

CHIMERIC ANTIGEN RECEPTOR TO TARGET TROP-2-POSITIVE CANCERS

[0001] This application claims priority to U.S. Provisional Patent Application Serial No. 63/220,283, filed July 9, 2021, which is incorporated by reference herein in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on July 7, 2022, is named MDAC_P1304WO_Sequence_Listing.xml and is 103,067 bytes in size.

BACKGROUND**I. Technical field**

[0003] Embodiments of the disclosure include at least the fields of cell biology, molecular biology, immunology, and medicine, including cancer medicine.

II. Background

[0004] Genetic reprogramming of Natural Killer (NK) cells for adoptive cancer immunotherapy has clinically relevant applications and benefits such as 1) innate anti-tumor surveillance without prior need for sensitization; 2) allogeneic efficacy without graft versus host reactivity; and 3) direct cell-mediated cytotoxicity and cytolysis of target tumors. Human NK cell development and acquisition of self-tolerance, alloreactivity, and effector functions is an adaptive process of licensing, calibration, and arming. At the molecular level, specific activating and inhibitory receptors direct NK cellular functions by aggregating, balancing, and integrating extracellular signals into distinct effector functions. The functional activity of NK cells and responsiveness to extrinsic stimuli follow the ‘rheostat’ model of continuous education and thus are amenable to reprogramming. Genetic modification of NK cells to redirect their effector functions is an effective method to harness their cytotoxic capability to kill tumor cells.

[0005] TROP-2 is a type-I transmembrane glycoprotein. TROP-2 is expressed on a variety of human epithelial cancer cells, including breast, lung, urothelial, gastric, colorectal, pancreatic, prostatic, cervical, head and neck, and ovarian carcinomas. TROP-2 is expressed at

low levels on the surface of skin and oral mucosa; otherwise TROP-2 is not expressed on normal tissue.

[0006] There exists a need in the field of cancer biology for methods and compositions concerning genetic engineering of cells, including human NK cells, for cell therapy to target cancers including TROP-2-positive tumors.

SUMMARY

[0007] Embodiments of the disclosure encompass methods and compositions related to engineered cellular receptors, including chimeric antigen receptors (CARs) that target TROP-2 (also known as tumor-associated calcium signal transducer 2, trophoblast cell surface antigen 2, or EGP-1, for example). In specific embodiments, the engineered receptors that target TROP-2 are in the form of polynucleotides, polypeptides, and/or are comprised on the surface of cells of any kind, including immune cells. In specific cases, the cells are immune cells, and in certain embodiments the immune cells are NK cells, NK T cells, invariant NKT cells, gamma delta T cells, alpha beta T cells, regulatory T cells, B cells, macrophages, mesenchymal stromal cells (MSCs), dendritic cells, and so forth, from any source. In some embodiments, the immune cells are NK cells. In certain embodiments, reprogrammed NK cells from cord blood (CB-NK) are encompassed for targeting cancers expressing TROP-2 molecules.

[0008] TROP-2 (also “TROP2” or “Trop2”) is utilized as a target antigen for aspects of the disclosed methods and compositions at least in part because it is expressed on multiple cancers, including breast, lung, urothelial, gastric, colorectal, pancreatic, prostatic, cervical, head and neck, and ovarian carcinomas.

[0009] The present disclosure includes a number of novel CAR molecules including fusion of an scFv targeting human TROP-2 (including, for example, an scFv from an antibody such as an RS7 antibody (e.g., mRS7, hRS7), Pr1E11, TrMab-29, datopotamab, 2G10, 2EF, etc.), including in some cases that incorporate either CD3 ζ alone or in combination with costimulatory or adaptor signaling domains, such as from NKG2D, OX-40, CD27, 41BB, CD28, DAP10, DAP12, and/or 2B4. In specific cases, allogeneic CB-NK cells are retrovirally transduced to express a TROP-2 CAR. In particular embodiments, immune cells of the disclosure encompassing TROP-2 CAR molecules also express one or more proteins that support their survival and proliferation. In specific cases, the immune cells are engineered to express one or more cytokines that facilitate the cells' expansion and persistence. In specific cases, the one or more cytokines are interleukin 15 (IL-15), IL-2, IL-7, IL-12, IL-18, IL-21,

and/or IL-23. In certain aspects, a vector that encodes the CAR also encodes the cytokine, and each ultimately are produced as separate polypeptides. In other aspects, the CAR and the cytokine are encoded on separate vectors.

[0010] Particular embodiments of the disclosure allow for the use of off-the-shelf immune cells, including at least NK cells, that are allogeneic with respect to a recipient individual, that target TROP-2-positive cells of any kind, and that also may or may not be transduced to express one or more cytokines, such as IL-15, IL-2, IL-21, IL-12, IL-23, IL-7, and/or IL-18.

[0011] In specific embodiments of the disclosure, expression of one or more endogenous genes in the immune cell has been modified, for example the expression may be partially or fully reduced in expression. Although the modification may occur by any means, in specific embodiments expression of the one or more genes has been modified, such as by being reduced in expression levels, and this may occur by any suitable means including at least CRISPR. Merely as examples, the endogenous gene may be selected from the group consisting of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, CD7, CTLA-4, TDAG8, CD38, and a combination thereof.

[0012] Embodiments of the disclosure include polynucleotides that encode an anti-TROP-2 chimeric antigen receptor (CAR), the CAR comprising an anti-TROP-2 antigen binding region of a TROP-2 specific antibody, a transmembrane domain, and an intracellular domain. In some embodiments, the TROP-2 specific antibody is an RS7 antibody. In some embodiments, the RS7 antibody is a murine RS7 (mRS7). In some embodiments, the RS7 antibody is a humanized RS7 (hRS7). In some embodiments, the anti-TROP-2 antigen binding region comprises a sequence having at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with SEQ ID NO:9. In some embodiments, the anti-TROP-2 antigen binding region comprises a sequence having at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with SEQ ID NO:14. In some embodiments, the anti-TROP-2 antigen binding region comprises a sequence having at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with SEQ ID NO:9 and a sequence having at least 85%, at least 90%, at least 95%, or at least 99% identity with SEQ ID NO:14. In some embodiments, the anti-TROP-2 antigen binding region comprises SEQ ID NO:9 and SEQ ID NO:14. In some embodiments, the anti-TROP-2 antigen binding region comprises a sequence having at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with SEQ ID NO:10. In some embodiments, the anti-TROP-2 antigen binding region comprises a sequence having at least

85%, at least 90%, at least 95%, or at least 99% sequence identity with SEQ ID NO:15. In some embodiments, the anti-TROP-2 antigen binding region comprises a sequence having at least 85%, at least 90%, at least 95%, or at least 99% sequence identity with SEQ ID NO:10 and a sequence having at least 85%, at least 90%, at least 95%, or at least 99% identity with SEQ ID NO:15. In some embodiments, the anti-TROP-2 antigen binding region comprises SEQ ID NO:10 and SEQ ID NO:15. The anti-TROP-2 antigen binding region may be codon-optimized.

[0013] In some embodiments, the TROP-2 specific antibody is a 2G10 antibody. In some embodiments, the 2G10 antibody is a murine 2G10 (m2G10). In some embodiments, the 2G10 antibody is a humanized 2G10 (h2G10).

[0014] In some embodiments, the TROP-2 specific antibody is a 2EF antibody. In some embodiments, the 2EF antibody is a murine 2EF antibody (m2EF). In some embodiments, the 2EF antibody is a humanized 2EF antibody (h2EF).

[0015] The transmembrane domain may be a transmembrane domain from, for example, CD28, the alpha chain of the T- cell receptor, beta chain of the T- cell receptor, zeta chain of the T- cell receptor, CD3 zeta, CD3 epsilon, CD3 gamma, CD3 delta, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD27, CD33, CD37, CD64, CD80, CD86, CD 134, CD137, CD154, ICOS/CD278, GITR/CD357, NKG2D, DAP10, DAP12, or any combination thereof. In some embodiments, the transmembrane domain is a CD27 transmembrane domain. The CD27 transmembrane domain may comprise SEQ ID NO:22. In some embodiments, the transmembrane domain is a CD28 transmembrane domain. The CD28 transmembrane domain may comprise SEQ ID NO:23. In some embodiments, the transmembrane domain is a CD8 transmembrane domain. The CD8 transmembrane domain may comprise SEQ ID NO:24.

[0016] The intracellular domain may be an intracellular domain from, for example, CD3 zeta, CD27, CD28, 4-1BB, DAP12, NKG2D, OX-40 (CD134), DAP10, CD40L, 2B4, DNAM, CS1, CD48, NKp30, NKp44, NKp46, or NKp80, or any combination thereof. In some embodiments, the intracellular domain is a CD3 zeta intracellular domain. The CD3 zeta intracellular domain may comprise SEQ ID NO:29. In some embodiments, the intracellular domain is a CD28 intracellular domain. The CAR may comprise two or more, or three or more, intracellular domains. In certain aspect, the two or more intracellular domains comprise a CD3 zeta intracellular domain and an additional intracellular domain selected from a CD28, DAP10, DAP12, 4-1BB, NKG2D, and 2B4 intracellular domain. In a specific case, the two or more intracellular domains comprise a CD3 zeta intracellular domain and a CD28 intracellular domain.

[0017] In some embodiments, the CAR further comprises a signal peptide. In certain aspects, the signal peptide is from CD8, CD27, granulocyte-macrophage colony-stimulating factor receptor (GMSCF-R), Ig heavy chain (IgH), CD3, or CD4. In some embodiments, the signal peptide is an IgH signal peptide, The IgH signal peptide may comprise SEQ ID NO:20. In some embodiments, the signal peptide is a GMSCF-R signal peptide. The GMSCF-R signal peptide may comprise SEQ ID NO:21. In some embodiments, the signal peptide is a CD8 signal peptide. In certain aspects, the CAR does not comprise a signal peptide.

[0018] In certain embodiments, a polynucleotide encoding a CAR of the disclosure further encodes an additional polypeptide of interest. The sequence encoding the additional polypeptide of interest and the sequence encoding the CAR may be separated on the polynucleotide by a 2A element, such as an E2A element. In certain aspects, the polypeptide of interest is a therapeutic protein or a protein that enhances cell activity, expansion, and/or persistence. In some embodiments, the additional polypeptide of interest is a suicide gene product, a cytokine, or a human or viral protein that enhances proliferation, expansion and/or metabolic fitness. In certain embodiments the additional polypeptide of interest is a cytokine, for example IL-15, IL-2, IL-12, IL-18, IL-21, IL-23, or IL-7. In a specific embodiment, the cytokine is IL-15. In some embodiments, the additional polypeptide of interest is a suicide gene product. In some embodiments, the suicide gene product is Caspase 9. The suicide gene product may be an inducible suicide gene product. In some embodiments, a polynucleotide of the disclosure encodes, in addition to a CAR, a cytokine (e.g., IL-15) and a suicide gene product (e.g., caspase 9, such as inducible caspase 9).

[0019] Aspects of the disclosure are directed to a polypeptide encoding a CAR comprising a sequence having at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity with SEQ ID NO:2, 4, 6, or 8. In some embodiments, the CAR comprises SEQ ID NO:2, 4, 6, or 8. In some embodiments, the CAR comprises SEQ ID NO:2. In some embodiments, the CAR comprises SEQ ID NO:4. In some embodiments, the CAR comprises SEQ ID NO:6. In some embodiments, the CAR comprises SEQ ID NO:8. In some aspects, the polynucleotide comprises a sequence having at least 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.1%, 99.2%, 99.3%, 99.4%, 99.5%, 99.6%, 99.7%, 99.8%, or 99.9% sequence identity with SEQ ID NO:1, 3, 5, or 7. In some embodiments, the polynucleotide comprises SEQ ID NO:1, 3, 5, or 7. In some embodiments, the polynucleotide comprises SEQ ID NO:1. In some

embodiments, the polynucleotide comprises SEQ ID NO:3. In some embodiments, the polynucleotide comprises SEQ ID NO:5. In some embodiments, the polynucleotide comprises SEQ ID NO:7.

[0020] Also presented herein are vectors comprising a polynucleotide of the disclosure. Vectors contemplated herein include viral vectors (e.g., adenoviral vectors, adeno-associated viral vectors, lentiviral vectors, and retroviral vectors) and non-viral vectors (e.g., plasmids).

[0021] Embodiments of the disclosure include immune cells of any kind comprising any polynucleotide and/or polypeptide encompassed herein. In specific embodiments, the immune cell is a NK cell, T cell, gamma delta ($\gamma\delta$) T cell, alpha beta ($\alpha\beta$) T cell, invariant NKT (iNKT) cell, B cell, macrophage, MSC, dendritic cell, or mixture thereof. In cases wherein the immune cell is an NK cell, the NK cell may be derived from cord blood (including pooled cord blood units), peripheral blood, induced pluripotent stem cells, bone marrow, and/or from a cell line. In specific aspects, the NK cell line is NK-92 cell line or another NK cell line derived from a tumor or from a healthy NK cell or a progenitor cell.

[0022] In specific embodiments, the immune cell is an NK cell, such as one derived from cord blood, such as from a cord blood mononuclear cell. The NK cell may be a CD56⁺ NK cell, in specific cases. The NK cells may express one or more exogenously provided cytokines, such as IL-15, IL-2, IL-12, IL-18, IL-21, IL-23, IL-7, or a combination thereof. Particular embodiments include populations of immune cells of any kind of the disclosure, and the cells may be present in a suitable medium or a suitable carrier of any kind.

[0023] Methods of treating or preventing cancer of any kind are encompassed herein, including by administering cells expressing particular anti-TROP-2 CARs at a therapeutically effective amount to ameliorate or prevent the cancer, or reduce the risk of the cancer, reduce the severity of the cancer, prevent metastasis or risk thereof, or delay the onset of the cancer.

[0024] In some embodiments, disclosed is a method of killing TROP-2-positive cells in an individual comprising administering to the individual an effective amount of cells harboring any polynucleotide and/or polypeptide of the disclosure (e.g., a TROP-2 CAR of the disclosure). In specific embodiments, the cells are NK cells, T cells, gamma delta T cells, alpha beta T cells, invariant NKT (iNKT) cells, B cells, macrophages, mesenchymal stromal cells (MSCs), or dendritic cells. NK cells may be derived from cord blood, peripheral blood, induced pluripotent stem cells, hematopoietic stem cells, bone marrow, or from a cell line. NK cells may be derived from cord blood mononuclear cells. In some cases, the TROP-2-positive cells are cancer cells, including from hematopoietic cancers or solid tumors. The cells may be

allogeneic or autologous with respect to the individual, who may or may not be a human. The cells may be administered to the individual by injection, intravenously, intraarterially, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, intracranially, percutaneously, subcutaneously, regionally, by perfusion, in a tumor microenvironment, or a combination thereof.

[0025] In particular embodiments of the methods, the cells may be administered to the individual once or more than once. The duration of time between administrations of the cells to the individual may be 1-24 hours, 1-7 days, 1-4 weeks, 1-12 months, or 1 or more years. The methods may further comprise the step of providing to the individual an effective amount of an additional therapy, such as surgery, radiation, gene therapy, immunotherapy, and/or hormone therapy. The additional therapy may comprise one or more antibodies or antibody-based agents, in some cases. In some aspects to the methods, they may further comprising the step of identifying TROP-2-positive cells in the individual.

[0026] It is contemplated that any embodiment discussed in this specification can be implemented with respect to any method or composition of the invention, and vice versa. Furthermore, compositions of the invention can be used to achieve methods of the invention.

[0027] The foregoing has outlined rather broadly the features and technical advantages of the present disclosure in order that the detailed description that follows may be better understood. Additional features and advantages will be described hereinafter which form the subject of the claims herein. It should be appreciated by those skilled in the art that the conception and specific embodiments disclosed may be readily utilized as a basis for modifying or designing other structures for carrying out the same purposes of the present designs. It should also be realized by those skilled in the art that such equivalent constructions do not depart from the spirit and scope as set forth in the appended claims. The novel features which are believed to be characteristic of the designs disclosed herein, both as to the organization and method of operation, together with further objects and advantages will be better understood from the following description when considered in connection with the accompanying figures. It is to be expressly understood, however, that each of the figures is provided for the purpose of illustration and description only and is not intended as a definition of the limits of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0028] The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

[0029] FIGs. 1A-1C show results demonstrating that Trop2 is an attractive target in PDAC. (A) The Trop2 expression at mRNA level was analyzed for various cancer types, using the TCGA dataset, which shows that PDAC has relatively high expression of Trop2. (B) Kaplan-Meier survival curve showing that patients with high-Trop2 expression had significantly shorter overall survival than those with low-Trop2 expression ($p = 0.033$). (C) The surface expression of Trop2 was determined using flow cytometry. The representative histograms show that PDAC cell lines have higher expression of Trop2 on their surface. Mean Fluorescence Intensity (MFI) corresponds to the Trop2 expression.

[0030] FIGs. 2A-2B show results demonstrating that Trop2 is an attractive target in Ovarian cancer. (A) Trop2 expression at mRNA level was analyzed for various cancer types, using the TCGA dataset, which shows that ovarian cancer has relatively high expression of Trop2. (B) The surface expression of Trop2 was determined using flow cytometry and the representative histograms show that ovarian cancer cell lines have higher expression of Trop2 on their surface. Mean Fluorescence Intensity (MFI) corresponds to the Trop2 expression.

[0031] FIG. 3 shows results demonstrating that Trop2 is expressed in various colorectal cancer (CRC) cell lines. The surface expression of Trop2 was determined using flow cytometry and the representative histograms show that several CRC cell lines (such as HCT-15, SW403, SW1116, WiDr, Sw480) have high expression of Trop2 on their surface. CMS: Consensus molecular subtypes (CMS1: MSI Immune; CMS2: Canonical; CMS3: Metabolic; and CMS4: Mesenchymal).

[0032] FIGs. 4A-4B show design and information regarding a chimeric antigen receptor construct against Trop2. (A) Trop2 CAR sequence were generated using the single-chain variable fragment (scFv) sequence derived from Sacituzumab govitecan-hziy (RS7) antibody sequence. The top panel shows the schematic representation of the CAR design for various constructs and bottom panel shows the representative ID's for these CAR constructs used in FIGs. 4A-10C. (B) 293 T cell transfection efficiency with the different Trop2 CAR constructs. Transfection efficiency was determined by checking the surface expression of TROP2 in 293T cells post virus collection using flow cytometry. Trop2 antigen tagged with histidine (His) is

added to the cells for 20 mins, and later anti-His antibody is used to check the transfection efficiency. Non transduced (NT) cells were used as control.

[0033] FIGs. 5A-5B show results demonstrating that cord blood (CB) NK cells transduced with Trop2 CAR constructs showed high transduction efficiency. The retroviral supernatants collected from the transfection experiments were used to transduce CBNK cells. Forty eight hours post transduction, the transduction efficiency was assessed by flow cytometry. Non transduced (NT) cells were used as control. CBNK cells transduced with all Trop2 constructs showed high expression of the CAR on their surface. (A) Representative histogram of the CAR staining. (B) CAR transduction efficiency was quantified from three different donors.

[0034] FIG. 6 shows results demonstrating that Trop2 CAR engineering of CBNK cells enhances their cytotoxicity against Trop2 expressing PDAC cell lines. NK cells were derived from cord blood and were transduced with various Trop2 CAR constructs. Non-transduced (NT) CBNK cells were used as controls. CFPAC1, PATC148 and PANC1 cells, which have high, high, and low/no CD70 expression respectively, were labelled with chromium-51 and co-cultured with various CAR CBNK cells at various effector to target ratios for a 4-hour chromium release assay. Compared to NT CBNK cells, all Trop2 CAR NK cells showed increased cytotoxicity against CFPAC1 (left) and PATC148 (middle) cells, which have high Trop2 expression. On the other hand, there was no difference in the cytotoxicity of Trop2 CAR NK cells compared to NT-NK cells against PANC1 cells (right) which have low/no Trop2 expression.

[0035] FIG. 7 shows results demonstrating that Trop2 CAR engineering of CBNK cells enhances their cytotoxicity against Trop2 expressing ovarian cancer cell lines. NK cells were derived from cord blood and were transduced with various Trop2 CARs. Non-transduced (NT) CBNK cells were used as controls. SKOV3 cell line, which have high CD70 expression, was labelled with chromium-51 and co-cultured with CAR CBNK cells at various effector to target ratios for four hours and chromium release was measured, which corresponds to the cytotoxicity of the cancer cells. Compared to NT NK cells, all Trop2 CAR CBNK cells showed increased cytotoxicity against SKOV3 cells.

[0036] FIGs. 8A-8D show results demonstrating that Trop2 CAR engineering of CBNK cells enhances their cytotoxicity against Trop2 expressing CRC cell lines. NK cells were derived from cord blood and were transduced with various Trop2 CARs. Non-transduced (NT) CBNK cells were used as controls. Cell lines with high Trop2 expression (SW403 and WiDR) and low/no Trop2 expression (RKO and LoVo) were subjected to chromium release assay. Compared to NT NK cells, all Trop2 CAR CBNK cells (especially Trop2-CAR #2) showed

increased cytotoxicity against SW403 (top left) and WiDr (top right) cells, which have high Trop2 expression. On the other hand, there was no difference in the cytotoxicity of Trop2 CAR NK cells compared to NT-NK cells against against RKO (bottom left) and LoVo (bottom right) cells which have low/noTrop2 expression.

[0037] FIGs. 9A-9B show results demonstrating that Trop2 CAR engineering of CBNK cells enhances their cytotoxicity against Trop2 expressing CRC cell lines, as shown by the IncuCyte cytotoxicity assay. To analyze the real-time cytotoxic activity of CBNK cells transduced with the different Trop2 CAR constructs, we performed an IncuCyte cytotoxicity assay. Trop2 CAR-CBNK cells were co-cultured at 1:1 ratio with either WiDr (Trop2 high) or RKO (Trop2 low/no) cells, and real-time cytotoxicity of NK cells against the tumor cell lines measured every hour over 120 hours. Compared to NT NK cells, all Trop2 CAR NK cells showed increased cytotoxicity against WiDr (A), which has high Trop2 expression. On the other hand, there was no difference in the cytotoxicity of Trop2 CAR NK cells compared to NT-NK cells against RKO (B) cells which have low/noTrop2 expression.

[0038] FIGs. 10A-10C show results demonstrating that CBNK cells transduced with Trop2 CAR showed effective anti-tumor activity against Trop2 positive ovarian cancer cells *in vivo*. NSG mice were engrafted with 0.5M firefly luciferase-labelled SKOV3 (SKOV3 FFluc) with high Trop2 expression. (A) Bioluminescence imaging of the tumor showing that CBNK cells transduced with Trop2 CARs were able to reduce the tumor burden, when compared to tumor alone group or NT CBNK group. (B) Graph plotting average radiance of BLI data and comparing the difference among groups of mice shown in panel 10A. (C) Survival curve showing the significant survival benefit of a single dose of CBNK cells transduced with the various Trop2 CARs compared to the tumor alone or NT CBNK control groups.

[0039] FIGs. 11A-11B show generation and expression of Trop2 CAR constructs with varying co-stimulatory domain. (A) Schematic map representing the various regions of the CAR construct and the different costimulatory molecules (DAP10 vs CD28). (B) Representative histogram of the CAR staining with TROP2 CAR constructs incorporating different costimulatory molecules.

[0040] FIGs. 12A-12B show results demonstrating that Trop2 CAR CBNK cells have superior cytotoxicity against PATC148 cells when compared to non-transduced CBNK cells. (A) CBNK cells were derived from cord blood and transduced with Trop2 CARs incorporating different costimulatory molecules. Non-transduced (NT) CBNK cells and 20% SDS (which causes the full lysis of the cells) were used as controls. PATC148 cells, which have high Trop2 expression, were grown in 96 well RTCA E-Plates overnight and the different NK groups were

added the next day at 2:1 effector to target (E:T) ratio. The cancer cell growth was measured continuously by Xcelligence assay and represented as normalized cell index. Compared to NT-NK cells, Trop2 CAR NK cells with either CD28 or DAP10 co-stimulation showed increased cytotoxicity against PATC148 cells. Arrow (↓) denotes the time when CBNK cells or SDS were added to the tumor cells. (B) To analyze the real-time cytotoxic activity of Trop2 CAR CBNK cells against cancer cells, an IncuCyte live-imaging cytotoxicity assay was performed. Trop2 CAR transduced CBNK cells and PATC148 cells were co-cultured at 1:1 ratio, and real-time cytotoxicity of CBNK cells against PATC148 cells measured every hour over a 60-hour period. Compared to NT NK cells, Trop2 CAR NK cells with either CD28 or DAP10 co-stimulation showed increased cytotoxicity against PATC148 cells.

[0041] FIGs. 13A-13B show results demonstrating that Trop2 CAR-transduced CBNK cells and Trop2 CAR-transduced T cells have superior cytotoxicity against ovarian cancer cells compared to their non-transduced counterparts. (A) CBNK cells were derived from cord blood and transduced with Trop2 CARs with either CD28 or DAP10 co-stimulation. SKOV3 cells, which have high Trop2 expression, were grown in 96 well RTCA E-Plates overnight and CBNK cells were added the next day at 2:1 effector to target (E:T) ratio. The cancer cell growth was measured continuously by the Xcelligence machine and represented as normalized cell index. Compared to NT CAR CBNK cells, Trop2 CAR NK cells with either CD28 or DAP10 co-stimulation showed increased cytotoxicity against SKOV3 cells. Arrow (↓) denotes the time when CBNK cells were added to the SKOV3 cells. (B) T cells were derived from the peripheral blood of healthy donors and transduced with the different Trop2 CARs. HEYA8 cells (a Trop2-expressing ovarian cancer cell line) were grown in 96 well RTCA E-Plates overnight and CAR T cells transduced with the different CAR constructs were added the next day at 1:1 effector to target (E:T) ratio. The cancer cell growth was measured continuously by the Xcelligence machine and represented as normalized cell index. Compared to NT T-cells, Trop2 CAR T cells showed increased cytotoxicity against HEYA8 cells. Arrow (↓) denotes the time when T cells were added to the HEYA8 cells.

[0042] FIGs. 14A-14C show results demonstrating that CBNK cells transduced with Trop2 CAR expressing CD28 or DAP10 costimulation showed effective anti-tumor activity and survival benefit against Trop2 positive ovarian cancer cells *in vivo*. NSG mice were engrafted with 0.5M firefly luciferase labelled SKOV3 (SKOV3 FFluc) which have high Trop2 expression. (A) Bioluminescence imaging of the tumor showing that CBNK cells transduced with Trop2 CARs with either CD28 or DAP10 costimulation were able to reduce tumor burden of Trop2-expressing SKOV3 cells compared to tumor alone group, NT or IL15 transduced

CBNK groups. (B) Graph plotting average radiance of BLI data comparing the different groups of mice shown in the panel 14A. (C) Survival curve showing the significant survival benefit of a single infusion of Trop2 CAR NK cells with either CD28 or DAP10 costimulation in NSG mice engrafted with SKOV3, compared to tumor alone group, NT or IL15 transduced CBNK groups.

[0043] FIG. 15 shows results demonstrating that TROP2 constructs derived from hRS7 or 2G10 antibody clones result in high transfection efficiency in 293T cells. 293T cells were transfected with various TROP2 constructs, as shown, using Fugene as a transfection reagent. The transfection efficiency was determined by checking the surface expression of chimeric antigen receptor (CAR) in 293T cells post virus collection using flow cytometry. Mean Fluorescence Intensity (MFI) corresponds to the transfection efficiency. Non transduced (NT) cells were used as negative control.

[0044] FIG. 16 shows results demonstrating that TROP2 constructs derived from hRS7 or 2G10 antibody clones result in high transduction efficiency in T cells. The retroviral supernatant collected from transfection experiments were used to transduce T cells isolated from peripheral blood (PB) and Retronectin was used to enhance transduction efficiency. Forty eight hours post transduction, the transduction efficiency was measured by checking the surface expression of TROP2 CAR in T cells using flow cytometry. Supernatant from non transduced (NT) cells were used as negative control. PB1 (left) and PB2 (Right) are peripheral blood from two different donors.

[0045] FIG. 17 shows results demonstrating that 2G10 derived TROP2 CAR T cells have improved cytotoxicity against HEY8 ovarian cancer cells when compared to non-transduced T cells but have less cytotoxic activity compared to hRS7-TROP2 CAR T cells. T cells were derived from peripheral blood and were transduced with various Trop2 constructs to generate Trop2 CAR cells. Non-transduced (NT) T cells and T cells transduced with hRS7-TROP2 CAR T cells (hRS7-TROP2-DAP10 and hRS7-TROP2-CD28) were used as controls. HEY8 cells were grown in 96 well RTCA E-Plates for overnight and various Trop2 CAR T cells were added the next day at 1:1 effector to target (E:T) ratio. The cytotoxicity was measured continuously by the Xcelligence machine and represented as normalized cell index. Compared to NT T cells, 2G10 derived TROP2 CAR T cells could kill HEY8 ovarian cancer but hRS7-TROP2 CAR T cells showed superior cytotoxicity, indicating that the cytotoxic activity of 2G10 derived Trop2 CAR T cells against HEY8 is lower than that of hRS7-TROP2 CAR T cells. Experiments were performed with CAR T cells generated from one donor.

[0046] FIGs. 18A-18B show results demonstrating that 2G10 derived TROP2 CAR T cells have improved cytotoxicity against SW480 cancer cells when compared to non-transduced T cells but are less cytotoxic than hRS7-TROP2 CAR T cells. T cells were derived from peripheral blood and were transduced with various Trop2 constructs to generate Trop2 CAR cells. Non-transduced (NT) T cells and T cells transduced with hRS7-TROP2 CAR T cells (hRS7-TROP2-DAP10 and hRS7-TROP2-CD28) were used as controls. SW480 cells were grown in 96 well RTCA E-Plates overnight and various Trop2 CAR T cells were added the next day at 1:1 effector to target (E:T) ratio. The cytotoxicity was measured continuously by the Xcelligence machine and represented as normalized cell index. Compared to NT T cells, 2G10 derived and hRS7-TROP2 CAR T cells showed improved cytotoxicity against SW480 cancer cells. Experiments were performed with CAR T cells generated from one donor.

[0047] FIGs. 19A-19B show results demonstrating that 2G10 derived TROP2 CAR T cells and hRS7-TROP2 CAR T cells show comparable cytotoxicity against PATC148 PDAC cancer cells. T cells were derived from peripheral blood and were transduced with various Trop2 constructs to generate Trop2 CAR cells. Non-transduced (NT) T cells and T cells transduced with hRS7-TROP2 CAR T cells (hRS7-TROP2-DAP10 and hRS7-TROP2-CD28) were used as controls. PATC148 cells were grown in 96 well RTCA E-Plates for overnight and various Trop2 CAR T cells were added the next day at 1:1 effector to target (E:T) ratio. The cytotoxicity was measured continuously by Xcelligence and represented as normalized cell index. 2G10 derived TROP2 CAR T cells had comparable cytotoxicity against PATC148 ovarian cancer cells to hRS7-TROP2 CAR T cells indicating that both 2G10 derived TROP2 CAR T cells and hRS7-TROP2 CAR T cells have superior cytotoxic activity against PATC148 compared to NT T cells. Experiments were performed with CAR T cells generated from two donors (FIG. 19A shows results from cells generated from the first donor, FIG. 19B shows results from cells generated from the second donor).

[0048] FIGs. 20A-20B show results demonstrating that 2G10 derived TROP2 CAR T cells have lower cytotoxicity against SKOV3 ovarian cancer cells when compared to hRS7-TROP2 CAR T cells. T cells were derived from peripheral blood and were transduced with various Trop2 CAR constructs to generate Trop2 CAR T cells. Non-transduced (NT) T cells and T cells transduced with hRS7-TROP2 CAR T cells (hRS7-TROP2-DAP10 and hRS7-TROP2-CD28) were used as controls. SKOV3 cells were grown in 96 well RTCA E-Plates overnight and various Trop2 CAR T cells were added the next day at 1:1 effector to target (E:T) ratio. The cytotoxicity was measured continuously by the Xcelligence machine and represented as normalized cell index. 2G10 derived TROP2 CAR T cells and NT-T cells failed to kill SKOV3

cells whereas hRS7-TROP2 CAR T could effectively kill SKOV3 cells. Experiments were performed with CAR T cells generated from two donors (FIG. 20A shows results from cells generated from the first donor, FIG. 20B shows results from cells generated from the second donor).

[0049] FIGs. 21A-21B show results demonstrating that 2G10 derived TROP2 CAR T cells show comparable cytotoxicity against OVCAR5 ovarian cancer cells when compared to hRS7-TROP2 CAR T cells. T cells were derived from peripheral blood and were transduced with various Trop2 CAR constructs to generate Trop2 CAR T cells. Non-transduced (NT) T cells and T cells transduced with hRS7-TROP2 CAR T cells (hRS7-TROP2-DAP10 and hRS7-TROP2-CD28) were used as controls. OVCAR5 cells were grown in 96 well RTCA E-Plates for overnight and various Trop2 CAR T cells were added the next day at 1:1 effector to target (E:T) ratio. The cytotoxicity was measured continuously by the Xcelligence machine and represented as normalized cell index. 2G10 derived TROP2 CAR T cells and hRS7-TROP2 CAR T cells had comparable cytotoxicity against OVCAR mRS7 or hRS7, CD28 hinge and transmembrane domain, CD28 co-stimulatory domain, CD3zeta intracellular domain, and 5 ovarian cancer cells, indicating that both 2G10 derived TROP2 CAR T cells and hRS7-TROP2 CAR T cells can efficiently kill OVCAR5 cells. Experiments were performed with CAR T cells generated from two donors.

DETAILED DESCRIPTION

I. Examples of Definitions

[0050] In keeping with long-standing patent law convention, the words “a” and “an” when used in the present specification in concert with the word comprising, including the claims, denote “one or more.” Some embodiments of the disclosure may consist of or consist essentially of one or more elements, method steps, and/or methods of the disclosure. It is contemplated that any method or composition described herein can be implemented with respect to any other method or composition described herein and that different embodiments may be combined.

[0051] Throughout this specification, unless the context requires otherwise, the words “comprise”, “comprises” and “comprising” will be understood to imply the inclusion of a stated step or element or group of steps or elements but not the exclusion of any other step or element or group of steps or elements. By “consisting of” is meant including, and limited to, whatever follows the phrase “consisting of.” Thus, the phrase “consisting of” indicates that the listed

elements are required or mandatory, and that no other elements may be present. By “consisting essentially of” is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase “consisting essentially of” indicates that the listed elements are required or mandatory, but that no other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0052] Reference throughout this specification to “one embodiment,” “an embodiment,” “a particular embodiment,” “a related embodiment,” “a certain embodiment,” “an additional embodiment,” or “a further embodiment” or combinations thereof means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearances of the foregoing phrases in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more embodiments.

[0053] As used herein, the terms “or” and “and/or” are utilized to describe multiple components in combination or exclusive of one another. For example, “x, y, and/or z” can refer to “x” alone, “y” alone, “z” alone, “x, y, and z,” “(x and y) or z,” “x or (y and z),” or “x or y or z.” It is specifically contemplated that x, y, or z may be specifically excluded from an embodiment.

[0054] Throughout this application, the term “about” is used according to its plain and ordinary meaning in the area of cell and molecular biology to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

[0055] The term “engineered” as used herein refers to an entity that is generated by the hand of man, including a cell, nucleic acid, polypeptide, vector, and so forth. In at least some cases, an engineered entity is synthetic and comprises elements that are not naturally present or configured in the manner in which it is utilized in the disclosure.

[0056] The term “isolated” as used herein refers to molecules or biologicals or cellular materials being substantially free from other materials. In one aspect, the term “isolated” refers to nucleic acid, such as DNA or RNA, or protein or polypeptide, or cell or cellular organelle, or tissue or organ, separated from other DNAs or RNAs, or proteins or polypeptides, or cells or cellular organelles, or tissues or organs, respectively, such as that are present in the natural source. The term “isolated” also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA

techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an "isolated nucleic acid" is meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term "isolated" is also used herein to refer to polypeptides that are isolated from other cellular proteins and is meant to encompass both purified and recombinant polypeptides. The term "isolated" is also used herein to refer to cells or tissues that are isolated from other cells or tissues and is meant to encompass both cultured and engineered cells or tissues.

[0057] As used herein, "prevent," and similar words such as "prevented," "preventing" *etc.*, indicate an approach for preventing, inhibiting, or reducing the likelihood of the occurrence or recurrence of, a disease or condition, *e.g.*, cancer. It also refers to delaying the onset or recurrence of a disease or condition or delaying the occurrence or recurrence of the symptoms of a disease or condition. As used herein, "prevention" and similar words also includes reducing the intensity, effect, symptoms and/or burden of a disease or condition prior to onset or recurrence of the disease or condition.

[0058] The term "sample," as used herein, generally refers to a biological sample. The sample may be taken from tissue or cells from an individual. In some examples, the sample may comprise, or be derived from, a tissue biopsy, blood (*e.g.*, whole blood), blood plasma, extracellular fluid, dried blood spots, cultured cells, discarded tissue. The sample may have been isolated from the source prior to collection. Non-limiting examples include blood, cerebral spinal fluid, pleural fluid, amniotic fluid, lymph fluid, saliva, urine, stool, tears, sweat, or mucosal excretions, and other bodily fluids isolated from the primary source prior to collection. In some examples, the sample is isolated from its primary source (cells, tissue, bodily fluids such as blood, environmental samples, *etc.*) during sample preparation. The sample may or may not be purified or otherwise enriched from its primary source. In some cases the primary source is homogenized prior to further processing. The sample may be filtered or centrifuged to remove buffy coat, lipids, or particulate matter. The sample may also be purified or enriched for nucleic acids, or may be treated with RNases. The sample may contain tissues or cells that are intact, fragmented, or partially degraded.

[0059] The term "subject," as used herein, generally refers to an individual having a biological sample that is undergoing processing or analysis and, in specific cases, has or is suspected of having cancer. The subject can be any organism or animal subject that is an object of a method or material, including mammals, *e.g.*, humans, laboratory animals (*e.g.*, primates, rats, mice, rabbits), livestock (*e.g.*, cows, sheep, goats, pigs, turkeys, and chickens), household pets (*e.g.*, dogs, cats, and rodents), horses, and transgenic non-human animals. The subject can

be a patient, *e.g.*, have or be suspected of having a disease (that may be referred to as a medical condition), such as benign or malignant neoplasias, or cancer. The subject may be undergoing or having undergone treatment. The subject may be asymptomatic. The subject may be healthy individuals but that are desirous of prevention of cancer. The term “individual” may be used interchangeably, in at least some cases. The “subject” or “individual”, as used herein, may or may not be housed in a medical facility and may be treated as an outpatient of a medical facility. The individual may be receiving one or more medical compositions *via* the internet. An individual may comprise any age of a human or non-human animal and therefore includes both adult and juveniles (*i.e.*, children) and infants and includes *in utero* individuals. It is not intended that the term connote a need for medical treatment, therefore, an individual may voluntarily or involuntarily be part of experimentation whether clinical or in support of basic science studies.

[0060] As used herein “treatment” or “treating,” includes any beneficial or desirable effect on the symptoms or pathology of a disease or pathological condition, and may include even minimal reductions in one or more measurable markers of the disease or condition being treated, *e.g.*, cancer. Treatment can involve optionally either the reduction or amelioration of symptoms of the disease or condition, or the delaying of the progression of the disease or condition. “Treatment” does not necessarily indicate complete eradication or cure of the disease or condition, or associated symptoms thereof.

[0061] Any method in the context of a therapeutic, diagnostic, or physiologic purpose or effect may also be described in “use” claim language such as “Use of” any compound, composition, or agent discussed herein for achieving or implementing a described therapeutic, diagnostic, or physiologic purpose or effect.

[0062] The present disclosure concerns methods and compositions directed to therapies for TROP-2-positive cancers, particularly utilizing adoptive cell therapy that targets TROP-2-positive cancer cells. In particular embodiments, genetically engineered mammalian immune cells of any kind (including at least human NK cells) are generated to target TROP-2-positive cancers. The disclosure encompasses a genetically engineered receptor of any kind (including a chimeric antigen receptor (CAR)) that is directed against TROP-2. In specific embodiments there are provided a number of novel expression constructs, including retroviral constructs, that express a TROP-2-targeting extracellular domain (including an antigen-binding domain or portion thereof from an anti-TROP-2 antibody such as mRS7 or hRS7) used in a CAR and, in some cases, that also express one or more cytokines, such as IL-15, and/or one or more suicide

gene products, such as Caspase 9. In some embodiments, the CAR is a fusion of an scFV from an RS7 antibody and one or more additional domains (e.g., transmembrane domain, intracellular domain).

II. Genetically Engineered Receptors

[0063] The immune cells of the present disclosure can be genetically engineered to express one or more antigen-binding receptors that target TROP-2, such as engineered CARs or, alternatively, engineered TCRs. For example, the immune cells may be immune cells that are modified to express a CAR and/or TCR having antigenic specificity for TROP-2. Other CARs and/or TCRs may be expressed by the same cells as the TROP-2 antigen receptor-expressing cells, and they may be directed to different antigens. In some aspects, the immune cells are engineered to express the TROP-2-specific CAR or TROP-2-specific TCR by knock-in of the CAR or TCR using CRISPR/Cas technology.

[0064] Suitable methods of modification of cells are known in the art. See, for instance, Sambrook and Ausubel, *supra*. For example, the cells may be transduced to express a CAR or TCR having antigenic specificity for a cancer antigen using transduction techniques described in Heemskerk *et al.*, 2008 and Johnson *et al.*, 2009.

[0065] In some embodiments, the cells comprise one or more nucleic acids introduced *via* genetic engineering that encode one or more antigen-targeting receptors (at least one of which is directed against TROP-2), and genetically engineered products of such nucleic acids. In some embodiments, the nucleic acids are heterologous, *i.e.*, normally not present in a cell or sample obtained from the cell, such as one obtained from another organism or cell, which for example, is not ordinarily found in the cell being engineered and/or an organism from which such cell is derived. In some embodiments, the nucleic acids are not naturally occurring, such as a nucleic acid not found in nature (*e.g.*, chimeric).

[0066] Exemplary antigen receptors, including CARs and recombinant TCRs, as well as methods for engineering and introducing the receptors into cells, include those described, for example, in international patent application publication numbers WO200014257, WO2013126726, WO2012/129514, WO2014031687, WO2013/166321, WO2013/071154, WO2013/123061 U.S. patent application publication numbers US2002131960, US2013287748, US20130149337, U.S. Patent Nos.: 6,451,995, 7,446,190, 8,252,592, 8,339,645, 8,398,282, 7,446,179, 6,410,319, 7,070,995, 7,265,209, 7,354,762, 7,446,191, 8,324,353, and 8,479,118, and European patent application number EP2537416, and/or those

described by Sadelain *et al.*, 2013; Davila *et al.*, 2013; Turtle *et al.*, 2012; Wu *et al.*, 2012. In some aspects, the genetically engineered antigen receptors include a CAR as described in U.S. Patent No.: 7,446,190, and those described in International Patent Application Publication No.: WO/2014055668 A1.

A. Chimeric Antigen Receptors

[0067] In particular embodiments, a TROP-2-specific CAR is utilized that comprises at least: a) one or more intracellular signaling domains, b) a transmembrane domain, and c) an extracellular domain comprising at least one antigen binding region that targets, including specifically binds, TROP-2. In some embodiments the antigen binding region is an antibody or functional fragment thereof. In other cases the antigen binding region of the CAR is not an antibody or functional fragment thereof (such as a ligand for TROP-2). In some embodiments, the antigen binding region comprises a portion of the murine RS7 antibody. In some embodiments, the antigen binding region is an scFv of the murine RS7 antibody. In some embodiments, the antigen binding region comprises a portion of a humanized RS7 antibody. In some embodiments, the antigen binding region is an scFv of the humanized RS7 antibody. As used herein, an “RS7 antibody” includes a murine RS7 antibody (mRS7) and a humanized RS7 antibody (hRS7); such antibodies are described in, for example, Stein *et al.*, Cancer Res. 50: 1330 (1990) and U.S. Patent 8,574,575, each incorporated herein by reference in their entirety.

[0068] In some embodiments, the antigen binding region comprises a portion of the murine 2G10 antibody. In some embodiments, the antigen binding region is an scFv of the murine 2G10 antibody. In some embodiments, the antigen binding region comprises a portion of a humanized 2G10 antibody. In some embodiments, the antigen binding region is an scFv of the humanized 2G10 antibody. As used herein, a “2G10 antibody” includes a murine 2G10 antibody (m2G10) and a humanized 2G10 antibody (h2G10; also “Hu2G10” or “Hu-2G10”); such antibodies are described in, for example, U.S. Patent 10,501,555, incorporated herein by reference in its entirety.

[0069] In some embodiments, the antigen binding region comprises a portion of the murine 2EF antibody. In some embodiments, the antigen binding region is an scFv of the murine 2EF antibody. In some embodiments, the antigen binding region comprises a portion of a humanized 2EF antibody. In some embodiments, the antigen binding region is an scFv of the humanized 2EF antibody. As used herein, a “2EF antibody” includes a murine 2EF antibody

(m2EF) and a humanized 2EF antibody (h2EF; also “Hu2EF” or “Hu-2EF”); such antibodies are described in, for example, U.S. Patent 10,501,555, incorporated herein by reference in its entirety.

[0070] In some embodiments, the TROP-2-specific CAR binds only TROP-2, whereas in other cases the CAR as a single polypeptide is bispecific by comprising two or more antigen binding domains, one of which binds TROP-2 and the other of which binds another, non-identical antigen.

[0071] In some embodiments, the engineered antigen receptors include CARs, including activating or stimulatory CARs, or costimulatory CARs (see WO2014/055668). The CARs generally include an extracellular antigen (or ligand) binding domain linked to one or more intracellular signaling components, in some aspects *via* linkers and/or transmembrane domain(s). Such molecules typically mimic or approximate a signal through a natural antigen receptor, a signal through such a receptor in combination with a costimulatory receptor, and/or a signal through a costimulatory receptor alone.

[0072] It is contemplated that the chimeric construct can be introduced into immune cells as naked DNA or in a suitable vector. Methods of stably transfecting cells by electroporation using naked DNA are known in the art. See, *e.g.*, U.S. Patent No. 6,410,319. Naked DNA generally refers to the DNA encoding a chimeric receptor contained in a plasmid expression vector in proper orientation for expression.

[0073] Alternatively, a viral vector (*e.g.*, a retroviral vector, adenoviral vector, adeno-associated viral vector, or lentiviral vector) can be used to introduce the chimeric CAR construct into immune cells. Suitable vectors for use in accordance with the method of the present disclosure are non-replicating in the immune cells. A large number of vectors are known that are based on viruses, where the copy number of the virus maintained in the cell is low enough to maintain the viability of the cell, such as, for example, vectors based on HIV, SV40, EBV, HSV, or BPV.

[0074] Certain embodiments of the present disclosure concern the use of nucleic acids, including nucleic acids encoding a TROP-2-specific CAR polypeptide, including in some cases a CAR that has been humanized to reduce immunogenicity (hCAR), comprising at least one intracellular signaling domain, a transmembrane domain, and an extracellular domain comprising one or more signaling motifs. In certain embodiments, the TROP-2-specific CAR may recognize an epitope comprising the shared space between one or more antigens. In certain embodiments, the binding region can comprise complementary determining regions of a monoclonal antibody, variable regions of a monoclonal antibody, and/or antigen binding

fragments thereof. In another embodiment, that specificity is derived from a peptide (*e.g.*, cytokine) that binds to a receptor.

[0075] It is contemplated that the human TROP-2 CAR nucleic acids may be human genes used to enhance cellular immunotherapy for human patients. In a specific embodiment, the disclosure includes a full-length TROP-2-specific CAR cDNA or coding region. The antigen binding regions or domain can comprise a fragment of the V_H and V_L chains of a single-chain variable fragment (scFv) derived from a particular human monoclonal antibody, such as those described in U.S. Patent 7,109,304, incorporated herein by reference. The fragment can also be any number of different antigen binding domains of a human antigen-specific antibody. In a more specific embodiment, the fragment is a TROP-2-specific scFv encoded by a sequence that is optimized for human codon usage for expression in human cells.

[0076] The arrangement could be multimeric, such as a diabody or multimers. The multimers are most likely formed by cross pairing of the variable portion of the light and heavy chains into a diabody. The hinge portion of the construct can have multiple alternatives from being totally deleted, to having the first cysteine maintained, to a proline rather than a serine substitution, to being truncated up to the first cysteine. The Fc portion can be deleted. Any protein that is stable and/or dimerizes can serve this purpose. One could use just one of the Fc domains, *e.g.*, either the CH2 or CH3 domain from human immunoglobulin. One could also use the hinge, CH2 and CH3 region of a human immunoglobulin that has been modified to improve dimerization. One could also use just the hinge portion of an immunoglobulin. One could also use portions of CD8alpha.

[0077] In some embodiments, TROP-2-specific CAR is constructed with specificity for TROP-2, such as TROP-2 being expressed on a diseased cell type. Thus, the CAR typically includes in its extracellular portion one or more TROP-2-binding molecules, such as one or more antigen-binding fragments, domains, antibody variable domains, and/or antibody molecules of any kind. An example of a human TROP-2 nucleic acid is at National Center for Biotechnology Information's GenBank® database at Accession No. NM_002353. An example of a human TROP-2 polypeptide is at GenBank® Accession No. NP_002344. One of skill in the art is able to generate antibodies, including scFvs against TROP-2 based on knowledge at least of the polypeptide and routine practices, although numerous anti-TROP-2 scFvs and monoclonal antibodies are already present in the art. In some embodiments, the TROP-2-specific scFv is an scFV from one or more of antibody clones.

[0078] In some embodiments, the TROP-2-specific CAR includes an antigen-binding portion or portions of an antibody molecule, such as a single-chain antibody fragment (scFv)

derived from the variable heavy (VH) and variable light (VL) chains of a monoclonal antibody (mAb). In specific embodiments, the antibody or functional fragment thereof is or is derived from an RS7 antibody (e.g., mRS7, hRS7), Pr1E11, TrMab-29, or datopotamab. In some embodiments, the antibody or functional fragment thereof is or is derived from a RS7 antibody (e.g., mRS7, hRS7). In some embodiments, the antibody or functional fragment thereof is or is derived from a 2G10 antibody (e.g., m2G10, h2G10). In some embodiments, the antibody or functional fragment thereof is or is derived from a 2EF antibody (e.g., m2EF, h2EF). The antibody may also be one that is generated *de novo* against TROP-2, and the scFv sequence may be obtained, or derived, from such *de novo* antibodies.

[0079] In certain embodiments, the anti-TROP-2 CAR comprises an extracellular domain that is or comprises a ligand for TROP-2. In some embodiments, the anti-TROP-2 CAR comprises an extracellular domain that is or comprises a VH and/or VL from an anti-TROP-2 antibody. In specific embodiments, the anti-TROP-2 CAR comprises a VH and VL from an RS7 antibody. In some embodiments, the anti-TROP-2 CAR comprises an extracellular domain comprising SEQ ID NO:9. In some embodiments, the anti-TROP-2 CAR comprises an extracellular domain comprising SEQ ID NO:14. In some embodiments, the anti-TROP-2 CAR comprises an extracellular domain comprising SEQ ID NO:9 and SEQ ID NO:14. In some embodiments, the anti-TROP-2 CAR comprises an extracellular domain comprising SEQ ID NO:10. In some embodiments, the anti-TROP-2 CAR comprises an extracellular domain comprising SEQ ID NO:15. In some embodiments, the anti-TROP-2 CAR comprises an extracellular domain comprising SEQ ID NO:10 and SEQ ID NO:15. In some embodiments, the anti-TROP-2 CAR comprises a VH and/or VL from a 2G10 antibody. In some embodiments, the anti-TROP-2 CAR comprises a VH and/or VL from a 2EF antibody.

[0080] The sequence of the open reading frame encoding the chimeric receptor can be obtained from a genomic DNA source, a cDNA source, or can be synthesized (*e.g., via PCR*), or combinations thereof. Depending upon the size of the genomic DNA and the number of introns, it may be desirable to use cDNA or a combination thereof, as it is found that introns stabilize the mRNA. Also, it may be further advantageous to use endogenous or exogenous non-coding regions to stabilize the mRNA.

[0081] In some aspects, the antigen-specific binding, or recognition component is linked to one or more transmembrane and intracellular signaling domains. In some embodiments, the CAR includes a transmembrane domain fused to the extracellular domain of the CAR. In one embodiment, the transmembrane domain that naturally is associated with one of the domains in the CAR is used. In some instances, the transmembrane domain is selected or modified by

amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins to minimize interactions with other members of the receptor complex. The transmembrane domain in some embodiments is derived either from a natural or from a synthetic source. Where the source is natural, the domain in some aspects is derived from any membrane-bound or transmembrane protein. Transmembrane regions include those derived from (*i.e.* comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T- cell receptor, CD28, DAP12, DAP10, NKG2D, CD3 zeta, CD3 epsilon, CD3 gamma, CD3 delta, CD45, CD4, CD5, CD8, CD9, CD 16, CD22, CD33, CD37, CD64, CD80, CD86, CD 134, CD137, CD154, ICOS/CD278, GITR/CD357, and so forth. Alternatively the transmembrane domain in some embodiments is synthetic. In some aspects, the synthetic transmembrane domain comprises predominantly hydrophobic residues such as leucine and valine. In some aspects, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain.

[0082] In some embodiments, the TROP-2 CAR nucleic acid comprises a sequence encoding other costimulatory receptors, such as a transmembrane domain and one or more intracellular signaling domains. In addition to a primary T cell activation signal, such as may be initiated by CD3 ζ and/or Fc ϵ RI γ , an additional stimulatory signal for immune effector cell proliferation and effector function following engagement of the chimeric receptor with the target antigen may be utilized. For example, part or all of a human costimulatory receptor for enhanced activation of cells may be utilized that could help improve *in vivo* persistence and improve the therapeutic success of the adoptive immunotherapy. Examples include costimulatory domains from molecules such as DAP12, DAP10, NKG2D, CD2, CD28, CD27, 4-1BB, (CD137), OX40, ICOS, (CD278), CD30, HVEM, CD40, LFA-1 (CD11a/CD18), and/or ICAM-1, although in specific alternative embodiments any one of these listed may be excluded from use in the CAR.

[0083] In certain embodiments, the platform technologies disclosed herein to genetically modify immune cells, such as NK cells, comprise (i) non-viral gene transfer using an electroporation device (*e.g.*, a nucleofector), (ii) CARs that signal through endodomains (*e.g.*, CD28/CD3- ζ , CD137/CD3- ζ , or other combinations), (iii) CARs with variable lengths of extracellular domains connecting the TROP-2-recognition domain to the cell surface, and, in some cases, (iv) artificial antigen presenting cells (aAPC) derived from K562 to be able to robustly and numerically expand CAR⁺ immune cells (Singh *et al.*, 2008; Singh *et al.*, 2011).

B. Examples of Specific CAR Embodiments

[0084] In particular embodiments, specific TROP-2 CAR molecules are encompassed herein. In some cases, the TROP-2 binding domain of the CAR is a scFv, and any scFv that binds to TROP-2 may be utilized herein. In some cases, the TROP-2 binding domain of the CAR is a binding domain from a ligand of TROP-2, and any domain that binds TROP-2 may be utilized herein. In cases wherein an anti-TROP-2 scFv is utilized in the extracellular domain of the CAR, the variable heavy chain and the variable light chain for the scFv may be in any order in N-terminal to C-terminal direction. For example, the variable heavy chain may be on the N-terminal side of the variable light chain, or vice versa. The scFv and/or ligand that binds TROP-2 in the CAR may or may not be codon optimized. In particular embodiments, a vector encodes a TROP-2-specific CAR and also encodes one or more other molecules. For example, a vector may encode a TROP-2-specific CAR and also may encode another protein of interest, such as another engineered antigen receptor, a suicide gene, and/or a particular cytokine.

[0085] On the same molecule, the TROP-2-specific CAR may comprise one or more antigen-specific extracellular domains, a specific hinge, a specific transmembrane domain, one or more specific costimulatory domains, and one or more specific activation signals. When more than one antigen-specific extracellular domain is utilized, such as for targeting two different antigens (one of which is TROP-2), there may be a linker between the two antigen-specific extracellular domains.

[0086] In particular embodiments of specific CAR molecules, a CAR may utilize DAP10, DAP12, 4-1BB, NKG2D, or other costimulatory domains (which may be referred to herein as an intracytoplasmic domain). In some cases, CD3zeta is utilized without any costimulatory domains. In particular embodiments of specific CAR molecules, a CAR may utilize any suitable transmembrane domain, such as from DAP12, DAP10, 4-1BB, 2B4, OX40, CD27, NKG2D, CD8, or CD28.

[0087] In particular embodiments, there is an expression construct comprising sequence that encodes a particular TROP-2-specific engineered receptor.

[0088] Examples of specific sequence embodiments are provided below.

1. Antigen-specific extracellular domains

[0089] Examples of specific sequence embodiments are provided below.

[0090] In specific embodiments, an anti-TROP-2 extracellular domain nucleotide sequence is utilized, as follows:

GACATCCAGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAG
ACAGAGTCAGCATCACCTGCAAGGCCAGTCAGGATGTGAGTATTGCTGTAGCCT
GGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCTGATCTACTCGGCATCCT
ACCGGTACACTGGAGTCCCTGATAGGTTCAAGTGGCAGTGGATCTGGGACAGATTT
CACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAGTTTATTACTGTCAG
CAACATTATATTACTCCGCTCACGTTCCGGTGCTGGGACCAAGGTGGAGATCAAAC
GTTTGGAATAAAGGGCTCTACAAGCGGCTCAGGAAAACCTGGATCAGGCGAAG
GGTCTACGCAGGTCCAACCTGCAGCAATCTGGGTCTGAGTTGAAGAAGCCTGGGG
CCTCAGTGAAGGTTTCCTGCAAGGCTTCTGGATACACCTTCACAACTATGGAAT
GAACTGGGTGAAGCAGGCCCCCTGGACAAGGGCTTAAATGGATGGGCTGGATAAA
CACCTACACTGGAGAGCCAACATATACTGATGACTTCAAGGGACGTTTTGCCTTC
TCCTTGACACCTCTGTCAGCACGGCATATCTCCAGATCAGCAGCCTAAAGGCTG
ACGACACTGCCGTGTATTTCTGTGCAAGAGGGGGGTTCCGGTAGTAGCTACTGGTA
CTTCGATGTCTGGGGCCAAGGGTCCCTGGTCACCGTCTCCTCA (SEQ ID NO:43)

ATGGAGTTCGGCTTGAGTTGGTTGTTTCCTTGTGGCGATACTCAAAGGC
GTTCAATGTCAAGTGCAGCTCGTCCAGTCTGGAGCTGAAGTCAAAAAGCCTGGG
GCTTCAGTGAAAGTCTCCTGCAAGGCTTCTGGCTTCACCTTCAGCAGTAGCTATA
TCAGTTGGTTGAGGCAGGCCCCCTGGACAGAGACTTGAGTGGATTGCATGGATTTA
TGCTGGAAGTGGCGGAACTAGCTATAATCAGAAGTTCACAGGCAAGGCCACACT
GACTGTAGACACATCCGCCAGCACAGCCTACATGGAAGTTCAGCAGCCTGAGATC
TGAGGACACTGCCGTCTATTACTGTGCAAGACATAACCCTCGTTACTATGCTATG
GACTACTGGGGCCAAGGAACCACAGTCACCGTCTCCTCATTGGAAATAAAGGGC
TCTACAAGCGGCTCAGGAAAACCTGGATCAGGCGAAGGGTCTACGGACACCCAG
ATGACCCAGTCTCCAAGCTCCCTGTCCGCCAGCGTGGGAGATAGAGTCAACATCA
CATGCATCACCAGCACTGATATTGATGATGATATGAACTGGTACCAGCAGAAGC
CAGGGAAAGCTCCTAAGCTCCTGATTTTCAAGGCAATACTCTGCGCCCTGGAGT
CCCATCCCGATTCTCCGGCAGTGGCTATGGAACAGATTTTACCTTTACAATTAGC
TCCCTGCAGCCAGAAGATATTGCAACCTACTACTGTTTGCAAAGTGATAACCTGC
CCTACACCTTCGGAGGGGGGACCAAAGTCGAAATCAA (SEQ ID NO:45)

ATGGAGTTCGGCTTGAGTTGGTTGTTTCCTTGTGGCGATACTCAAAGGC
GTTCAATGTCAAGTGCAGCTCGTCCAGTCTGGAGCTGAAGTCAAAAAGCCTGGG
GCTTCAGTGAAAGTCTCCTGCAAGGCTTCTGGCTTCACCTTCAGCAGTAGCTATA

TCAGTTGGTTGAGGCAGGCCCTGGACAGAGACTTGAGTGGATTGCATGGATTTA
TGCTGGAAGTGGCGGAAGTACTAGCTATAATCAGAAAGTTCACAGGCAGAGTCACACT
GACTGTAGACACATCCGCCAGCACAGCCTACATGGAACTCAGCAGCCTGAGATC
TGAGGACACTGCCGTCTATTACTGTGCAAGACATAACCCTCGTTACTATGCTATG
GACTACTGGGGCCAAGGAACACAGTCACCGTCTCCTCATTGGAAATAAAGGGC
TCTACAAGCGGCTCAGGAAAACCTGGATCAGGCGAAGGGTCTACGGACACCCAG
ATGACCCAGTCTCCAAGCTCCCTGTCCGCCAGCGTGGGAGATAGAGTCACCATCA
CATGCATCACCAGCACTGATATTGATGATGATATGAACTGGTACCAGCAGAAGC
CAGGGAAAGCTCCTAAGCTCCTGATTTTCAAGGCAATACTCTGCGCCCTGGAGT
CCCATCCCGATTCTCCGGCAGTGGCTATGGAACAGATTTTACCTTTACAATTAGC
TCCCTGCAGCCAGAAGATATTGCAACCTACTACTGTTTGCAAAGTGATAACCTGC
CCTACACCTTCGGAGGGGGGACCAAAGTCGAAATCAAA (SEQ ID NO:47)

ATGGAGTTCGGCTTGAGTTGGTTGTTTCCTTGTGGCGATACTCAAAGGC
GTTCAATGTCAAGTCCAGCTCGTGCAGTCTGGAGCTGAAGTGAAGAAACCTGGG
GCTTCAGTGAAGGTGTCTTGCAAGGCTTCTGGATACACCTTCACTAACTACTGGA
TCGGATGGGTCAAACAGGCCCTGGACAGGGCCTCGAGTGGATTGGAGATATTT
ACCCTGGAGGAGGCTATACTAACTACAATGAGAAGTTCAAGGGCAGAGCCACAC
TGAAGTGCAGACACATCCGCCAGCACAGCCTACATGGAGCTCAGCAGCCTGAGAT
CTGAGGACACTGCCGTGTATTACTGTGCAAGAGGAACTGGAGGCGGAGACTACT
GGGGCCAAGGGACTCTGGTCACTGTCTCTTTCATTGGAAATAAAGGGCTCTACAAG
CGGCTCAGGAAAACCTGGATCAGGCGAAGGGTCTACGGACATTGTGCTGACACA
GTCTCCTGACTCCCTGGCTGTGTCTCTGGGGGAGAGGGGCCACCATCAACTGCAGG
GCCAGCCAAAGTGTCAGTACATCTAGCTATAGTTATATGCACTGGTACCAACAGA
AACCAGGACAGCCACCCAACTCCTCATCAAGTATGCATCCAACCTGGAATCTG
GGGTCCCTGACAGATTCAGTGGCAGTGGGTCTGGGACAGACTTCACCCTCACCAT
CAGCTCCCTGCAGGCCGAGGATGTGGCAGTCTATTACTGTCAGCACAGTTGGGAG
ATTCCCTACACCTTCGGAGGCGGGACCAAAGCTGGAAATCAAA (SEQ ID NO:49)

[0091] Any polynucleotide encompassed by the present disclosure may comprise SEQ ID NO:43, 45, 47, or 49 or a sequence that is at least 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or more % identical to SEQ ID NO:43, 45, 47, or 49.

[0092] Example anti-TROP-2 extracellular domain amino acid sequences are as follows:

DIQLTQSPSSLSASVGDRVSITCKASQDVSIABAWYQQKPGKAPKLLIYSA
 SYRYTGVPDRFSGSGSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRLE
 IKGSTSGSGKPGSGEGSTQVQLQQSGSELKKPGASVKVSKASGYTFTNYGMNWVK
 QAPGQGLKWMGWINTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVY
 FCARGGFGSSYWFVDVWGQGS�TVSS (SEQ ID NO:19)

MEFGLSWLFLVAILKGVQCQVQLVQSGAEVKKPGASVKVSKASGFTFSS
 SYISWLRQAPGQRLEWIAWIYAGTGGTSYNQKFTGKATLTVDTASTAYMELSSLRS
 EDTAVYYCARHNPRYYAMDYWGQGTITVTVSSLEIKGSTSGSGKPGSGEGSTDTQMT
 QSPSSLSASVGDRVTITCITSTDIDDDMNWYQQKPGKAPKLLISEGNTLRPGVPSRFSG
 SGYGTDFTFITISSLQPEDATYYCLQSDNLPYTFGGGKVEIKR (SEQ ID NO:44)

MEFGLSWLFLVAILKGVQCQVQLVQSGAEVKKPGASVKVSKASGFTFSS
 SYISWLRQAPGQRLEWIAWIYAGTGGTSYNQKFTGRVTLTVDTASTAYMELSSLRS
 EDTAVYYCARHNPRYYAMDYWGQGTITVTVSSLEIKGSTSGSGKPGSGEGSTDTQMT
 QSPSSLSASVGDRVTITCITSTDIDDDMNWYQQKPGKAPKLLISEGNTLRPGVPSRFSG
 SGYGTDFTFITISSLQPEDATYYCLQSDNLPYTFGGGKVEI (SEQ ID NO:46)

PMEFGLSWLFLVAILKGVQCQVQLVQSGAEVKKPGASVKVSKASGYTF
 TNYWIGWVKQAPGQGLEWIGDIYPGGGYTNYNEKFKGRATLTADTASTAYMELSS
 LRSEDTAVYYCARGTGGGDYWGQGTITVTVSSLEIKGSTSGSGKPGSGEGSTDIVLTQ
 SPDSLAVSLGERATINCRASQSVSTSSYSYMHWYQQKPGQPPKLLIKYASNLESGVP
 DRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSWEIPYTFGGGKLEIK (SEQ ID NO:48)

[0093] Any polypeptide encompassed by the present disclosure may comprise SEQ ID NO:19, 44, 46, or 48 or a sequence that is at least 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or more % identical to SEQ ID NO:19, 44, 46, or 48.

[0094] In specific examples, the region of an anti-TROP-2 antibody (e.g., RS7, 2G10, 2EF) that is utilized in the CAR molecule comprises, consists of, or consists essentially of amino acids 1-50, 1-51, 1-52, 1-53, 1-54, 1-55, 1-56, 1-57, 1-58, 1-59, 1-60, 1-61, 1-62, 1-63, 1-64, 1-65, 1-66, 1-67, 1-68, 1-69, 1-70, 1-71, 1-72, 1-73, 1-74, 1-75, 1-76, 1-77, 1-78, 1-79, 1-80, 1-81, 1-82, 1-83, 1-84, 1-85, 1-86, 1-87, 1-88, 1-89, 1-90, 1-91, 1-92, 1-93, 1-94, 1-95, 1-96, 1-97, 1-98, 1-99, 1-100, 1-101, 1-102, 1-103, 1-104, 1-105, 1-106, 1-107, 1-108, 1-109, 1-110, 1-111, 1-112, 1-113, 1-114, 1-115, 1-116, 1-117, 1-118, 1-119, 1-120, 1-121, 1-122, 1-123, 1-124, 1-125, 1-126, 1-127, 1-128, 1-129, 1-130, 1-131, 1-132, 1-133, 1-134, 1-135, 1-136, 1-

137, 1-138, 1-139, 1-140, 1-141, 1-142, 1-143, 1-144, 1-145, 1-146, 1-147, 1-148, 1-149, 1-150, 1-151, 1-152, 1-153, 1-154, 1-155, 1-156, 1-157, 1-158, 1-159, 1-160, 1-161, 1-162, 1-163, 1-164, 1-165, 1-166, 1-167, 1-168, 1-169, 1-170, 1-171, 1-172, 1-173, 1-174, 1-175, 1-176, 1-177, 1-178, 1-179, 1-180, 1-181, 1-182, 1-183, 1-184, 1-185, 1-186, 1-187, 1-188, 1-189, 1-190, 1-191, 1-192, 1-193, 1-194, 1-195, 1-196, 1-197, 1-198, 1-199, 1-200, 1-201, 1-202, 1-203, 1-204, 1-205, 1-206, 1-207, 1-208, 1-209, 1-210, 1-211, 1-212, 1-213, 1-214, 1-215, 1-216, 1-217, 1-218, 1-219, 1-220, or all of SEQ ID NO:9, 10, 14, 15, 19, 44, 46, and/or 48; in specific embodiments, such amino acids in these ranges are contiguous. In some embodiments, a region of SEQ ID NO:9, 10, 14, 15, 19, 44, 46, and/or 48 is utilized that has truncation at the N-terminus, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids from the N-terminus. In certain cases, there is truncation at that N-terminus of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more amino acids and there is truncation at the C-terminus.

2. Transmembrane Domains

[0095] Any suitable transmembrane domain may be utilized in a TROP-2-specific CAR of the disclosure. Examples include at least transmembrane domains from DAP10, DAP12, CD28, NKG2D, CD3 epsilon, CD4, CD5, CD8, CD9, CD16, CD22, CD28, CD33, CD37, CD45, CD64, CD80, CD86, CD134, CD137, or CD154, from a T-cell receptor alpha (α) or beta (β) chain, from a CD3 zeta (ζ) chain, from ICOS, functional derivatives thereof, and combinations thereof. In specific cases, a transmembrane domain from DAP10, DAP12, CD28, CD8, or NKG2D is utilized. Examples of particular transmembrane domain sequences may be used, as follows:

[0096] CD27 transmembrane domain amino acid sequence:

[0097] ILVIFSGMFLVFTLAGALFLH (SEQ ID NO:22)

[0098] CD28 transmembrane domain amino acid sequence:

FWVLVVVGGLACYSLLVTVAFIIFWV (SEQ ID NO:23)

[0099] CD8 transmembrane domain amino acid sequence:

IYIWAPLAGTCGVLLSLVIT (SEQ ID NO:24)

[0100] 4-1BB transmembrane domain amino acid sequence:

IISFFLALTSTALLFLLFLLTLRFSVV (SEQ ID NO:25)

[0101] DAP10 transmembrane domain amino acid sequence:

LLAGLVAADAVASLLIVGAVF (SEQ ID NO:26)

[0102] DAP12 transmembrane domain amino acid sequence:

GVLAGIVMGDLVLTVLIALAV (SEQ ID NO:27)

[0103] NKG2D transmembrane domain amino acid sequence:

AVMIIFRIGMAVAIFCCFFFP (SEQ ID NO:28)

[0104] Any polypeptide encompassed by the present disclosure may comprise SEQ ID NO:22, 23, 24, 25, 26, 27, or 28 or a sequence that is at least 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or more % identical to SEQ ID NO: 22, 23, 24, 25, 26, 27, or 28.

3. Intracellular domains

[0105] One or more intracellular domains (which may also be referred to herein as signal activation domains or costimulatory domains, in appropriate cases) may or may not be utilized in specific anti-TROP-2 CARs of the disclosure. Specific examples include intracellular domains from CD3 zeta, 4-1BB, NKG2D, OX-40, CD27, DAP10, DAP12, B7-1/CD80, CD28, 2B4, 4-1BBL, B7-2/CD86, CTLA-4, B7-H1/PD-L1, ICOS, B7-H2, PD-1, B7-H3, PD-L2, B7-H4, PDCD6, BTLA, or a combination thereof.

[0106] Examples of particular intracellular domains which may be used in a CAR of the disclosure are as follows:

[0107] An example CD3zeta intracellular domain amino acid sequence:

[0108] RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPPEMGGK
PRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDA
LHMQUALPPRG (SEQ ID NO:29)

[0109] 4-1BB intracellular domain amino acid sequence:

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO:30)

[0110] DAP10 intracellular domain amino acid sequence:

LCARPRRSPAQEDGKVYINMPGRG (SEQ ID NO:31)

[0111] DAP12 intracellular domain amino acid sequence:

YFLGRLVPRGRGAAEAATRKQRITETESPYQELQGQRSDVYSDLNTQRPYYK (SEQ
ID NO:32)

[0112] NKG2D intracellular domain amino acid sequence:

SANERCKSKVPCRQKQWRTSFDSKKLDLNYNHFESMEWSHRSRRGRIWGM (SEQ
ID NO:33)

[0113] Any polypeptide encompassed by the present disclosure may comprise SEQ ID NO:29, 30, 31, 32, or 33 or a sequence that is at least 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or more % identical to SEQ ID NO: 29, 30, 31, 32, or 33.

4. Hinge

[0114] In some embodiments of the CARs, there is a hinge region between the one or more extracellular antigen binding domains and the transmembrane domain. In specific embodiments, the hinge is of a particular length, such as 10-20, 10-15, 11-20, 11-15, 12-20, 12-15, or 15-20 amino acids in length, for example. The hinge may be any suitable hinge and includes a hinge from IgG, or CD28, in some cases. In specific embodiments, the hinge is a small flexible polypeptide that connects CH2-CH3 and CH1 domains of IgG Fc. For example, one may utilize CH2-CH3 hinge (part or all) from various IgG subclasses (IgG1-4, either modified or not). However, in some cases the entire CH2-CH3 hinge is not utilized but instead a portion of the hinge is used (such as CH3 by itself or part of CH3 by itself). In particular embodiments, the CH2-CH3 hinge derived from IgG1 is utilized, and in some cases the entire CH2-CH3 hinge is used (all 229 amino acids), only the CH3 hinge (119 amino acids) is used, or a short hinge (12 amino acids) is used.

[0115] In specific cases, one can modify the identity or length of the spacer and/or hinge to optimize efficiency of the CAR. See, for example, Hudecek et al. (2014) and Jonnalagadda et al. (2015). In specific embodiments, the TROP-2 CAR utilizes IgG4 hinge+CH3 or utilizes CD8a stalk, for example.

[0116] Thus, in specific embodiments the IgG hinge region that is utilized is typically IgG1 or IgG4, and in some cases the CAR comprises the CH2-CH3 domain of IgG Fc. The use of the IgG Fc domain can provide flexibility to the CAR, has low immunogenicity, facilitates detection of CAR expression using anti-Fc reagents, and allows removal of one or more CH2 or CH3 modules to accommodate different spacer lengths. However, in one embodiment mutations in certain spacers to avoid FcγR binding may improve CAR+ T cell engraftment and antitumor efficacy to avoid binding of soluble and cell surface Fc gamma receptors, for example, yet maintain the activity to mediate antigen-specific lysis. For example, one can employ IgG4-Fc spacers that have either been modified in the CH2 region. For example, the CH2 region may be mutated, including point mutations and/or deletions. Specific modifications have been demonstrated at two sites (L235E; N297Q) within the CH2 region and/or incorporate a CH2 deletion (Jonnalagadda et al, 2015). In specific embodiments, one may employ the IgG4

hinge-CH2-CH3 domain (229 aa in length) or only the hinge domain (12 aa in length) (Hudececk et al., 2015).

[0117] In specific embodiments, the hinge is from IgG, CD28, CD-8 alpha, 4-1BB, OX40, CD3-zeta, T cell receptor a or b chain, a CD3 zeta chain, CD28, CD3e, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, ICOS, or CD154.

[0118] Examples of specific sequences of hinges that may be utilized include at least the following:

[0119] IgG Hinge amino acid sequence:

TVTSSQDPAEPKSPDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV
DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSD
IAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSMHEAL
(SEQ ID NO:34)

[0120] CD28 Hinge amino acid sequence:

IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFLPGPSKP (SEQ ID NO:35)

[0121] CD8 α Hinge amino acid sequence:

SCLCSCPPSPPPPLPLDLPPQPQQSPASLCPCGPKPVDLLPAEPCTPEAWISPA (SEQ ID NO:36)

[0122] Any polypeptide encompassed by the present disclosure may comprise SEQ ID NO:34, 35, or 36 or a sequence that is at least 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, or more % identical to SEQ ID NO: 34, 35, or 36.

5. Other Proteins

[0123] In some embodiments, one or more other proteins are utilized with an anti-TROP-2 CAR of the disclosure. The one or more other proteins may be utilized for any reason, including to facilitate efficacy of the CAR itself and/or of any kind of cells expressing the CAR. In some cases, the other protein facilitates treatment of an individual receiving cells expressing the CAR as therapy, whether or not the other protein(s) directly or indirectly impact activity of the CAR or the cells. In some cases, the other protein is a suicide gene, one or more cytokines, or both. In specific embodiments, one or more other proteins are produced from a vector and

ultimately are produced as two separate polypeptides. For example, the anti-TROP-2 CAR and the other protein(s) may be separated by a 2A sequence or by an IRES, for example.

[0124] In specific embodiments, a cytokine such as IL-15 is utilized in conjunction with the anti-TROP-2 CAR.

[0125] IL-15 amino acid sequence:

ISKPHLRISISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKIED
LIQSMHIDATLYTESDVHPSCCKVTAMKCFLELQVISLESGDASIHDTVENLIILANNS
LSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:37)

[0126] In specific embodiments, a suicide gene product such as caspase 9 (e.g., inducible caspase 9) is utilized in conjunction with the anti-TROP-2 CAR.

[0127] Example caspase 9 amino acid sequence:

MLEGVQVETISPGDGRTPFKRGQTCVVHYTGMLEDGKKVDSSDRNKPFKFMLGK
QEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLESG
GGSGVDGFGDVGALESLRGNADLAYILSMEPCGHCLIINNPNFCRESGLRTRTGSNID
CEKLRRRFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQAS
HLQFPGAVYGTGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVAS
TSPEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFGFVSWRDPKSG
SWYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTS
AS (SEQ ID NO: 42)

[0128] In cases where the CAR and another protein in the same vector are intended to be produced into two different polypeptides, a specific 2A sequence may be utilized.

[0129] E2A amino acid sequence may be utilized as follows:

QCTNYALLKLAGDVESNPGP (SEQ ID NO:38)

[0130] Other 2A examples may be utilized and are as follows:

[0131] T2A: EGRGSLTTCGDVEENPGP (SEQ ID NO:39)

[0132] P2A: ATNFSLLKQAGDVESNPGP (SEQ ID NO:40)

[0133] F2A: VKQTLNFDLLKLAGDVESNPGP (SEQ ID NO:41)

[0134] The disclosure also encompasses specific CAR molecules, including for expression in any type of immune effector cells.

[0135] In a vector, the CAR may be expressed with IL-15, such as may be separated from the CAR by a 2A sequence. In a specific example, such a CAR and IL-15 construct may have the following nucleotide sequence:

[0136] iC9mRs7VLVH28H28z15

ATGCTCGAGGGAGTGCAGGTGGAACCATCTCCCCAGGCGACGGGCG
CACCTTCCCCAAGCGCGGCCAGACCTGCGTGGTGCCTACACCGGGATGCTTGAA
GATGGAAAGAAAGTTGATTCCCTCCCGGGACAGAAACAAGCCCTTTAAGTTTATG
CTAGGCAAGCAGGAGGTGATCCGAGGCTGGGAAGAAGGGGTGCCCAGATGAGT
GTGGGTCAGAGAGCCAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTG
GGCACCCAGGCATCATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCT
AAAACCTGGAATCTGGCGGTGGATCCGGAGTCGACGGATTTGGTGATGTCGGTGC
TCTTGAGAGTTTGAGGGGAAATGCAGATTTGGCTTACATCCTGAGCATGGAGCCC
TGTGGCCACTGCCTCATTATCAACAATGTGAACTTCTGCCGTGAGTCCGGGCTCC
GCACCCGCACTGGCTCCAACATCGACTGTGAGAAGTTGCGGCGTCGCTTCTCCTC
GCTGCATTTTCATGGTGGAGGTGAAGGGCGACCTGACTGCCAAGAAAATGGTGCT
GGCTTTGCTGGAGCTGGCGCAGCAGGACCACGGTGCTCTGGACTGCTGCGTGGTG
GTCATTCTCTCTCACGGCTGTCAGGCCAGCCACCTGCAGTCCCAGGGGCTGTCT
ACGGCACAGATGGATGCCCTGTGTCGGTCGAGAAGATTGTGAACATCTTCAATG
GGACCAGCTGCCCCAGCCTGGGAGGGAAGCCCAAGCTCTTTTTTCATCCAGGCCTG
TGGTGGGGAGCAGAAAGATCATGGGTTTGAGGTGGCCTCCACTTCCCCTGAAGA
CGAGTCCCCTGGCAGTAACCCCGAGCCAGATGCCACCCCGTTCCAGGAAGGTTTG
AGGACCTTCGACCAGCTGGACGCCATATCTAGTTTGCCACACCCAGTGACATCT
TTGTGTCCTACTCTACTTTCCCAGGTTTTGTTTCTGAGGGACCCCAAGAGTGGC
TCCTGGTACGTTGAGACCCTGGACGACATCTTTGAGCAGTGGGCTCACTCTGAAG
ACCTGCAGTCCCTCCTGCTTAGGGTCGCTAATGCTGTTTCGGTGAAAGGGATTTA
TAAACAGATGCCTGGTTGCTTTAATTTCTCCGAAAAAACTTTTCTTTAAACAT
CAGCTTCGCGAGCCGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGG
AAAATCCCGGGCCCATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTTGGCTATTTTA
AAAGGTGTCCAGTGCTCTAGAGagGACATTCAGCTGACCCAGTCTCACAAATTCA
TGTCACATCAGTAGGAGACAGGGTCAGCATCACCTGCAAGGCCAGTCAGGATG
TGAGTATTGCTGTAGCCTGGTATCAACAGAAACCAGGACAATCTCCTAACTACT
GATTTACTCGGCATCCTACCGGTACACTGGAGTCCCTGATCGCTTCACTGGCAGT
GGATCTGGGACGGATTTCACTTTACCATCAGCAGTGTGCAGGCTGAAGACCTGG
CAGTTTATTACTGTCAGCAACATTATATTACTCCGCTCACGTTCCGGTGCTGGGACC
AAGCTGGAGCTGAAACGGTTGGAAATAAAGGGCTCTACAAGCGGCTCAGGAAAA
CCTGGATCAGGCGAAGGGTCTACGGTGAAGCTGCAGGAGTCAGGACCTGAGCTG
AAGAAGCCTGGAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGATATACCTTC

ACAAACTATGGAATGAACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGG
ATGGGCTGGATAAACACCTACACTGGAGAGCCAACATATACTGATGACTTCAAG
GGACGGTTTGCCTTCTCTTTGGAAACCTCTGCCACCACTGCCTATTTGCAGATCAA
CAACCTCAAAAGTGAGGACATGGCTACATATTTCTGTGCAAGAGGGGGGTTCGG
TAGTAGCTACTGGTACTTCGATGTCTGGGGCCAAGGGACCACGGTCACCGTCTCC
TCAcCGTACGCCATTGAAGTTATGTATCCTCCTCCTTACCTAGACAATGAGAAGA
GCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCCAAGTCCCCTATT
TCCCGGACCTTCTAAGCCCAAATTTTGGGTGCTGGTGGTGGTTGGTGGAGTCTCG
GCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTA
AGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCG
GGCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTA
TCGCTCACGCGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGG
CCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGT
TTTGGACAAAAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGA
AGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAG
GCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGGCAAGGGGACGA
TGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTAC
ATGCAGGCCCTGCCCCCTCGCGGACCGCAGTGTACTAATTATGCTCTCTTGAAAT
TGGCTGGAGATGTTGAGAGCAATCCCGGGCCCATGCGCATTAGCAAGCCCCACC
TGCGGAGCATCAGCATCCAGTGCTACCTGTGCCTGCTGCTGAACAGCCACTTCT
GACCGAGGCCGGCATCCACGTGTTTCATCCTGGGCTGCTTCAGCGCCGGACTGCCC
AAGACCGAGGCCAACTGGGTGAACGTGATCAGCGACCTGAAGAAGATCGAGGA
CCTGATCCAGAGCATGCACATCGACGCCACCCTGTACACCGAGAGCGACGTGCA
CCCCAGCTGCAAGGTGACCGCCATGAAGTGCTTTCTGCTGGAACTGCAGGTGATC
AGCCTGGAAAGCGGCGACGCCAGCATCCACGACACCGTGGAGAACCTGATCATC
CTGGCCAAACAACAGCCTGAGCAGCAACGGCAACGTGACCGAGAGCGGCTGCAAA
GAGTGCGAGGAACTGGAAGAGAAGAACATCAAAGAGTTTCTGCAGAGCTTCGTG
CACATCGTGCAGATGTTTCATCAACACCAGCTGA (SEQ ID NO:1)

[0137] A corresponding amino acid sequence for iC9mRs7VLVH28H28z15 is as follows:

MLEGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKP
KFMLGKQEVIRGWEEGVAQMSVGQRAKL TISPDYAYGATGHPGIIPPHATLVFDVEL
LKLESGGSGVDGFGDVGALSLRGNADLAYILSMEPCGHCLIINNPNFCRESGLRT
RTGSNIDCEKLRRRFSSLHFMVEVKGDLTAKKMVLAALLELAQQDHGALDCCVVVIL

SHGCQASHLQFPGAVYGTGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKD
HGFEVASTPEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVS
WRDPKSGSWYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRK
KLFFKTSASRAEGRGSLTTCGDVEENPGPMEFGLSWLFLVAILKGVQCSREDIQLTQS
HKFMSTSVGDRVSITCKASQDVSIATAVWYQQKPGQSPKLLIYSASYRYTGVPDRFTG
SGSGTDFTFITSSVQAEDLAVYYCQQHYYITPLTFGAGTKLELKRLEIKGSTSGSGKPGS
GEGSTVKLQESGPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWI
NTYTGEPTYTDDFKGRFAFSLETSATTAYLQINNLSKSEDMATYFCARGGFGSSYWF
DVWGGQTTVTVSSPYAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPKFW
VLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRGPQCTNYALLKLAGDVESNPGPMRISKPHLRSISIQCYLCLLNSH
FLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLELQVISLESGDASIHDTVENLILANNSLSSNGNVTESGCKECEEELEEK
NIKEFLQSFVHIVQMFINTS (SEQ ID NO:2)

[0138] iC9mRS7VHVL28H28icz15

ATGCTCGAGGGAGTGCAGGTGGAACCATCTCCCCAGGCGACGGGCG
CACCTTCCCCAAGCGCGGCCAGACCTGCGTGGTGCCTACACCGGGATGCTTGAA
GATGGAAAGAAAGTTGATTCTCTCCCGGGACAGAAACAAGCCCTTTAAGTTTATG
CTAGGCAAGCAGGAGGTGATCCGAGGCTGGGAAGAAGGGGTTGCCAGATGAGT
GTGGGTCAGAGAGCCAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTG
GGCACCCAGGCATCATCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCT
AAAACCTGGAATCTGGCGGTGGATCCGGAGTCGACGGATTTGGTGATGTGGGTGC
TCTTGAGAGTTTGAGGGGAAATGCAGATTTGGCTTACATCCTGAGCATGGAGCCC
TGTGGCCACTGCCTCATTATCAACAATGTGAACTTCTGCCGTGAGTCCGGGCTCC
GCACCCGCACTGGCTCCAACATCGACTGTGAGAAGTTGCGGCGTCGCTTCTCCTC
GCTGCATTTTCATGGTGGAGGTGAAGGGCGACCTGACTGCCAAGAAAATGGTGCT
GGCTTTGCTGGAGCTGGCGCAGCAGGACCACGGTGCTCTGGACTGCTGCGTGGTG
GTCATTCTCTCTCACGGCTGTCAGGCCAGCCACCTGCAGTTCCCAGGGGCTGTCT
ACGGCACAGATGGATGCCCTGTGTCGGTTCGAGAAGATTGTGAACATCTTCAATG
GGACCAGCTGCCCCAGCCTGGGAGGGAAGCCCAAGCTCTTTTTCATCCAGGCCTG
TGGTGGGGAGCAGAAAGATCATGGGTTTGAGGTGGCCTCCACTTCCCCTGAAGA

CGAGTCCCCTGGCAGTAACCCCGAGCCAGATGCCACCCCGTTCCAGGAAGGTTTG
AGGACCTTCGACCAGCTGGACGCCATATCTAGTTTGCCACACCCAGTGACATCT
TTGTGTCCTACTCTACTTTCCCAGGTTTTGTTTCCTGGAGGGACCCCAAGAGTGGC
TCCTGGTACGTTGAGACCCTGGACGACATCTTTGAGCAGTGGGCTCACTCTGAAG
ACCTGCAGTCCCTCCTGCTTAGGGTCGCTAATGCTGTTTCGGTGAAAGGGATTTA
TAAACAGATGCCTGGTTGCTTTAATTTCCCTCCGGAAAAAACTTTTCTTTAAAACAT
CAGCTTCGCGAGCCGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGG
AAAATCCCCGGGCCCATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTA
AAAGGTGTCCAGTGCTCTAGAGagGTGAAGCTGCAGGAGTCAGGACCTGAGCTGA
AGAAGCCTGGAGAGACAGTCAAGATCTCCTGCAAGGCTTCTGGATATACCTTCAC
AAACTATGGAATGAACTGGGTGAAGCAGGCTCCAGGAAAGGGTTTAAAGTGGAT
GGGCTGGATAAACACCTACACTGGAGAGCCAACATATACTGATGACTTCAAGGG
ACGGTTTGCCTTCTCTTTGGAAACCTCTGCCACCACTGCCTATTTGCAGATCAACA
ACCTCAAAAGTGAGGACATGGCTACATATTTCTGTGCAAGAGGGGGGTTTCGGTA
GTAGCTACTGGTACTTCGATGTCTGGGGCCAAGGGACCACGGTCACCGTCTCCTC
ATTGGAAATAAAGGGCTCTACAAGCGGCTCAGGAAAACCTGGATCAGGCGAAGG
GTCTACGGACATTCAGCTGACCCAGTCTCACAAATTCATGTCCACATCAGTAGGA
GACAGGGTCAGCATCACCTGCAAGGCCAGTCAGGATGTGAGTATTGCTGTAGCC
TGGTATCAACAGAAACCAGGACAATCTCCTAAACTACTGATTTACTCGGCATCCT
ACCGGTACACTGGAGTCCCTGATCGCTTCACTGGCAGTGGATCTGGGACGGATTT
CACTTTCACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAG
CAACATTATATTACTCCGCTCACGTTTCGGTGCTGGGACCAAGCTGGAGCTGAAAC
GGcCGTACGCCATTGAAGTTATGTATCCTCCTCCTTACCTAGACAATGAGAAGAG
CAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCCAAGTCCCCTATTT
CCCGGACCTTCTAAGCCCAAATTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGG
CTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAA
GAGGAGCAGGCTCCTGCACAGTGAATGACATGAACATGACTCCCCGCCGCCCGG
GCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTAT
CGCTCACGCGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGC
CAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTT
TTGGACAAAAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAA
GAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGC
CTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGGCAAGGGGCACGATG
GCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACAT

GCAGGCCCTGCCCCCTCGCGGACCGCAGTGTACTAATTATGCTCTCTTGAAATTG
GCTGGAGATGTTGAGAGCAATCCCGGGCCCATGCGCATTAGCAAGCCCCACCTG
CGGAGCATCAGCATCCAGTGCTACCTGTGCCTGCTGCTGAACAGCCACTTCCTGA
CCGAGGCCCGGCATCCACGTGTTTCATCCTGGGCTGCTTCAGCGCCGGACTGCCCAA
GACCGAGGCCAACTGGGTGAACGTGATCAGCGACCTGAAGAAGATCGAGGACCT
GATCCAGAGCATGCACATCGACGCCACCCTGTACACCGAGAGCGACGTGCACCC
CAGCTGCAAGGTGACCGCCATGAAGTGCTTTCTGCTGGAAGTGCAGGTGATCAGC
CTGGAAAGCGGCGACGCCAGCATCCACGACACCGTGGAGAACCTGATCATCCTG
GCCAACAACAGCCTGAGCAGCAACGGCAACGTGACCGAGAGCGGCTGCAAAGA
GTGCGAGGAACTGGAAGAGAAGAACATCAAAGAGTTTCTGCAGAGCTTCGTGCA
CATCGTGCAGATGTTTCATCAACACCAGCTGA (SEQ ID NO:3)

[0139] A corresponding amino acid sequence for iC9mRS7VHVL28H28icz15 is as follows:

MLEGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKP
KFMLGKQEVIRGWEEGVAQMSVGQRAKLITSPDYAYGATGHPGIIPPHATLVFDVEL
LKLESGGGSGVDGFGDVGALESRLGNADLAYILSMEPCGHCLIINNPNFCRESGLRT
RTGSNIDCEKLRRRFFSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVIL
SHGCQASHLQFPGAVYGTGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKD
HGFEVASTPEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVS
WRDPKSGSWYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRK
KLFFKTSASRAEGRGSLTTCGDVEENPGPMEFGLSWLFLVAILKGVQCSREVKLQES
GPELKKPGETVKISCKASGYTFTNYGMNWVKQAPGKGLKWMGWINTYTGEPTYTD
DFKGRFAFSLETSATTAYLQINNPKSEDMATYFCARGGFGSSYWFYFDVWGQGT
VSSLEIKGSTSGSGKPGSGEGSTDIQLTQSHKFMSTSVGDRVSITCKASQDVSI
YQKPGQSPKLLIYSASYRYTGVPDRFTGSGSGTDFTFITSSVQAEDLAVYYCQ
TPLTFGAGTKLELKPYPYAEVMPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPKF
WVLVVVGGVLACYSLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPY
APPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRGRDPE
MGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATK
DTYDALHMQALPPRGPCQCTNYALLKLAGDVESNPGPMRISKPHLRSISIQCYLCLLN
SHFLTEAGIHVFILGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
SCKVTAMKCFLELQVISLESGLDASIHTVENLILANNSLSSNGNVTESGCKECEELE
EKNIKEFLQSFVHIVQMFINTS (SEQ ID NO:4)

[0140] iC9hRs7VLVH28H28z15

ATGCTCGAGGGAGTGCAGGTGGAACCATCTCCCCAGGCGACGGGCG
CACCTTCCCCAAGCGCGGCCAGACCTGCGTGGTGCCTACACCGGGATGCTTGAA
GATGGAAAGAAAGTTGATTCCCTCCCGGGACAGAAACAAGCCCTTTAAGTTTATG
CTAGGCAAGCAGGAGGTGATCCGAGGCTGGGAAGAAGGGGTGCCCAGATGAGT
GTGGGTCAGAGAGCCAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTG
GGCACCCAGGCATCATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCT
AAAACCTGGAATCTGGCGGTGGATCCGGAGTCGACGGATTTGGTGATGTCGGTGC
TCTTGAGAGTTTGAGGGGAAATGCAGATTTGGCTTACATCCTGAGCATGGAGCCC
TGTGGCCACTGCCTCATTATCAACAATGTGAACTTCTGCCGTGAGTCCGGGCTCC
GCACCCGCACTGGCTCCAACATCGACTGTGAGAAGTTGCGGCGTCGCTTCTCCTC
GCTGCATTTTCATGGTGGAGGTGAAGGGCGACCTGACTGCCAAGAAAATGGTGCT
GGCTTTGCTGGAGCTGGCGCAGCAGGACCACGGTGCTCTGGACTGCTGCGTGGTG
GTCATTCTCTCTCACGGCTGTCAGGCCAGCCACCTGCAGTTCACAGGGGCTGTCT
ACGGCACAGATGGATGCCCTGTGTCGGTCGAGAAGATTGTGAACATCTTCAATG
GGACCAGCTGCCCCAGCCTGGGAGGGAAGCCCAAGCTCTTTTTTCATCCAGGCCTG
TGGTGGGGAGCAGAAAGATCATGGGTTTGAGGTGGCCTCCACTTCCCCTGAAGA
CGAGTCCCCTGGCAGTAACCCCGAGCCAGATGCCACCCCGTTCCAGGAAGGTTTG
AGGACCTTCGACCAGCTGGACGCCATATCTAGTTTGCCACACCCAGTGACATCT
TTGTGTCCTACTCTACTTTCCCAGGTTTTGTTTCCTGGAGGGACCCCAAGAGTGGC
TCCTGGTACGTTGAGACCCTGGACGACATCTTTGAGCAGTGGGCTCACTCTGAAG
ACCTGCAGTCCCTCCTGCTTAGGGTCGCTAATGCTGTTTCGGTGAAAGGGATTTA
TAAACAGATGCCTGGTTGCTTTAATTTCTCCGAAAAAACTTTTCTTTAAACAT
CAGCTTCGCGAGCCGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGG
AAAATCCCGGGCCCATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTTGGCTATTTTA
AAAGGTGTCCAGTGCTCTAGAGagGACATCCAGCTGACCCAGTCTCCATCCTCCCT
GTCTGCATCTGTAGGAGACAGAGTCAGCATCACCTGCAAGGCCAGTCAGGATGT
GAGTATTGCTGTAGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCTAAGCTCCT
GATCTACTCGGCATCCTACCGGTACACTGGAGTCCCTGATAGGTTCAGTGGCAGT
GGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTG
CAGTTTATTACTGTCAGCAACATTATATTACTCCGCTCACGTTCCGGTGCTGGGACC
AAGGTGGAGATCAAACGTTTGGAATAAAGGGCTCTACAAGCGGCTCAGGAAAA
CCTGGATCAGGCGAAGGGTCTACGCAGGTCCAACCTGCAGCAATCTGGGTCTGAG
TTGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCTTCTGGATACACCT

TCACAACTATGGAATGAACTGGGTGAAGCAGGCCCTGGACAAGGGCTTAAAT
GGATGGGCTGGATAAACACCTACACTGGAGAGCCAACATATACTGATGACTTCA
AGGGACGGTTTGCCTTCTCCTTGGACACCTCTGTCAGCACGGCATATCTCCAGAT
CAGCAGCCTAAAGGCTGACGACACTGCCGTGTATTTCTGTGCAAGAGGGGGGTT
CGGTAGTAGCTACTGGTACTTCGATGTCTGGGGCCAAGGGTCCCTGGTCACCGTC
TCCTCAcCGTACGCCATTGAAGTTATGTATCCTCCTCCTTACCTAGACAATGAGAA
GAGCAATGGAACCATTATCCATGTGAAAGGGAAACACCTTTGTCCAAGTCCCCTA
TTTCCCGGACCTTCTAAGCCCAAATTTTGGGTGCTGGTGGTGGTTGGTGGAGTCC
TGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGT
AAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCC
GGGCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCT
ATCGCTCACGCGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGGTACCAGCAGG
GCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATG
TTTTGGACAAAAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGG
AAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGA
GGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACG
ATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCA
CATGCAGGCCCTGCCCCCTCGCGGACCGCAGTGTACTAATTATGCTCTCTTGA
TTGGCTGGAGATGTTGAGAGCAATCCCGGGCCCATGCGCATTAGCAAGCCCCAC
CTGCGGAGCATCAGCATCCAGTGCTACCTGTGCCTGCTGCTGAACAGCCACTTCC
TGACCGAGGCCGGCATCCACGTGTTATCCTGGGCTGCTTCAGCGCCGGACTGCC
CAAGACCGAGGCCAACTGGGTGAACGTGATCAGCGACCTGAAGAAGATCGAGG
ACCTGATCCAGAGCATGCACATCGACGCCACCCTGTACACCGAGAGCGACGTGC
ACCCAGCTGCAAGGTGACCGCCATGAAGTGCTTTCTGCTGGAAGTGCAGGTGAT
CAGCCTGGAAAGCGGCGACGCCAGCATCCACGACACCGTGGAGAACCTGATCAT
CCTGGCCAACAACAGCCTGAGCAGCAACGGCAACGTGACCGAGAGCGGCTGCAA
AGAGTGCGAGGAACTGGAAGAGAAGAACATCAAAGAGTTTCTGCAGAGCTTCGT
GCACATCGTGCAGATGTTTCATCAACACCAGCTGA (SEQ ID NO:5)

[0141] A corresponding amino acid sequence for iC9hRs7VLVH28H28z15 is as follows:

MLEGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKP
KFMLGKQEVIRGWEEGVAQMSVGQRAKL TISPDYAYGATGHPGIIPPHATLVFDVEL
LKLESGGSGVDGFGDVGALESLRGNADLAYILSMEPCGHCLIINNPNFCRESGLRT
RTGSNIDCEKLRRRFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVIL

SHGCQASHLQFPGAVYGTGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKD
HGFEVASTPEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVS
WRDPKSGSWYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRK
KLFFKTSASRAEGRGSLTTCGDVEENPGPMEFGLSWLFLVAILKGVQCSREDIQLTQS
PSSLSASVGDRVSITCKASQDVSI AVAWYQQKPGKAPKLLIYSASYRYTGVPDRFSGS
GSGTDFTLTISSLQPEDFAVYYCQQHYITPLTFGAGTKVEIKRLEIKGSTSGSGKPGSG
EGSTQVQLQQSGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGW
INTYTGEPTYTDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWYF
DVWGGQSLVTVSSPYAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLPFGPSKPKFW
VLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYA
PPRDFAA YRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVL DKRRGRDPEM
GGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDT
YDALHMQALPPRGPQCTNYALLKLAGDVESNPGPMRISKPHLRSISIQCYLCLLNSH
FLTEAGIHVFILGCF SAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSC
KVTAMKCFLELQVISLESGDASIHTVENLILANNSLSSNGNVTESGCKECEEELEEK
NIKEFLQSFVHIVQMFINTS (SEQ ID NO:6)

[0142] iC9TROP2VLVH28H28z15

ATGCTCGAGGGAGTGCAGGTGGA AACCATCTCCCCAGGCGACGGGCG
CACCTTCCCCAAGCGCGGCCAGACCTGCGTGGTGC ACTACACCGGGATGCTTGAA
GATGGAAAGAAAGTTGATTCTCTCCCGGGACAGAAACAAGCCCTTTAAGTTTATG
CTAGGCAAGCAGGAGGTGATCCGAGGCTGGGAAGAAGGGGTTGCCAGATGAGT
GTGGGTCAGAGAGCCAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTG
GGCACCCAGGCATCATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCT
AAA ACTGGAATCTGGCGGTGGATCCGGAGTCGACGGATTTGGTGATGTGGGTGC
TCTTGAGAGTTTGAGGGGAAATGCAGATTTGGCTTACATCCTGAGCATGGAGCCC
TGTGGCCACTGCCTCATTATCAACAATGTGAACTTCTGCCGTGAGTCCGGGCTCC
GCACCCGCACTGGCTCCAACATCGACTGTGAGAAGTTGCGGCGTCGCTTCTCCTC
GCTGCATTT CATGGTGGAGGTGAAGGGCGACCTGACTGCCAAGAAAATGGTGCT
GGCTTTGCTGGAGCTGGCGCAGCAGGACCACGGTGCTCTGGACTGCTGCGTGGTG
GTCATTCTCTCTCACGGCTGTCAGGCCAGCCACCTGCAGTTCCCAGGGGCTGTCT
ACGGCACAGATGGATGCCCTGTGTCGGTCGAGAAGATTGTGAACATCTTCAATG
GGACCAGCTGCCCCAGCCTGGGAGGGAAGCCCAAGCTCTTTTTCATCCAGGCCTG
TGGTGGGGAGCAGAAAGATCATGGGTTTGAGGTGGCCTCCACTTCCCCTGAAGA

CGAGTCCCCTGGCAGTAACCCCGAGCCAGATGCCACCCCGTTCCAGGAAGGTTTG
AGGACCTTCGACCAGCTGGACGCCATATCTAGTTTGCCACACCCAGTGACATCT
TTGTGTCCTACTCTACTTTCCCAGGTTTTGTTTCCTGGAGGGACCCCAAGAGTGGC
TCCTGGTACGTTGAGACCCCTGGACGACATCTTTGAGCAGTGGGCTCACTCTGAAG
ACCTGCAGTCCCTCCTGCTTAGGGTCGCTAATGCTGTTTCGGTGAAAGGGATTTA
TAAACAGATGCCTGGTTGCTTTAATTTCCCTCCGGAAAAAACTTTTCTTTAAAACAT
CAGCTTCGCGAGCCGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGG
AAAATCCCGGGGCCATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTA
AAAGGTGTCCAGTGCTCTAGAGagCAGGTCCAAGTGCAGCAATCTGGGTCTGAGT
TGAAGAAGCCTGGGGCCTCAGTGAAGGTTTCCTGCAAGGCTTCTGGATACACCTT
CACAACTATGGAATGAACTGGGTGAAGCAGGCCCTGGACAAGGGCTTAAATG
GATGGGCTGGATAAACACCTACACTGGAGAGCCAACATATACTGATGACTTCAA
GGGACGGTTTGCCTTCTCCTTGGACACCTCTGTCAGCACGGCATATCTCCAGATC
AGCAGCCTAAAGGCTGACGACACTGCCGTGTATTTCTGTGCAAGAGGGGGGTTT
GGTAGTAGCTACTGGTACTTCGATGTCTGGGGCCAAGGGTCCCTGGTCACCGTCT
CCTCATTGGAATAAAGGGCTCTACAAGCGGCTCAGGAAAACCTGGATCAGGCG
AAGGGTCTACGGACATCCAGCTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGT
AGGAGACAGAGTCAGCATCACCTGCAAGGCCAGTCAGGATGTGAGTATTGCTGT
AGCCTGGTATCAGCAGAAACCAGGGAAAGCCCCCTAAGCTCCTGATCTACTCGGC
ATCCTACCGGTACACTGGAGTCCCTGATAGGTTTCAAGTGGCAGTGGATCTGGGACA
GATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAGTTTATTACTG
TCAGCAACATTATATTACTCCGCTCACGTTTCGGTGCTGGGACCAAGGTGGAGATC
AAACGTcCGTACGCCATTGAAGTTATGTATCCTCCTCCTTACCTAGACAATGAGA
AGAGCAATGGAACCATTTATCCATGTGAAAGGGAAACACCTTTGTCCAAGTCCCT
ATTTCCCGGACCTTCTAAGCCCAAATTTTGGGTGCTGGTGGTGGTGGTGGAGTC
CTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAG
TAAGAGGAGCAGGCTCCTGCACAGTGAATGACATGAACATGACTCCCCGCCGCC
CGGGCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCC
TATCGCTCACGCGTGAAGTTCAGCAGGAGCGCAGACGCCCCGCGTACCAGCAG
GGCCAGAACCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGAT
GTTTTGGACAAAAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAG
GAAGAACCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGG
AGGCCTACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGGCAAGGGGCAC
GATGGCCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTC

ACATGCAGGCCCTGCCCCCTCGCGGACCGCAGTGTACTAATTATGCTCTCTTGAA
ATTGGCTGGAGATGTTGAGAGCAATCCCGGGCCCATGCGCATTAGCAAGCCCCA
CCTGCGGAGCATCAGCATCCAGTGCTACCTGTGCCTGCTGCTGAACAGCCACTTC
CTGACCGAGGCCGGCATCCACGTGTTTCATCCTGGGCTGCTTCAGCGCCGGACTGC
CCAAGACCGAGGCCAACTGGGTGAACGTGATCAGCGACCTGAAGAAGATCGAGG
ACCTGATCCAGAGCATGCACATCGACGCCACCCTGTACACCGAGAGCGACGTGC
ACCCAGCTGCAAGGTGACCGCCATGAAGTGCTTTCTGCTGGAAGTGCAGGTGAT
CAGCCTGGAAAGCGGCGACGCCAGCATCCACGACACCGTGGAGAACCTGATCAT
CCTGGCCAACAACAGCCTGAGCAGCAACGGCAACGTGACCGAGAGCGGCTGCAA
AGAGTGCGAGGAACTGGAAGAGAAGAACATCAAAGAGTTTCTGCAGAGCTTCGT
GCACATCGTGCAGATGTTTCATCAACACCAGCTGA (SEQ ID NO:7)

[0143] A corresponding amino acid sequence for iC9TROP2VLVH28H28z15 is as follows:

MLEGVQVETISPGDGRTFPKRGQTCVVHYTGMLDGKKVDSSRDRNKP
KFMLGKQEVIRGWEEGVAQMSVGQRAKLITSPDYAYGATGHPGIIPPHATLVFDVEL
LKLESGGGSGVDGFGDVGALESRLGNADLAYILSMEPCGHCLIINNVNFCRESGLRT
RTGSNIDCEKLRRRFFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVIL
SHGCQASHLQFPGAVYGTGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKD
HGFEVASTPEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVS
WRDPKSGSWYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRK
KLFFKTSASRAEGRGSLTTCGDVEENPGPMEFGLSWLFLVAILKGVQCSREQVQLQQ
SGSELKKPGASVKVSCKASGYTFTNYGMNWVKQAPGQGLKWMGWINTYTGEPTY
TDDFKGRFAFSLDTSVSTAYLQISSLKADDTAVYFCARGGFGSSYWFYDVGQGS
VTVSSLEIKGSTSGSGKPGSGEGSTDIQLTQSPSSLSASVGDRVSITCKASQDVSI
AVA WYQQKPGKAPKLLIYSASYRYTGVPDRFSGSGSGTDFTLTISLQPEDFAVYYCQ
QH YITPLTFGAGTKVEIKRPAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSP
LPGPSKP KFWVLVVVGGLACYSLLVTVAFIHFWVRSKRSRLLHSDYMNMT
PRRPGPTRKHYQ PYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR
REEYDVLDKRRGRDP EMGGKPRRKNPQEGLYNELQKDKMAEAYSEIGMKGER
RRGKGHDGLYQGLSTATK DTYDALHMQALPPRG
PQCTNYALLKLAGDVESNPGPMRISKPHLRSISIQCYLC
LLN SHFLTEAGIHVFILGCF
SAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHP
SCKVTAMKCFLELQVISLES
GDASIHTVENLILANNSLSSNGNVTESGCKECEELE
EKNIKEFLQSFVHIVQMFIN
TS (SEQ ID NO:8)

iC9h2EF-7VHL28H28icZ15

ATGGGAGTGCAGGTGGAAACCATCTCCCCAGGCGACGGGCGCACCTTCCCCAAG
CGCGGCCAGACCTGCGTGGTGCCTACACCGGGATGCTTGAAGATGGAAAGAAA
GTTGATTCTCTCCCGGGACAGAAACAAGCCCTTTAAGTTTATGCTAGGCAAGCAGG
AGGTGATCCGAGGCTGGGAAGAAGGGGTTGCCCAGATGAGTGTGGGTCAGAGAG
CCAAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCACCCAGGCAT
CATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAACTGGAATCT
GGCGGTGGATCCGGAGTCGACGGATTTGGTGATGTCCGGTGCTCTTGAGAGTTTGA
GGGGAAATGCAGATTTGGCTTACATCCTGAGCATGGAGCCCTGTGGCCACTGCCT
CATTATCAACAATGTGAACTTCTGCCGTGAGTCCGGGCTCCGCACCCGCACTGGC
TCCAACATCGACTGTGAGAAGTTGCGGCGTCGCTTCTCCTCGCTGCATTTTCATGG
TGGAGGTGAAGGGCGACCTGACTGCCAAGAAAATGGTGCTGGCTTTGCTGGAGC
TGGCGCAGCAGGACCACGGTGCTCTGGACTGCTGCGTGGTGGTCATTCTCTCTCA
CGGCTGTCAGGCCAGCCACCTGCAGTTCCCAGGGGCTGTCTACGGCACAGATGG
ATGCCCTGTGTCGGTCGAGAAGATTGTGAACATCTTCAATGGGACCAGCTGCCCC
AGCCTGGGAGGGAAGCCCAAGCTCTTTTTTCATCCAGGCCTGTGGTGGGGAGCAG
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GTAACCCCGAGCCAGATGCCACCCCGTTCCAGGAAGGTTTGAGGACCTTCGACC
AGCTGGACGCCATATCTAGTTTGCCACACCCAGTGACATCTTTGTGTCTACTCT
ACTTTCCCAGGTTTTGTTCCTGGAGGGACCCCAAGAGTGGCTCCTGGTACGTTG
AGACCCTGGACGACATCTTTGAGCAGTGGGCTCACTCTGAAGACCTGCAGTCCCT
CCTGCTTAGGGTCGCTAATGCTGTTTCGGTGAAAGGGATTTATAAACAGATGCCT
GGTTGCTTTAATTTCTCCGGAATAAACTTTTCTTTAAACATCAGCTTCGCGAGC
CGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCCCGGGCC
CATGGAGTTCGGCTTGAGTTGGTTGTTCTTGTGGCGATACTCAAAGGCGTTCAA
TGTCAAGTCCAGCTCGTGCAGTCTGGAGCTGAAGTGAAGAAACCTGGGGCTTCA
GTGAAGGTGTCCTGCAAGGCTTCTGGATACACCTTCACTAACTACTGGATCGGAT
GGGTCAAACAGGCCCTGGACAGGGCCTCGAGTGGATTGGAGATATTTACCCTG
GAGGAGGCTATACTAACTACAATGAGAAGTTCAAGGGCAGAGCCCACTGACTG
CAGACACATCCGCCAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGG
AACTGCCGTGTATTACTGTGCAAGAGGAACTGGAGGCGGAGACTACTGGGGCC
AAGGGACTCTGGTCACTGTCTCTTCATTGGAAATAAAGGGCTCTACAAGCGGCTC
AGGAAAACCTGGATCAGGCGAAGGGTCTACGGACATTGTGCTGACACAGTCTCC

TGACTCCCTGGCTGTGTCTCTGGGGGAGAGGGCCACCATCAACTGCAGGGCCAG
CCAAAGTGTCAGTACATCTAGCTATAGTTATATGCACTGGTACCAACAGAAACCA
GGACAGCCACCCAACTCCTCATCAAGTATGCATCCAACCTGGAATCTGGGGTCC
CTGACAGATTCAAGTGGCAGTGGGTCTGGGACAGACTTCACCCTCACCATCAGCTC
CCTGCAGGCCGAGGATGTGGCAGTCTATTACTGTCAGCACAGTTGGGAGATTCCC
TACACCTTCGGAGGCGGGACCAAGCTGGAAATCAAACGTACGATTGAAGTTATG
TATCCTCCTCCTTACCTAGACAATGAGAAGAGCAATGGAACCATTATCCATGTGA
AAGGGAAACACCTTTGTCCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTG
GGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTG
GCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACT
ACATGAACATGACTCCCCGCCGCCCGGGCCACCCGCAAGCATTACCAGCCCTA
TGCCCCACCACGCGACTTCGCAGCCTATCGCTCAACGCGTGTGAAGTTCAGCAGG
AGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTC
AATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAAAGACGTGGCCGGGAC
CCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAA
TGAAGTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAG
GCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAG
CCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCGGACC
GCAGTGTACTAATTATGCTCTCTTGAAATTGGCTGGAGATGTTGAGAGCAATCCC
GGGCCCATGCGCATTAGCAAGCCCCACCTGCGGAGCATCAGCATCCAGTGCTAC
CTGTGCCTGCTGCTGAACAGCCACTTCCTGACCGAGGCCGGCATCCACGTGTTCA
TCCTGGGCTGCTTCAGCGCCGACTGCCCAAGACCGAGGCCAACTGGGTGAACG
TGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCGACG
CCACCCTGTACACCGAGAGCGACGTGCACCCAGCTGCAAGGTGACCGCCATGA
AGTGCTTTCTGCTGGAAGTGCAGGTGATCAGCCTGGAAAGCGGCGACGCCAGCA
TCCACGACACCGTGGAGAACCTGATCATCCTGGCCAACAACAGCCTGAGCAGCA
ACGGCAACGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAG
AACATCAAAGAGTTTCTGCAGAGCTTCGTGCACATCGTGCAGATGTTTCATCAACA
CCAGCTGA (SEQ ID NO:50)

A corresponding amino acid sequence for iC9h2EF-7VHL28H28icZ15 is as follows:
MGVQVETISPGDGRTPKRGQTCVVHYTGMLDGKKVDSSDRNPKFKFMLGKQE
VIRGWEEGVAQMSVGQRAKLITSPDYAYGATGHPGIIPPHATLVFDVELLKLESGGG
SGVDGFGDVGALSLRGNADLAYILSMEPCGHCLINNVNFCRESGLRTRTGSNIDCE

KLRRRFSSLHFMVEVKGDLTAKKMVLAALLELAQQDHGALDCCVVVILSHGCQASHL
QFPGAVYGTGCPVSVVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTS
PEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGS
WYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTSA
SRAEGRGSLLTCDGVEENPGPMEFGLSWLFLVAILKGVQCQVQLVQSGAEVKKPGA
SVKVSCASGYTFTNYWIGWVKQAPGQGLEWIGDIYPGGGYTNYNEKFKGRATLT
ADTSASTAYMELSSLRSEDVAVYYCARGTGGGDYWGQGTLLTVSSLEIKGSTSGSG
KPGSGEGSTDIVLTQSPDSLAVSLGERATINCRASQSVSTSSYSYMHWYQQKPGQPP
KLLIKYASNLESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQHSWEIPYTFGGGT
KLEIKRTIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLEFPGPSKPFVVLVVVGGVLA
CYSLLVTVAFIIFWVRSKRSRLHSDYMNMTPRRPGPTRKHYPYAPPRDFAAYRST
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRRGRDPGEMGGKPRRKNPQ
EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP
PRGPQCTNYALLKLAGDVESNPGPMRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFI
LGCFSAGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCCKVTAMKCFL
LELQVISLESGLDASIHDTVENLILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFV
HIVQMFINTS (SEQ ID NO:51)

iC9h2EF-7VHL28H10icZ15

ATGGGAGTGCAGGTGGAAACCATCTCCCCAGGCGACGGGCGCACCTTCCCCAAG
CGCGGCCAGACCTGCGTGGTGCCTACACCGGGATGCTTGAAGATGGAAAGAAA
GTTGATTCTCTCCCGGACAGAAACAAGCCCTTTAAGTTTATGCTAGGCAAGCAGG
AGGTGATCCGAGGCTGGGAAGAAGGGGTTGCCAGATGAGTGTGGGTCAGAGAG
CCAAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCACCCAGGCAT
CATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAACTGGAATCT
GGCGGTGGATCCGGAGTCGACGGATTTGGTGATGTGCGGTGCTCTTGAGAGTTTGA
GGGGAAATGCAGATTTGGCTTACATCCTGAGCATGGAGCCCTGTGGCCACTGCCT
CATTATCAACAATGTGAACTTCTGCCGTGAGTCCGGGCTCCGCACCCGCACTGGC
TCCAACATCGACTGTGAGAAGTTGCGGCGTCGCTTCTCCTCGCTGCATTTTCATGG
TGGAGGTGAAGGGCGACCTGACTGCCAAGAAAATGGTGCTGGCTTTGCTGGAGC
TGGCGCAGCAGGACCACGGTGCTCTGGACTGCTGCGTGGTGGTCATTCTCTCTCA
CGGCTGTCAGGCCAGCCACCTGCAGTTCCCAGGGGCTGTCTACGGCACAGATGG
ATGCCCTGTGTCGGTCGAGAAGATTGTGAACATCTTCAATGGGACCAGCTGCCCC
AGCCTGGGAGGGAAGCCCAAGCTCTTTTTCATCCAGGCCTGTGGTGGGGAGCAG

AAAGATCATGGGTTTGAGGTGGCCTCCACTTCCCCTGAAGACGAGTCCCCTGGCA
GTAACCCCGAGCCAGATGCCACCCCGTTCCAGGAAGGTTTGAGGACCTTCGACC
AGCTGGACGCCATATCTAGTTTGCCACACCCAGTGACATCTTTGTGTCTACTCT
ACTTTCCCAGGTTTTGTTCCTGGAGGGACCCCAAGAGTGGCTCCTGGTACGTTG
AGACCCTGGACGACATCTTTGAGCAGTGGGCTCACTCTGAAGACCTGCAGTCCCT
CCTGCTTAGGGTCGCTAATGCTGTTTCGGTGAAAGGGATTATATAAACAGATGCCT
GGTTGCTTTAATTTCTCCGAAAAAACTTTTCTTTAAAACATCAGCTTCGCGAGC
CGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCCCGGGCC
CATGGAGTTCGGCTTGAGTTGGTTGTTCTTGTGGCGATACTCAAAGGCGTTCAA
TGTCAGTCCAGCTCGTGCAGTCTGGAGCTGAAGTGAAGAAACCTGGGGCTTCA
GTGAAGGTGTCCTGCAAGGCTTCTGGATACACCTTCACTAACTACTGGATCGGAT
GGGTCAAACAGGCCCTGGACAGGGCCTCGAGTGGATTGGAGATATTTACCCTG
GAGGAGGCTATACTAACTACAATGAGAAGTTCAAGGGCAGAGCCACACTGACTG
CAGACACATCCGCCAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGG
AACTGCCGTGTATTACTGTGCAAGAGGAACTGGAGGCGGAGACTACTGGGGCC
AAGGGACTCTGGTCACTGTCTCTTCATTGGAAATAAAGGGCTCTACAAGCGGCTC
AGGAAAACCTGGATCAGGCGAAGGGTCTACGGACATTGTGCTGACACAGTCTCC
TGACTCCCTGGCTGTGTCTCTGGGGGAGAGGGCCACCATCAACTGCAGGGCCAG
CCAAAGTGTCAGTACATCTAGCTATAGTTATATGCACTGGTACCAACAGAAACCA
GGACAGCCACCCAACTCCTCATCAAGTATGCATCCAACCTGGAATCTGGGGTCC
CTGACAGATTCAGTGGCAGTGGGTCTGGGACAGACTTCACCCTCACCATCAGCTC
CCTGCAGGCCGAGGATGTGGCAGTCTATTACTGTCAGCACAGTTGGGAGATTCCC
TACACCTTCGGAGGCGGGACCAAGCTGGAAATCAAACGTACGATTGAAGTTATG
TATCCTCCTCCTTACCTAGACAATGAGAAGAGCAATGGAACCATATCCATGTGA
AAGGGAAACACCTTTGTCCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTG
GGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTG
GCCTTTATTATTTTCTGGGTGCTTTGCGCACGCCCACGCCGCAGCCCCGCCCAAG
AAGATGGCAAAGTCTACATCAACATGCCAGGCAGGGGCACGCGTGTGAAGTTCA
GCAGGAGCGCAGACGCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACG
AGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAAAGACGTGGCC
GGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTG
TACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATG
AAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGT
ACAGCCACCAAGGACACCTACGACGCCCTTACATGCAGGCCCTGCCCCCTCGCG

GACCGCAGTGTACTAATTATGCTCTCTTGAAATTGGCTGGAGATGTTGAGAGCAA
TCCCGGGCCCATGCGCATTAGCAAGCCCCACCTGCGGAGCATCAGCATCCAGTGC
TACCTGTGCCTGCTGCTGAACAGCCACTTCCTGACCGAGGCCGGCATCCACGTGT
TCATCCTGGGCTGCTTCAGCGCCGACTGCCCAAGACCGAGGCCAACTGGGTGA
ACGTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCG
ACGCCACCCTGTACACCGAGAGCGACGTGCACCCCAGCTGCAAGGTGACCGCCA
TGAAGTGCTTTCTGCTGGAAGTGCAGGTGATCAGCCTGGAAGCGGCGACGCCA
GCATCCACGACACCGTGGAGAACCTGATCATCCTGGCCAACAACAGCCTGAGCA
GCAACGGCAACGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAG
AAGAACATCAAAGAGTTTCTGCAGAGCTTCGTGCACATCGTGCAGATGTTTCATCA
ACACCAGCTGA (SEQ ID NO:52)

A corresponding amino acid sequence for iC9h2EF-7VHL28H10icZ15 is as follows:
MGVQVETISPGDGRFTFPRGQTCVVHYTGMLEDGKKVDSSDRNPKFKFMLGKQE
VIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKLESGGG
SGVDGFGDVGALSLRGNADLAYILSMEPCGHCLINNPNFCRESGLRTRTGSNIDCE
KLRRRFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHL
QFPGAVYGTGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFVASTS
PEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTSDIFVSYSTFPGFVSWRDPKSGS
WYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTSA
SRAEGRGSLTTCGDVEENPGPMEFGLSWLFLVAILKGVQCQVQLVQSGAEVKKPGA
SVKVSKASGYTFTNYWIGWVKQAPGQGLEWIGDIYPGGGYTNYNEKFKGRATLT
ADTSASTAYMELSSLRSEDVAVYYCARGTGGGDYWGQGLVTVSSLEIKGSTSGSG
KPGSGEGSTDIVLTQSPDSLAVSLGERATINCRASQSVSTSSYSYMHWYQQKPGQPP
KLLIKYASNLESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQHSWEIPYTFGGGT
KLEIKRTIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVVGGLA
CYSLLVTVAFIIFWVLCARPRRSPAQEDGKVYINMPGRGTRVKFSRSADAPAYQQGQ
NQLYNELNLGRREEYDVLDRRGRDPGEMGGKPRRKNPQGLYNELQKDKMAEAYS
EIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQALPPRGPQCTNYALLKLAGD
VESNPGPMRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFSAGLPKTEANWVN
VISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLESGLDASIHDTV
ENLIILANNSLSSNGNVTESGCKECEELEEKNIKEFLQSFVHIVQMFINTS (SEQ ID
NO:53)

iC9h2G10-5VHL28H28icZ15

ATGGGAGTGCAGGTGGAAACCATCTCCCCAGGCGACGGGCGCACCTTCCCCAAG
CGCGGCCAGACCTGCGTGGTGCCTACACCGGGATGCTTGAAGATGGAAAGAAA
GTTGATTCTCTCCCGGACAGAAACAAGCCCTTTAAGTTTATGCTAGGCAAGCAGG
AGGTGATCCGAGGCTGGGAAGAAGGGGTGCCCAGATGAGTGTGGGTCAGAGAG
CCAAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCACCCAGGCAT
CATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAACTGGAATCT
GGCGGTGGATCCGGAGTCGACGGATTGGTGATGTCGGTGCTCTTGAGAGTTTGA
GGGGAATGCAGATTTGGCTTACATCCTGAGCATGGAGCCCTGTGGCCACTGCCT
CATTATCAACAATGTGAACTTCTGCCGTGAGTCCGGGCTCCGCACCCGCACTGGC
TCCAACATCGACTGTGAGAAGTTGCGGCGTCGCTTCTCCTCGCTGCATTTTCATGG
TGGAGGTGAAGGGCGACCTGACTGCCAAGAAAATGGTGCTGGCTTTGCTGGAGC
TGGCGCAGCAGGACCACGGTGCTCTGGACTGCTGCGTGGTGGTCATTCTCTCTCA
CGGCTGTCAGGCCAGCCACCTGCAGTTCCAGGGGCTGTCTACGGCACAGATGG
ATGCCCTGTGTCGGTCGAGAAGATTGTGAACATCTTCAATGGGACCAGCTGCCCC
AGCCTGGGAGGGAAGCCCAAGCTCTTTTTCATCCAGGCCTGTGGTGGGGAGCAG
AAAGATCATGGGTTTGAGGTGGCCTCCACTTCCCCTGAAGACGAGTCCCCTGGCA
GTAACCCCGAGCCAGATGCCACCCCGTTCCAGGAAGGTTTGAGGACCTTCGACC
AGCTGGACGCCATATCTAGTTTGCCACACCCAGTGACATCTTTGTGTCTACTCT
ACTTTCCAGGTTTTTGTTCCTGGAGGGACCCCAAGAGTGGCTCCTGGTACGTTG
AGACCCTGGACGACATCTTTGAGCAGTGGGCTCACTCTGAAGACCTGCAGTCCCT
CCTGCTTAGGGTCGCTAATGCTGTTTCGGTGAAAGGGATTTATAAACAGATGCCT
GGTTGCTTTAATTTCTCCGGAATAAACTTTTCTTTAAACATCAGCTTCGCGAGC
CGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCCCGGGCC
CATGGAGTTCGGCTTGAGTTGGTTGTTCTTGTGGCGATACTCAAAGGCGTTCAA
TGTCAGTGCAGCTCGTCCAGTCTGGAGCTGAAGTCAAAAAGCCTGGGGCTTCA
GTGAAAGTCTCCTGCAAGGCTTCTGGCTTCACCTTCAGCAGTAGCTATATCAGTT
GGTTGAGGCAGGCCCTGGACAGAGACTTGAGTGGATTGCATGGATTTATGCTG
GAACTGGCGGAACTAGCTATAATCAGAAGTTCACAGGCAAGGCCACACTGACTG
TAGACACATCCGCCAGCACAGCCTACATGGAACCTCAGCAGCCTGAGATCTGAGG
ACACTGCCGTCTATTACTGTGCAAGACATAACCCTCGTTACTATGCTATGGACTA
CTGGGGCCAAGGAACCACAGTCACCGTCTCCTCATTGGAAATAAAGGGCTCTAC
AAGCGGCTCAGGAAAACCTGGATCAGGCGAAGGGTCTACGGACACCCAGATGAC
CCAGTCTCCAAGCTCCCTGTCCGCCAGCGTGGGAGATAGAGTCACCATCACATGC

ATCACCAGCACTGATATTGATGATGATATGAACTGGTACCAGCAGAAGCCAGGG
AAAGCTCCTAAGCTCCTGATTTTCAGAAGGCAATACTCTGCGCCCTGGAGTCCCAT
CCCGATTCTCCGGCAGTGGCTATGGAACAGATTTTACCTTTACAATTAGCTCCCT
GCAGCCAGAAGATATTGCAACCTACTACTGTTTGCAAAGTGATAACCTGCCCTAC
ACCTTCGGAGGGGGGACCAAAGTCGAAATCAAACGTACGATTGAAGTTATGTAT
CCTCCTCCTTACCTAGACAATGAGAAGAGCAATGGAACCATTATCCATGTGAAAG
GGAAACACCTTTGTCCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTG
CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCT
TTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCTGCACAGTGA CTACAT
GAACATGACTCCCCGCCGCCCGGGCCACCCGCAAGCATTACCAGCCCTATGCC
CCACCACGCGACTTCGCAGCCTATCGCTCAACGCGTGTGAAGTTCAGCAGGAGC
GCAGACGCCCCCGCGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCTCAAT
CTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAAAGACGTGGCCGGGACCCT
GAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACAATGA
ACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAAGGCG
AGCGCCGGAGGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACAGCCA
CCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCGGACCGCA
GTGTACTAATTATGCTCTCTTGAAATTGGCTGGAGATGTTGAGAGCAATCCCGGG
CCCATGCGCATTAGCAAGCCCCACCTGCGGAGCATCAGCATCCAGTGCTACCTGT
GCCTGCTGCTGAACAGCCACTTCCTGACCGAGGCCGGCATCCACGTGTTTCATCCT
GGGCTGCTTCAGCGCCGACTGCCCAAGACCGAGGCCAACTGGGTGAACGTGAT
CAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCGACGCCAC
CCTGTACACCGAGAGCGACGTGCACCCCAGCTGCAAGGTGACCGCCATGAAGTG
CTTTCTGCTGGAACCTGCAGGTGATCAGCCTGGAAAGCGGCGACGCCAGCATCCA
CGACACCGTGGAGAACCTGATCATCCTGGCCAACAACAGCCTGAGCAGCAACGG
CAACGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAAGAACA
TCAAAGAGTTTCTGCAGAGCTTCGTGCACATCGTGCAGATGTTTCATCAACACCAG
CTGA (SEQ ID NO:54)

A corresponding amino acid sequence for iC9h2G10-5VHL28H28icZ15 is as follows:
MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFFMLGKQE
VIRGWEEGVAQMSVGQRAKL TISPDYAYGATGHPGIIPPHATLVFDVELLKLESGGG
SGVDGFGDVGALSLRGNADLAYILSMEPCGHCLINNVNFCRESGLRTRTGSNIDCE
KLRRRFSSLHFMVEVKGDLTAKKMVLALLELAQQDHGALDCCVVVILSHGCQASHL

QFPGAVYGTGCPVSVVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFEVASTS
PEDESPGSNPEPDATPFQEGLRTFDQLDAISSLPTPSDIFVSYSTFPGFVSWRDPKSGS
WYVETLDDIFEQWAHSEDLQSLLLRVANAVSVKGIYKQMPGCFNFLRKKLFFKTSA
SRAEGRGSLLTGCDVEENPGPMEFGLSWLFLVAILKGVQCQVQLVQSGAEVKKPGA
SVKVSCKASGFTFSSSYISWLRQAPGQRLEWIAWIYAGTGGTSYNQKFTGKATLTV
TSASTAYMELSSLRSEDTAVYYCARHNPRYYAMDYWGQGTTVTVSSLEIKGSTSGS
GKPGSGEGSTDTQMTQSPSSLSASVGDRVTTITCITSTDIDDDMNWYQQKPGKAPKLLI
SEGNTLRPGVPSRFSGSGYGTDFTFITSSSLQPEDATYYCLQSDNLPYTFGGGKVEIK
RTIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPFPGPSKPFVVLVVVGGVLACYLL
VTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSTRVKFS
RSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYN
ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPRGPQ
CTNYALLKLAGDVESNPGPMRISKPHLRSISIQCYLCLLLNSHFLTEAGIHVFILGCFS
AGLPKTEANWVNVISDLKKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVI
SLESGDASIHTVENLIILANNSLSSNGNVTESGCKECEEELEEKNIKEFLQSFVHIVQM
FINTS (SEQ ID NO:55)

iC9hu2G10-5VHL28H10icZ15

ATGGGAGTGCAGGTGGAAACCATCTCCCCAGGCGACGGGCGCACCTTCCCCAAG
CGCGGCCAGACCTGCGTGGTGCCTACACCGGGATGCTTGAAGATGGAAAGAAA
GTTGATTCTCTCCCGGGACAGAAACAAGCCCTTTAAGTTTATGCTAGGCAAGCAGG
AGGTGATCCGAGGCTGGGAAGAAGGGGTGCCCAGATGAGTGTGGGTCAGAGAG
CCAAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCACCCAGGCAT
CATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAACTGGAATCT
GGCGGTGGATCCGGAGTCGACGGATTTGGTGATGTGCGGTGCTCTTGAGAGTTTGA
GGGGAAATGCAGATTTGGCTTACATCCTGAGCATGGAGCCCTGTGGCCACTGCCT
CATTATCAACAATGTGAACTTCTGCCGTGAGTCCGGGCTCCGCACCCGCACTGGC
TCCAACATCGACTGTGAGAAGTTGCGGCGTCGCTTCTCCTCGCTGCATTTTCATGG
TGGAGGTGAAGGGCGACCTGACTGCCAAGAAAATGGTGCTGGCTTTGCTGGAGC
TGGCGCAGCAGGACCACGGTGCTCTGGACTGCTGCGTGGTGGTCATTCTCTCTCA
CGGCTGTCAGGCCAGCCACCTGCAGTTCCCAGGGGCTGTCTACGGCACAGATGG
ATGCCCTGTGTCGGTCGAGAAGATTGTGAACATCTTCAATGGGACCAGCTGCCCC
AGCCTGGGAGGGAAGCCCAAGCTCTTTTTCATCCAGGCCTGTGGTGGGGAGCAG
AAAGATCATGGGTTTGAGGTGGCCTCCACTTCCCCTGAAGACGAGTCCCCTGGCA

GTAACCCCGAGCCAGATGCCACCCCGTTCCAGGAAGGTTTGAGGACCTTCGACC
AGCTGGACGCCATATCTAGTTTGCCACACCCAGTGACATCTTTGTGTCTACTCT
ACTTTCCCAGGTTTTGTTTCCTGGAGGGACCCCAAGAGTGGCTCCTGGTACGTTG
AGACCCTGGACGACATCTTTGAGCAGTGGGCTCACTCTGAAGACCTGCAGTCCCT
CCTGCTTAGGGTCGCTAATGCTGTTTCGGTGAAAGGGATTATATAACAGATGCCT
GGTTGCTTTAATTTCCCTCCGGAATAAATTTCTTTAAAACATCAGCTTCGCGAGC
CGAGGGCAGGGGAAGTCTTCTAACATGCGGGGACGTGGAGGAAAATCCCGGGCC
CATGGAGTTCGGCTTGAGTTGGTTGTTTCCTTGTGGCGATACTCAAAGGCGTTCAA
TGTCAAGTGCAGCTCGTCCAGTCTGGAGCTGAAGTCAAAAAGCCTGGGGCTTCA
GTGAAAGTCTCCTGCAAGGCTTCTGGCTTCACCTTCAGCAGTAGCTATATCAGTT
GGTTGAGGCAGGCCCTGGACAGAGACTTGAGTGGATTGCATGGATTTATGCTG
GAACTGGCGGAAGTACTAGCTATAATCAGAAGTTCACAGGCAAGGCCACACTGACTG
TAGACACATCCGCCAGCACAGCCTACATGGAAGTTCAGCAGCCTGAGATCTGAGG
ACACTGCCGTCTATTACTGTGCAAGACATAACCCTCGTTACTATGCTATGGACTA
CTGGGGCCAAGGAACCACAGTCACCGTCTCCTCATTGGAAATAAAGGGCTCTAC
AAGCGGCTCAGGAAAACCTGGATCAGGCGAAGGGTCTACGGACACCCAGATGAC
CCAGTCTCCAAGCTCCCTGTCCGCCAGCGTGGGAGATAGAGTCACCATCACATGC
ATCACCAGCACTGATATTGATGATGATATGAACTGGTACCAGCAGAAGCCAGGG
AAAGCTCCTAAGCTCCTGATTTTCTAGAAGGCAATACTCTGCGCCCTGGAGTCCCAT
CCCGATTCTCCGGCAGTGGCTATGGAACAGATTTTACCTTTACAATTAGCTCCCT
GCAGCCAGAAGATATTGCAACCTACTACTGTTTGCAAAGTGATAACCTGCCCTAC
ACCTTCGGAGGGGGGACCAAAGTCGAAATCAAACGTACGATTGAAGTTATGTAT
CCTCCTCCTTACCTAGACAATGAGAAGAGCAATGGAACCATATCCATGTGAAAG
GGAAACACCTTTGTCCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTTGGGTG
CTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACAGTGGCCT
TTATTATTTTCTGGGTGCTTTGCGCACGCCCACGCCGCAGCCCCGCCCAAGAAGA
TGGCAAAGTCTACATCAACATGCCAGGCAGGGGCACGCGTGTGAAGTTCAGCAG
GAGCGCAGACGCCCCCGGTACCAGCAGGGCCAGAACCAGCTCTATAACGAGCT
CAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGACAAAAGACGTGGCCGGGA
CCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCCTCAGGAAGGCCTGTACA
ATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAGTGAGATTGGGATGAAA
GGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTACCAGGGTCTCAGTACA
GCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCCCTGCCCCCTCGCGGAC
CGCAGTGTACTAATTATGCTCTCTTGAAATTGGCTGGAGATGTTGAGAGCAATCC

CGGGCCCATGCGCATTAGCAAGCCCCACCTGCGGAGCATCAGCATCCAGTGCTA
CCTGTGCCTGCTGCTGAACAGCCACTTCCTGACCGAGGCCGGCATCCACGTGTTC
ATCCTGGGCTGCTTCAGCGCCGGACTGCCCAAGACCGAGGCCAACTGGGTGAAC
GTGATCAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCGAC
GCCACCCTGTACACCGAGAGCGACGTGCACCCCAAGCTGCAAGGTGACCGCCATG
AAGTGCTTTCTGCTGGAAGTGCAGGTGATCAGCCTGGAAAGCGGCGACGCCAGC
ATCCACGACACCGTGGAGAACCTGATCATCCTGGCCAACAACAGCCTGAGCAGC
AACGGCAACGTGACCGAGAGCGGCTGCAAAGAGTGCGAGGAACTGGAAGAGAA
GAACATCAAAGAGTTTCTGCAGAGCTTCGTGCACATCGTGCAGATGTTTCATCAAC
ACCAGCTGA (SEQ ID NO:56)

A corresponding amino acid sequence for iC9hu2G10-5VHL28H10icZ15 is as follows:
MGVQVETISPGDGRTPKRGQTCVVHYTGMLDGKKVDSSDRNKPFFKMLGKQE
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QFPGAVYGTGCPVSVEKIVNIFNGTSCPSLGGKPKLFFIQACGGEQKDHGFVASTS
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KKIEDLIQSMHIDATLYTESDVHPSCKVTAMKCFLELQVISLES GDASIHDTVENLIL
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iC9hu2G10-6VHL28H28icZ15

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CAGCGACCTGAAGAAGATCGAGGACCTGATCCAGAGCATGCACATCGACGCCAC
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CTGA (SEQ ID NO:58)

A corresponding amino acid sequence for iC9hu2G10-6VHL28H28icZ15 is as follows:
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FINTS (SEQ ID NO:59)

iC9hu2G10 -6VHL28H10icZ15

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ACCAGCTGA (SEQ ID NO: 60)

A corresponding amino acid sequence for iC9hu2G10 -6VHL28H10icZ15 is as follows:
MGVQVETISPGDGRTPKRGQTCVVHYTGMLDGKKVDSSDRNPKPFKMLGKQE
VIRGWEEGVAQMSVGQRAKLITSPDYAYGATGHPGIIPPHATLVFDVELLKLESGGG
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C. T Cell Receptor (TCR)

[0144] In some embodiments, a TROP-2-targeting genetically engineered antigen receptor includes recombinant TCRs and/or TCRs cloned from naturally occurring T cells, or one or more portions thereof. A "T cell receptor" or "TCR" refers to a molecule that contains a variable α and β chains (also known as TCR α and TCR β , respectively) or a variable γ and δ chains (also known as TCR γ and TCR δ , respectively) and that is capable of specifically binding to an antigen peptide bound to a MHC receptor. In some embodiments, the TCR is in the $\alpha\beta$ form.

[0145] Typically, TCRs that exist in $\alpha\beta$ and $\gamma\delta$ forms are generally structurally similar, but T cells expressing them may have distinct anatomical locations or functions. A TCR can be found on the surface of a cell or in soluble form. Generally, a TCR is found on the surface of T cells (or T lymphocytes) where it is generally responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules. In some embodiments, a TCR also can contain a constant domain, a transmembrane domain and/or a short cytoplasmic tail (see, *e.g.*, Janeway *et al.*, 1997). For example, in some aspects, each chain of the TCR can possess one N-terminal immunoglobulin variable domain, one immunoglobulin constant domain, a transmembrane region, and a short cytoplasmic tail at the C-terminal end. In some embodiments, a TCR is associated with invariant proteins of the CD3 complex involved in mediating signal transduction. Unless otherwise stated, the term "TCR" should be understood to encompass functional TCR fragments thereof. The term also encompasses intact or full-length TCRs, including TCRs in the $\alpha\beta$ form or $\gamma\delta$ form.

[0146] Thus, for purposes herein, reference to a TCR includes any TCR or functional fragment, such as an antigen-binding portion of a TCR that binds to a specific antigenic peptide bound in an MHC molecule, *i.e.* MHC-peptide complex. An "antigen-binding portion" or antigen-binding fragment" of a TCR, which can be used interchangeably, refers to a molecule that contains a portion of the structural domains of a TCR, but that binds the antigen (*e.g.* MHC-peptide complex) to which the full TCR binds. In some cases, an antigen-binding portion contains the variable domains of a TCR, such as variable α chain and variable β chain of a TCR, sufficient to form a binding site for binding to a specific MHC-peptide complex, such as generally where each chain contains three complementarity determining regions.

[0147] In some embodiments, the variable domains of the TCR chains associate to form loops, or complementarity determining regions (CDRs) analogous to immunoglobulins, which confer antigen recognition and determine peptide specificity by forming the binding site of the TCR molecule and determine peptide specificity. Typically, like immunoglobulins, the CDRs

are separated by framework regions (FRs) (see, *e.g.*, Jores *et al.*, 1990; Chothia *et al.*, 1988; Lefranc *et al.*, 2003). In some embodiments, CDR3 is the main CDR responsible for recognizing processed antigen, although CDR1 of the alpha chain has also been shown to interact with the N-terminal part of the antigenic peptide, whereas CDR1 of the beta chain interacts with the C-terminal part of the peptide. CDR2 is thought to recognize the MHC molecule. In some embodiments, the variable region of the β -chain can contain a further hypervariability (HV4) region.

[0148] In some embodiments, the TCR chains contain a constant domain. For example, like immunoglobulins, the extracellular portion of TCR chains (*e.g.*, α -chain, β -chain) can contain two immunoglobulin domains, a variable domain (*e.g.*, V_α or V_β ; typically amino acids 1 to 116 based on Kabat numbering Kabat *et al.*, "Sequences of Proteins of Immunological Interest, US Dept. Health and Human Services, Public Health Service National Institutes of Health, 1991, 5th ed.) at the N-terminus, and one constant domain (*e.g.*, α -chain constant domain or C_α , typically amino acids 117 to 259 based on Kabat, β -chain constant domain or C_β , typically amino acids 117 to 295 based on Kabat) adjacent to the cell membrane. For example, in some cases, the extracellular portion of the TCR formed by the two chains contains two membrane-proximal constant domains, and two membrane-distal variable domains containing CDRs. The constant domain of the TCR domain contains short connecting sequences in which a cysteine residue forms a disulfide bond, making a link between the two chains. In some embodiments, a TCR may have an additional cysteine residue in each of the α and β chains such that the TCR contains two disulfide bonds in the constant domains.

[0149] In some embodiments, the TCR chains can contain a transmembrane domain. In some embodiments, the transmembrane domain is positively charged. In some cases, the TCR chains contains a cytoplasmic tail. In some cases, the structure allows the TCR to associate with other molecules like CD3. For example, a TCR containing constant domains with a transmembrane region can anchor the protein in the cell membrane and associate with invariant subunits of the CD3 signaling apparatus or complex.

[0150] Generally, CD3 is a multi-protein complex that can possess three distinct chains (γ , δ , and ϵ) in mammals and the ζ -chain. For example, in mammals the complex can contain a CD3 γ chain, a CD3 δ chain, two CD3 ϵ chains, and a homodimer of CD3 ζ chains. The CD3 γ , CD3 δ , and CD3 ϵ chains are highly related cell surface proteins of the immunoglobulin superfamily containing a single immunoglobulin domain. The transmembrane regions of the CD3 γ , CD3 δ , and CD3 ϵ chains are negatively charged, which is a characteristic that allows

these chains to associate with the positively charged T cell receptor chains. The intracellular tails of the CD3 γ , CD3 δ , and CD3 ϵ chains each contain a single conserved motif known as an immunoreceptor tyrosine -based activation motif or ITAM, whereas each CD3 ζ chain has three. Generally, ITAMs are involved in the signaling capacity of the TCR complex. These accessory molecules have negatively charged transmembrane regions and play a role in propagating the signal from the TCR into the cell. The CD3- and ζ -chains, together with the TCR, form what is known as the T cell receptor complex.

[0151] In some embodiments, the TCR may be a heterodimer of two chains α and β (or optionally γ and δ) or it may be a single chain TCR construct. In some embodiments, the TCR is a heterodimer containing two separate chains (α and β chains or γ and δ chains) that are linked, such as by a disulfide bond or disulfide bonds. In some embodiments, a TCR for a target antigen (*e.g.*, a cancer antigen) is identified and introduced into the cells. In some embodiments, nucleic acid encoding the TCR can be obtained from a variety of sources, such as by polymerase chain reaction (PCR) amplification of publicly available TCR DNA sequences. In some embodiments, the TCR is obtained from a biological source, such as from cells such as from a T cell (*e.g.* cytotoxic T cell), T cell hybridomas or other publicly available source. In some embodiments, the T cells can be obtained from *in vivo* isolated cells. In some embodiments, a high-affinity T cell clone can be isolated from a patient, and the TCR isolated. In some embodiments, the T cells can be a cultured T cell hybridoma or clone. In some embodiments, the TCR clone for a target antigen has been generated in transgenic mice engineered with human immune system genes (*e.g.*, the human leukocyte antigen system, or HLA). See, *e.g.*, tumor antigens (see, *e.g.*, Parkhurst *et al.*, 2009 and Cohen *et al.*, 2005). In some embodiments, phage display is used to isolate TCRs against a target antigen (see, *e.g.*, Varela-Rohena *et al.*, 2008 and Li, 2005). In some embodiments, the TCR or antigen-binding portion thereof can be synthetically generated from knowledge of the sequence of the TCR.

III. Cytokines

[0152] One or more cytokines may be utilized with one or more TROP-2-targeting genetically engineered receptors, such as TROP-2-specific CARs. In some cases, one or more cytokines are present on the same vector molecule as the engineered receptor, although in other cases they are on separate vector molecules. In particular embodiments, one or more cytokines are co-expressed from the same vector as the engineered receptor. One or more cytokines may be produced as a separate polypeptide from the TROP-2-specific receptor. As one example,

Interleukin-15 (IL-15), is utilized. IL-15 may be employed because, for example, it is tissue restricted and only under pathologic conditions is it observed at any level in the serum, or systemically. IL-15 possesses several attributes that are desirable for adoptive therapy. IL-15 is a homeostatic cytokine that induces development and cell proliferation of natural killer cells, promotes the eradication of established tumors *via* alleviating functional suppression of tumor-resident cells, and inhibits activation-induced cell death. In addition to IL-15, other cytokines are envisioned. These include, but are not limited to, cytokines, chemokines, and other molecules that contribute to the activation and proliferation of cells used for human application. As one example, the one or more cytokines are IL-15, IL-12, IL-2, IL-18, IL-21, IL-23, IL-7, or combination thereof. NK cells expressing IL-15 may be utilized and are capable of continued supportive cytokine signaling, which is useful for their survival post-infusion.

[0153] In specific embodiments, NK cells express one or more exogenously provided cytokines. The cytokine may be exogenously provided to the NK cells because it is expressed from an expression vector within the cell and/or because it is provided in a culture medium of the cells. In an alternative case, an endogenous cytokine in the cell is upregulated upon manipulation of regulation of expression of the endogenous cytokine, such as genetic recombination at the promoter site(s) of the cytokine. In cases wherein the cytokine is provided on an expression construct to the cell, the cytokine may be encoded from the same vector as a suicide gene. The cytokine may be expressed as a separate polypeptide molecule from a suicide gene and as a separate polypeptide from an engineered receptor of the cell. In some embodiments, the present disclosure concerns co-utilization of CAR and/or TCR vectors with IL-15, particularly in NK cells.

IV. Suicide Genes

[0154] In particular embodiments, a suicide gene is utilized in conjunction with cell therapy of any kind to control its use and allow for termination of the cell therapy at a desired event and/or time. The suicide gene is employed in transduced cells for the purpose of eliciting death for the transduced cells when needed. The TROP-2-targeting cells of the present disclosure that have been modified to harbor a vector encompassed by the disclosure may comprise one or more suicide genes. In some embodiments, the term “suicide gene” as used herein is defined as a gene which, upon administration of a prodrug or other agent, effects transition of a gene product to a compound which kills its host cell. In other embodiments, a suicide gene encodes a gene product that is, when desired, targeted by an agent (such as an antibody) that targets the

suicide gene product. A “suicide gene product” describes a protein or polypeptide encoded by a suicide gene.

[0155] Examples of suicide gene/prodrug combinations which may be used are Herpes Simplex Virus-thymidine kinase (HSV-tk) and ganciclovir, acyclovir, or FIAU; oxidoreductase and cycloheximide; cytosine deaminase and 5-fluorocytosine; thymidine kinase thymidilate kinase (Tdk::Tmk) and AZT; and deoxycytidine kinase and cytosine arabinoside. The *E.coli* purine nucleoside phosphorylase, a so-called suicide gene that converts the prodrug 6-methylpurine deoxyriboside to toxic purine 6-methylpurine, may be used. Other examples of suicide genes used with prodrug therapy are the *E. coli* cytosine deaminase gene and the HSV thymidine kinase gene.

[0156] Exemplary suicide genes also include CD20, CD52, EGFRv3, or inducible caspase 9. In one embodiment, a truncated version of EGFR variant III (EGFRv3) may be used as a suicide antigen that can be ablated by Cetuximab. Further suicide genes known in the art that may be used in the present disclosure include Purine nucleoside phosphorylase (PNP), Cytochrome p450 enzymes (CYP), Carboxypeptidases (CP), Carboxylesterase (CE), Nitroreductase (NTR), Guanine Ribosyltransferase (XGRTP), Glycosidase enzymes, Methionine- α,γ -lyase (MET), and Thymidine phosphorylase (TP). In some embodiments, an inducible caspase 9 (iC9) is used. An example iC9 is described in, for example, Yagyu S, et al. Mol Ther. 2015 Sep;23(9):1475-85, incorporated by reference herein in its entirety.

[0157] In particular embodiments, vectors that encode the TROP-2-targeting CAR, or any vector in a NK cell encompassed herein, include one or more suicide genes. The suicide gene may or may not be on the same vector as a TROP-2-targeting CAR. In cases wherein the suicide gene is present on the same vector as the TROP-2-targeting CAR, the suicide gene and the CAR may be separated by an IRES or 2A element, for example.

V. Vectors

[0158] The TROP-2-targeting CARs may be delivered to the recipient immune cells by any suitable vector, including by a viral vector or by a non-viral vector. Examples of viral vectors include at least retroviral, lentiviral, adenoviral, or adeno-associated viral vectors. Examples of non-viral vectors include at least plasmids, transposons, lipids, nanoparticles, and so forth.

[0159] In cases wherein the immune cell is transduced with a vector encoding the TROP-2-targeting receptor and also requires transduction of another gene or genes into the cell, such as a suicide gene and/or cytokine and/or an optional therapeutic gene product, the TROP-2-

targeting receptor, suicide gene, cytokine, and optional therapeutic gene may or may not be comprised on or with the same vector. In some cases, the TROP-2-targeting CAR, suicide gene, cytokine, and optional therapeutic gene are expressed from the same vector molecule, such as the same viral vector molecule. In such cases, the expression of the TROP-2-targeting CAR, suicide gene, cytokine, and optional therapeutic gene may or may not be regulated by the same regulatory element(s). When the TROP-2-targeting CAR, suicide gene, cytokine, and optional therapeutic gene are on the same vector, they may or may not be expressed as separate polypeptides. In cases wherein they are expressed as separate polypeptides, they may be separated on the vector by a 2A element or IRES element (or both kinds may be used on the same vector once or more than once), for example.

A. General Embodiments

[0160] One of skill in the art would be well-equipped to construct a vector through standard recombinant techniques (see, for example, Sambrook *et al.*, 2001 and Ausubel *et al.*, 1996, both incorporated herein by reference) for the expression of the antigen receptors of the present disclosure.

1. Regulatory Elements

[0161] Expression cassettes included in vectors useful in the present disclosure in particular contain (in a 5'-to-3' direction) a eukaryotic transcriptional promoter operably linked to a protein-coding sequence, splice signals including intervening sequences, and a transcriptional termination/polyadenylation sequence. The promoters and enhancers that control the transcription of protein encoding genes in eukaryotic cells may be comprised of multiple genetic elements. The cellular machinery is able to gather and integrate the regulatory information conveyed by each element, allowing different genes to evolve distinct, often complex patterns of transcriptional regulation. A promoter used in the context of the present disclosure includes constitutive, inducible, and tissue-specific promoters, for example. In cases wherein the vector is utilized for the generation of cancer therapy, a promoter may be effective under conditions of hypoxia.

2. Promoter/Enhancers

[0162] The expression constructs provided herein comprise a promoter to drive expression of the antigen receptor and other cistron gene products. A promoter generally comprises a

sequence that functions to position the start site for RNA synthesis. The best known example of this is the TATA box, but in some promoters lacking a TATA box, such as, for example, the promoter for the mammalian terminal deoxynucleotidyl transferase gene and the promoter for the SV40 late genes, a discrete element overlying the start site itself helps to fix the place of initiation. Additional promoter elements regulate the frequency of transcriptional initiation. Typically, these are located in the region upstream of the start site, although a number of promoters have been shown to contain functional elements downstream of the start site as well. To bring a coding sequence “under the control of” a promoter, one positions the 5' end of the transcription initiation site of the transcriptional reading frame “downstream” of (*i.e.*, 3' of) the chosen promoter. The “upstream” promoter stimulates transcription of the DNA and promotes expression of the encoded RNA.

[0163] 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 tk promoter, for example, 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. A promoter may or may not be used in conjunction with an “enhancer,” which refers to a cis-acting regulatory sequence involved in the transcriptional activation of a nucleic acid sequence.

[0164] A promoter may be one naturally associated with a nucleic acid sequence, as may be obtained by isolating the 5' non-coding sequences located upstream of the coding segment and/or exon. Such a promoter can be referred to as “endogenous.” Similarly, an enhancer may be one naturally associated with a nucleic acid sequence, located either downstream or upstream of that sequence. Alternatively, certain advantages will be gained by positioning the coding nucleic acid segment under the control of a recombinant or heterologous promoter, which refers to a promoter that is not normally associated with a nucleic acid sequence in its natural environment. A recombinant or heterologous enhancer refers also to an enhancer not normally associated with a nucleic acid sequence in its natural environment. Such promoters or enhancers may include promoters or enhancers of other genes, and promoters or enhancers isolated from any other virus, or prokaryotic or eukaryotic cell, and promoters or enhancers not “naturally occurring,” *i.e.*, containing different elements of different transcriptional regulatory regions, and/or mutations that alter expression. For example, promoters that are most commonly used in recombinant DNA construction include the β -lactamase (penicillinase), lactose and tryptophan (trp-) promoter systems. In addition to producing nucleic acid

sequences of promoters and enhancers synthetically, sequences may be produced using recombinant cloning and/or nucleic acid amplification technology, including PCR™, in connection with the compositions disclosed herein. Furthermore, it is contemplated that the control sequences that direct transcription and/or expression of sequences within non-nuclear organelles such as mitochondria, chloroplasts, and the like, can be employed as well.

[0165] Naturally, it will be important to employ a promoter and/or enhancer that effectively directs the expression of the DNA segment in the organelle, cell type, tissue, organ, or organism chosen for expression. Those of skill in the art of molecular biology generally know the use of promoters, enhancers, and cell type combinations for protein expression, (*see*, for example Sambrook *et al.* 1989, incorporated herein by reference). The promoters employed may be constitutive, tissue-specific, inducible, and/or useful under the appropriate conditions to direct high level expression of the introduced DNA segment, such as is advantageous in the large-scale production of recombinant proteins and/or peptides. The promoter may be heterologous or endogenous.

[0166] Additionally, any promoter/enhancer combination (as per, for example, the Eukaryotic Promoter Data Base EPDB, through world wide web at epd.isb-sib.ch/) could also be used to drive expression. Use of a T3, T7 or SP6 cytoplasmic expression system is another possible embodiment. Eukaryotic cells can support cytoplasmic transcription from certain bacterial promoters if the appropriate bacterial polymerase is provided, either as part of the delivery complex or as an additional genetic expression construct.

[0167] Non-limiting examples of promoters include early or late viral promoters, such as, SV40 early or late promoters, cytomegalovirus (CMV) immediate early promoters, Rous Sarcoma Virus (RSV) early promoters; eukaryotic cell promoters, such as, *e. g.*, beta actin promoter, GADPH promoter, metallothionein promoter; and concatenated response element promoters, such as cyclic AMP response element promoters (*cre*), serum response element promoter (*sre*), phorbol ester promoter (TPA) and response element promoters (*tre*) near a minimal TATA box. It is also possible to use human growth hormone promoter sequences (*e.g.*, the human growth hormone minimal promoter described at GenBank®, accession no. X05244, nucleotide 283-341) or a mouse mammary tumor promoter (available from the ATCC, Cat. No. ATCC 45007). In certain embodiments, the promoter is CMV IE, dectin-1, dectin-2, human CD11c, F4/80, SM22, RSV, SV40, Ad MLP, beta-actin, MHC class I or MHC class II promoter, however any other promoter that is useful to drive expression of the therapeutic gene is applicable to the practice of the present disclosure.

[0168] In certain aspects, methods of the disclosure also concern enhancer sequences, *i.e.*, nucleic acid sequences that increase a promoter's activity and that have the potential to act in cis, and regardless of their orientation, even over relatively long distances (up to several kilobases away from the target promoter). However, enhancer function is not necessarily restricted to such long distances as they may also function in close proximity to a given promoter.

3. Initiation Signals and Linked Expression

[0169] A specific initiation signal also may be used in the expression constructs provided in the present disclosure for efficient translation of coding sequences. These signals include the ATG initiation codon or adjacent sequences. Exogenous translational control signals, including the ATG initiation codon, may need to be provided. One of ordinary skill in the art would readily be capable of determining this and providing the necessary signals. It is well known that the initiation codon must be "in-frame" with the reading frame of the desired coding sequence to ensure translation of the entire insert. The exogenous translational control signals and initiation codons can be either natural or synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements.

[0170] In certain embodiments, the use of internal ribosome entry sites (IRES) elements are used to create multigene, or polycistronic messages. IRES elements are able to bypass the ribosome scanning model of 5' methylated Cap dependent translation and begin translation at internal sites. IRES elements from two members of the picornavirus family (polio and encephalomyocarditis) have been described, as well an IRES from a mammalian message. IRES elements can be linked to heterologous open reading frames. Multiple open reading frames can be transcribed together, each separated by an IRES, creating polycistronic messages. By virtue of the IRES element, each open reading frame is accessible to ribosomes for efficient translation. Multiple genes can be efficiently expressed using a single promoter/enhancer to transcribe a single message.

[0171] As detailed elsewhere herein, certain 2A sequence elements could be used to create linked- or co-expression of genes in the constructs provided in the present disclosure. For example, cleavage sequences could be used to co-express genes by linking open reading frames to form a single cistron. An exemplary cleavage sequence is the equine rhinitis A virus (E2A) or the F2A (Foot-and-mouth disease virus 2A) or a "2A-like" sequence (*e.g.*, *Thosea asigna* virus 2A; T2A) or porcine teschovirus-1 (P2A). In specific embodiments, in a single vector

the multiple 2A sequences are non-identical, although in alternative embodiments the same vector utilizes two or more of the same 2A sequences. Examples of 2A sequences are provided in US 2011/0065779 which is incorporated by reference herein in its entirety.

4. Origins of Replication

[0172] In order to propagate a vector in a host cell, it may contain one or more origins of replication sites (often termed “ori”), for example, a nucleic acid sequence corresponding to oriP of EBV as described above or a genetically engineered oriP with a similar or elevated function in programming, which is a specific nucleic acid sequence at which replication is initiated. Alternatively a replication origin of other extra-chromosomally replicating virus as described above or an autonomously replicating sequence (ARS) can be employed.

5. Selection and Screenable Markers

[0173] In some embodiments, NK cells comprising a TROP-2-targeting receptor construct of the present disclosure may be identified *in vitro* or *in vivo* by including a marker in the expression vector. Such markers would confer an identifiable change to the cell permitting easy identification of cells containing the expression vector. Generally, a selection marker is one that confers a property that allows for selection. A positive selection marker is one in which the presence of the marker allows for its selection, while a negative selection marker is one in which its presence prevents its selection. An example of a positive selection marker is a drug resistance marker.

[0174] Usually the inclusion of a drug selection marker aids in the cloning and identification of transformants, for example, genes that confer resistance to neomycin, puromycin, hygromycin, DHFR, GPT, zeocin and histidinol are useful selection markers. In addition to markers conferring a phenotype that allows for the discrimination of transformants based on the implementation of conditions, other types of markers including screenable markers such as GFP, whose basis is colorimetric analysis, are also contemplated. Alternatively, screenable enzymes as negative selection markers such as herpes simplex virus thymidine kinase (*tk*) or chloramphenicol acetyltransferase (CAT) may be utilized. One of skill in the art would also know how to employ immunologic markers, possibly in conjunction with FACS analysis. The marker used is not believed to be important, so long as it is capable of being expressed simultaneously with the nucleic acid encoding a gene product. Further examples of selection and screenable markers are well known to one of skill in the art.

B. Multicistronic Vectors

[0175] In particular embodiments, the TROP-2-targeting receptor, optional suicide gene, optional cytokine, and/or optional therapeutic gene are expressed from a multicistronic vector (The term “cistron” as used herein refers to a nucleic acid sequence from which a gene product may be produced). In specific embodiments, the multicistronic vector encodes the TROP-2-targeting receptor, the suicide gene, and at least one cytokine, and/or engineered receptor, such as a T-cell receptor and/or an additional non- TROP-2-targeting CAR. In some cases, the multicistronic vector encodes at least one TROP-2-targeting CAR, at least one TNF-alpha mutant, and at least one cytokine. The cytokine may be of a particular type of cytokine, such as human or mouse or any species. In specific cases, the cytokine is IL15, IL12, IL2, IL18, and/or IL21.

[0176] In certain embodiments, the present disclosure provides a flexible, modular system (the term “modular” as used herein refers to a cistron or component of a cistron that allows for interchangeability thereof, such as by removal and replacement of an entire cistron or of a component of a cistron, respectively, for example by using standard recombination techniques) utilizing a polycistronic vector having the ability to express multiple cistrons at substantially identical levels. The system may be used for cell engineering allowing for combinatorial expression (including overexpression) of multiple genes. In specific embodiments, one or more of the genes expressed by the vector includes one, two, or more antigen receptors. The multiple genes may comprise, but are not limited to, CARs, TCRs, cytokines, chemokines, homing receptors, CRISPR/Cas9-mediated gene mutations, decoy receptors, cytokine receptors, chimeric cytokine receptors, and so forth. The vector may further comprise: (1) one or more reporters, for example fluorescent or enzymatic reporters, such as for cellular assays and animal imaging; (2) one or more cytokines or other signaling molecules; and/or (3) a suicide gene.

[0177] In specific cases, the vector may comprise at least 4 cistrons separated by cleavage sites of any kind, such as 2A cleavage sites. The vector may or may not be Moloney Murine Leukemia Virus (MoMLV or MMLV)-based including the 3' and 5' LTR with the psi packaging sequence in a pUC19 backbone. The vector may comprise 4 or more cistrons with three or more 2A cleavage sites and multiple ORFs for gene swapping. The system allows for combinatorial overexpression of multiple genes (7 or more) that are flanked by restriction site(s) for rapid integration through subcloning, and the system also includes at least three 2A self-cleavage sites, in some embodiments. Thus, the system allows for expression of multiple

CARs, TCRs, signaling molecules, cytokines, cytokine receptors, and/or homing receptors. This system may also be applied to other viral and non-viral vectors, including but not limited to lentivirus, adenovirus AAV, as well as non-viral plasmids.

[0178] The modular nature of the system also enables efficient subcloning of a gene into each of the 4 cistrons in the polycistronic expression vector and the swapping of genes, such as for rapid testing. Restriction sites strategically located in the polycistronic expression vector allow for swapping of genes with efficiency.

[0179] Embodiments of the disclosure encompass systems that utilize a polycistronic vector wherein at least part of the vector is modular, for example by allowing removal and replacement of one or more cistrons (or component(s) of one or more cistrons), such as by utilizing one or more restriction enzyme sites whose identity and location are specifically selected to facilitate the modular use of the vector. The vector also has embodiments wherein multiple of the cistrons are translated into a single polypeptide and processed into separate polypeptides, thereby imparting an advantage for the vector to express separate gene products in substantially equimolar concentrations.

[0180] The vector of the disclosure is configured for modularity to be able to change one or more cistrons of the vector and/or to change one or more components of one or more particular cistrons. The vector may be designed to utilize unique restriction enzyme sites flanking the ends of one or more cistrons and/or flanking the ends of one or more components of a particular cistron.

[0181] Embodiments of the disclosure include polycistronic vectors comprising at least two, at least three, or at least four cistrons each flanked by one or more restriction enzyme sites, wherein at least one cistron encodes for at least one antigen receptor. In some cases, two, three, four, or more of the cistrons are translated into a single polypeptide and cleaved into separate polypeptides, whereas in other cases multiple of the cistrons are translated into a single polypeptide and cleaved into separate polypeptides. Adjacent cistrons on the vector may be separated by a self cleavage site, such as a 2A self cleavage site. In some cases each of the cistrons express separate polypeptides from the vector. On particular cases, adjacent cistrons on the vector are separated by an IRES element.

[0182] In certain embodiments, the present disclosure provides a system for cell engineering allowing for combinatorial expression, including overexpression, of multiple cistrons that may include one, two, or more antigen receptors, for example. In particular embodiments, the use of a polycistronic vector as described herein allows for the vector to produce equimolar levels of multiple gene products from the same mRNA. The multiple genes

may comprise, but are not limited to, CARs, TCRs, cytokines, chemokines, homing receptors, CRISPR/Cas9-mediated gene mutations, decoy receptors, cytokine receptors, chimeric cytokine receptors, and so forth. The vector may further comprise one or more fluorescent or enzymatic reporters, such as for cellular assays and animal imaging. The vector may also comprise a suicide gene product for termination of cells harboring the vector when they are no longer needed or become deleterious to a host to which they have been provided.

[0183] In specific embodiments, the vector is a viral vector (retroviral vector, lentiviral vector, adenoviral vector, or adeno-associated viral vector, for example) or a non-viral vector. The vector may comprise a Moloney Murine Leukemia Virus (MMLV) 5' LTR, 3' LTR, and/or psi packaging element. In specific cases, the psi packaging is incorporated between the 5' LTR and the antigen receptor coding sequence. The vector may or may not comprise pUC19 sequence. In some aspects of the vector, at least one cistron encodes for a cytokine (IL-15, IL-7, IL-21, IL-23, IL-18, IL-12, or IL-2, for example), chemokine, cytokine receptor, and/or homing receptor.

[0184] When 2A cleavage sites are utilized in the vector, the 2A cleavage site may comprise a P2A, T2A, E2A and/or F2A site.

[0185] A restriction enzyme site may be of any kind and may include any number of bases in its recognition site, such as between 4 and 8 bases; the number of bases in the recognition site may be at least 4, 5, 6, 7, 8, or more. The site when cut may produce a blunt cut or sticky ends. The restriction enzyme may be of Type I, Type II, Type III, or Type IV, for example. Restriction enzyme sites may be obtained from available databases, such as Integrated relational Enzyme database (IntEnz) or BRENDA (The Comprehensive Enzyme Information System).

[0186] Exemplary vectors may be circular and by convention, where position 1 (12 o'clock position at the top of the circle, with the rest of the sequence in clock-wise direction) is set at the start of 5' LTR.

[0187] In embodiments wherein self-cleaving 2A peptides are utilized, the 2A peptides may be 18–22 amino-acid (aa)-long viral oligopeptides that mediate “cleavage” of polypeptides during translation in eukaryotic cells. The designation “2A” refers to a specific region of the viral genome and different viral 2As have generally been named after the virus they were derived from. The first discovered 2A was F2A (foot-and-mouth disease virus), after which E2A (equine rhinitis A virus), P2A (porcine teschovirus-1 2A), and T2A (thossea asigna virus 2A) were also identified. The mechanism of 2A-mediated “self-cleavage” was discovered to

be ribosome skipping the formation of a glycyl-prolyl peptide bond at the C-terminus of the 2A.

[0188] In specific cases, the vector may be a γ -retroviral transfer vector. The retroviral transfer vector may comprise a backbone based on a plasmid, such as the pUC19 plasmid (large fragment (2.63kb) in between HindIII and EcoRI restriction enzyme sites). The backbone may carry viral components from Moloney Murine Leukemia Virus (MoMLV) including 5' LTR, psi packaging sequence, and 3' LTR. LTRs are long terminal repeats found on either side of a retroviral provirus, and in the case of a transfer vector, brackets the genetic cargo of interest, such as TROP-2-targeting CARs and associated components. The psi packaging sequence, which is a target site for packaging by nucleocapsid, is also incorporated in *cis*, sandwiched between the 5' LTR and the CAR coding sequence. Thus, the basic structure of an example of a transfer vector can be configured as such: pUC19 sequence – 5' LTR – psi packaging sequence – genetic cargo of interest – 3' LTR – pUC19 sequence. This system may also be applied to other viral and non-viral vectors, including but not limited to lentivirus, adenovirus AAV, as well as non-viral plasmids.

VI. Cells

[0189] The present disclosure encompasses immune cells or stem cells of any kind that harbor at least one vector that encodes a TROP-2-targeting receptor and that also may encode at least one cytokine and/or at least one suicide gene. In some cases, different vectors encode the CAR vs. encodes the suicide gene and/or cytokine. The immune cells, including NK cells, may be derived from cord blood (including pooled cord blood from multiple sources), peripheral blood, induced pluripotent stem cells (iPSCs), hematopoietic stem cells (HSCs), bone marrow, or a mixture thereof. The NK cells may be derived from a cell line such as, but not limited to, NK-92 cells, for example. The NK cell may be a cord blood mononuclear cell, such as a CD56⁺ NK cell.

[0190] The present disclosure encompasses immune or other cells of any kind, including conventional T cells, gamma-delta T cells, NKT and invariant NK T cells, regulatory T cells, macrophages, B cells, dendritic cells, mesenchymal stromal cells (MSCs), or a mixture thereof.

[0191] In some cases, the cells have been expanded in the presence of an effective amount of universal antigen presenting cells (UAPCs), including in any suitable ratio. The cells may be cultured with the UAPCs at a ratio of 10:1 to 1:10; 9:1 to 1:9; 8:1 to 1:8; 7:1 to 1:7; 6:1 to 1:6; 5:1 to 1:5; 4:1 to 1:4; 3:1 to 1:3; 2:1 to 1:2; or 1:1, including at a ratio of 1:2, for example.

In some cases, the NK cells were expanded in the presence of IL-2, such as at a concentration of 10-500, 10-400, 10-300, 10-200, 10-100, 10-50, 100-500, 100-400, 100-300, 100-200, 200-500, 200-400, 200-300, 300-500, 300-400, or 400-500 U/mL.

[0192] Following genetic modification with the vector(s), the NK cells may be immediately infused or may be stored. In certain aspects, following genetic modification, the cells may be propagated for days, weeks, or months *ex vivo* as a bulk population within about 1, 2, 3, 4, 5 days or more following gene transfer into cells. In a further aspect, the transfectants are cloned and a clone demonstrating presence of a single integrated or episomally maintained expression cassette or plasmid, and expression of the TROP-2-targeting CAR is expanded *ex vivo*. The clone selected for expansion demonstrates the capacity to specifically recognize and lyse TROP-2-expressing target cells. The recombinant immune cells may be expanded by stimulation with IL-2, or other cytokines that bind the common gamma-chain (*e.g.*, IL-7, IL-12, IL-15, IL-21, IL-23, and others). The recombinant immune cells may be expanded by stimulation with artificial antigen presenting cells. In a further aspect, the genetically modified cells may be cryopreserved.

[0193] Embodiments of the disclosure encompass cells that express one or more TROP-2-targeting CARs and one or more suicide genes as encompassed herein. The NK cell comprises a recombinant nucleic acid that encodes one or more TROP-2-targeting CARs and one or more engineered nonsecretable, membrane bound TNF-alpha mutant polypeptides, in specific embodiments. In specific embodiments, in addition to expressing one or more TROP-2-targeting CARs and TNF-alpha mutant polypeptides, the cell also comprises a nucleic acid that encodes one or more therapeutic gene products.

[0194] The cells may be obtained from an individual directly or may be obtained from a depository or other storage facility. The cells as therapy may be autologous or allogeneic with respect to the individual to which the cells are provided as therapy.

[0195] The cells may be from an individual in need of therapy for a medical condition, and following their manipulation to express the TROP-2-targeting CAR, optional suicide gene, optional cytokine(s), and optional therapeutic gene product(s) (using standard techniques for transduction and expansion for adoptive cell therapy, for example), they may be provided back to the individual from which they were originally sourced. In some cases, the cells are stored for later use for the individual or another individual.

[0196] The immune cells may be comprised in a population of cells, and that population may have a majority that are transduced with one or more TROP-2-targeting receptors and/or one or more suicide genes and/or one or more cytokines. A cell population may comprise 51,

52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% of immune cells that are transduced with one or more TROP-2-targeting receptors and/or one or more suicide genes and/or one or more cytokines. The one or more TROP-2-targeting receptors and/or one or more suicide genes and/or one or more cytokines may be separate polypeptides.

[0197] The immune cells may be produced with the one or more TROP-2-targeting receptors and/or one or more suicide genes and/or one or more cytokines for the intent of being modular with respect to a specific purpose. For example, cells may be generated, including for commercial distribution, expressing a TROP-2-targeting CARs and/or one or more suicide genes and/or one or more cytokines (or distributed with a nucleic acid that encodes the mutant for subsequent transduction), and a user may modify them to express one or more other genes of interest (including therapeutic genes) dependent upon their intended purpose(s). For instance, an individual interested in treating TROP-2-positive cells, including TROP-2-positive cancer, may obtain or generate suicide gene-expressing cells (or heterologous cytokine-expressing cells) and modify them to express a receptor comprising a TROP-2-specific scFv, or *vice versa*.

[0198] In particular embodiments, NK cells are utilized, and the genome of the transduced NK cells expressing the one or more TROP-2-targeting CARs and/or one or more suicide genes and/or one or more cytokines may be modified. The genome may be modified in any manner, but in specific embodiments the genome is modified by CRISPR gene editing, for example. The genome of the cells may be modified to enhance effectiveness of the cells for any purpose.

VII. Gene Editing of TROP-2-specific CAR Cells

[0199] In particular embodiments, cells comprising at least a TROP-2-specific engineered receptor are gene edited to modify expression of one or more endogenous genes in the cell. In specific cases, the TROP-2-specific CAR cells are modified to have reduced levels of expression of one or more endogenous genes, including inhibition of expression of one or more endogenous genes (that may be referred to as knocked out). Such cells may or may not be expanded.

[0200] In particular cases, one or more endogenous genes of the TROP-2-specific CAR cells are modified, such as disrupted in expression where the expression is reduced in part or in full. In specific cases, one or more genes are knocked down or knocked out using processes

of the disclosure. In specific cases, multiple genes are knocked down or knocked out, and this may or may not occur in the same step in their production. The genes that are edited in the TROP-2-specific CAR cells may be of any kind, but in specific embodiments the genes are genes whose gene products inhibit activity and/or proliferation of the TROP-2-specific CAR cells, including TROP-2-specific CAR NK cells, such as those derived from cord blood, as one example. In specific cases the genes that are edited in the TROP-2-specific CAR cells allow the TROP-2-specific CAR cells to work more effectively in a tumor microenvironment. In specific cases, the genes are one or more of NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFBR2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, and CD7. In specific embodiments, the TGFBR2 gene is knocked out or knocked down in the TROP-2-specific CAR cells.

[0201] In some embodiments, the gene editing is carried out using one or more DNA-binding nucleic acids, such as alteration *via* an RNA-guided endonuclease (RGEN). For example, the alteration can be carried out using clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins; in some embodiments, CpF1 is utilized instead of Cas9. In general, "CRISPR system" refers collectively to transcripts and other elements involved in the expression of or directing the activity of CRISPR-associated ("Cas") genes, including sequences encoding a Cas gene, a tracr (trans-activating CRISPR) sequence (*e.g.*, tracrRNA or an active partial tracrRNA), a tracr-mate sequence (encompassing a "direct repeat" and a tracrRNA-processed partial direct repeat in the context of an endogenous CRISPR system), a guide sequence (also referred to as a "spacer" in the context of an endogenous CRISPR system), and/or other sequences and transcripts from a CRISPR locus.

[0202] The CRISPR/Cas nuclease or CRISPR/Cas nuclease system can include a non-coding RNA molecule (guide) RNA, which sequence-specifically binds to DNA, and a Cas protein (*e.g.*, Cas9), with nuclease functionality (*e.g.*, two nuclease domains). One or more elements of a CRISPR system can derive from a type I, type II, or type III CRISPR system, *e.g.*, derived from a particular organism comprising an endogenous CRISPR system, such as *Streptococcus pyogenes*.

[0203] In some aspects, a Cas nuclease and gRNA (including a fusion of crRNA specific for the target sequence and fixed tracrRNA) are introduced into the cell. In general, target sites at the 5' end of the gRNA target the Cas nuclease to the target site, *e.g.*, the gene, using complementary base pairing. The target site may be selected based on its location immediately 5' of a protospacer adjacent motif (PAM) sequence, such as typically NGG, or NAG. In this

respect, the gRNA is targeted to the desired sequence by modifying the first 20, 19, 18, 17, 16, 15, 14, 14, 12, 11, or 10 nucleotides of the guide RNA to correspond to the target DNA sequence. In general, a CRISPR system is characterized by elements that promote the formation of a CRISPR complex at the site of a target sequence. Typically, "target sequence" generally refers to a sequence to which a guide sequence is designed to have complementarity, where hybridization between the target sequence and a guide sequence promotes the formation of a CRISPR complex. Full complementarity is not necessarily required, provided there is sufficient complementarity to cause hybridization and promote formation of a CRISPR complex.

[0204] The CRISPR system can induce double stranded breaks (DSBs) at the target site, followed by disruptions or alterations as discussed herein. In other embodiments, Cas9 variants, deemed "nickases," are used to nick a single strand at the target site. Paired nickases can be used, *e.g.*, to improve specificity, each directed by a pair of different gRNAs targeting sequences such that upon introduction of the nicks simultaneously, a 5' overhang is introduced. In other embodiments, catalytically inactive Cas9 is fused to a heterologous effector domain such as a transcriptional repressor or activator, to affect gene expression.

[0205] The target sequence may comprise any polynucleotide, such as DNA or RNA polynucleotides. The target sequence may be located in the nucleus or cytoplasm of the cell, such as within an organelle of the cell. Generally, a sequence or template that may be used for recombination into the targeted locus comprising the target sequences is referred to as an "editing template" or "editing polynucleotide" or "editing sequence". In some aspects, an exogenous template polynucleotide may be referred to as an editing template. In some aspects, the recombination is homologous recombination.

[0206] Typically, in the context of an endogenous CRISPR system, formation of the CRISPR complex (comprising the guide sequence hybridized to the target sequence and complexed with one or more Cas proteins) results in cleavage of one or both strands in or near (*e.g.* within 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or more base pairs from) the target sequence. The tracr sequence, which may comprise or consist of all or a portion of a wild-type tracr sequence (*e.g.* about or more than about 20, 26, 32, 45, 48, 54, 63, 67, 85, or more nucleotides of a wild-type tracr sequence), may also form part of the CRISPR complex, such as by hybridization along at least a portion of the tracr sequence to all or a portion of a tracr mate sequence that is operably linked to the guide sequence. The tracr sequence has sufficient complementarity to a tracr mate sequence to hybridize and participate in formation of the CRISPR complex, such as at least 50%, 60%, 70%, 80%, 90%, 95% or 99% of sequence complementarity along the length of the tracr mate sequence when optimally aligned.

[0207] One or more vectors driving expression of one or more elements of the CRISPR system can be introduced into the cell such that expression of the elements of the CRISPR system direct formation of the CRISPR complex at one or more target sites. Components can also be delivered to cells as proteins and/or RNA. For example, a Cas enzyme, a guide sequence linked to a tracr-mate sequence, and a tracr sequence could each be operably linked to separate regulatory elements on separate vectors. Alternatively, two or more of the elements expressed from the same or different regulatory elements, may be combined in a single vector, with one or more additional vectors providing any components of the CRISPR system not included in the first vector. The vector may comprise one or more insertion sites, such as a restriction endonuclease recognition sequence (also referred to as a "cloning site"). In some embodiments, one or more insertion sites are located upstream and/or downstream of one or more sequence elements of one or more vectors. When multiple different guide sequences are used, a single expression construct may be used to target CRISPR activity to multiple different, corresponding target sequences within a cell.

[0208] A vector may comprise a regulatory element operably linked to an enzyme-coding sequence encoding the CRISPR enzyme, such as a Cas protein. Non-limiting examples of Cas proteins include Cas1, Cas1B, Cas2, Cas3, Cas4, Cas5, Cas6, Cas7, Cas8, Cas9 (also known as Csn1 and Csx12), Cas10, Csy1, Csy2, Csy3, Cse1, Cse2, Csc1, Csc2, Csa5, Csn2, Csm2, Csm3, Csm4, Csm5, Csm6, Cmr1, Cmr3, Cmr4, Cmr5, Cmr6, Csb1, Csb2, Csb3, Csx17, Csx14, Csx10, Csx16, CsaX, Csx3, Csx1, Csx15, Csf1, Csf2, Csf3, Csf4, Cpf1 (Cas12a) homologs thereof, or modified versions thereof. These enzymes are known; for example, the amino acid sequence of *S. pyogenes* Cas9 protein may be found in the SwissProt database under accession number Q99ZW2.

[0209] The CRISPR enzyme can be Cas9 (*e.g.*, from *S. pyogenes* or *S. pneumonia*). In some cases, Cpf1 (Cas12a) may be used as an endonuclease instead of Cas9. The CRISPR enzyme can direct cleavage of one or both strands at the location of a target sequence, such as within the target sequence and/or within the complement of the target sequence. The vector can encode a CRISPR enzyme that is mutated with respect to a corresponding wild-type enzyme such that the mutated CRISPR enzyme lacks the ability to cleave one or both strands of a target polynucleotide containing a target sequence. For example, an aspartate-to-alanine substitution (D10A) in the RuvC I catalytic domain of Cas9 from *S. pyogenes* converts Cas9 from a nuclease that cleaves both strands to a nickase (cleaves a single strand). In some embodiments, a Cas9 nickase may be used in combination with guide sequence(s), *e.g.*, two guide sequences,

which target respectively sense and antisense strands of the DNA target. This combination allows both strands to be nicked and used to induce NHEJ or HDR.

[0210] In some embodiments, an enzyme coding sequence encoding the CRISPR enzyme is codon optimized for expression in particular cells, such as eukaryotic cells. The eukaryotic cells may be those of or derived from a particular organism, such as a mammal, including but not limited to human, mouse, rat, rabbit, dog, or non-human primate. In general, codon optimization refers to a process of modifying a nucleic acid sequence for enhanced expression in the host cells of interest by replacing at least one codon of the native sequence with codons that are more frequently or most frequently used in the genes of that host cell while maintaining the native amino acid sequence. Various species exhibit particular bias for certain codons of a particular amino acid. Codon bias (differences in codon usage between organisms) often correlates with the efficiency of translation of messenger RNA (mRNA), which is in turn believed to be dependent on, among other things, the properties of the codons being translated and the availability of particular transfer RNA (tRNA) molecules. The predominance of selected tRNAs in a cell is generally a reflection of the codons used most frequently in peptide synthesis. Accordingly, genes can be tailored for optimal gene expression in a given organism based on codon optimization.

[0211] In general, a guide sequence is any polynucleotide sequence having sufficient complementarity with a target polynucleotide sequence to hybridize with the target sequence and direct sequence-specific binding of the CRISPR complex to the target sequence. In some embodiments, the degree of complementarity between a guide sequence and its corresponding target sequence, when optimally aligned using a suitable alignment algorithm, is about or more than about 50%, 60%, 75%, 80%, 85%, 90%, 95%, 97%, 99%, or more.

[0212] Optimal alignment may be determined with the use of any suitable algorithm for aligning sequences, non-limiting example of which include the Smith-Waterman algorithm, the Needleman-Wunsch algorithm, algorithms based on the Burrows-Wheeler Transform (*e.g.* the Burrows Wheeler Aligner), Clustal W, Clustal X, BLAT, Novoalign (Novocraft Technologies, ELAND (Illumina, San Diego, Calif.), SOAP (available at soap.genomics.org.cn), and Maq (available at maq.sourceforge.net).

[0213] The CRISPR enzyme may be part of a fusion protein comprising one or more heterologous protein domains. A CRISPR enzyme fusion protein may comprise any additional protein sequence, and optionally a linker sequence between any two domains. Examples of protein domains that may be fused to a CRISPR enzyme include, without limitation, epitope tags, reporter gene sequences, and protein domains having one or more of the following

activities: methylase activity, demethylase activity, transcription activation activity, transcription repression activity, transcription release factor activity, histone modification activity, RNA cleavage activity and nucleic acid binding activity. Non-limiting examples of epitope tags include histidine (His) tags, V5 tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Examples of reporter genes include, but are not limited to, glutathione-S-transferase (GST), horseradish peroxidase (HRP), chloramphenicol acetyltransferase (CAT) beta galactosidase, beta-glucuronidase, luciferase, green fluorescent protein (GFP), HcRed, DsRed, cyan fluorescent protein (CFP), yellow fluorescent protein (YFP), and autofluorescent proteins including blue fluorescent protein (BFP). A CRISPR enzyme may be fused to a gene sequence encoding a protein or a fragment of a protein that bind DNA molecules or bind other cellular molecules, including but not limited to maltose binding protein (MBP), S-tag, Lex A DNA binding domain (DBD) fusions, GAL4A DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. Additional domains that may form part of a fusion protein comprising a CRISPR enzyme are described in US 20110059502, incorporated herein by reference.

VIII. Methods of Treatment

[0214] In various embodiments, diseased or other cells expressing endogenous TROP-2 on their surface are targeted for the purpose of improving a medical condition in an individual that has the medical condition or for the purpose of reducing the risk or delaying the severity and/or onset of the medical condition in an individual. In specific cases, cancer cells expressing endogenous TROP-2 are targeted for the purpose of killing the cancer cells.

[0215] TROP-2-targeting CAR constructs, nucleic acid sequences, vectors, immune cells and so forth as contemplated herein, and/or pharmaceutical compositions comprising the same, are used for the prevention, treatment or amelioration of a cancerous disease, such as a tumorous disease. In particular embodiments, the pharmaceutical composition of the present disclosure may be particularly useful in preventing, ameliorating and/or treating cancer, including cancers that express TROP-2 and that may or may not be solid tumors, for example.

[0216] The immune cells for which the TROP-2-targeting receptor is utilized may be NK cells, T cells, gamma delta T cells, alpha beta T cells, or NKT or invariant NKT (iNKT), or invariant NKT cells engineered for cell therapy for mammals, in particular embodiments. In such cases where the cells are NK cells, the NK cell therapy may be of any kind and the NK cells may be of any kind. In specific embodiments, the cells are NK cells that have been

engineered to express one or more TROP-2-targeting CARs and/or one or more suicide genes and/or one or more cytokines. In specific embodiments, the cells are NK cells that are transduced with a TROP-2-targeting CAR.

[0217] In particular embodiments, the present disclosure contemplates, in part, TROP-2 CAR-expressing cells, TROP-2-targeting CAR constructs, TROP-2-targeting CAR nucleic acid molecules and TROP-2-targeting CAR vectors that can be administered either alone or in any combination using standard vectors and/or gene delivery systems, and in at least some aspects, together with a pharmaceutically acceptable carrier or excipient. In certain embodiments, subsequent to administration, the nucleic acid molecules or vectors may be stably integrated into the genome of the subject.

[0218] In specific embodiments, viral vectors may be used that are specific for certain cells or tissues and persist in NK cells. Suitable pharmaceutical carriers and excipients are well known in the art. The compositions prepared according to the disclosure can be used for the prevention or treatment or delaying the above identified diseases.

[0219] Furthermore, the disclosure relates to a method for the prevention, treatment or amelioration of a tumorous disease comprising the step of administering to a subject in the need thereof an effective amount of cells that express a TROP-2-targeting CAR, a nucleic acid sequence, a vector, as contemplated herein and/or produced by a process as contemplated herein.

[0220] Possible indications for administration of the composition(s) of the exemplary TROP-2-targeting CAR cells are cancerous diseases, including tumorous diseases, including B cell malignancies, multiple myeloma, breast cancer, glioblastoma, renal cancer, pancreatic cancer, or lung cancer, for example. Exemplary indications for administration of the composition(s) of TROP-2-targeting CAR cells are cancerous diseases, including any malignancies that express TROP-2. The administration of the composition(s) of the disclosure is useful for all stages (I, II, III, or IV) and types of cancer, including for minimal residual disease, early cancer, advanced cancer, and/or metastatic cancer and/or refractory cancer, for example.

[0221] The disclosure further encompasses co-administration protocols with other compounds, *e.g.* bispecific antibody constructs, targeted toxins or other compounds, which act *via* immune cells. The clinical regimen for co-administration of the inventive compound(s) may encompass co-administration at the same time, before or after the administration of the other component. Particular combination therapies include chemotherapy, radiation, surgery, hormone therapy, or other types of immunotherapy.

[0222] Embodiments relate to a kit comprising a TROP-2-targeting CAR construct as defined herein, a nucleic acid sequence as defined herein, a vector as defined herein and/or a host cell (such as an immune cell) as defined herein. It is also contemplated that the kit of this disclosure comprises a pharmaceutical composition as described herein above, either alone or in combination with further medicaments to be administered to an individual in need of medical treatment or intervention.

A. Pharmaceutical Compositions

[0223] Also provided herein are pharmaceutical compositions and formulations comprising transduced NK cells and a pharmaceutically acceptable carrier. The transduced cells may be comprised in a media suitable for transfer to an individual and/or media suitable for preservation, such as cryopreservation, including prior to transfer to an individual.

[0224] Pharmaceutical compositions and formulations as described herein can be prepared by mixing the active ingredients (such as the cells) having the desired degree of purity with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 22nd edition, 2012), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include interstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX[®], Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including

rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

B. Combination Therapies

[0225] In certain embodiments, the compositions and methods of the present embodiments involve an immune cell population (including NK cell population) in combination with at least one additional therapy. The additional therapy may be radiation therapy, surgery (*e.g.*, lumpectomy and a mastectomy), chemotherapy, gene therapy, DNA therapy, viral therapy, RNA therapy, immunotherapy, bone marrow transplantation, nanotherapy, monoclonal antibody therapy, hormone therapy, oncolytic viruses, or a combination of the foregoing. The additional therapy may be in the form of adjuvant or neoadjuvant therapy.

[0226] In some embodiments, the additional therapy is the administration of small molecule enzymatic inhibitor or anti-metastatic agent. In some embodiments, the additional therapy is the administration of side-effect limiting agents (*e.g.*, agents intended to lessen the occurrence and/or severity of side effects of treatment, such as anti-nausea agents, *etc.*). In some embodiments, the additional therapy is radiation therapy. In some embodiments, the additional therapy is surgery. In some embodiments, the additional therapy is a combination of radiation therapy and surgery. In some embodiments, the additional therapy is gamma irradiation. In some embodiments, the additional therapy is therapy targeting PBK/AKT/mTOR pathway, HSP90 inhibitor, tubulin inhibitor, apoptosis inhibitor, and/or chemopreventative agent. The additional therapy may be one or more of the chemotherapeutic agents known in the art.

[0227] In particular embodiments, in addition to the inventive cell therapy of the disclosure, the individual may have been provided, may be provided, and/or may will be provided a specific additional therapy for cancer, including one or more of surgery, radiation, immunotherapy (other than the cell therapy of the present disclosure), hormone therapy, gene therapy, chemotherapy, and so forth.

[0228] An immune cell therapy may be administered before, during, after, or in various combinations relative to an additional cancer therapy. The administrations may be in intervals ranging from concurrently to minutes to days to weeks. In embodiments where the immune cell therapy is provided to a patient separately from an additional therapeutic agent, one would generally ensure that a significant period of time did not expire between the time of each

delivery, such that the two compounds would still be able to exert an advantageously combined effect on the patient. In such instances, it is contemplated that one may provide a patient with the antibody therapy and the anti-cancer therapy within about 12 to 24 or 72 h of each other and, more particularly, within about 6-12 h of each other. In some situations it may be desirable to extend the time period for treatment significantly where several days (2, 3, 4, 5, 6, or 7) to several weeks (1, 2, 3, 4, 5, 6, 7, or 8) lapse between respective administrations.

[0229] Various combinations may be employed. For the example below an immune cell therapy is “A” and an anti-cancer therapy is “B”:

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B

B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A

B/A/B/A B/A/A/B A/A/A/B B/A/A/A A/B/A/A A/A/B/A

[0230] Administration of any compound or cell therapy of the present embodiments to a patient will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the agents. Therefore, in some embodiments there is a step of monitoring toxicity that is attributable to combination therapy.

1. Chemotherapy

[0231] A wide variety of chemotherapeutic agents may be used in accordance with the present embodiments. The term “chemotherapy” refers to the use of drugs to treat cancer. A “chemotherapeutic agent” is used to connote a compound or composition that is administered in the treatment of cancer. These agents or drugs are categorized by their mode of activity within a cell, for example, whether and at what stage they affect the cell cycle. Alternatively, an agent may be characterized based on its ability to directly cross-link DNA, to intercalate into DNA, or to induce chromosomal and mitotic aberrations by affecting nucleic acid synthesis.

[0232] Examples of chemotherapeutic agents include alkylating agents, such as thiotepa and cyclophosphamide; alkyl sulfonates, such as busulfan, improsulfan, and piposulfan; aziridines, such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines, including altretamine, triethylenemelamine, trietylenephosphoramidate, triethylenethiophosphoramidate, and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin

(including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratiastatin; a sarcodictyin; spongistatin; nitrogen mustards, such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard; nitrosureas, such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics, such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin omegaII); dynemicin, including dynemicin A; bisphosphonates, such as clodronate; an esperamicin; as well as neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authrarnycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycinis, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins, such as mitomycin C, mycophenolic acid, nogalarnycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; anti-metabolites, such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues, such as denopterin, pteropterin, and trimetrexate; purine analogs, such as fludarabine, 6-mercaptapurine, thiamiprine, and thioguanine; pyrimidine analogs, such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens, such as calusterone, dromostanolone propionate, epitio stanol, mepitio stanane, and testolactone; anti-adrenals, such as mitotane and trilostane; folic acid replenisher, such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids, such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSKpolysaccharide complex; razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; taxoids, *e.g.*, paclitaxel and docetaxel gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes, such as cisplatin, oxaliplatin, and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; vinorelbine; novantrone; teniposide;

edatrexate; daunomycin; aminopterin; xeloda; ibandronate; irinotecan (*e.g.*, CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids, such as retinoic acid; capecitabine; carboplatin, procarbazine, plicomycin, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatin, and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

2. Radiotherapy

[0233] Other factors that cause DNA damage and have been used extensively include what are commonly known as γ -rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated, such as microwaves, proton beam irradiation (U.S. Patents 5,760,395 and 4,870,287), and UV-irradiation. It is most likely that all of these factors affect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000 roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

3. Immunotherapy

[0234] The skilled artisan will understand that additional immunotherapies may be used in combination or in conjunction with methods of the embodiments. In the context of cancer treatment, immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. Rituximab (RITUXAN®) is such an example. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually affect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc.) and serve as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells.

[0235] Antibody-drug conjugates have emerged as a breakthrough approach to the development of cancer therapeutics. Cancer is one of the leading causes of deaths in the world. Antibody-drug conjugates (ADCs) comprise monoclonal antibodies (MAbs) that are

covalently linked to cell-killing drugs. This approach combines the high specificity of MAb against their antigen targets with highly potent cytotoxic drugs, resulting in “armed” MAb that deliver the payload (drug) to tumor cells with enriched levels of the antigen. Targeted delivery of the drug also minimizes its exposure in normal tissues, resulting in decreased toxicity and improved therapeutic index. The approval of two ADC drugs, ADCETRIS® (brentuximab vedotin) in 2011 and KADCYLA® (trastuzumab emtansine or T-DM1) in 2013 by FDA validated the approach. There are currently more than 30 ADC drug candidates in various stages of clinical trials for cancer treatment (Leal *et al.*, 2014). As antibody engineering and linker-payload optimization are becoming more and more mature, the discovery and development of new ADCs are increasingly dependent on the identification and validation of new targets that are suitable to this approach and the generation of targeting MAb. Two criteria for ADC targets are upregulated/high levels of expression in tumor cells and robust internalization.

[0236] In one aspect of immunotherapy, the tumor cell must bear some marker that is amenable to targeting, *i.e.*, is not present on the majority of other cells. Many tumor markers exist and any of these may be suitable for targeting in the context of the present embodiments. Common tumor markers include CD20, carcinoembryonic antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, laminin receptor, erb B, and p155. An alternative aspect of immunotherapy is to combine anticancer effects with immune stimulatory effects. Immune stimulating molecules also exist including: cytokines, such as IL-2, IL-4, IL-12, GM-CSF, gamma-IFN, chemokines, such as MIP-1, MCP-1, IL-8, and growth factors, such as FLT3 ligand.

[0237] Examples of immunotherapies currently under investigation or in use are immune adjuvants, *e.g.*, *Mycobacterium bovis*, *Plasmodium falciparum*, dinitrochlorobenzene, and aromatic compounds (U.S. Patents 5,801,005 and 5,739,169; Hui and Hashimoto, 1998; Christodoulides *et al.*, 1998); cytokine therapy, *e.g.*, interferons α , β and γ , IL-1, GM-CSF, and TNF (Bukowski *et al.*, 1998; Davidson *et al.*, 1998; Hellstrand *et al.*, 1998); gene therapy, *e.g.*, TNF, IL-1, IL-2, and p53 (Qin *et al.*, 1998; Austin-Ward and Villaseca, 1998; U.S. Patents 5,830,880 and 5,846,945); and monoclonal antibodies, *e.g.*, anti-CD20, anti-ganglioside GM2, and anti-p185 (Hollander, 2012; Hanibuchi *et al.*, 1998; U.S. Patent 5,824,311). It is contemplated that one or more anti-cancer therapies may be employed with the antibody therapies described herein.

[0238] In some embodiments, the immunotherapy may be an immune checkpoint inhibitor. Immune checkpoints either turn up a signal (*e.g.*, co-stimulatory molecules) or turn down a signal. Inhibitory immune checkpoints that may be targeted by immune checkpoint blockade include adenosine A2A receptor (A2AR), B7-H3 (also known as CD276), B and T lymphocyte attenuator (BTLA), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4, also known as CD152), indoleamine 2,3-dioxygenase (IDO), killer-cell immunoglobulin (KIR), lymphocyte activation gene-3 (LAG3), programmed death 1 (PD-1), T-cell immunoglobulin domain and mucin domain 3 (TIM-3) and V-domain Ig suppressor of T cell activation (VISTA). In particular, the immune checkpoint inhibitors target the PD-1 axis and/or CTLA-4.

[0239] The immune checkpoint inhibitors may be drugs such as small molecules, recombinant forms of ligand or receptors, or, in particular, are antibodies, such as human antibodies (*e.g.*, International Patent Publication WO2015016718; Pardoll, *Nat Rev Cancer*, 12(4): 252-64, 2012; both incorporated herein by reference). Known inhibitors of the immune checkpoint proteins or analogs thereof may be used, in particular chimerized, humanized or human forms of antibodies may be used. As the skilled person will know, alternative and/or equivalent names may be in use for certain antibodies mentioned in the present disclosure. Such alternative and/or equivalent names are interchangeable in the context of the present disclosure. For example it is known that lambrolizumab is also known under the alternative and equivalent names MK-3475 and pembrolizumab.

[0240] In some embodiments, the PD-1 binding antagonist is a molecule that inhibits the binding of PD-1 to its ligand binding partners. In a specific aspect, the PD-1 ligand binding partners are PDL1 and/or PDL2. In another embodiment, a PDL1 binding antagonist is a molecule that inhibits the binding of PDL1 to its binding partners. In a specific aspect, PDL1 binding partners are PD-1 and/or B7-1. In another embodiment, the PDL2 binding antagonist is a molecule that inhibits the binding of PDL2 to its binding partners. In a specific aspect, a PDL2 binding partner is PD-1. The antagonist may be an antibody, an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide. Exemplary antibodies are described in U.S. Patent Nos. US8735553, US8354509, and US8008449, all incorporated herein by reference. Other PD-1 axis antagonists for use in the methods provided herein are known in the art such as described in U.S. Patent Application No. US20140294898, US2014022021, and US20110008369, all incorporated herein by reference.

[0241] In some embodiments, the PD-1 binding antagonist is an anti-PD-1 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody). In some embodiments, the anti-PD-1 antibody is selected from the group consisting of nivolumab, pembrolizumab, and

CT-011. In some embodiments, the PD-1 binding antagonist is an immunoadhesin (*e.g.*, an immunoadhesin comprising an extracellular or PD-1 binding portion of PDL1 or PDL2 fused to a constant region (*e.g.*, an Fc region of an immunoglobulin sequence). In some embodiments, the PD-1 binding antagonist is AMP-224. Nivolumab, also known as MDX-1106-04, MDX-1106, ONO-4538, BMS-936558, and OPDIVO[®], is an anti-PD-1 antibody described in WO2006/121168. Pembrolizumab, also known as MK-3475, Merck 3475, lambrolizumab, KEYTRUDA[®], and SCH-900475, is an anti-PD-1 antibody described in WO2009/114335. CT-011, also known as hBAT or hBAT-1, is an anti-PD-1 antibody described in WO2009/101611. AMP-224, also known as B7-DCIg, is a PDL2-Fc fusion soluble receptor described in WO2010/027827 and WO2011/066342.

[0242] Another immune checkpoint that can be targeted in the methods provided herein is the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), also known as CD152. The complete cDNA sequence of human CTLA-4 has the Genbank accession number L15006. CTLA-4 is found on the surface of T cells and acts as an “off” switch when bound to CD80 or CD86 on the surface of antigen-presenting cells. CTLA4 is a member of the immunoglobulin superfamily that is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells. CTLA4 is similar to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 and CD86, also called B7-1 and B7-2 respectively, on antigen-presenting cells. CTLA4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Intracellular CTLA4 is also found in regulatory T cells and may be important to their function. T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules.

[0243] In some embodiments, the immune checkpoint inhibitor is an anti-CTLA-4 antibody (*e.g.*, a human antibody, a humanized antibody, or a chimeric antibody), an antigen binding fragment thereof, an immunoadhesin, a fusion protein, or oligopeptide.

[0244] Anti-human-CTLA-4 antibodies (or VH and/or VL domains derived therefrom) suitable for use in the present methods can be generated using methods well known in the art. Alternatively, art recognized anti-CTLA-4 antibodies can be used. For example, the anti-CTLA-4 antibodies disclosed in: US 8,119,129, WO 01/14424, WO 98/42752; WO 00/37504 (CP675,206, also known as tremelimumab; formerly ticilimumab), U.S. Patent No. 6,207,156; Hurwitz *et al.* (1998) *Proc Natl Acad Sci USA* 95(17): 10067-10071; Camacho *et al.* (2004) *J Clin Oncology* 22(145): Abstract No. 2505 (antibody CP-675206); and Mokyr *et al.* (1998) *Cancer Res* 58:5301-5304 can be used in the methods disclosed herein. The teachings of each of the aforementioned publications are hereby incorporated by reference. Antibodies that

compete with any of these art-recognized antibodies for binding to CTLA-4 also can be used. For example, a humanized CTLA-4 antibody is described in International Patent Application No. WO2001014424, WO2000037504, and U.S. Patent No. 8,017,114; all incorporated herein by reference.

[0245] An exemplary anti-CTLA-4 antibody is ipilimumab (also known as 10D1, MDX-010, MDX-101, and Yervoy®) or antigen binding fragments and variants thereof (see, *e.g.*, WO 01/14424). In other embodiments, the antibody comprises the heavy and light chain CDRs or VRs of ipilimumab. Accordingly, in one embodiment, the antibody comprises the CDR1, CDR2, and CDR3 domains of the VH region of ipilimumab, and the CDR1, CDR2 and CDR3 domains of the VL region of ipilimumab. In another embodiment, the antibody competes for binding with and/or binds to the same epitope on CTLA-4 as the above-mentioned antibodies. In another embodiment, the antibody has at least about 90% variable region amino acid sequence identity with the above-mentioned antibodies (*e.g.*, at least about 90%, 95%, or 99% variable region identity with ipilimumab).

[0246] Other molecules for modulating CTLA-4 include CTLA-4 ligands and receptors such as described in U.S. Patent Nos. US5844905, US5885796 and International Patent Application Nos. WO1995001994 and WO1998042752; all incorporated herein by reference, and immunoadhesins such as described in U.S. Patent No. US8329867, incorporated herein by reference.

4. Surgery

[0247] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative, and palliative surgery. Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed and may be used in conjunction with other therapies, such as the treatment of the present embodiments, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy, and/or alternative therapies. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically-controlled surgery (Mohs' surgery).

[0248] Upon excision of part or all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection, or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated,

for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well.

5. Other Agents

[0249] It is contemplated that other agents may be used in combination with certain aspects of the present embodiments to improve the therapeutic efficacy of treatment. These additional agents include agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers, or other biological agents. Increases in intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with certain aspects of the present embodiments to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present embodiments. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with certain aspects of the present embodiments to improve the treatment efficacy.

IX. Proteins

[0250] As used herein, a “protein” or “polypeptide” refers to a molecule comprising at least three amino acid residues. As used herein, the term “wild-type” refers to the endogenous version of a molecule that occurs naturally in an organism. In some embodiments, wild-type versions of a protein or polypeptide are employed, however, in many embodiments of the disclosure, a modified protein or polypeptide is employed. The terms described above may be used interchangeably. A “modified protein” or “modified polypeptide” or a “variant” refers to a protein or polypeptide whose chemical structure, particularly its amino acid sequence, is altered with respect to the wild-type protein or polypeptide. In some embodiments, a modified/variant protein or polypeptide has at least one modified activity or function (recognizing that proteins or polypeptides may have multiple activities or functions). It is specifically contemplated that a modified/variant protein or polypeptide may be altered with respect to one activity or function yet retain a wild-type activity or function in other respects.

[0251] Where a protein is specifically mentioned herein, it is in general a reference to a native (wild-type) or recombinant (modified) protein or, optionally, a protein in which any signal sequence has been removed. The protein may be isolated directly from the organism of which it is native, produced by recombinant DNA/exogenous expression methods, or produced by solid-phase peptide synthesis (SPPS) or other in vitro methods. In particular embodiments, there are isolated nucleic acid segments and recombinant vectors incorporating nucleic acid sequences that encode a polypeptide (e.g., an antibody or fragment thereof). The term “recombinant” may be used in conjunction with a polypeptide or the name of a specific polypeptide, and this generally refers to a polypeptide produced from a nucleic acid molecule that has been manipulated in vitro or that is a replication product of such a molecule.

[0252] In certain embodiments the size of a protein or polypeptide (wild-type or modified) may comprise, but is not limited to, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, 575, 600, 625, 650, 675, 700, 725, 750, 775, 800, 825, 850, 875, 900, 925, 950, 975, 1000, 1100, 1200, 1300, 1400, 1500, 1750, 2000, 2250, 2500 amino acid residues or greater, and any range derivable therein, or derivative of a corresponding amino sequence described or referenced herein. It is contemplated that polypeptides may be mutated by truncation, rendering them shorter than their corresponding wild-type form, also, they might be altered by fusing or conjugating a heterologous protein or polypeptide sequence with a particular function (e.g., for targeting or localization, for enhanced immunogenicity, for purification purposes, etc.). As used herein, the term “domain” refers to any distinct functional or structural unit of a protein or polypeptide, and generally refers to a sequence of amino acids with a structure or function recognizable by one skilled in the art.

[0253] The polypeptides, proteins, or polynucleotides encoding such polypeptides or proteins of the disclosure may include 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 (or any derivable range therein) or more variant amino acids or nucleic acid substitutions or be at least 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% (or any derivable range therein) similar, identical, or homologous with at least, or at most 3, 4,

5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 300, 400, 500, 550, 1000 or more contiguous amino acids or nucleic acids, or any range derivable therein, of SEQ ID NOs:1-61.

[0254] In some embodiments, the protein, polypeptide, or polynucleotide may comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388,

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[0255] In some embodiments, the polypeptide, protein, or polynucleotide may comprise at least, at most, or exactly 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500, 501, 502, 503, 504, 505, 506, 507, 508, 509, 510, 511, 512, 513, 514, 515, 516, 517, 518, 519, 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, 540, 541, 542, 543, 544, 545, 546, 547, 548, 549, 550, 551, 552, 553, 554, 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, 575, 576, 577, 578, 579, 580, 581, 582, 583, 584, 585, 586, 587, 588, 589, 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, 610, 611, 612, 613, 614, 615, 616, 617, 618, 619, 620, 621, 622, 623, 624, 625, 626, 627, 628, 629, 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648,

649, 650, 651, 652, 653, 654, 655, 656, 657, 658, 659, 660, 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 681, 682, 683, 684, 685, 686, 687, 688, 689, 690, 691, 692, 693, 694, 695, 696, 697, 698, 699, 700, 701, 702, 703, 704, 705, 706, 707, 708, 709, 710, 711, 712, 713, 714, 715, 716, 717, 718, 719, 720, 721, 722, 723, 724, 725, 726, 727, 728, 729, 730, 731, 732, 733, 734, 735, 736, 737, 738, 739, 740, 741, 742, 743, 744, 745, 746, 747, 748, 749, 750, 751, 752, 753, 754, 755, 756, 757, 758, 759, 760, 761, 762, 763, 764, 765, 766, 767, 768, 769, 770, 771, 772, 773, 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784, 785, 786, 787, 788, 789, 790, 791, 792, 793, 794, 795, 796, 797, 798, 799, 800, 801, 802, 803, 804, 805, 806, 807, 808, 809, 810, 811, 812, 813, 814, 815, 816, 817, 818, 819, 820, 821, 822, 823, 824, 825, 826, 827, 828, 829, 830, 831, 832, 833, 834, 835, 836, 837, 838, 839, 840, 841, 842, 843, 844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, or 1000 (or any derivable range therein) contiguous amino acids or nucleotides of SEQ ID NOS: 1-61 that are at least, at most, or exactly 60%, 61%, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% (or any derivable range therein) similar, identical, or homologous with one of SEQ ID NOS: 1-61.

[0256] In some aspects there is a nucleic acid molecule or polypeptide starting at position 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197,

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844, 845, 846, 847, 848, 849, 850, 851, 852, 853, 854, 855, 856, 857, 858, 859, 860, 861, 862, 863, 864, 865, 866, 867, 868, 869, 870, 871, 872, 873, 874, 875, 876, 877, 878, 879, 880, 881, 882, 883, 884, 885, 886, 887, 888, 889, 890, 891, 892, 893, 894, 895, 896, 897, 898, 899, 900, 901, 902, 903, 904, 905, 906, 907, 908, 909, 910, 911, 912, 913, 914, 915, 916, 917, 918, 919, 920, 921, 922, 923, 924, 925, 926, 927, 928, 929, 930, 931, 932, 933, 934, 935, 936, 937, 938, 939, 940, 941, 942, 943, 944, 945, 946, 947, 948, 949, 950, 951, 952, 953, 954, 955, 956, 957, 958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976, 977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995, 996, 997, 998, 999, or 1000 of any of SEQ ID NOS:1-61 and comprising at least, at most, or exactly 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 455, 456, 457, 458, 459, 460, 461, 462, 463, 464, 465, 466, 467, 468, 469, 470, 471, 472, 473, 474, 475, 476, 477, 478, 479, 480, 481, 482, 483, 484, 485, 486, 487, 488, 489, 490, 491, 492, 493, 494, 495, 496, 497, 498, 499, 500,

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[0257] The nucleotide as well as the protein, polypeptide, and peptide sequences for various genes have been previously disclosed, and may be found in the recognized computerized databases. Two commonly used databases are the National Center for Biotechnology Information's Genbank and GenPept databases (on the World Wide Web at ncbi.nlm.nih.gov/) and The Universal Protein Resource (UniProt; on the World Wide Web at

uniprot.org). The coding regions for these genes may be amplified and/or expressed using the techniques disclosed herein or as would be known to those of ordinary skill in the art.

[0258] It is contemplated that in compositions of the disclosure, there is between about 0.001 mg and about 10 mg of total polypeptide, peptide, and/or protein per ml. The concentration of protein in a composition can be about, at least about or at most about 0.001, 0.010, 0.050, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0 mg/ml or more (or any range derivable therein).

X. Kits of the Disclosure

[0259] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, cells, reagents to produce cells, vectors, and reagents to produce vectors and/or components thereof may be comprised in a kit. In certain embodiments, NK cells may be comprised in a kit, and they may or may not yet express a TROP-2-targeting receptor, an optional cytokine, or an optional suicide gene. Such a kit may or may not have one or more reagents for manipulation of cells. Such reagents include small molecules, proteins, nucleic acids, antibodies, buffers, primers, nucleotides, salts, and/or a combination thereof, for example. Nucleotides that encode one or more TROP-2-targeting CARs, suicide gene products, and/or cytokines may be included in the kit. Proteins, such as cytokines or antibodies, including monoclonal antibodies, may be included in the kit. Nucleotides that encode components of engineered CAR receptors may be included in the kit, including reagents to generate same.

[0260] In particular aspects, the kit comprises the NK cell therapy of the disclosure and also another cancer therapy. In some cases, the kit, in addition to the cell therapy embodiments, also includes a second cancer therapy, such as chemotherapy, hormone therapy, and/or immunotherapy, for example. The kit(s) may be tailored to a particular cancer for an individual and comprise respective second cancer therapies for the individual.

[0261] The kits may comprise suitably aliquoted compositions of the present disclosure. The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit, the kit also may generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be

comprised in a vial. The kits of the present invention also will typically include a means for containing the composition and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

XI. Examples

[0262] The following examples are included to demonstrate certain embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute certain modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

TROP-2-TARGETING CHIMERIC ANTIGEN RECEPTORS

[0263] Expression constructs were generated for production of TROP-2-targeting chimeric antigen receptor (CAR) in addition to production of the cytokine, IL-15, and suicide gene, inducible caspase 9 (iC9). The expression constructs were:

Construct	SEQ ID NO
iC9mRs7VLVH28H28z15 (DNA)	1
iC9mRs7VLVH28H28z15 (Polypeptide)	2
iC9mRS7VHVL28H28icz15 (DNA)	3
iC9mRS7VHVL28H28icz15 (Polypeptide)	4
iC9hRs7VLVH28H28z15 (DNA)	5
iC9hRs7VLVH28H28z15 (Polypeptide)	6
iC9TROP2VLVH28H28z15 (DNA)	7
iC9TROP2VLVH28H28z15 (Polypeptide)	8

EXAMPLE 2

EVALUATING TROP-2 AS A TARGET IN PDAC AND OVARIAN CANCER

[0264] mRNA expression of Trop2 was analyzed for various cancer types using the TCGA dataset, showing that pancreatic ductal adenocarcinoma (PDAC) and ovarian cancer have high expression of Trop 2 (FIGs. 1A and 2A). Surface expression of Trop2 was measured in various PDAC (FIG. 1C), ovarian cancer (FIG. 2B), and colorectal cancer (FIG. 3) cell lines, showing that these cells express Trop2.

EXAMPLE 3

DESIGN AND TESTING OF RS7-DERIVED TROP2 CAR NK CELLS

[0265] Four CAR constructs were generated as shown in FIG. 4A, each comprising an iCas9 suicide gene, scFv from mRS7 or hRS7, CD28 hinge and transmembrane domain, CD28 co-stimulatory domain, CD3zeta intracellular domain, and IL15 gene. FIGs. 4B-10C show results from experiments demonstrating transfection transduction efficiency of the generated CARs as well as *in vitro* and *in vivo* cytotoxicity of NK cells expressing the CAR constructs against various Trop2-expressing cancer cells.

[0266] CAR construct “#2” was modified to replace the CD28 co-stimulatory domain with DAP10 co-stimulatory domain (shown in FIG. 11A). FIGs. 11B-14C show results from experiments demonstrating transfection and transduction efficiency of the generated CARs as well as *in vitro* and *in vivo* cytotoxicity of NK cells expressing the CAR constructs against Trop2-expressing cancer cells.

EXAMPLE 4

DESIGN AND TESTING OF 2G10-DERIVED TROP2 CAR NK CELLS

[0267] CAR constructs were generated using the scFv sequence derived from the 2G10 antibody sequence. FIGs. 15 and 16 show results demonstrating transfection and transduction efficiency of the various 2G10-derived constructs compared with the hRS7-derived constructs described in Example 3. FIGs. 17-21B show results demonstrating *in vitro* and *in vivo* cytotoxicity of NK cells expressing the 2G10-derived CAR constructs against Trop2-expressing cancer cells compared with NK cells expressing the hRS7-derived constructs.

* * *

[0268] All of the methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

CLAIMS

What is claimed is:

1. A polynucleotide that encodes an anti-TROP-2 chimeric antigen receptor (CAR), the CAR comprising (i) an anti-TROP-2 antigen binding region of a TROP-2 specific antibody, (ii) a transmembrane domain, and (iii) an intracellular region.
2. The polynucleotide of claim 1, wherein the TROP-2 specific antibody is an RS7 antibody.
3. The polynucleotide of claim 2, wherein the RS7 antibody is murine RS7 (mRS7).
4. The polynucleotide of claim 2, wherein the RS7 antibody is humanized RS7 (hRS7).
5. The polynucleotide of any of claims 1-4, wherein the anti-TROP-2 antigen binding region comprises a sequence having at least 85% sequence identity with SEQ ID NO:9.
6. The polynucleotide of any of claims 1-5, wherein the anti-TROP-2 antigen binding region comprises a sequence having at least 85% sequence identity with SEQ ID NO:14.
7. The polynucleotide of any of claims 1-6, wherein the anti-TROP-2 antigen binding region comprises a first sequence having at least 85% sequence identity with SEQ ID NO:9 and a second sequence having at least 85% sequence identity with SEQ ID NO:14.
8. The polynucleotide of any of claims 1-7, wherein the anti-TROP-2 antigen binding region comprises SEQ ID NO:9.
9. The polynucleotide of any of claims 1-8, wherein the anti-TROP-2 antigen binding region comprises SEQ ID NO:14.
10. The polynucleotide of any of claims 1-9, wherein the anti-TROP-2 antigen binding region comprises SEQ ID NO:9 and SEQ ID NO:14.
11. The polynucleotide of claim 1, wherein the TROP-2 specific antibody is a 2G10 antibody.
12. The polynucleotide of claim 11, wherein the 2G10 antibody is murine 2G10 (m2G10).
13. The polynucleotide of claim 11, wherein the 2G10 antibody is human 2G10 (h2G10).

14. The polynucleotide of any of claims 11-13, wherein the anti-TROP-2 antigen binding region comprises SEQ ID NO:44.
15. The polynucleotide of any of claims 11-13, wherein the anti-TROP-2 antigen binding region comprises SEQ ID NO:46.
16. The polynucleotide of any of claims 1-15, wherein the anti-TROP-2 antigen binding region is a codon optimized anti-TROP-2 antigen binding region.
17. The polynucleotide of any of claims 1-16, wherein the transmembrane domain is a transmembrane domain from CD28, the alpha chain of the T- cell receptor, beta chain of the T- cell receptor, zeta chain of the T- cell receptor, CD3 zeta, CD3 epsilon, CD3 gamma, CD3 delta, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD27, CD33, CD37, CD64, CD80, CD86, CD 134, CD137, CD154, ICOS/CD278, GITR/CD357, NKG2D, DAP10, or DAP12.
18. The polynucleotide of claim 17, wherein the transmembrane domain is a CD27 transmembrane domain.
19. The polynucleotide of claim 18, wherein the transmembrane domain comprises SEQ ID NO:22.
20. The polynucleotide of claim 17, wherein the transmembrane domain is a CD28 transmembrane domain.
21. The polynucleotide of claim 20, wherein the transmembrane domain comprises SEQ ID NO:23.
22. The polynucleotide of claim 17, wherein the transmembrane domain is a CD8 transmembrane domain.
23. The polynucleotide of claim 22, wherein the transmembrane domain comprises SEQ ID NO:24.
24. The polynucleotide of any of claims 1-23, wherein the intracellular domain is an intracellular domain from CD3 zeta, CD27, CD28, 4-1BB, DAP12, NKG2D, OX-40 (CD134), DAP10, CD40L, 2B4, DNAM, CS1, CD48, NKp30, NKp44, NKp46, or NKp80.

25. The polynucleotide of claim 24, wherein the intracellular domain is a CD3 zeta intracellular domain.
26. The polynucleotide of claim 25, wherein the intracellular domain comprises SEQ ID NO:29.
27. The polynucleotide of claim 24, wherein the intracellular domain is a CD28 intracellular domain.
28. The polynucleotide of any of claims 1-26, wherein the CAR comprises two or more intracellular domains.
29. The polynucleotide of claim 28, wherein the two or more intracellular domains comprise a CD3 zeta intracellular domain and an additional intracellular domain selected from a CD28, DAP10, DAP12, 4-1BB, NKG2D, and 2B4 intracellular domain.
30. The polynucleotide of claim 28 or 29, wherein the two or more intracellular domains comprise a CD3 zeta intracellular domain and a CD28 intracellular domain.
31. The polynucleotide of claim 28 or 29, wherein the two or more intracellular domains comprise a CD3 zeta intracellular domain and a DAP10 intracellular domain.
32. The polynucleotide of any of claims 1-31, further comprising a signal peptide.
33. The polynucleotide of claim 32, wherein the signal peptide is a signal peptide from CD8, CD27, granulocyte-macrophage colony-stimulating factor receptor (GMSCF-R), Ig heavy chain (IgH), CD3, or CD4.
34. The polynucleotide of claim 33, wherein the signal peptide is a GMSCF-R signal peptide.
35. The polynucleotide of claim 33 or 34, wherein the signal peptide comprises SEQ ID NO:21.
36. The polynucleotide of claim 33, wherein the signal peptide is a IgH signal peptide.
37. The polynucleotide of claim 36, wherein the signal peptide comprises SEQ ID NO:20.

38. The polynucleotide of any of claims 1-37, wherein the CAR comprises an IgH signal peptide, an anti-TROP-2 antigen binding region of an RS7 antibody, a CD28 transmembrane domain, and a CD3 zeta intracellular domain.
39. The polynucleotide of any of claims 1-38, wherein the polynucleotide further encodes an additional polypeptide of interest.
40. The polynucleotide of claim 39, wherein the sequence encoding the additional polypeptide of interest and the sequence encoding the CAR are separated on the polynucleotide by a 2A element.
41. The polynucleotide of claim 40, wherein the 2A element is an E2A element.
42. The polynucleotide of claim 41, wherein the E2A element comprises SEQ ID NO:38.
43. The polynucleotide of any of claims 39-42, wherein the additional polypeptide of interest is a therapeutic protein or a protein that enhances cell activity, expansion, and/or persistence.
44. The polynucleotide of any of claims 39-43, wherein the additional polypeptide of interest is a suicide gene product, a cytokine, or a human or viral protein that enhances proliferation, expansion and/or metabolic fitness.
45. The polynucleotide of any of claims 39-44, wherein the additional polypeptide of interest is a cytokine.
46. The polynucleotide of claim 45, wherein the cytokine is IL-15, IL-2, IL-12, IL-18, IL-21, IL-23, or IL-7.
47. The polynucleotide of claim 46, wherein the cytokine is IL-15.
48. The polynucleotide of claim 47, wherein the cytokine comprises SEQ ID NO:37.
49. The polynucleotide of any of claims 39-48, wherein the additional polypeptide of interest is a suicide gene product.
50. The polynucleotide of claim 49, wherein the suicide gene product is Caspase 9.
51. The polynucleotide of claim 49 or 50, wherein the polynucleotide encodes for a sequence having at least 95% identity with SEQ ID NO:42.

52. The polynucleotide of claim 51, wherein the polynucleotide encodes for SEQ ID NO:42.
53. The polynucleotide of any of claims 1-52, wherein the polynucleotide encodes for a sequence having at least 95% sequence identity with SEQ ID NO:2.
54. The polynucleotide of claim 53, wherein the polynucleotide encodes for SEQ ID NO:2.
55. The polynucleotide of claim 54, wherein the polynucleotide comprises SEQ ID NO:1.
56. The polynucleotide of any of claims 1-52, wherein the polynucleotide encodes for a sequence having at least 95% sequence identity with SEQ ID NO:4.
57. The polynucleotide of claim 56, wherein the polynucleotide encodes for SEQ ID NO:4.
58. The polynucleotide of claim 57, wherein the polynucleotide comprises SEQ ID NO:3.
59. The polynucleotide of any of claims 1-52, wherein the polynucleotide encodes for a sequence having at least 95% sequence identity with SEQ ID NO:6.
60. The polynucleotide of claim 59, wherein the polynucleotide encodes for SEQ ID NO:6.
61. The polynucleotide of claim 60, wherein the polynucleotide comprises SEQ ID NO:5.
62. The polynucleotide of any of claims 1-52, wherein the polynucleotide encodes for a sequence having at least 95% sequence identity with SEQ ID NO:8.
63. The polynucleotide of claim 62, wherein the polynucleotide encodes for SEQ ID NO:8.
64. The polynucleotide of claim 63, wherein the polynucleotide comprises SEQ ID NO:7.
65. The polynucleotide of any of claims 1-52, wherein the polynucleotide encodes for a sequence having at least 95% sequence identity with SEQ ID NO:51.
66. The polynucleotide of claim 65, wherein the polynucleotide encodes for SEQ ID NO:51.
67. The polynucleotide of claim 66, wherein the polynucleotide comprises SEQ ID NO:50.
68. The polynucleotide of any of claims 1-52, wherein the polynucleotide encodes for a sequence having at least 95% sequence identity with SEQ ID NO:53.
69. The polynucleotide of claim 68, wherein the polynucleotide encodes for SEQ ID NO:53.

70. The polynucleotide of claim 70, wherein the polynucleotide comprises SEQ ID NO:52.
71. The polynucleotide of any of claims 1-52, wherein the polynucleotide encodes for a sequence having at least 95% sequence identity with SEQ ID NO:55.
72. The polynucleotide of claim 71, wherein the polynucleotide encodes for SEQ ID NO:55.
73. The polynucleotide of claim 72, wherein the polynucleotide comprises SEQ ID NO:54.
74. The polynucleotide of any of claims 1-52, wherein the polynucleotide encodes for a sequence having at least 95% sequence identity with SEQ ID NO:57.
75. The polynucleotide of claim 74, wherein the polynucleotide encodes for SEQ ID NO:57.
76. The polynucleotide of claim 75, wherein the polynucleotide comprises SEQ ID NO:56.
77. The polynucleotide of any of claims 1-52, wherein the polynucleotide encodes for a sequence having at least 95% sequence identity with SEQ ID NO:59.
78. The polynucleotide of claim 77, wherein the polynucleotide encodes for SEQ ID NO:59.
79. The polynucleotide of claim 78, wherein the polynucleotide comprises SEQ ID NO:58.
80. The polynucleotide of any of claims 1-52, wherein the polynucleotide encodes for a sequence having at least 95% sequence identity with SEQ ID NO:61.
81. The polynucleotide of claim 80, wherein the polynucleotide encodes for SEQ ID NO:61.
82. The polynucleotide of claim 81, wherein the polynucleotide comprises SEQ ID NO:60.
83. A vector comprising the polynucleotide of any one of claims 1-82.
84. The vector of claim 83, wherein the vector is a viral vector.
85. The vector of claim 84, wherein the viral vector is an adenoviral vector, adeno-associated viral vector, lentiviral vector, or retroviral vector.
86. The vector of claim 83, wherein the vector is a non-viral vector.
87. The vector of claim 86, wherein the non-viral vector is a plasmid.

88. An immune cell comprising the polynucleotide of any one of claims 1-55 or the vector of any one of claims 83-87.
89. The immune cell of claim 88, wherein the immune cell is a natural killer (NK) cell, T cell, gamma delta T cell, alpha beta T cell, invariant NKT (iNKT) cell, B cell, macrophage, mesenchymal stromal cell, or dendritic cell.
90. The immune cell of claim 89, wherein the immune cell is a NK cell
91. The immune cell of claim 90, wherein the NK cell is derived from cord blood, peripheral blood, induced pluripotent stem cells, hematopoietic stem cells, bone marrow, or from a cell line.
92. The immune cell of claim 91, wherein the NK cell is derived from a cell line, wherein the NK cell line is NK-92.
93. The immune cell of claim 91, wherein the NK cell is derived from a cord blood mononuclear cell.
94. The immune cell of any of claims 90-93, wherein the NK cell is a CD56⁺ NK cell.
95. The immune cell of any of claims 90-94, wherein the NK cell expresses a recombinant cytokine.
96. The immune cell of claim 95, wherein the cytokine is IL-15, IL-2, IL-12, IL-18, IL-21, IL-7, or IL-23.
97. The immune cell of claim 96, wherein the cytokine is IL-15.
98. The immune cell of any of claims 88-97, wherein expression of one or more endogenous genes in the immune cell has been modified.
99. The immune cell of claim 98, wherein the one or more genes comprise NKG2A, SIGLEC-7, LAG3, TIM3, CISH, FOXO1, TGFB2, TIGIT, CD96, ADORA2, NR3C1, PD1, PDL-1, PDL-2, CD47, SIRPA, SHIP1, ADAM17, RPS6, 4EBP1, CD25, CD40, IL21R, ICAM1, CD95, CD80, CD86, IL10R, CD5, CD7, CTLA-4, TDAG8, CD38, or a combination thereof.
100. A population of immune cells comprising the immune cell of any one of claims 88-99.

101. A method of killing TROP-2-positive cells in an individual, comprising administering to the individual an effective amount of cells harboring the polynucleotide of any one of claims 1-55.
102. The method of claim 101, wherein the cells harboring the polynucleotide are immune cells.
103. The method of claim 102, wherein the immune cells are NK cells, T cells, gamma delta T cells, alpha beta T cells, invariant NKT (iNKT) cells, B cells, macrophages, dendritic cells, or a mixture thereof.
104. The method of claim 103, wherein the immune cells comprise NK cells, wherein the NK cells are derived from cord blood, peripheral blood, induced pluripotent stem cells, hematopoietic stem cells, bone marrow, from a cell line, or a mixture thereof.
105. The method of claim 104, wherein the NK cells are derived from cord blood mononuclear cells.
106. The method of any of claims 102-105, wherein the immune cells are allogeneic with respect to the individual.
107. The method of any of claims 102-105, wherein the immune cells are autologous with respect to the individual.
108. The method of any of claims 101-107, wherein the individual is human.
109. The method of any of claims 101-108, wherein the individual has cancer.
110. The method of claim 109, wherein the individual has breast cancer, acute myeloid leukemia, multiple myeloma, or glioblastoma.
111. The method of any of claims 101-110, wherein the cells harboring the polynucleotide are administered to the individual once or more than once.
112. The method of claim 111, wherein the duration of time between administrations of the cells harboring the polynucleotide to the individual is 1-24 hours, 1-7 days, 1-4 weeks, 1-12 months, or one or more years.

113. The method of any of claims 101-112, further comprising the step of providing to the individual an effective amount of an additional therapy.

114. The method of claim 113, wherein the additional therapy comprises surgery, radiation, gene therapy, immunotherapy, or hormone therapy.

115. The method of any of claims 101-114, wherein the cells harboring the polynucleotide are administered to the individual by injection, intravenously, intraarterially, intraperitoneally, intratracheally, intratumorally, intramuscularly, endoscopically, intralesionally, intracranially, percutaneously, subcutaneously, regionally, by perfusion, in a tumor microenvironment, or a combination thereof.

116. The method of any of claims 101-115, further comprising identifying TROP-2-positive cancer in the individual.

117. The method of any of claims 101-116, further comprising producing the cells harboring the polynucleotide.

118. A polynucleotide that encodes:

(a) an anti-TROP-2 chimeric antigen receptor (CAR), the CAR comprising (i) an anti-TROP-2 antigen binding region of an RS7 antibody, (ii) a transmembrane domain, and (iii) an intracellular region;

(b) IL-15; and

(c) Caspase 9.

119. The polynucleotide of claim 118, wherein the RS7 antibody is a murine RS7 antibody.

120. The polynucleotide of claim 118, wherein the RS7 antibody is a humanized RS7 antibody.

121. A polynucleotide that encodes:

(a) an anti-TROP-2 chimeric antigen receptor (CAR), the CAR comprising (i) an anti-TROP-2 antigen binding region of a 2G10 antibody, (ii) a transmembrane domain, and (iii) an intracellular region;

(b) IL-15; and

(c) Caspase 9.

122. The polynucleotide of claim 121, wherein the 2G10 antibody is a murine 2G10 antibody.

123. The polynucleotide of claim 121, wherein the 2G10 antibody is a humanized 2G10 antibody.

124. An NK cell comprising the polynucleotide of any of claims 118-123.

125. A natural killer cell comprising:

(a) a first polynucleotide encoding an anti-TROP-2 chimeric antigen receptor;

(b) a second polynucleotide encoding IL-15; and

(c) a third polynucleotide encoding Caspase 9.

126. The natural killer cell of claim 125, wherein the first polynucleotide, the second polynucleotide, and the third polynucleotide are present on the same nucleic acid molecule.

127. The natural killer cell of claim 125, wherein the first polynucleotide, the second polynucleotide, and the third polynucleotide are present on two different nucleic acid molecules.

128. The natural killer cell of claim 125, wherein the first polynucleotide, the second polynucleotide, and the third polynucleotide are present on three different nucleic acid molecules.

129. A polynucleotide that encodes for SEQ ID NO:2.

130. The polynucleotide of claim 129, wherein the polynucleotide comprises a sequence having at least 90% identity with SEQ ID NO:1.

131. The polynucleotide of claim 130, wherein the polynucleotide comprises SEQ ID NO:1.

132. A polynucleotide that encodes for SEQ ID NO:4.

133. The polynucleotide of claim 132, wherein the polynucleotide comprises a sequence having at least 90% identity with SEQ ID NO:3.
134. The polynucleotide of claim 133, wherein the polynucleotide comprises SEQ ID NO:3.
135. A polynucleotide that encodes for SEQ ID NO:6.
136. The polynucleotide of claim 135, wherein the polynucleotide comprises a sequence having at least 90% identity with SEQ ID NO:5.
137. The polynucleotide of claim 136, wherein the polynucleotide comprises SEQ ID NO:5.
138. A polynucleotide that encodes for SEQ ID NO:8.
139. The polynucleotide of claim 138, wherein the polynucleotide comprises a sequence having at least 90% identity with SEQ ID NO:7.
140. The polynucleotide of claim 139, wherein the polynucleotide comprises SEQ ID NO:7.