TVSSGGGGSG GGGSGGGSD
 161 IELTQSPATL SVTPGDSVSL SCRASQISN NLHWYQKSH ESPRLIKSA SQSISGIPSR FSGSGGTDF TLSINSVETE
 241 DFGMYFCQOS NSWPYTFGGG TKLEIKRTTT PAPRPPTAP TIASQPLSLR PEACRPAAG AVHTRGLDFA CDIYIWAPLA
 321 GTCGVLLLSL VITLYCKRGR KKLLYIFKQP FMRPVQTTQE EDGSCRFPE EEEGGCELRV KFSRSADAPA YQQGONOLYN
 401 ELNLGRREY DVLDKRRGRD PEMGKPRK NPOEGLYNEL OKDKMAEAYS EIGMKERRR GKGGHGLYQG LSTATKDTYD
 481 ALHMQALPPR GSGATNFSLL KQAGDVEENP G

FIG. 46

Anti-human CD7 (TH69) CAR-p2A- Anti-human CD7 (TH69) PEBL (SEQ ID NO:95)

1 MALPVTALLL PLALLHAAR PAAVKDIQMT QTTSSLSASL GDRVTISCSA SQGISNYLNV YQKPDGTVK LLIYTTSSLH
 81 SGVPSRFSGS GSGTDYSLTI SNLEPEDIAT YYCQYSKLP YTFGGGTKLE IKRGGGGSGG GSGGGGGSGG GGSEVQLVES
 161 GGGLVKPGGS LKLSCAASGL TFSSYAMSWV RQTPEKRLEW VASISSGGFT YYPDSVKGRF TISRDNARNI LYLQMSLLRS
 241 EDTAMYICAR DEVRGYLDVW GAGTTVTVSS TTTAPAPRPPT PAPTIASQPL SLRPEACRPA AGGAVHTRGL DFACDIYIWA
 321 PLAGTCGVLL LSLVITLYCK RGRKKLLYIF KQPFMRPVQT TQEEEDGCSQR FPEEEEGGCE LRVKFSRSAD APAYQQGQNG
 401 LYNELNLGRR EYDVLDRRR GRDPENMGKPK RRKNPQEGLY NELQDKMAE AYSEIGMKGE RRRKGHDGL YQGLSTATKD
 481 TYDALHMQAL PPRGSGATNF SLKQAGDVE ENPGPMALPV TALLPLALL LHAARPAAYK DIQMTQTTSS LSASLGDRVT
 561 ISCSAQGIS NYLNWYQKP DGTVKLLIY TSSLHSGVPS RFSGSGGTD YSLTISNLEP EDIATYYCQQ YSKLPYTFGG
 641 GTKLEIKRGG GGSGGGGSGG GGSGGGGSEV QLVESGGGLV KPGGSLKLS C AASGLTFSSY AMSWVRQTP PE KRLEWVASIS
 721 SGGFTYYPDS VKGRFTISR D NARNILYLQ M SSLRSEDTAM YYCARDEV RG YLDVWGAGTT VTVSSEOKLI SEEDLKDEL