

(12) STANDARD PATENT APPLICATION (11) Application No. AU 2026201842 A1
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Engineered cells, T cell immune modulating antibodies and methods for using the same

(51) International Patent Classification(s)
A61K 39/00 (2006.01)

(21) Application No: **2026201842** (22) Date of Filing: **2026.03.11**

(43) Publication Date: **2026.04.02**

(43) Publication Journal Date: **2026.04.02**

(62) Divisional of:
2019235900

(71) Applicant(s)
Dana-Farber Cancer Institute, Inc.

(72) Inventor(s)
MARASCO, Wayne A.;ZHU, Quan Karen;KUIPER, Emily

(74) Agent / Attorney
Spruson & Ferguson, GPO Box 3898, Sydney, NSW, 2001, AU

ABSTRACT

This invention is directed to engineered cells and methods for using the same. In embodiments, the engineered cell comprises a nucleic acid encoding a chimeric antigen receptor and a polypeptide, wherein the chimeric antigen receptor is specific for two or more antigens on the surface of a cancer cell, and wherein the polypeptide comprises an antibody or fragment thereof that can be secreted from the engineered cell.

2026201842 11 Mar 2026

**ENGINEERED CELLS, T CELL IMMUNE MODULATING ANTIBODIES AND
METHODS FOR USING THE SAME**

[0001] The present application is a divisional application of Australian Patent Application No. 2019235900 which claims priority from U.S. Provisional Patent Application No. 62/643,040, filed on March 14, 2018, and U.S. Provisional Patent Application No. 62/657,151, filed on April 13, 2018, the contents of each of which are incorporated herein by reference in its entirety.

[0002] All patents, patent applications and publications cited herein are hereby incorporated by reference in their entirety. The disclosures of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art as known to those skilled therein as of the date of the invention described and claimed herein.

[0003] This patent disclosure contains material that is subject to copyright protection. The copyright owner has no objection to the facsimile reproduction by anyone of the patent document or the patent disclosure as it appears in the U.S. Patent and Trademark Office patent file or records, but otherwise reserves any and all copyright rights.

SEQUENCE LISTING

[0003a] Preceding applications contained a sequence listing which was originally submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, is named 5031461-043-WO1 and is 77 kilo bytes in size. The instant application contains a sequence listing which has been submitted electronically as an XML document in the ST26 format and is hereby incorporated by reference in its entirety. Said XML copy, created on 9 March 2026, is named P0023723AUD1 ST26.xml and is 164 kilo bytes in size.

GOVERNMENT INTERESTS

[0004] This invention was made with government support under Grant No. T32-CA207021 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

[0005] This invention is directed to engineered cells and methods for using the same. In embodiments, the engineered cell comprises a nucleic acid encoding a chimeric antigen

receptor and a polypeptide, wherein the chimeric antigen receptor is specific for two or more antigens on the surface of a cancer cell, and wherein the polypeptide comprises an antibody or fragment thereof that can be secreted from the engineered cell.

BACKGROUND OF THE INVENTION

[0006] No targeted treatments exist for triple-negative breast cancer (TNBC), therefore chemotherapy remains the only treatment for patients. Immunotherapies like anti-PD-L1 had

[Text continues on page 2]

limited success (18% response rate) for TNBC, indicating that existing therapies are not an effective treatment.

SUMMARY OF THE INVENTION

[0007] Aspects of the invention are directed towards an engineered cell comprising a nucleic acid encoding a chimeric antigen receptor and a polypeptide.

[0008] In embodiments, the engineered cell is an engineered T cell. Non-limiting examples comprise a cytotoxic T cell (also known as TC, Cytotoxic T Lymphocyte, CTL, T-Killer cell, cytolytic T cell, CD8+ T-cells or killer T cell); CD4+ T cells; NK cells; and NKT cells

[0009] In embodiments, the chimeric antigen receptor comprise an antigen-recognition domain and a signaling and/or stimulatory domain.

[0010] In embodiments, the antigen-recognition domain is specific for two or more antigens on the surface of a cell. For example, the cell is a cancer cell. For example, the antigens comprise C-X-C chemokine receptor type 4 and claudin-4. In embodiments, the chimeric antigen receptor (and/or antigen-recognition domain) comprises one or more antibody fragments. In embodiments, the chimeric antigen receptor comprises scFv. In embodiments, the chimeric antigen receptor comprises scFv directed to (targeted to) C-X-C chemokine receptor type 4 and claudin-4.

[0011] In embodiments, the signaling and/or stimulatory domain comprises CD28, 41BB, CD3-zeta intracellular signaling domains, or fragments thereof.

[0012] In embodiments, the polypeptide comprises an antibody or fragment thereof that can be secreted from the engineered cell. In embodiments, the secreted polypeptide modulates the immune system of a subject.

[0013] In embodiments, the polypeptide comprises an antibody or fragment thereof, such as a mini-body. Non-limiting examples of antibodies comprise monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies), humanized antibodies, fully human antibodies. In embodiments, the polypeptide comprises a monospecific antibody, a bispecific antibody, a trispecific antibody, or a multi-specific antibody. Non-limiting examples of antibody fragments comprise Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. In one embodiment, the invention comprise scFVs directed towards (and specific for) a target antigen.

[0014] In embodiments, the polypeptide is expressed from an expression construct separate than that which expresses the CAR, but which is a component of the same DNA vector as the CAR. In embodiments, the polypeptide is expressed from an expression construct separate than that which expresses the CAR, and which is a component of a different DNA vector as the CAR.

[0015] In embodiments, the constructs are cloned into one or more viral vectors. A non-limiting example comprises a lentiviral vector.

[0016] Aspects of the invention are further directed towards a method for treating a subject afflicted with cancer.

[0017] Aspects of the invention are still further directed towards a method of reducing progression or promoting regression of a cancer in a subject

[0018] Still further, aspects of the invention are directed towards a method of reducing cellular proliferation of a cancer cell in a subject.

[0019] Further, aspects of the invention are directed towards a method of inducing cytotoxicity of a cell, preferably a cancer cell.

[0020] In embodiments, the method comprises administering to the subject a therapeutically effective amount of the engineered cell as described herein, such as an engineered T cell.

[0021] In other embodiments, the method comprises administering to the subject a therapeutically effective amount of a secreted polypeptide as described herein.

[0022] Non-limiting examples of cancer comprise carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, smallcell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

[0023] In embodiments, cancer comprises triple-negative breast cancer (TNBC).

[0024] Aspects of the invention are yet further directed towards a method for assessing the killing capability of engineered CAR T cells.

[0025] In embodiments, the method comprises obtaining cells from one or more types of cancer; admixing the cells with a dye, so as to stain the cells; seeding the cells in a multi-well plate; incubating the admixture for a period of time; adding different T cell types to the

admixture to create a second admixture; co-culturing the second admixture for a period of time; and assessing the killing capability of the engineered CAR T cells. In embodiments, assessing comprises scanning and analyzing the plate. In embodiments, the plates can be analyzed using the bright field and blue fluorescent channels.

[0026] In embodiments, the cells comprise one or more types of cancer cells. In embodiments, the cells comprise one or more of cells isolated from the kidney (e.g., HEK293T cells), breast cancer cells (e.g., MDA-MB-231 cells, MDA-MB-468 cells, HCC38 cells), and kidney cancer cells (sk-rc-59 cells).

[0027] In embodiments the dy comprises ViaStain™ Tracer Blue dye.

[0028] In embodiments, the plate comprises a multi-well plate with 4-, 6-, 8-, 12-, 24-, 48- 96- or greater than 96 wells.

[0029] In embodiments, period of time refers to about 2 hours, 4 hours 6 hours, 8 hours, 12 hours, 16 hours, 24 hours, 36 hours, 48 hours, or longer than 48 hours.

[0030] Other objects and advantages of this invention will become readily apparent from the ensuing description.

BRIEF DESCRIPTION OF THE FIGURES

[0031] FIG. 1 shows anti-CXCR4 single-chain variable fragment expressing bacteriophage, 2N and 6R bind CXCR4 positive cells. Dilution series of CXCR4-recognizing single-chain variable fragments (scFv), expressed as bacteriophage, binding to CXCR4-expressing stable HEK 293T cells. Binding was analyzed using flow cytometry analysis with a PE-anti-M13 bacteriophage secondary antibody. All scFvs, except 12G5, were discovered in the Marasco laboratory. 12G5 is a commercially available CXCR-4 recognizing antibody from a mouse hybridoma.

[0032] FIG. 2 shows specific cytotoxicity of CXCR-4 expressing cells by CXCR4-targeting chimeric antigen receptor T cells by measurement of target-cell adherence after CAR T-cell addition at 40 hours. *A*, Schematic diagram of CAR gene. A leader sequence targets the CAR through cell endoplasmic reticulum for membrane expression. Here, a monospecific scfv is fused to CD8 hinge, 41BB transmembrane and intracellular signaling domain and CD3 zeta signaling domain. In the second expression cassette either fluorescent molecule or immune-modulating minibody or antibody can be expressed. *B,C* Loss of target cell adherence due to CAR-T cell killing of MDA-MB-231 CXCR4 target cell line or MDA-MB-231 (no CXCR4), respectively. Controls include untransduced T-cells (un) and irrelevant CAR (G36) and target cells without T cells (black line) *C*, IFN- γ enzyme-linked

immunosorbant assay (ELISA) measuring CAR-T cell activation in the presence of MDA-MB-231 (grey) and MDA-MB-231-CXCR4 expressing target cells (black) demonstrating specific activation of CAR-T cells only in presence of CXCR4 expressing target cells. Target cell/CAR description as above.

[0033] FIG. 3 shows Specific cytotoxicity of CXCR-4 expressing target cells by CXCR4-targeting chimeric antigen receptor (CAR) T cells by quantification of fluorescently labeled target cells. Enumeration of mCardinal fluorescently labeled target cells (*TOP GRAPH*) MDA-MB-231-CXCR4, or (*BOTTOM GRAPH*) MDA-MB-231 alone (time -1 hour), directly after T-cell addition (0 hour), at 24 and 48 hours post T-cell addition. Loss of target cells at 24 and 48 hours by 2N, 6R, X33 and X48 only in A, and not B demonstrate, specific killing due to recognition of CXCR4 on target cells. Controls include untransduced T cells (Un), irrelevant CAR (G36) and no T cells (cells only)

[0034] FIG. 4 shows *Clostridium perfringens* endotoxin (CPE) -FC fusion protein recognizes claudin-4 on cells. *A*, Linear schematic of *Clostridium perfringens* endotoxin protein. Cytotoxic domain (black), claudin-4 targeting domain (green), amino acid domain used in CPE-FC fusion (185-319) underlined. *B*, Linear schematic of CPE-FC fusion protein with claudin-4-binding domain expressed in frame to IgG1 hinge (H), CH2 and CH3 FC domains. *C*, SDS-PAGE of CPE-FC with and without reducing agents after purification on protein A column. *D*, Flow cytometry analysis of CPE-FC and FITC anti-human-FC secondary binding to HEK 293 (grey) and claudin-4 transfected HEK (black).

[0035] FIG. 5 shows Lead claudin-4 (cldn4) specific scFv EK01 binds claudin-4 expressing cells. *A*, Analysis of cldn4 on TNBC cell line HCC38 by flow cytometry staining with CPE-FC. *B*, Claudin-4 targeting scFv EK01 specifically binds HCC38 cells compared to HEK.

[0036] FIG. 6 shows Anti-T-cell immunoreceptor with Ig and ITIM domains (TIGIT) as part of a secreted mini/antibody for bispecific triple-negative breast cancer CAR. Sensorgrams of discovered anti-TIGIT antibodies (TIG1 (red), TIG6 (green)) and commercially available anti-TIGIT antibody MAB7898 (blue) binding to His-tagged TIGIT using biolayer interferometry. Dissociation rates – TIG1 3.12 nM, TIG6 13.3 nM.

[0037] FIG. 7 is a photomicrograph showing the Quantification of fluorescent target cells in co-culture with CAR-T cells using Celigo.

[0038] FIG. 8 are graphs showing the Enumeration of MDA-MB-231 +/- CXCR4 target cells in co-culture with untransduced (Un, black), irrelevant CAR (IR, orange), CXCR4 targeting CARs (CX1-4) at effector to target (E:T) ratio 10:1. (n=2 donors)

[0039] FIG. 9 are graphs showing the Enumeration of MDA-MB-231 +/- CXCR4 target cells in co-culture with untransduced (Un, black), irrelevant CAR (IR, orange), CXCR4 targeting CARs (CX1-4) at effector to target (E:T) ratio 2:1. (n=2 donors)

[0040] FIG. 10 shows *in vitro* evaluation of CXCR4-targeted CAR Killing. Graphs depict killing of HCC38 +/- CXCR4 at 10:1 (E:T). (n=2 donors)

[0041] FIG. 11 shows immune infiltrate of TNBC xenografts in a humanized mouse model. Fig. 11A is a graph showing the percentage of human CD45+ 17 weeks post HSC implantation. Fig. 11B is a graph showing tumor growth of four TNBC cell line-derived xenografts (n=4). Fig. 11C is a graph showing the percentage of human CD45+ events in tumors. Fig. 11D is a graph showing the percentage of CD3+ T-cells of CD45+ parent population. Fig. 11E is a graph showing the percentage of CD3+ myeloid cells of CD45+ parent population.

[0042] FIG. 12 is a table showing the tumor weight.

[0043] FIG. 13 shows the amino acid sequences of the following clones: 2N, 6R, X18, X19, X20, X33, and X48. The residues shown in bold represent the consensus amino acid sequence. In the consensus sequence, four or more clones having the same amino acid at a given position are designated as that amino acid. Framework Regions 1-4 (FW1-4), and Complementarity Determining Regions 1-3 (CDR1-3) for both the variable region of heavy chain ("VH") and the variable region of light chain ("VL") are shown for each clone. The VH and VL family designations are also provided.

[0044] FIG. 14 is a schematic showing the amino acid sequence of the human CXCR4 receptor. Also provided are helical wheel (TOP) and serpentine diagrams (BOTTOM) of the human CXCR4 receptor.

[0045] FIG. 15 shows the amino acid sequences of the anti-CLDN4 clone EK01. Panel A shows the amino acid sequence of the anti-CLDN4 clone EK01. Panel B shows the amino acid sequence of anti-CLDN4 clone EK01 with germline comparison.

[0046] FIG. 16 shows the identification of three additional anti-TIGIT antibodies from new PMPL panning as determined by ELISA assay. ELISA plates were coated with 1µg/ml soluble PD1 for 2 hours at 37°C. The plates were then washed and blocked with 2% BSA/PBS at 37°C for 1 hour. The blocking solution was removed and 3x serial dilutions of the antibodies were added to each well (100µl), starting with 6µg/ml. The plates were then incubated at RT with gentle shaking, washed 6x with PBS-T, and the secondary anti-human Fc-HRP (1:150k, Bethyl) was added. The plates were again incubated at room temperature with gentle shaking for 1 hour before being washed 6x with PBS-T. TMB substrate was

added and the plate was incubated at 30°C for 10 min to accelerate the HRP reaction. The signal was then quenched with TMB stop solution and read at 450nm. Samples P5-E10 and P6-G7 were run in quadruplicate and P6-G7 and TIG1 were run in triplicate.

[0047] **FIG. 17** shows the amino acid sequences and germline alignment of anti-TIGIT antibodies (sol protein & PMPL).

[0048] **FIG. 18** shows coexpression of CXCR4 and CLDN4 on breast cell lines. As described herein, TNBC cell lines tested include DU4475 and HCC1187, which are primary TNBC cell lines with different level of CXCR4 and CLDN4 endogenous cell surface expression. Data include CXCR4 and CLDN4 surface expression determined by flow cytometry, *in vitro* CART killing assays analyzed with Cellego, and *in vivo* tumor growth in humanized mice and TIL analysis by flow cytometry.

[0049] **FIG. 19** shows flow cytometry analysis of endogenous CXCR4 and Claudin-4 protein expression of DU4475 and HCC1187. CLDN4 expression was determined with CPE-Fc and FITC anti-human-FC secondary antibody; and CXCR4 expression was determined using anti-CXCR4 antibody followed by APC conjugated secondary antibody.

[0050] **FIG. 20** shows X48 CART kills CXCR4 expressing cell lines HCC1187 and DU4475. Cytotoxicity of HCC1187, DU4475, and MDA-MB-231 +/- CXCR4 target cells in co-culture with untransduced (Un, blue), irrelevant CAR (G36, red), CXCR4 targeting CARs (X48, green) and no T cells (cells only, black) at effector to target (E:T) ratio 10:1 and 2:1 is shown. Enumeration of mCardinal fluorescently labeled target cells were determined with Celego directly after T-cell addition (0 hour), at 24 and 48 hours post T-cell addition.

[0051] **FIG. 21** shows IFN- γ production by CAR-T co-cultured with endogenous expressing CXCR4 cell lines HCC1187 and DU 4475. IFN- γ enzyme-linked immunosorbant assay (ELISA) measuring CAR-T cell activation in the presence of MDA-MB-231 (blue), MDA-MB-231-CXCR4 expressing target cells (red), HCC1187 (green) and DUC4475 (purple) demonstrating specific activation of CAR-T cells only in presence of CXCR4 expressing target cells. Target cell/CAR description same as in FIG. 20. 231 CXCR4-exogenous gene expression.

[0052] **FIG. 22** shows HC1187 tumor growth in humanized mouse model. Shown herein is immune infiltrate of TNBC xenografts in a humanized mouse model. Panel A shows the tumor image at the end of study (33 days post engraftment). Panel B is a graph showing tumor growth in each mouse (n=5) as measured by caliber. Panel C shows the percentage of human CD45+ events as well as other cell subpopulations events in HC1187 TNBC tumors

vs. spleen. Panel D is a graph showing the percentage of PD-1+, TIGIT+ or TIGIT+/PD-1+ T-cells of the CD3+ parent population in tumor vs. spleen.

DETAILED DESCRIPTION OF THE INVENTION

[0053] Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer with poor clinical prognosis. While treated with chemotherapies, the high incidence of relapse signifies the need for novel, targeted therapies.

[0054] Chimeric-antigen receptor (CAR) T-cell therapies redirect a patient's T-cells to kill tumor cells by the exogenous expression of a CAR. A CAR can be a membrane spanning fusion protein that links the antigen recognition domain of an antibody to the intracellular signaling domains of the T-cell receptor and co-receptor. Solid tumors offer unique challenges for CAR-T therapies. Unlike blood cancers, tumor-associated target proteins are overexpressed between the tumor and healthy tissue resulting in on-target/off-tumor T-cell killing of healthy tissues. Furthermore, immune repression in the tumor microenvironment (TME) limits the activation of CAR-T cells towards killing the tumor.

[0055] The present invention relates to engineered chimeric antigen receptor (CAR) T-cell factories that secrete antibodies for TNBC. Without wishing to be bound by theory, a bispecific CAR targeting two antigens on the solid tumor, such as TNBC, will mitigate on-target/off-tumor T-cell killing, and that the secretion of a checkpoint blockade antibody will remove repression in the tumor microenvironment. Following local immune restoration, the CAR-T cells and other cells in the TME will work synergistically to shrink and clear tumors.

[0056] Abbreviations and Definitions

[0057] Detailed descriptions of one or more preferred embodiments are provided herein. It is to be understood, however, that the present invention may be embodied in various forms. Therefore, specific details disclosed herein are not to be interpreted as limiting, but rather as a basis for the claims and as a representative basis for teaching one skilled in the art to employ the present invention in any appropriate manner.

[0058] The singular forms "a", "an" and "the" include plural reference unless the context clearly dictates otherwise. The use of the word "a" or "an" when used in conjunction with the

term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

[0059] Wherever any of the phrases “for example,” “such as,” “including” and the like are used herein, the phrase “and without limitation” is understood to follow unless explicitly stated otherwise. Similarly “an example,” “exemplary” and the like are understood to be nonlimiting.

[0060] The term “substantially” allows for deviations from the descriptor that do not negatively impact the intended purpose. Descriptive terms are understood to be modified by the term “substantially” even if the word “substantially” is not explicitly recited.

[0061] The terms “comprising” and “including” and “having” and “involving” (and similarly “comprises”, “includes,” “has,” and “involves”) and the like are used interchangeably and have the same meaning. Specifically, each of the terms is defined consistent with the common United States patent law definition of “comprising” and is therefore interpreted to be an open term meaning “at least the following,” and is also interpreted not to exclude additional features, limitations, aspects, etc. Thus, for example, “a process involving steps a, b, and c” means that the process includes at least steps a, b and c. Wherever the terms “a” or “an” are used, “one or more” is understood, unless such interpretation is nonsensical in context.

[0062] As used herein the term “about” is used herein to mean approximately, roughly, around, or in the region of. When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 20 percent up or down (higher or lower).

[0063] *Engineered CAR T cells*

[0064] Chimeric-antigen receptor (CAR) T-cell therapies redirect a patient’s T-cells to kill tumor cells by the exogenous expression of a CAR. A CAR is a membrane spanning fusion protein that links the antigen recognition domain of an antibody or fragment to the intracellular signaling domains of the T-cell receptor and co-receptor. For example, chimeric antigen receptors fuse antigen-specific antibody fragments to T-cell co-stimulatory domains and the CD3 zeta intracellular signaling domain, allowing for the re-direction of T-cells towards an antigen presented on a cell of interest, for example, onto tumor cells.

[0065] The term “antibody” herein is used in the broadest sense and refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig)

molecules, i.e., molecules that contain an antigen binding site that specifically binds (immunoreacts with) an antigen. “specifically binds” or “immunoreacts with” can refer to the antibody reacting with one or more antigenic determinants of the desired antigen and does not react with other polypeptides. Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, humanized, fully human, bispecific, multispecific, chimeric, dAb (domain antibody), single chain antibodies, Fab, Fab' and F(ab')₂ fragments, scFvs, diabodies, minibodies, scFv-Fc fusions, and Fab expression libraries. Unless specified to the contrary, any reference to “antibody” or “antibodies” made herein encompasses, for example, any (or all) of these molecules so long as they exhibit the desired antigen-binding activity.

[0066] A single chain Fv (“scFv”) polypeptide molecule is a covalently linked VH:VL heterodimer, which can be expressed from a gene fusion including VH- and VL-encoding genes linked by a peptide-encoding linker. (See Huston et al. (1988) Proc Nat Acad Sci USA 85(16):5879-5883). A number of methods have been described to discern chemical structures for converting the naturally aggregated, but chemically separated, light and heavy polypeptide chains from an antibody V region into an scFv molecule, which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, e.g., U.S. Pat. Nos. 5,091,513; 5,132,405; and 4,946,778.

[0067] Bispecific antibodies refer to antibodies that have binding specificities for at least two different antigens. For example, bispecific antibodies can be monoclonal antibodies, such as human or humanized antibodies. In the present case, one of the binding specificities is CXCR4 and/or Claudin-4. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit. For example, one of the binding specificities is for CXCR4 and the second binding specificity is for claudin-4.

[0068] Bispecific antibodies can be prepared as full length antibodies or antibody fragments (e.g. F(ab')₂ bispecific antibodies). Methods for making bispecific antibodies are known in the art. See for example U.S. patent 8,329,178, which is incorporated herein by reference in its entirety.

[0069] In general, antibody molecules obtained from humans relate to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain.

[0070] The term “antigen-binding site,” or “binding portion” refers to the part of the immunoglobulin molecule that participates in antigen binding. The antigen binding site is

formed by amino acid residues of the N-terminal variable (“V”) regions of the heavy (“H”) and light (“L”) chains. Three highly divergent stretches within the V regions of the heavy and light chains, referred to as “hypervariable regions,” are interposed between more conserved flanking stretches known as “framework regions,” or “FRs”. Thus, the term “FR” refers to amino acid sequences which are naturally found between, and adjacent to, hypervariable regions in immunoglobulins. In an antibody molecule, the three hypervariable regions of a light chain and the three hypervariable regions of a heavy chain are disposed relative to each other in three dimensional space to form an antigen-binding surface. The antigen-binding surface is complementary to the three-dimensional surface of a bound antigen, and the three hypervariable regions of each of the heavy and light chains are referred to as “complementarity-determining regions,” or “CDRs.”

[0071] Solid tumors offer unique challenges for CAR-T therapies. Unlike blood cancers, tumor-associated target proteins are overexpressed between the tumor and healthy tissue resulting in on-target/off-tumor T-cell killing of healthy tissues. Furthermore, immune repression in the tumor microenvironment (TME) limits the activation of CAR-T cells towards killing the tumor. Aspects of the invention address these problems. For example, embodiments comprise T cells comprising a bispecific CAR that (a) targets two antigens on a cancer cell to mitigate on-target/off-tumor T-cell killing, and (b) secretes a checkpoint blockade antibody that removes repression in the tumor microenvironment.

[0072] An emerging mechanism associated with the progression of tumors is the immune checkpoint pathway, which include cellular interactions that prevent excessive activation of T cells under normal conditions, allowing T cell function in a self-limited manner. As an evasion mechanism, many tumors are able to stimulate the expression of immune checkpoint molecules, resulting in an anergic phenotype of T cells that cannot restrain tumor progression. For example, emerging clinical data highlight the importance of one inhibitory ligand and receptor pair as an immune checkpoint: the programmed death-ligand 1 (PD-L1; B7-H1 and CD274) and programmed death receptor-1 (PD-1; CD279), in preventing killing of cancer cells by cytotoxic T-lymphocytes. PD1 receptor is expressed by many cell types like T cells, B cells, Natural Killer cells (NK) and host tissues. Tumors and Antigen-presenting cells (APC) expressing PD-L1 can block T cell receptor (TCR) signaling of cytotoxic T-lymphocytes through binding to receptor PD-1, decreasing the production of cytokines and T cell proliferation. PD-L1 overexpression can be found in many tumor types and may also mediate an immunosuppressive function through its interaction with other

proteins, including CD80 (B7.1), blocking its ability to activate T cells through binding to CD28.

[0073] Genetic engineering of human lymphocytes to express tumor-directed chimeric antigen receptors (CAR) can produce antitumor effector cells that bypass tumor immune escape mechanisms that are due to abnormalities in protein-antigen processing and presentation. Moreover, these transgenic receptors can be directed to tumor-associated antigens that are not protein-derived. In certain embodiments of the invention, there are lymphocytes (CARTS) that are modified to comprise at least a CAR, and in particular embodiments of the invention, a single CAR targets two or more antigens. In some embodiments, the CARTS are further modified to express and secrete one or more polypeptides, such as for example an antibody or a cytokine. Such CARTS are referred to herein as armed CARTS or CAR factories. Armed CARTS allow for simultaneous secretion of the polypeptide locally at the targeted site (i.e., tumor site).

[0074] A modified TCR called chimeric antigen receptor (CAR) containing single chain variable antibody fragment (scFv) previously selected by high affinity against a specific tumor associated antigen is a powerful new approach against cancer. The scFv presented in the CAR is linked to an intracellular signaling block that includes CD3 ζ to induce T cell activation followed by antigen binding. This structure is characteristic for first-generation CARs, which were improved to second- and generation CARs that link the signaling co-stimulatory endodomains of CD28, 4-1BB, or OX40 to CD3 or 3rd-generation CARs that links two elements to CD3 ζ in tandem. These endodomains are required for complete T cell activation during TCR recognition by antigen-presenting cells (APCs), improving cytokine production and proliferation of CAR-T cells. The effect of CART cells has heretofore been modest for the treatment of solid tumors, due to difficulty in finding unique tumor associated antigens, inefficient homing of T cells to tumor locations, low persistence of T cells in the body and the immunosuppressive microenvironment of solid tumors.

[0075] In particular cases, the lymphocytes can include a receptor that is chimeric, non-natural and engineered at least in part by the hand of man. In particular cases, the engineered chimeric antigen receptor (CAR) has one, two, three, four, or more components, and in some embodiments the one or more components facilitate targeting or binding of the lymphocyte to one or more tumor antigen-comprising cancer cells.

[0076] The CAR according to the invention generally comprises at least one transmembrane polypeptide comprising at least one extracellular ligand-binding domain and;

one transmembrane polypeptide comprising at least one intracellular signaling domain; such that the polypeptides assemble together to form a Chimeric Antigen Receptor. Exemplary CARS useful in aspects of the invention include those disclosed in for example PCT/US2015/067225.

[0077] The term "extracellular ligand-binding domain" as used herein can refer to an oligo- or polypeptide that is capable of binding a ligand. The domain can be capable of interacting with a cell surface molecule. For example, the extracellular ligand-binding domain may be chosen to recognize a ligand that acts as a cell surface marker on target cells associated with a particular disease state.

[0078] In particular, the extracellular ligand-binding domain can comprise an antigen binding domain or antigen recognition domain derived from an antibody against an antigen of the target. The antigen binding domain or antigen recognition domain can be an antibody fragment. An "antibody fragment" can be a molecule other than an intact antibody that comprises a portion of an intact antibody that binds the antigen to which the intact antibody binds. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')₂; diabodies; linear antibodies; single-chain antibody molecules (e.g. scFv); and multispecific antibodies formed from antibody fragments. Referring to FIG. 2, for example, one embodiment comprises a CAR with an scFv as the antigen recognition domain..

[0079] The antigen recognition domain can be directed towards any antigen target of interest. In embodiments, the antigen target of interest is on the surface of a cell, such as the surface of a cancer cell. Non-limiting examples of antigen targets comprise C-X-C chemokine receptor type 4 (CXCR-4) and/or claudin-4.

[0080] As non limiting examples, the antigen of the target can be a tumor-associated surface antigen, such as ErbB2 (HER2/neu), carcinoembryonic antigen (CEA), epithelial cell adhesion molecule (EpCAM), epidermal growth factor receptor (EGFR), EGFR variant III (EGFRvIII), CD19, CD20, CD30, CD40, disialoganglioside GD2, ductal-epithelial mucine, gp36, TAG-72, glycosphingolipids, glioma-associated antigen, .beta.-human chorionic gonadotropin, alphafetoprotein (AFP), lectin-reactive AFP, thyroglobulin, RAGE-1, MN-CA IX, human telomerase reverse transcriptase, RU1, RU2 (AS), intestinal carboxyl esterase, mut hsp70-2, M-CSF, prostase, prostase specific antigen (PSA), PAP, NY-ESO-1, LAGA-1a, p53, prostein, PSMA, surviving and telomerase, prostate-carcinoma tumor antigen-1 (PCTA-1), MAGE, ELF2M, neutrophil elastase, ephrin B2, CD22, insulin growth factor (IGF1)-I, IGF-II, IGF1 receptor, mesothelia, a major histocompatibility complex (MHC) molecule presenting a tumor-specific peptide epitope, 5T4, ROR1, Nkp30, NKG2D, tumor stromal

antigens, the extra domain A (EDA) and extra domain B (EDB) of fibronectin and the A1 domain of tenascin-C (TnC A1) and fibroblast associated protein (fap); a lineage-specific or tissue specific antigen such as CD3, CD4, CD8, CD24, CD25, CD33, CD34, CD133, CD138, CTLA-4, B7-1 (CD80), B7-2 (CD86), endoglin, a major histocompatibility complex (MHC) molecule, BCMA (CD269, TNFRSF 17), or a virus-specific surface antigen such as an HIV-specific antigen (such as HIV gp120); an EBV-specific antigen, a CMV-specific antigen, a HPV-specific antigen, a Lasse Virus-specific antigen, an Influenza Virus-specific antigen as well as any derivate or variant of these surface markers.

[0081] In some embodiments, the CAR is specific for CXCR4 and/or claudin-4.

[0082] In embodiments, said extracellular ligand-binding domain is a single chain antibody fragment (scFv) comprising the light (V_L) and the heavy (V_H) variable fragment of a target antigen specific monoclonal antibody joined by a flexible linker. For example, the scFv antibody is specific for CXCR4 and/or claudin-4.

[0083] Non-limiting examples of antibodies useful in constructing the CAR according to the invention includes antibodies disclosed in for example: WO/2005/060520 , WO/2006/089141, WO/2007/065027, WO/2009/086514, WO/2009/079259, WO/2011/153380, WO/2014/055897, WO 2015/143194, WO 2015/164865, WO 2013/166500, and WO 2014/144061; PCT/US2015/054202, PCT/US2015/054010, PCT/US2015/067225, and PCT/US2016/026800, the contents of which are hereby incorporated by reference in their entireties.

[0084] The antigen recognition domain useful in constructing the CAR-Ts, for example scFVs directed toward C-X-C chemokine receptor type 4 (CXCR-4) and/or claudin-4, can be synthesized, engineered, and/or produced using nucleic acids (e.g., DNA). The DNA encoding the antigen recognition domain can be cloned in frame to DNA encoding necessary CAR-T elements such as, but not limited to, CD8 hinge regions, transmembrane domains, co-stimulatory domains of molecules of immunological interest such as, but not limited to, CD28 and 41BB and CD3-zeta intracellular signaling domains. See FIG. 2, for example.

[0085] *CXCR4*

[0086] Chemokine (C-X-C motif) receptor 4 (“CXCR4”) (also known as fusin, LESTR, or HUMSTR) is a G protein-coupled, 7-transmembrane domain chemokine receptor that is normally embedded within the membrane of a cell. CXCR4 is one of the best-characterized chemokine receptors. The 352 amino acid sequence (along with helical wheel and serpentine diagrams) of the human CXCR4 receptor is shown in FIGS. 14 (Top and Bottom panels). As

shown in FIG. 14, bottom panel, CXCR4 is characterized by four different extracellular regions: the N terminal domain, ECL1, ECL2, and ECL3.

[0087] CXCR4 is expressed at least in dendritic cells; naïve, non-memory T-cells; regulatory T cells; neurons and microglia; fresh primary monocytes; endothelial cells; neutrophils and B-cells; tumor cells, including but not limited to breast cancer cells, renal cell carcinoma cells, non-small cell lung cancer cells, prostate cancer cells, and glioblastoma cells; and CD34⁺ hematopoietic stem cells. CXCR4 is essential for leukocyte trafficking; chemotaxis; B cell lymphopoiesis and myelopoiesis; stem cell migration; tumor or cancer cell metastasis; tumor cell angiogenesis; gastrointestinal tract vascularization; neuronal and germ cell migration; and X4-tropic HIV invasion of host cells. (See Li et al., *Cancer Cell* 6:459-69 (2004); Hernandez et al., *Nat. Genet.* 34:70-74 (2003); Nagasawa et al., *Nature* 382:635-38 (1996); Knaut et al., *Nature* 421:279-82 (2003); Kunwar et al., *Nature* 421:226-27 (2003); Connor et al., *J. Exp. Med.* 185:621-28 (1997); and Scarlatti et al., *Nat. Med.* 3:1259-65 (1997)).

[0088] The alpha-chemokine stromal cell-derived factor (SDF-1) (also known as CXCL12) is the natural ligand for CXCR4. SDF-1 α is the only chemokine that has just one chemokine receptor. (See Imitola et al., *Proc. Natl. Acad. Sci. USA* 101(52):18117-22 (2004); Lu et al., *Proc. Natl. Acad. Sci. USA* 99:2090-95 (2002). SDF-1 binding to CXCR4 activates multiple pathways that function to regulate cell invasion and migration. (See Benovic et al., *Cancer Cell* 6:429-30 (2004)). For example, in response to binding its ligand, CXCR4 triggers the migration and recruitment of immune cells. Additionally, this ligand-receptor pair may also play a role in the development of the nervous system. SDF-1 binding to CXCR4 also plays an important role in hematopoiesis and organogenesis. (See Nagasawa et al., *Nature* 382:635 (1996)). CXCR4 is also recognized by an antagonistic chemokine, the viral macrophage inflammatory protein II (vMIP-II) encoded by human herpesvirus type III. (See, Zhou et al., 9th Conference on Retroviruses and Opportunistic Infections, Session 39 Poster Session, Abstract 189-M (2002)).

[0089] Embodiments of the present invention can comprise anti-CXCR4 antibodies. Exemplary anti-CXCR4 antibodies include those described in U.S. patent 8,329,178, which is incorporated herein by reference in its entirety.

[0090] For example, anti-CXCR4 antibodies can comprise mAb18, mAb 19, mAb 20, mAb 33, mAb 48, mAb2N, or mAb 6R. The amino acid sequences of the VH and VL regions of mAb 18, mAb 19, mAb 20, mAb 33, mAb 48, mAb 2N, and mAb 6R are provided in FIG. 13. FIG. 13 also provides a consensus sequence (e.g., bolded letters; see also U.S. patent

8,329,178, which is incorporated herein by reference in its entirety). In this consensus, when four or more clones have the same amino acid at a given position, that position in the consensus is designated by that amino acid. The anti-CXCR4 antibody can also comprise 12G5. 12G5 is a commercially available CXCR-4 recognizing antibody from a mouse hybridoma.

[0091] As shown in FIG. 13, CDR1 of the VH region of the mAb 2N heavy chain has the sequence: SYGMH (SEQ ID NO:17); CDR2 of the VH region of the mAb 2N heavy chain has the sequence: VISYDGSNKYYADSVKG (SEQ ID NO:18); CDR3 of the VH region of the mAb 2N heavy chain has the sequence: DLVAAAGTAFDI (SEQ ID NO:19); CDR1 of the VL region of the mAb 2N light chain has the sequence TGTISDVGGHNFVS (SEQ ID NO:20); CDR2 of the VL region of the mAb 2N light chain has the sequence: EVTKRPA (SEQ ID NO:21); and CDR3 of the VL region of the mAb 2N light chain has the sequence: SSYGGSNDVI (SEQ ID NO:22).

[0092] Moreover, as shown in FIG. 13, CDR1 of the VH region of the mAb 6R heavy chain has the sequence: SNFVAWN (SEQ ID NO:23); CDR2 of the VH region of the mAb 6R heavy chain has the sequence: RTYYRSRWYNDYAVSVQS (SEQ ID NO:24); CDR3 of the VH region of the mAb 6R heavy chain has the sequence: GQHSGFDF (SEQ ID NO:25); CDR1 of the VL region of the mAb 6R light chain has the sequence TGNSNNVGNQGAA (SEQ ID NO:26); CDR2 of the VL region of the mAb 6R light chain has the sequence: RNNNRPS (SEQ ID NO:27); and CDR3 of the VL region of the mAb 6R light chain has the sequence: SAWDNRLKTYV (SEQ ID NO:28).

[0093] As also shown in FIG. 13, CDR1 of the VH region of the mAb 18 heavy chain has the sequence: SYGIS (SEQ ID NO:29); CDR2 of the VH region of the mAb 18 heavy chain has the sequence: WISAYNGNTNYAQKLQG (SEQ ID NO:30); CDR3 of the VH region of the mAb 18 heavy chain has the sequence: DTPGIAARRYYYYGMDV (SEQ ID NO:31); CDR1 of the VL region of the mAb 18 light chain has the sequence QGDSLRLKFFAS (SEQ ID NO:32); CDR2 of the VL region of the mAb 18 light chain has the sequence: GKNSRPS (SEQ ID NO:33); and CDR3 of the VL region of the mAb 18 light chain has the sequence: NSRDSRDNHQV (SEQ ID NO:34).

[0094] Similarly, as shown in FIG. 13, CDR1 of the VH region of the mAb 19 heavy chain has the sequence: SYPMH (SEQ ID NO:35); CDR2 of the VH region of the mAb 19 heavy chain has the sequence: VISSDGRNKYYPDSVKG (SEQ ID NO:36); CDR3 of the VH region of the mAb 19 heavy chain has the sequence: GGYHDFWSGPDY (SEQ ID NO:37); CDR1 of the VL region of the mAb 19 light chain has the sequence

RASQSVNTNLA (SEQ ID NO:38); CDR2 of the VL region of the mAb 19 light chain has the sequence: GASSRAT (SEQ ID NO:39); and CDR3 of the VL region of the mAb 19 light chain has the sequence: QHYGSSPLT (SEQ ID NO:40).

[0095] As shown in FIG. 13, CDR1 of the VH region of the mAb 20 heavy chain has the sequence: SYAMS (SEQ ID NO:41); CDR2 of the VH region of the mAb 20 heavy chain has the sequence: NIKQDGSEKYYVDSVKG (SEQ ID NO:42); CDR3 of the VH region of the mAb 20 heavy chain has the sequence: DQVSGITIFGGKWRSPDV (SEQ ID NO:43); CDR1 of the VL region of the mAb 20 light chain has the sequence QGDSLRSYYAS (SEQ ID NO:44); CDR2 of the VL region of the mAb 20 light chain has the sequence: GKNNRPS (SEQ ID NO:45); and CDR3 of the VL region of the mAb 20 light chain has the sequence: NSRSGSQRV (SEQ ID NO:46).

[0096] Moreover, CDR1 of the VH region of the mAb 33 heavy chain has the sequence: NYGLH (SEQ ID NO:47); CDR2 of the VH region of the mAb 33 heavy chain has the sequence: VISHDGTTKYYADSVKG (SEQ ID NO:48); CDR3 of the VH region of the mAb 33 heavy chain has the sequence: DGGYCSGGRCYSYGMDV (SEQ ID NO:49); CDR1 of the VL region of the mAb 33 light chain has the sequence SGSRSNIGSNTVN (SEQ ID NO:50); CDR2 of the VL region of the mAb 33 light chain has the sequence: TNNQRPS (SEQ ID NO:51); and CDR3 of the VL region of the mAb 33 light chain has the sequence: LSFDSLSYV (SEQ ID NO:52).

[0097] Likewise, as also shown in FIG. 13, CDR1 of the VH region of the mAb 48 heavy chain has the sequence: RYGMH (SEQ ID NO:53); CDR2 of the VH region of the mAb 48 heavy chain has the sequence: LISYDGSKTFYGESVKG (SEQ ID NO: 54); CDR3 of the VH region of the mAb 48 heavy chain has the sequence: ATVTTDGYYYMDV (SEQ ID NO: 55); CDR1 of the VL region of the mAb 48 light chain has the sequence SGSRSNIGGNTVN (SEQ ID NO:56); CDR2 of the VL region of the mAb 48 light chain has the sequence: ANNQRPS (SEQ ID NO: 57); and CDR3 of the VL region of the mAb 48 light chain has the sequence: AAWDDNLSGHVV (SEQ ID NO: 58).

[0098] In embodiments, the anti-CXCR4 antibody can comprise a heavy chain with a VH comprising a CDR1 of SEQ ID NO: 17, 23, 29, 35, 41, 47, or 53; a CDR2 of SEQ ID NO: 18, 24, 30, 36, 42, 48, or 54; and a CDR3 of SEQ ID NO: 19, 25, 31, 37, 43, 49, or 55; or any combination thereof.

[0099] In embodiments, the anti- CXCR4 antibody can comprise a light chain with a VL comprising a CDR1 of SEQ ID NO: 20, 26, 32, 38, 44, 50, or 56; a CDR2 of SEQ ID NO: 21,

27, 33, 39, 45, 51, or 57; and a CDR3 of SEQ ID NO: 22, 28, 34, 40, 46, 52, or 58; or any combination thereof.

[00100] The invention also encompasses single chain antibodies. For example, the invention encompasses scFvs 18, 19, 20, 33, 48, 2N, and 6R as well as any other scFvs identified according to the methods disclosed herein..

[00101] *Claudin-4*

[00102] Claudins are transmembrane proteins that belong to the multigene adhesion molecule family and are found in cellular tight junctions. These proteins are considered to be responsible for the establishment of a paracellular barrier that controls the flow of molecules across the intracellular spaces of the epithelium. Claudins are believed to regulate cell proliferation and differentiation because they also bind cell-signaling ligands. There are ~24 known claudins that have a tissue-specific expression pattern and any change in their pattern or distribution has been suggested to have a role in the pathology of various disorders, including cancer. Gene expression profiling showed that claudin 4 was overexpressed in pancreatic duct adenocarcinoma, and it is also expressed in normal tissues of the breast, ovaries, prostate, bladder, and gastrointestinal mucosa. However, because the level of expression of this protein is significantly higher in primary and metastatic pancreatic cancers, the possible use of claudin 4 as an early marker or therapeutic target for imaging of this cancer was suggested. See [¹²⁵I]Anti-claudin 4 monoclonal antibody from The MICAD Research Team; June 28, 2007.

[00103] As shown in FIG. 15, CDR1 of the VH region of the EK01 heavy chain has the sequence GFTV.... SSNY (SEQ ID NO: 59); CDR2 of the VH region has the sequence IYSG...GST (SEQ ID NO: 60); and CDR3 of the VH region has the sequence ARDNPLSAFDI (SEQ ID NO: 61); CDR1 of the VL region of the EK01 light chain has the sequence QSI.....NSW (SEQ ID NO: 62); CDR2 of the VL region has the sequence KA.....S (SEQ ID NO: 63); and CDR3 of the VL region has the sequence QQYDDLPLT (SEQ ID NO: 64).

[00104] Other binding domain than scFv can also be used for predefined targeting of lymphocytes, such as camelid single-domain antibody fragments or receptor ligands, antibody binding domains, antibody hypervariable loops or CDRs as non limiting examples.

[00105] In a preferred embodiment said transmembrane domain further comprises a stalk region between said extracellular ligand-binding domain and said transmembrane domain. The term "stalk region" used herein can mean any oligo- or polypeptide that functions to link

the transmembrane domain to the extracellular ligand-binding domain. In particular, stalk region are used to provide more flexibility and accessibility for the extracellular ligand-binding domain. A stalk region may comprise up to 300 amino acids, preferably 10 to 100 amino acids and most preferably 25 to 50 amino acids. Stalk region may be derived from all or part of naturally occurring molecules, such as from all or part of the extracellular region of CD8, CD4 or CD28, or from all or part of an antibody constant region. Alternatively the stalk region may be a synthetic sequence that corresponds to a naturally occurring stalk sequence, or may be an entirely synthetic stalk sequence. In an embodiment said stalk region is a part of human CD8 alpha chain

[00106] The signal transducing domain or intracellular signaling domain of the CAR of the invention is responsible for intracellular signaling following the binding of extracellular ligand binding domain to the target resulting in the activation of the immune cell and immune response. In other words, the signal transducing domain is responsible for the activation of at least one of the normal effector functions of the immune cell in which the CAR is expressed. For example, the effector function of a T cell can be a cytolytic activity or helper activity including the secretion of cytokines. Thus, the term "signal transducing domain" can refer to the portion of a protein which transduces the effector signal function signal and directs the cell to perform a specialized function.

[00107] Signal transduction domain can comprise two distinct classes of cytoplasmic signaling sequence, those that initiate antigen-dependent primary activation, and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal. Primary cytoplasmic signaling sequence can comprise signaling motifs which are known as immunoreceptor tyrosine-based activation motifs of ITAMs. ITAMs are well defined signaling motifs found in the intracytoplasmic tail of a variety of receptors that serve as binding sites for syk/zap70 class tyrosine kinases. Examples of ITAM used in the invention can include as non limiting examples those derived from TCR zeta, FcR gamma, FcR beta, FcR epsilon, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b and CD66d. In a preferred embodiment, the signaling transducing domain of the CAR can comprise the CD3 zeta signaling domain, or the intracytoplasmic domain of the Fc epsilon RI beta or gamma chains. In another preferred embodiment, the signaling is provided by CD3 zeta together with co-stimulation provided by CD28 and a tumor necrosis factor receptor (TNFr), such as 4-1BB or OX40), for example.

[00108] In an embodiment the intracellular signaling domain of the CAR of the present invention comprises a co-stimulatory signal molecule. In some embodiments the intracellular

signaling domain contains 2, 3, 4 or more co-stimulatory molecules in tandem. A co-stimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient immune response.

[00109] "Co-stimulatory ligand" can refer to a molecule on an antigen presenting cell that specifically binds a cognate co-stimulatory molecule on a T-cell, thereby providing a signal which, in addition to the primary signal provided by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, mediates a T cell response, including, but not limited to, proliferation activation, differentiation and the like. A co-stimulatory ligand can include but is not limited to CD7, B7-1 (CD80), B7-2 (CD86), PD-L1, PD-L2, 4-1BBL, OX40L, inducible costimulatory ligand (ICOS-L), intercellular adhesion molecule (ICAM, CD30L, CD40, CD70, CD83, HLA-G, MICA, M1CB, HVEM, lymphotoxin beta receptor, 3/TR6, ILT3, ILT4, an agonist or antibody that binds Toll ligand receptor and a ligand that specifically binds with B7-H3. A co-stimulatory ligand also encompasses, inter alia, an antibody that specifically binds with a co-stimulatory molecule present on a T cell, such as but not limited to, CD27, CD28, 4-1BB, OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LTGHT, NKG2C, B7-H3, a ligand that specifically binds with CD83.

[00110] A "co-stimulatory molecule" can refer to the cognate binding partner on a T-cell that specifically binds with a co-stimulatory ligand, thereby mediating a co-stimulatory response by the cell, such as, but not limited to proliferation. Co-stimulatory molecules include, but are not limited to an MHC class 1 molecule, BTLA and Toll ligand receptor. Examples of costimulatory molecules include CD27, CD28, CD8, 4-1BB (CD137), OX40, CD30, CD40, PD-1, ICOS, lymphocyte function-associated antigen-1 (LFA-1), CD2, CD7, LIGHT, NKG2C, B7-H3 and a ligand that specifically binds with CD83 and the like.

[00111] In another particular embodiment, said signal transducing domain is a TNFR-associated Factor 2 (TRAF2) binding motifs, intracytoplasmic tail of costimulatory TNFR member family. Cytoplasmic tail of costimulatory TNFR family member contains TRAF2 binding motifs consisting of the major conserved motif (P/S/A)X(Q/E)E or the minor motif (PXQXXD), wherein X is any amino acid. TRAF proteins are recruited to the intracellular tails of many TNFRs in response to receptor trimerization.

[00112] Chimeric antigen receptors fuse antigen-recognition domains to signaling domains (also referred to as stimulatory domains) that modulate (i.e., stimulate) cell signaling. Non-limiting examples of such stimulatory domains comprise those of CD28, 41BB, and/or CD3-zeta intracellular signaling domains. See FIG. 2, for example.

[00113] The distinguishing features of appropriate transmembrane polypeptides comprise the ability to be expressed at the surface of an immune cell, in particular lymphocyte cells or Natural killer (NK) cells, and to interact together for directing cellular response of immune cell against a predefined target cell. The different transmembrane polypeptides of the CAR of the present invention comprising an extracellular ligand-binding domain and/or a signal transducing domain interact together to take part in signal transduction following the binding with a target ligand and induce an immune response. The transmembrane domain can be derived either from a natural or from a synthetic source. The transmembrane domain can be derived from any membrane-bound or transmembrane protein.

[00114] The term "a part of" used herein can refer to any subset of the molecule, that is a shorter peptide. Alternatively, amino acid sequence functional variants of the polypeptide can be prepared by mutations in the DNA which encodes the polypeptide. Such variants or functional variants include, for example, deletions from, or insertions or substitutions of, residues within the amino acid sequence. Any combination of deletion, insertion, and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity, especially to exhibit a specific anti-target cellular immune activity. The functionality of the CAR of the invention within a host cell is detectable in an assay suitable for demonstrating the signaling potential of said CAR upon binding of a particular target. Such assays are available to the skilled person in the art. For example, this assay allows the detection of a signaling pathway, triggered upon binding of the target, such as an assay involving measurement of the increase of calcium ion release, intracellular tyrosine phosphorylation, inositol phosphate turnover, or interleukin (IL) 2, interferon gamma., GM-CSF, IL-3, IL-4 production thus effected.

[00115] Cells

[00116] Embodiments of the invention include cells that express a CAR (i.e., CARTS). The cell may be of any kind, including an immune cell capable of expressing the CAR for cancer therapy or a cell, such as a bacterial cell, that harbors an expression vector that encodes the CAR. As used herein, the terms "cell," "cell line," and "cell culture" may be used interchangeably. All of these terms also include their progeny, which is any and all subsequent generations. It is understood that all progeny may not be identical due to deliberate or inadvertent mutations. In the context of expressing a heterologous nucleic acid sequence, "host cell" refers to a eukaryotic cell that is capable of replicating a vector and/or expressing a heterologous gene encoded by a vector. A host cell can, and has been, used as a

recipient for vectors. A host cell may be "transfected" or "transformed," which refers to a process by which exogenous nucleic acid is transferred or introduced into the host cell. A transformed cell includes the primary subject cell and its progeny. As used herein, the terms "engineered" and "recombinant" cells or host cells are intended to refer to a cell into which an exogenous nucleic acid sequence, such as, for example, a vector, has been introduced.

Therefore, recombinant cells are distinguishable from naturally occurring cells which do not contain a recombinantly introduced nucleic acid. In embodiments of the invention, a host cell is a T cell, including a cytotoxic T cell (also known as TC, Cytotoxic T Lymphocyte, CTL, T-Killer cell, cytolytic T cell, CD8+ T-cells or killer T cell); NK cells and NKT cells are also encompassed in the invention.

[00117] Some vectors can employ control sequences that allow it to be replicated and/or expressed in both prokaryotic and eukaryotic cells. One of skill in the art would further understand the conditions under which to incubate all of the above described host cells to maintain them and to permit replication of a vector. Also understood and known are techniques and conditions that would allow large-scale production of vectors, as well as production of the nucleic acids encoded by vectors and their cognate polypeptides, proteins, or peptides.

[00118] The cells can be autologous cells, syngeneic cells, allogenic cells and even in some cases, xenogeneic cells.

[00119] In many situations one may wish to be able to kill the modified CTLs, where one wishes to terminate the treatment, the cells become neoplastic, in research where the absence of the cells after their presence is of interest, or other event. For this purpose one can provide for the expression of certain gene products in which one can kill the modified cells under controlled conditions, such as inducible suicide genes.

[00120] *Armed CARTS*

[00121] The invention further includes CARTs that are modified to secrete one or more polypeptides. Such CARTs can be referred to as CART factories or armed CARTs. The polypeptide can be, for example, an antibody or fragment thereof as described herein. For example, the polypeptide can be an antibody or cytokine. In embodiments, the antibody is specific for TIGIT, CAIX, GITR, PD-L1, PD-L2, PD-1, or CCR4.

[00122] For example, a second expression construct, which can be in the same DNA vector as that which encodes the CAR (e.g. the antigen-recognition domain) or in a second separate vector, can be used to encode a mini body (scFv-Fc) or antibody, or a fragment thereof, that

is directed against a single or multiple antigens of interest, and can be cloned after an internal ribosomal entry site (IRES). Referring to FIG. 2, the second expression cassette comprises either a fluorescent molecule or an immune-modulating minibody.

[00123] Armed CARTS have the advantage of simultaneously secreting a polypeptide at the targeted site, e.g. tumor site. Referring to FIGs. 6, 16 and 17, for example, armed CARTS can secrete anti-TIGIT antibodies or fragments thereof. TIGIT is a T-cell coinhibitory receptor that limits antitumor and other T-cell dependent chronic immune responses, such as CD8⁺ T cell-dependent immune responses. TIGIT is expressed on subsets of activated T cells and natural killer (NK) cells. For example, TIGIT is highly expressed on tumor-infiltrating T-cells. In cancer models, antibody blockade of TIGIT contributed to enhanced CD8⁺ T cell effector function and tumor clearance.

[00124] In embodiments, the anti-TIGIT antibody of the armed CART comprises one or more of the anti-TIGIT antibodyclones (or fragments thereof, such as FR1, FR2, FR3, FR4, CDR1, CDR2, CDR3, or any combinations of the framework and/or CDR regions therein) described in FIG. 17.

[00125] For example, the anti-TIGIT antibody can comprise a CDR1 of the VH region of having the sequence: GYTF...TSYG (SEQ ID NO:65); CDR2 of the VH region having the sequence: ISAY..NGNT (SEQ ID NO:66); CDR3 of the VH region having the sequence: ARDPGLWFG LTHDYFDY (SEQ ID NO:67); CDR1 of the VL region having the sequence SSNI...GSNT (SEQ ID NO:68); CDR2 of the VL region having the sequence: RN.....N (SEQ ID NO:69); and CDR3 of the VL region having the sequence: AAWDDSRSGPV (SEQ ID NO:70).

[00126] For example, the anti-TIGIT antibody can comprise a CDR1 of the VH region of having the sequence: GFTF....SDYS (SEQ ID NO:71); CDR2 of the VH region having the sequence: INSD..GSRT (SEQ ID NO: 72); CDR3 of the VH region having the sequence: ARGPGFFGFDI (SEQ ID NO: 73); CDR1 of the VL region having the sequence RSNI...GRNS (SEQ ID NO: 74); CDR2 of the VL region having the sequence: SN.....N (SEQ ID NO: 75); and CDR3 of the VL region having the sequence: AAWDARLTGPL (SEQ ID NO: 76).

[00127] For example, the anti-TIGIT antibody can comprise a CDR1 of the VH region of having the sequence: GYSF....TNYW (SEQ ID NO:77); CDR2 of the VH region having the sequence: INPV..NSRT (SEQ ID NO: 78); CDR3 of the VH region having the sequence: ARYYYYAMEV (SEQ ID NO: 79); CDR1 of the VL region having the sequence SSNI...GSNT (SEQ ID NO: 80); CDR2 of the VL region having the sequence: RN.....N

(SEQ ID NO: 81); and CDR3 of the VL region having the sequence: EAWDDSLNGPV (SEQ ID NO: 82).

[00128] For example, the anti-TIGIT antibody can comprise a CDR1 of the VH region of having the sequence: GYTF....TNYG (SEQ ID NO:83); CDR2 of the VH region having the sequence: VDNN..NGNI (SEQ ID NO: 84); CDR3 of the VH region having the sequence: ARGLFSSRWYLFDP (SEQ ID NO: 85); CDR1 of the VL region having the sequence SSDVG...GYNY (SEQ ID NO: 86); CDR2 of the VL region having the sequence: EV.....T (SEQ ID NO: 87); and CDR3 of the VL region having the sequence: SSYTRSSTSYVV (SEQ ID NO: 88).

[00129] For example, the anti-TIGIT antibody can comprise a CDR1 of the VH region of having the sequence: GGTF....SSYA (SEQ ID NO:89); CDR2 of the VH region having the sequence: ILPM..FGST (SEQ ID NO: 90); CDR3 of the VH region having the sequence: ARGRDIVAPNSGFDV (SEQ ID NO: 91); CDR1 of the VL region having the sequence SNNV....GNQG (SEQ ID NO: 92); CDR2 of the VL region having the sequence: RN.....D (SEQ ID NO: 93); and CDR3 of the VL region having the sequence: SAYDRSLNAWV (SEQ ID NO: 94).

[00130] In embodiments, the anti-TIGIT antibody can comprise a heavy chain with a VH comprising a CDR1 of SEQ ID NO: 65, 71, 77, 83, or 89; a CDR2 of SEQ ID NO: 66, 72, 78, 84, or 90; and a CDR3 of SEQ ID NO: 67, 73, 79, 85, or 91; or any combination thereof.

[00131] In embodiments, the anti-TIGIT antibody can comprise a light chain with a VL comprising a CDR1 of SEQ ID NO: 68, 74, 80, 86, or 92; a CDR2 of SEQ ID NO: 69, 75, 81, 87, or 93; and a CDR3 of SEQ ID NO: 70, 76, 82, 88, or 94; or any combination thereof.

[00132]

Armed CART can be constructed by including a nucleic acid encoding the polypeptide of interest after the intracellular signaling domain. Preferably, there is an internal ribosome entry site, (IRES), positioned between the intracellular signaling domain and the polypeptide of interest. One skilled in the art can appreciate that more than one polypeptide can be expressed by employing multiple IRES sequences in tandem.

[00133] In one embodiment, the methods and compositions presented herein provide a target-specific T cell, such as a T cell with specificity for CXCR4 and/or claudin-4, of second generation armed with the power to secrete polypeptides in the tumor microenvironment, for example to combat T cell exhaustion.

[00134] *Introduction of Constructs into CTLs*

[00135] Expression vectors that encode the CARs can be introduced as one or more DNA molecules or constructs, where there may be at least one marker that will allow for selection of host cells that contain the construct(s).

[00136] The constructs can be prepared in conventional ways, where the genes and regulatory regions can be isolated, as appropriate, ligated, cloned in an appropriate cloning host, analyzed by restriction or sequencing, or other convenient means. Particularly, using PCR, individual fragments including all or portions of a functional unit can be isolated, where one or more mutations may be introduced using "primer repair", ligation, in vitro mutagenesis, etc., as appropriate. The construct(s) once completed and demonstrated to have the appropriate sequences may then be introduced into the CTL by any convenient means. The constructs may be integrated and packaged into non-replicating, defective viral genomes like Adenovirus, Adeno-associated virus (AAV), or Herpes simplex virus (HSV) or others, including retroviral vectors or lentiviral vectors, for infection or transduction into cells. The constructs may include viral sequences for transfection, if desired. Alternatively, the construct may be introduced by fusion, electroporation, biolistics, transfection, lipofection, or the like. The host cells may be grown and expanded in culture before introduction of the construct(s), followed by the appropriate treatment for introduction of the construct(s) and integration of the construct(s). The cells are then expanded and screened by virtue of a marker present in the construct. Various markers that may be used successfully include hpvt, neomycin resistance, thymidine kinase, hygromycin resistance, etc.

[00137] In some instances, one may have a target site for homologous recombination, where it is desired that a construct be integrated at a particular locus. For example,) can knock-out an endogenous gene and replace it (at the same locus or elsewhere) with the gene encoded for by the construct using materials and methods as are known in the art for homologous recombination. For homologous recombination, one may use either .OMEGA. or O-vectors. See, for example, Thomas and Capecchi, Cell (1987) 51, 503-512; Mansour, et al., Nature (1988) 336, 348-352; and Joyner, et al., Nature (1989) 338, 153-156.

[00138] The constructs can be introduced as a single DNA molecule encoding at least the CAR and optionally another gene, or different DNA molecules having one or more genes. Other genes include genes that encode therapeutic molecules or suicide genes, for example. The constructs may be introduced simultaneously or consecutively, each with the same or different markers.

[00139] Vectors containing useful elements such as bacterial or yeast origins of replication, selectable and/or amplifiable markers, promoter/enhancer elements for expression in

prokaryotes or eukaryotes, etc. that may be used to prepare stocks of construct DNAs and for carrying out transfections are well known in the art, and many are commercially available.

[00140] *Methods of Use*

[00141] Aspects of the invention are directed towards methods of treating a subject afflicted with a cancer.

[00142] Aspects of the invention are further directed towards methods of stopping or reducing progression or promoting regression of a cancer in a subject.

[00143] Still further, aspects of the invention are directed towards a method of reducing cellular proliferation of a cancer cell in a subject.

[00144] Aspects of the invention are also directed towards methods of inducing cytotoxicity of cells. For example, referring to FIG. 2 and FIG. 3, specific cytotoxicity of CXCR-4 expressing cells is induced by CXCR-4 targeting chimeric antigen receptor T cells.

[00145] "Cancer" and "cancerous" refer to or describe, for example, the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small cell lung cancer, non-small cell lung cancer, adenocarcinoma of the lung, squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney cancer, liver cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma and various types of head and neck cancer.

[00146] In cancer, the normal intercellular interactions in tissues are disrupted, and the tumor microenvironment evolves to accommodate the growing tumor. The tumor microenvironment (TME) refers to the cellular environment in which a tumor exists, including components such as surrounding blood vessels, immune cells, fibroblasts, bone marrow-derived inflammatory cells, lymphocytes, signaling molecules and the extracellular matrix (ECM). Tumor microenvironment is complex and is heavily influenced by immune system.

[00147] This invention provides CAR-T cell therapy for triple-negative breast cancer, among others (such as those described herein). The secretion of a mono, bi-, or tri-specific minibody, antibody or minibody/antibody fusion protein by the CAR-T cell at the tumor site

could provide additional benefit by altering (i.e., modulating) the immune-repressive tumor microenvironment.

[00148] In embodiments, the method comprises administering to a subject afflicted with a cancer a therapeutically effective amount of an engineered cell as described herein.

Therapeutically effective amounts can depend on the severity and course of the cancer, previous therapy, the subject's health status, weight, and response to the drugs, and the judgment of the treating physician.

[00149] The subject can be afflicted with cancer such as liquid cancers (i.e., blood cancers) and/or solid cancers (i.e., tumors). The cancer can be benign or malignant, and can be one that is influenced by the immune system.

[00150] Embodiments as described herein can modulate the immune system so as to treat the subject afflicted with cancer. "Modulating" can refer to up-regulation, induction, stimulation, potentiation, and/or relief of inhibition, as well as inhibition, attenuation and/or down-regulation or suppression. In embodiments, the activity of the subject's immune system is modulated, the microenvironment surround the cancer cell and/or tumor is modulated, or both. For example, embodiments as described herein can alter the immune-repressive tumor microenvironment, reducing the microenvironment-dependent immune suppression, so as to modulate (or allow) the immune system to kill tumor cells.

[00151] One embodiment is directed towards methods of treating a subject afflicted with triple-negative breast cancer (TNBC), a highly aggressive subtype of breast cancer (e.g., a solid tumor) with poor clinical prognosis. While treated with chemotherapies, the high incidence of relapse signifies the need for novel, targeted therapies. Immune therapies, such as those described herein, offer an exciting therapeutic option for TNBC. For example, embodiments comprise engineering a chimeric-antigen receptor (CAR) T-cell factory, a CAR T-cell that secretes immune-modulating antibodies, for TNBC.

[00152] An "individual" or "subject" can be a mammal. Mammals include, but are not limited to, domesticated animals (e.g., cows, sheep, cats, dogs, and horses), primates (e.g., humans and non-human primates such as monkeys), rabbits, and rodents (e.g., mice and rats). In certain embodiments, the individual or subject is a human.

[00153] The cells according to the invention can be used for treating cancer in a patient in need thereof. In another embodiment, said isolated cell according to the invention can be used in the manufacture of a medicament for treatment of a cancer, viral infections of autoimmune disorders, in a patient in need thereof.

[00154] The present invention relies on methods for treating patients in need thereof, said method comprising at least one of the following steps: (a) providing a chimeric antigen receptor cells according to the invention and (b) administrating the cells to said patient.

[00155] Said treatment can be ameliorating, curative or prophylactic. It may be either part of an autologous immunotherapy or part of an allogenic immunotherapy treatment. By autologous, it is meant that cells, cell line or population of cells used for treating patients are originating from said patient or from a Human Leucocyte Antigen (HLA) compatible donor. By allogenic is meant that the cells or population of cells used for treating patients are not originating from said patient but from a donor.

[00156] The invention is particularly suited for allogenic immunotherapy, insofar as it enables the transformation of T-cells, typically obtained from donors, into non-alloreactive cells. This may be done under standard protocols and reproduced as many times as needed. The resulted modified T cells may be pooled and administrated to one or several patients, being made available as an "off the shelf" therapeutic product.

[00157] Cells that can be used with the disclosed methods are described in the previous section. Said treatment can be used to treat patients diagnosed with cancer. Cancers that may be treated include tumors that are not vascularized, or not yet substantially vascularized, as well as vascularized tumors. The cancers may comprise nonsolid tumors (such as hematological tumors, for example, leukemias and lymphomas) or may comprise solid tumors. Types of cancers to be treated with the CARs of the invention include, but are not limited to, carcinoma, blastoma, and sarcoma, and certain leukemia or lymphoid malignancies, benign and malignant tumors, and malignancies e.g., sarcomas, carcinomas, and melanomas. Adult tumors/cancers and pediatric tumors/cancers are also included.

[00158] It can be a treatment in combination with one or more therapies against cancer selected from the group of antibodies therapy, chemotherapy, cytokines therapy, dendritic cell therapy, gene therapy, hormone therapy, laser light therapy and radiation therapy.

[00159] According to an embodiment of the invention, said treatment can be administrated into patients undergoing an immunosuppressive treatment. Indeed, the present invention preferably relies on cells or population of cells, which have been made resistant to at least one immunosuppressive agent due to the inactivation of a gene encoding a receptor for such immunosuppressive agent. In this aspect, the immunosuppressive treatment should help the selection and expansion of the T-cells according to the invention within the patient.

[00160] In a further embodiment, the cell compositions of the present invention are administered to a patient in conjunction with (e.g., before, simultaneously or following) bone

marrow transplantation, T cell ablative therapy using either chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, or antibodies such as OKT3 or CAM PATH. In another embodiment, the cell compositions of the present invention are administered following B-cell ablative therapy such as agents that react with CD20, e.g., Rituxan. For example, in one embodiment, subjects may undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain embodiments, following the transplant, subjects receive an infusion of the expanded immune cells of the present invention. In an additional embodiment, expanded cells are administered before or following surgery. Said modified cells obtained by any one of the methods described here can be used in a particular aspect of the invention for treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD); therefore in the scope of the present invention is a method of treating patients in need thereof against Host versus Graft (HvG) rejection and Graft versus Host Disease (GvHD) comprising treating said patient by administering to said patient an effective amount of modified cells comprising inactivated TCR alpha and/or TCR beta genes.

[00161] Administration of Cells

[00162] The invention is particularly suited for allogenic immunotherapy, insofar as it enables the transformation of T-cells, typically obtained from donors, into non-alloreactive cells. This may be done under standard protocols and reproduced as many times as needed. The resulted modified T cells may be pooled and administrated to one or several patients, being made available as an "off the shelf" therapeutic product.

[00163] Depending upon the nature of the cells, the cells can be introduced into a host organism, e.g. a mammal, in a wide variety of ways. The cells can be introduced at the site of the tumor, in specific embodiments, although in alternative embodiments the cells hone to the cancer or are modified to hone to the cancer. The number of cells that are employed will depend upon a number of circumstances, the purpose for the introduction, the lifetime of the cells, the protocol to be used, for example, the number of administrations, the ability of the cells to multiply, the stability of the recombinant construct, and the like. The cells may be applied as a dispersion, generally being injected at or near the site of interest. The cells may be in a physiologically-acceptable medium.

[00164] In some embodiments, the cells are encapsulated to inhibit immune recognition and placed at the site of the tumor.

[00165] The cells can be administered as desired. Depending upon the response desired, the manner of administration, the life of the cells, the number of cells present, various protocols may be employed. The number of administrations will depend upon the factors described above at least in part.

[00166] The administration of the cells or population of cells according to the present invention can be carried out in any convenient manner, including by aerosol inhalation, injection, ingestion, transfusion, implantation or transplantation. The compositions described herein may be administered to a patient subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous or intralymphatic injection, or intraperitoneally. In one embodiment, the cell compositions of the present invention are preferably administered by intravenous injection.

[00167] The administration of the cells or population of cells can consist of the administration of 10^4 - 10^9 cells per kg body weight, preferably 10^5 to 10^6 cells/kg body weight including all integer values of cell numbers within those ranges. The cells or population of cells can be administered in one or more doses. In another embodiment, said effective amount of cells are administered as a single dose. In another embodiment, said effective amount of cells are administered as more than one dose over a period time. Timing of administration is within the judgment of managing physician and depends on the clinical condition of the patient. The cells or population of cells may be obtained from any source, such as a blood bank or a donor. While individual needs vary, determination of optimal ranges of effective amounts of a given cell type for a particular disease or conditions within the skill of the art. An effective amount means an amount which provides a therapeutic or prophylactic benefit. The dosage administered will be dependent upon the age, health and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment and the nature of the effect desired.

[00168] It should be appreciated that the system is subject to many variables, such as the cellular response to the ligand, the efficiency of expression and, as appropriate, the level of secretion, the activity of the expression product, the particular need of the patient, which may vary with time and circumstances, the rate of loss of the cellular activity as a result of loss of cells or expression activity of individual cells, and the like. Therefore, it is expected that for each individual patient, even if there were universal cells which could be administered to the population at large, each patient would be monitored for the proper dosage for the individual, and such practices of monitoring a patient are routine in the art.

[00169] *Nucleic Acid-Based Expression Systems*

[00170] The CARs of the present invention can be expressed from an expression vector. Recombinant techniques to generate such expression vectors are well known in the art.

[00171] DNA constructs, which can also be referred to as "DNA vectors", as described herein, can be cloned into a vector which will be used to transduce and produce chimeric-antigen receptor T-cells that secrete polypeptides and/or fragments thereof. For example, DNA constructs can be cloned into a lentiviral vector for production of lentivirus, which will be used to transduce and produce chimeric-antigen receptor T-cells that secrete a mono, bi- or tri-specific immune-modulating antibody/minibody and/or antibody-fusion protein at the tumor site.

[00172] *Vectors*

[00173] The term "vector" is used to refer to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. A nucleic acid sequence can be "exogenous," which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (e.g., YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques (see, for example, Maniatis et al., 1988 and Ausubel et al., 1994, both incorporated herein by reference).

[00174] The term "expression vector" refers to any type of genetic construct comprising a nucleic acid coding for a RNA capable of being transcribed. In some cases, RNA molecules are then translated into a protein, polypeptide, or peptide. In other cases, these sequences are not translated, for example, in the production of antisense molecules or ribozymes. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operably linked coding sequence in a particular host cell. In addition to control sequences that govern transcription and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well and are described infra.

[00175] *Promoters and Enhancers*

[00176] A "promoter" is a control sequence that is a region of a nucleic acid sequence at which initiation and rate of transcription are controlled. It may contain genetic elements at

which regulatory proteins and molecules may bind, such as RNA polymerase and other transcription factors, to initiate the specific transcription a nucleic acid sequence. The phrases "operatively positioned," "operatively linked," "under control," and "under transcriptional control" mean that a promoter is in a correct functional location and/or orientation in relation to a nucleic acid sequence to control transcriptional initiation and/or expression of that sequence.

[00177] 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 30-110 bp upstream of the start site, although a number of promoters have been shown to contain functional elements downstream of the start site as well. 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.

[00178] 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, 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.

[00179] A promoter may be one naturally associated with a nucleic acid sequence, as may be obtained by isolating the 5' prime' 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 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.TM., in connection with the compositions disclosed herein (see U.S. Pat. Nos. 4,683,202 and 5,928,906, each incorporated herein by reference). Furthermore, it is contemplated 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.

[00180] 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.

[00181] Additionally any promoter/enhancer combination 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.

[00182] The identity of tissue-specific promoters or elements, as well as assays to characterize their activity, is well known to those of skill in the art.

[00183] A specific initiation signal also may be required 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

[00184] In certain embodiments of the invention, the use of internal ribosome entry sites (IRES) elements are used to create multigene, or polycistronic, messages, and these may be used in the invention.

[00185] Vectors can include a multiple cloning site (MCS), which is a nucleic acid region that contains multiple restriction enzyme sites, any of which can be used in conjunction with standard recombinant technology to digest the vector. "Restriction enzyme digestion" refers to catalytic cleavage of a nucleic acid molecule with an enzyme that functions only at specific locations in a nucleic acid molecule. Many of these restriction enzymes are commercially available. Use of such enzymes is widely understood by those of skill in the art. Frequently, a vector is linearized or fragmented using a restriction enzyme that cuts within the MCS to enable exogenous sequences to be ligated to the vector. "Ligation" refers to the process of forming phosphodiester bonds between two nucleic acid fragments, which may or may not be contiguous with each other. Techniques involving restriction enzymes and ligation reactions are well known to those of skill in the art of recombinant technology.

[00186] Splicing sites, termination signals, origins of replication, and selectable markers may also be employed.

[00187] Plasmid Vectors

[00188] In certain embodiments, a plasmid vector is contemplated for use to transform a host cell. In general, plasmid vectors containing replicon and control sequences which are derived from species compatible with the host cell are used in connection with these hosts. The vector ordinarily carries a replication site, as well as marking sequences which are capable of providing phenotypic selection in transformed cells. In a non-limiting example, E. coli is often transformed using derivatives of pBR322, a plasmid derived from an E. coli species. pBR322 contains genes for ampicillin and tetracycline resistance and thus provides easy means for identifying transformed cells. The pBR plasmid, or other microbial plasmid or phage must also contain, or be modified to contain, for example, promoters which can be used by the microbial organism for expression of its own proteins.

[00189] In addition, phage vectors containing replicon and control sequences that are compatible with the host microorganism can be used as transforming vectors in connection with these hosts. For example, the phage lambda GEM.TM. 11 may be utilized in making a recombinant phage vector which can be used to transform host cells, such as, for example, E. coli LE392.

[00190] Further useful plasmid vectors include pIN vectors (Inouye et al., 1985); and pGEX vectors, for use in generating glutathione S transferase (GST) soluble fusion proteins for later purification and separation or cleavage. Other suitable fusion proteins are those with galactosidase, ubiquitin, and the like.

[00191] Bacterial host cells, for example, *E. coli*, comprising the expression vector, are grown in any of a number of suitable media, for example, LB. The expression of the recombinant protein in certain vectors may be induced, as would be understood by those of skill in the art, by contacting a host cell with an agent specific for certain promoters, e.g., by adding IPTG to the media or by switching incubation to a higher temperature. After culturing the bacteria for a further period, generally of between 2 and 24 h, the cells are collected by centrifugation and washed to remove residual media.

[00192] *Viral Vectors*

[00193] The ability of certain viruses to infect cells or enter cells via receptor mediated endocytosis, and to integrate into host cell genome and express viral genes stably and efficiently have made them attractive candidates for the transfer of foreign nucleic acids into cells (e.g., mammalian cells). Components of the present invention may be a viral vector that encodes one or more CARs of the invention. Non-limiting examples of virus vectors that may be used to deliver a nucleic acid of the present invention are described below.

[00194] *Adenoviral Vectors*

[00195] A particular method for delivery of the nucleic acid involves the use of an adenovirus expression vector. Although adenovirus vectors are known to have a low capacity for integration into genomic DNA, this feature is counterbalanced by the high efficiency of gene transfer afforded by these vectors. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to ultimately express a tissue or cell specific construct that has been cloned therein. Knowledge of the genetic organization of adenovirus, a 36 kb, linear, double stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus and Horwitz, 1992).

[00196] *AAV Vectors*

[00197] The nucleic acid may be introduced into the cell using adenovirus assisted transfection. Increased transfection efficiencies have been reported in cell systems using

adenovirus coupled systems (Kelleher and Vos, 1994; Cotten et al., 1992; Curiel, 1994). Adeno associated virus (AAV) is an attractive vector system for use in the cells of the present invention as it has a high frequency of integration and it can infect nondividing cells, thus making it useful for delivery of genes into mammalian cells, for example, in tissue culture (Muzyczka, 1992) or in vivo. AAV has a broad host range for infectivity (Tratschin et al., 1984; Laughlin et al., 1986; Lebkowski et al., 1988; McLaughlin et al., 1988). Details concerning the generation and use of rAAV vectors are described in U.S. Pat. Nos. 5,139,941 and 4,797,368, each incorporated herein by reference.

[00198] *Retroviral Vectors*

[00199] Retroviruses are useful as delivery vectors because of their ability to integrate their genes into the host genome, transferring a large amount of foreign genetic material, infecting a broad spectrum of species and cell types and of being packaged in special cell lines (Miller, 1992).

[00200] In order to construct a retroviral vector, a nucleic acid (e.g., one encoding the desired sequence) is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication defective. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed (Mann et al., 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into a special cell line (e.g., by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubenstein, 1988; Temin, 1986; Mann et al., 1983). The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression require the division of host cells (Paskind et al., 1975).

[00201] Lentiviruses are complex retroviruses, which, in addition to the common retroviral genes gag, pol, and env, contain other genes with regulatory or structural function. Lentiviral vectors are well known in the art (see, for example, Naldini et al., 1996; Zufferey et al., 1997; Blomer et al., 1997; U.S. Pat. Nos. 6,013,516 and 5,994,136). Some examples of lentivirus include the Human Immunodeficiency Viruses: HIV-1, HIV-2 and the Simian Immunodeficiency Virus: SIV. Lentiviral vectors have been generated by multiply

attenuating the HIV virulence genes, for example, the genes env, vif, vpr, vpu and nef are deleted making the vector biologically safe.

[00202] Recombinant lentiviral vectors are capable of infecting non-dividing cells and can be used for both in vivo and ex vivo gene transfer and expression of nucleic acid sequences. For example, recombinant lentivirus capable of infecting a non-dividing cell wherein a suitable host cell is transfected with two or more vectors carrying the packaging functions, namely gag, pol and env, as well as rev and tat is described in U.S. Pat. No. 5,994,136, incorporated herein by reference. One may target the recombinant virus by linkage of the envelope protein with an antibody or a particular ligand for targeting to a receptor of a particular cell-type. By inserting a sequence (including a regulatory region) of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target-specific.

[00203] Other Viral Vectors

[00204] Other viral vectors may be employed as vaccine constructs in the present invention. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Baichwal and Sugden, 1986; Coupar et al., 1988), sindbis virus, cytomegalovirus and herpes simplex virus may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Baichwal and Sugden, 1986; Coupar et al., 1988; Horwich et al., 1990).

[00205] Delivery Using Modified Viruses

[00206] A nucleic acid to be delivered may be housed within an infective virus that has been engineered to express a specific binding ligand. The virus particle will thus bind specifically to the cognate receptors of the target cell and deliver the contents to the cell. A novel approach designed to allow specific targeting of retrovirus vectors was developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification can permit the specific infection of hepatocytes via sialoglycoprotein receptors.

[00207] Another approach to targeting of recombinant retroviruses was designed in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled via the biotin components by using streptavidin (Roux et al., 1989). Using antibodies against major histocompatibility complex

class I and class II antigens, they demonstrated the infection of a variety of human cells that bore those surface antigens with an ecotropic virus in vitro (Roux et al., 1989).

[00208] Vector Delivery and Cell Transformation

[00209] Suitable methods for nucleic acid delivery for transfection or transformation of cells are known to one of ordinary skill in the art. Such methods include, but are not limited to, direct delivery of DNA such as by ex vivo transfection, by injection, and so forth. Through the application of techniques known in the art, cells may be stably or transiently transformed.

[00210] Ex Vivo Transformation

[00211] Methods for transfecting eukaryotic cells and tissues removed from an organism in an ex vivo setting are known to those of skill in the art. Thus, it is contemplated that cells or tissues may be removed and transfected ex vivo using nucleic acids of the present invention. In particular aspects, the transplanted cells or tissues may be placed into an organism. In preferred facets, a nucleic acid is expressed in the transplanted cells.

[00212] Kits of the Invention

[00213] Any of the compositions described herein may be comprised in a kit. In a non-limiting example, one or more cells for use in cell therapy and/or the reagents to generate one or more cells for use in cell therapy that harbors recombinant expression vectors may be comprised in a kit. The kit components are provided in suitable container means.

[00214] Some 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 will 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 components in close confinement for commercial sale. Such containers may include injection or blow molded plastic containers into which the desired vials are retained.

[00215] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly

ueful. In some cases, the container means may itself be a syringe, pipette, and/or other such like apparatus, from which the formulation may be applied to an infected area of the body, injected into an animal, and/or even applied to and/or mixed with the other components of the kit.

[00216] However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent.

[00217] In particular embodiments of the invention, cells that are to be used for cell therapy are provided in a kit, and in some cases the cells are essentially the sole component of the kit. The kit may comprise reagents and materials to make the desired cell. In specific embodiments, the reagents and materials include primers for amplifying desired sequences, nucleotides, suitable buffers or buffer reagents, salt, and so forth, and in some cases the reagents include vectors and/or DNA that encodes a CAR as described herein and/or regulatory elements therefor.

[00218] In particular embodiments, there are one or more apparatuses in the kit suitable for extracting one or more samples from an individual. The apparatus may be a syringe, scalpel, and so forth.

[00219] In some cases of the invention, the kit, in addition to 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.

[00220] **Combination Therapy**

[00221] In certain embodiments of the invention, methods of the present invention for clinical aspects are combined with other agents effective in the treatment of hyperproliferative disease, such as anti-cancer agents. An "anti-cancer" agent is capable of negatively affecting cancer in a subject, for example, by killing cancer cells, inducing apoptosis in cancer cells, reducing the growth rate of cancer cells, reducing the incidence or number of metastases, reducing tumor size, inhibiting tumor growth, reducing the blood supply to a tumor or cancer cells, promoting an immune response against cancer cells or a tumor, preventing or inhibiting the progression of cancer, or increasing the lifespan of a subject with cancer. More generally, these other compositions would be provided in a

combined amount effective to kill or inhibit proliferation of the cell. This process may involve contacting the cancer cells with the expression construct and the agent(s) or multiple factor(s) at the same time. This may be achieved by contacting the cell with a single composition or pharmacological formulation that includes both agents, or by contacting the cell with two distinct compositions or formulations, at the same time, wherein one composition includes the expression construct and the other includes the second agent(s).

[00222] Tumor cell resistance to chemotherapy and radiotherapy agents represents a major problem in clinical oncology. One goal of current cancer research is to find ways to improve the efficacy of chemo- and radiotherapy by combining it with other therapies. In the context of the present invention, it is contemplated that cell therapy could be used similarly in conjunction with chemotherapeutic, radiotherapeutic, or immunotherapeutic intervention, as well as pro-apoptotic or cell cycle regulating agents.

[00223] Alternatively, the present inventive therapy may precede or follow the other agent treatment by intervals ranging from minutes to weeks. In embodiments where the other agent and present invention are applied separately to the individual, one would generally ensure that a significant period of time did not expire between the time of each delivery, such that the agent and inventive therapy would still be able to exert an advantageously combined effect on the cell. In such instances, it is contemplated that one may contact the cell with both modalities within about 12-24 h of each other and, more preferably, within about 6-12 h of each other. In some situations, it may be desirable to extend the time period for treatment significantly, however, where several d (2, 3, 4, 5, 6 or 7) to several wk (1, 2, 3, 4, 5, 6, 7 or 8) lapse between the respective administrations.

[00224] It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, as well as surgical intervention, may be applied in combination with the inventive cell therapy.

[00225] Chemotherapy

[00226] Cancer therapies also include a variety of combination therapies with both chemical and radiation based treatments. Combination chemotherapies include, for example, abraxane, altretamine, docetaxel, herceptin, methotrexate, novantrone, zoladex, cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, gemcitabien, navelbine, farnesyl-protein transferase

inhibitors, transplatinum, 5-fluorouracil, vincristin, vinblastin and methotrexate, or any analog or derivative variant of the foregoing and also combinations thereof.

[00227] In specific embodiments, chemotherapy for the individual is employed in conjunction with the invention, for example before, during and/or after administration of the invention

[00228] Radiotherapy

[00229] Other factors that cause DNA damage and have been used extensively include what are commonly known as .gamma.-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 and UV-irradiation. It is most likely that all of these factors effect 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.

[00230] The terms "contacted" and "exposed," when applied to a cell, are used herein to describe the process by which a therapeutic construct and a chemotherapeutic or radiotherapeutic agent are delivered to a target cell or are placed in direct juxtaposition with the target cell. To achieve cell killing or stasis, both agents are delivered to a cell in a combined amount effective to kill the cell or prevent it from dividing.

[00231] Immunotherapy

[00232] Immunotherapeutics generally rely on the use of immune effector cells and molecules to target and destroy cancer cells. 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 effect 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 merely 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.

[00233] Immunotherapy other than the inventive therapy described herein could thus be used as part of a combined therapy, in conjunction with the present cell therapy. The general approach for combined therapy is discussed below. Generally, 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 invention. Common tumor markers include PD-1, PD-L1, CTLA4, carcinoembryonic antigen, prostate specific antigen, urinary tumor associated antigen, fetal antigen, tyrosinase (p97), gp68, TAG-72, HMFG, Sialyl Lewis Antigen, MucA, MucB, PLAP, estrogen receptor, laminin receptor, erb B and p155.

[00234] Genes

[00235] In yet another embodiment, the secondary treatment is a gene therapy in which a therapeutic polynucleotide is administered before, after, or at the same time as the present invention clinical embodiments. A variety of expression products are encompassed within the invention, including inducers of cellular proliferation, inhibitors of cellular proliferation, or regulators of programmed cell death.

[00236] Surgery

[00237] Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative and palliative surgery. Curative surgery is a cancer treatment that may be used in conjunction with other therapies, such as the treatment of the present invention, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy and/or alternative therapies.

[00238] Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed. 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). It is further contemplated that the present invention may be used in conjunction with removal of superficial cancers, precancers, or incidental amounts of normal tissue.

[00239] Upon excision of part of 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.

[00240] *Other Agents*

[00241] It is contemplated that other agents may be used in combination with the present invention to improve the therapeutic efficacy of treatment. These additional agents include immunomodulatory agents, agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, or agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers.

Immunomodulatory agents include tumor necrosis factor; interferon alpha, beta, and gamma; IL-2 and other cytokines; F42K and other cytokine analogs; or MIP-1, MIP-1beta, MCP-1, RANTES, and other chemokines. It is further contemplated that the upregulation of cell surface receptors or their ligands such as Fas/Fas ligand, DR4 or DR5/TRAIL would potentiate the apoptotic inducing abilities of the present invention by establishment of an autocrine or paracrine effect on hyperproliferative cells. Increases 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 the present invention to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present invention. 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 the present invention to improve the treatment efficacy.

[00242] *Methods of Assessing Activity of Engineered CAR T cells*

[00243] Aspects of the invention are further directed towards methods of and kits for assessing the killing capabilities of engineered CAR T cells. Specifically, embodiments are directed towards an immune complex analysis method and kits to determine the CAR T cell activity during co-culture with cancer cells. First, different target cancer cells (for example, HEK293T, MDA-MB-231, MDA-MB-468, HCC38, and skrc59) are stained with a dye, (for example, ViaStain™ Tracer Blue dye), seeded in a plate (for example a 96-well plate), and incubated for a period of time (for example 12 hours, or overnight). Next, different T cell types (for example, two different T cell types) are added to the wells (for example at a ratio

of 20:1 effector-to-target (E:T) ratios) and allowed to co-culture for a period of time (for example, 24 hours). Finally, the plate is scanned and analyzed (for example, using the bright field and blue fluorescent channels). The immune complexes were analyzed by confluence measurement and compared to the negative control of untransduced T cells. As a result, data plots displayed the CAR T cell activities for all of tested target and effector cells combinations. Utilization of an image cytometry platform can visually confirm interactions between effector and target cells, thus making results highly accurate and robust.

EXAMPLES

[00244] Examples are provided below to facilitate a more complete understanding of the invention. The following examples illustrate the exemplary modes of making and practicing the invention. However, the scope of the invention is not limited to specific embodiments disclosed in these Examples, which are for purposes of illustration only, since alternative methods can be utilized to obtain similar results.

EXAMPLE 1

[00245] *CXCR4 and claudin-4 bispecific chimeric antigen receptor T-cell therapy secreting an immune-modulating antibody*

[00246] Chimeric antigen receptors fuse antigen-specific antibody fragments to T-cell co-stimulatory domains and the CD3 zeta intracellular signaling, allowing for the re-direction of T-cells towards an antigen presented on a cell of interest including tumor cells. This invention combines antibody fragments, including, but not limited to, single-chain variable fragments (scFv) directed at C-X-C chemokine receptor type 4 and claudin-4. The DNA encoding the scFVs will be cloned in frame to DNA encoding necessary CAR-T elements such as, but not limited to, CD8 hinge regions, transmembrane, co-stimulatory domains of molecules of immunological interest such as, but not limited to, CD28 and 41BB and CD3-zeta intracellular signaling domains. Furthermore, in a second expression construct in the same DNA vector, a mini body (scFv-Fc) or antibody directed against a single or multiple antigens of interest are cloned after an internal ribosomal entry site (IRES). These DNA constructs are cloned into a lentiviral vector for production of lentivirus which will be used to transduce and produce chimeric-antigen receptor T-cells that secrete a mono, bi- or tri-specific immune-modulating antibody/minibody and/or antibody-fusion protein at the tumor site.

[00247] No targeted treatments exist for triple-negative breast cancer (TNBC), therefore chemotherapy remains the only treatment for patients. Immunotherapies like anti-PD_L1 had limited success (18% response rate) in for TNBC suggesting that these therapies alone is not an effective treatment. This invention would provide the first CAR-T cell therapy for triple-negative breast cancer. The secretion of a mono, bi-, or tri-specific minibody, antibody or minibody/antibody fusion protein by the CAR-T cell at the tumor site could provide additional benefit by altering the immune-repressive tumor microenvironment.

EXAMPLE 2

[00248] *High-throughput immune complex analysis method for CAR T cell-mediated cytotoxicity using the Celigo Image Cytometer*

[00249] Cancer immunotherapy has been gaining momentum in the field of cancer research. Advancements in combination immunotherapy as well as Chimeric Antigen Receptor (CAR) T technology have introduced new methods to combat various cancer diseases. Direct cell-mediated cytotoxicity assays are required to assess the killing capability of the engineered CAR T cells. Traditionally, these assays are conducted by measuring the amount of released Chromium, calcein AM, or Lactate dehydrogenase (LDH) molecules after the target cancer cells are killed with CAR T cells. These methods require a large amount of target cells which may not be ideal when working with donor primary samples. Additionally, they cannot specifically analyze the immune complexes formed during CAR T cell killing. Recent advancement in imaging technologies have developed novel methods for assessing these immune complexes. In this work, we demonstrated an immune complex analysis method using the Celigo Image Cytometer to determine the CAR T cell activity during co-culture with cancer cells. First, different target cancer cells (for example, HEK293T, MDA-MB-231, MDA-MB-468, HCC38, and skrc59) are stained with a dye, (for example, ViaStain™ Tracer Blue dye), seeded in a plate (for example a 96-well plate), and incubated for a period of time (for example 12 hours, or overnight). Next, different T cell types (for example, two different T cell types) are added to the wells (for example at a ratio of 20:1 effector-to-target (E:T) ratios) and allowed to co-culture for a period of time (for example, 24 hours). Finally, the plate is scanned and analyzed (for example, using the bright field and blue fluorescent channels). The immune complexes were analyzed by confluence measurement and compared to the negative control of untransduced T cells. As a result, data plots displayed the CAR T cell activities for all of tested target and effector cells combinations. Utilization of an image cytometry platform can visually confirm interactions between effector and target cells, thus making results highly accurate and robust. Unlike the traditional release assays, the ability to analyze the immune complexes formed during co-culture assays can provide additional important functionality information of the effector cells.

EXAMPLE 3

[00250] *A Bispecific Chimeric-Antigen Receptor T-cell Factory for Triple-Negative Breast Cancer*

[00251] Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer with poor clinical prognosis. While treated with chemotherapies, the high incidence of relapse signifies the need for novel, targeted therapies. Immune therapies offer an exciting therapeutic option for TNBC.

[00252] Embodiments as described herein comprise engineering a chimeric-antigen receptor (CAR) T-cell factory, a CAR T-cell that secretes immune-modulating antibodies, for TNBC.

[00253] Chimeric-antigen receptor (CAR) T-cell therapies redirect a patient's T-cells to kill tumor cells by the exogenous expression of a CAR. A CAR is a membrane spanning fusion protein that links the antigen recognition domain of an antibody to the intracellular signaling domains of the T-cell receptor and co-receptor. Solid tumors offer unique challenges for CAR-T therapies. Unlike blood cancers, tumor-associated target proteins are overexpressed between the tumor and healthy tissue resulting in on-target/off-tumor T-cell killing of healthy tissues. Furthermore, immune repression in the tumor microenvironment (TME) limits the activation of CAR-T cells towards killing the tumor.

[00254] Without wishing to be bound by theory, a bispecific CAR targeting two antigens on TNBC will mitigate on-target/off-tumor T-cell killing and that the secretion of a checkpoint blockade antibody will remove repression in the tumor microenvironment. Following local immune restoration, the CAR-T cells and other cells in the TME will work synergistically to shrink and clear tumors.

[00255] Our current work evaluates human single-chain variable fragments (scFvs) to serve as CAR-targeting moieties. We are evaluating the efficacy of scFvs to specifically kill target cells in vitro. This work will define lead scFvs used in the engineering of our bispecific CAR. Future work will evaluate this therapy in humanized mouse models of TNBC.

EXAMPLE 4

[00256] *A Bispecific CAR T-cell Factory for Triple-Negative Breast Cancer*

[00257] Triple-negative breast cancer (TNBC) is a highly aggressive subtype of breast cancer with poor clinical prognosis (1). While treated with chemotherapies, the high incidence of relapse signifies the need for novel, targeted therapies. Immune therapies offer an exciting therapeutic option for TNBC. The goal is to engineer a chimeric antigen receptor (CAR) T-cell factory, a CAR T-cell that secretes an immune-modulating antibody, for TNBC (2). CARs for solid tumors are thwarted by on-target/off-tumor killing and an immune-suppressive tumor microenvironment (TME, 3). Without being bound by theory, a bispecific

CAR targeting two antigens on TNBC can mitigate on-target/off-tumor T-cell killing and the secretion of a checkpoint blockade antibody can remove repression in the tumor microenvironment. Following local immune restoration, the CAR-T cells and other cells in the TME can work synergistically to shrink and clear tumors. This therapy will be evaluated in a humanized mouse model of TNBC.

[00258] *Objectives:* (1) Evaluate CXCR4 recognizing single-chain variable fragments (scfv) as CAR-T cell targeting domains; (2) Assess immune infiltration of TNBC xenografts in humanized mouse model.

[00259] *Methods:*

[00260] *In vitro* CAR-T killing. CD8⁺ T-cells were transduced with CAR gene via lentivirus. Stable fluorescent, CXCR4 transduced, TNBC cell lines were co-cultured with CAR T-cells. Target cells were enumerated using Nexcelom, Celigo Image Cytometer.

[00261] Humanized Mouse Model. Neonate NSG-SGM3 were implanted with human hematopoietic stem cells. Engraftment of humane immune system was confirmed at 12-20 weeks. Four million CXCR4-transduced TNBC cells were implanted into mammary fat pad. Flow cytometry analysis of tumor single-cell suspensions was conducted.

[00262] *Conclusions:* Four CXCR4-targeting CARS were identified with various killing kinetics on two CXCR4-transduced TNBC cell lines. HCC38 was identified as a highly immune infiltrated xenograft in a humanized mouse model.

[00263] *Other Embodiments:*

[00264] Bispecific CAR Development. In one embodiment, scFvs directed against second antigen for target cell killing can be evaluated. In another embodiment, a bispecific CAR can be engineered. The therapeutic efficacy of such a bispecific CAR-T factory can then be tested.

[00265] Mouse Model Development. In one embodiment, a transcriptome analysis of tumor and tumor-infiltrating lymphocytes (TIL) can be conducted. In another embodiment, a multi parameter IHC of the tumor microenvironment can be conducted. In some embodiments, the infiltrate can be confirmed with a second haematopoietic stem cell (HSC) donor.

[00266] *References for this Example:*

[00267] 1- Foulkes WD, *et al.* (2010) Triple-negative breast cancer. The New England journal of medicine. 2010;363(20):1938-48.

[00268] 2- Suarez ER, *et al.* (2016) Chimeric antigen receptor T cells secreting anti-PD-L1 antibodies more effectively regress renal cell carcinoma in a humanized mouse model.

Oncotarget 7(23):34341-34355.

[00269] 3- Kunert A, *et al.* (2018) Engineering T cells for adoptive therapy: outsmarting the tumor. *Current Opinion in Immunology* 51:133-139.

EXAMPLE 5

[00270] *CAR-T killing*

[00271] Stable fluorescent target cell lines were made by transduction with a lentivirus construct containing mCardinal with carboxyl-terminal nuclear localization signal. mCardinal expressing cells were sorted using flow cytometry to enrich for the fluorescent population.

Three-thousand target cells were plated per assay well.

[00272] To make CAR-T cells, CD8⁺ cells were purified from the PBMC from healthy donors and cultured in media containing interleukin-21 and CD3/CD28 costimulatory beads. After 24 hours in culture, T-cells were transduced with lentivirus containing the indicated CAR. After 5-9 days in culture, CAR-T cells were mixed with target cells at an T-cell (effector) to target (breast cancer cell line) of 10:1 or 2:1. Assays were imaged using a Celigo image cytometer at 0, 24 and 48 hours and fluorescent target cells counted.

EQUIVALENTS

[00273] Those skilled in the art will recognize, or be able to ascertain, using no more than routine experimentation, numerous equivalents to the specific substances and procedures described herein. Such equivalents are considered to be within the scope of this invention, and are covered by the following claims.

What is claimed:

1. An engineered cell comprising a nucleic acid encoding a chimeric antigen receptor and a polypeptide, wherein the chimeric antigen receptor is specific for two or more antigens on the surface of a cancer cell, and wherein the polypeptide comprises an antibody or fragment thereof that can be secreted from the engineered cell.
2. The engineered cell of claim 1, wherein the engineered cell comprises a T cell or an NK cell.
3. The engineered cell of claim 2, wherein the T cell is CD4⁺, CD8⁺, or a mixed population of CD4⁺ and CD8⁺ T cells.
4. The engineered cell of claim 1, wherein the two or more antigens comprise CXCR4 and claudin-4.
5. The engineered T-cell of claim 1, wherein the chimeric antigen receptor comprises a VH comprising SEQ ID NO: 1, 2, 3, 4, 5, 6, 7, or 8; a VL comprising SEQ ID NO: 9, 10, 11, 12, 13, 14, 15, or 16; or any combination thereof.
6. The engineered T-cell of claim 1, wherein the chimeric antigen receptor comprises:
 - a VH with CDR1 comprising SYGMH (SEQ ID NO:17); CDR2 comprising VISYDGSNKYYADSVKG (SEQ ID NO:18); CDR3 comprising DLVAAAGTAFDI (SEQ ID NO:19); and
 - a VL with CDR1 comprising TGTISDVGGHNFVS (SEQ ID NO:20); CDR2 comprising EVTKRPA (SEQ ID NO:21); CDR3 comprising SSYGGSNDVI (SEQ ID NO:22);
 - a VH with CDR1 comprising SNFVAWN (SEQ ID NO:23); CDR2 comprising RTYYRSRWYNDYAVSVQS (SEQ ID NO:24); CDR3 comprising GQHSGFDF (SEQ ID NO:25); and
 - a VL with CDR1 comprising TGNSNNVGNQGAA (SEQ ID NO:26); CDR2 comprising RNNNRPS (SEQ ID NO:27); and CDR3 comprising SAWDNRLKTYV (SEQ ID NO:28);
 - a VH with CDR1 comprising SYGIS (SEQ ID NO:29); CDR2 comprising WISAYNGNTNYAQKLQG (SEQ ID NO:30); CDR3 comprising DTPGIAARRYYYYGMDV (SEQ ID NO:31); and

a VL with CDR1 comprising QGDSLRLKFFAS (SEQ ID NO:32); CDR2 comprising GKNSRPS (SEQ ID NO:33); and CDR3 comprising NSRDSRDNHQV (SEQ ID NO:34);

a VH with CDR1 comprising SYPMH (SEQ ID NO:35); CDR2 comprising VISSDGRNKYYPDSVKG (SEQ ID NO:36); and CDR3 comprising GGYHDFWSPDY (SEQ ID NO:37); and

a VL with CDR1 comprising RASQSVNTNLA (SEQ ID NO:38); CDR2 comprising GASSRAT (SEQ ID NO:39); and CDR3 comprising QHYGSSPLT (SEQ ID NO:40);

a VH with CDR1 comprising SYAMS (SEQ ID NO:41); CDR2 comprising NIKQDGSEKYYVDSVKG (SEQ ID NO:42); and CDR3 comprising DQVSGITIFGGKWRSPDV (SEQ ID NO:43); and

a VL with CDR1 comprising QGDSLRSYYAS (SEQ ID NO:44); CDR2 comprising GKNNRPS (SEQ ID NO:45); and CDR3 comprising NSRSGSQRV (SEQ ID NO:46);

a VH with CDR1 comprising NYGLH (SEQ ID NO:47); a CDR2 comprising VISHDGTKKYYADSVKG (SEQ ID NO:48); and a CDR3 comprising DGGYCSGGRCYSYGMDV (SEQ ID NO:49); and

a VL with CDR1 comprising SGSRSNIGSNTVN (SEQ ID NO:50); CDR2 comprising TNNQRPS (SEQ ID NO:51); and CDR3 comprising LSFDSLSYV (SEQ ID NO:52);

a VH with CDR1 comprising RYGMH (SEQ ID NO:53); CDR2 comprising LISYDGSKTFYGESVKG (SEQ ID NO: 54); and CDR3 comprising ATVTTDGYYYMDV (SEQ ID NO: 55); and

a VL with CDR1 comprising SGSRSNIGGNTVN (SEQ ID NO:56); CDR2 comprising ANNQRPS (SEQ ID NO: 57); and CDR3 comprising AAWDDNLSGHVV (SEQ ID NO: 58);

or any combination thereof.

7. The engineered cell of claim 1, wherein the chimeric antigen receptor comprises

a VH with a CDR1 comprising GFTV.... SSNY (SEQ ID NO: 59); CDR2 comprising IYSG...GST (SEQ ID NO: 60); and CDR3 comprising ARDNPLSAFDI (SEQ ID NO: 61); and

a VL with a CDR1 comprising QSI.....NSW (SEQ ID NO: 62); CDR2 comprising KA.....S (SEQ ID NO: 63); and CDR3 comprising QQYDDLPLT (SEQ ID NO: 64).

8. The engineered cell of claim 1, wherein the secreted polypeptide modulates the immune system of a subject.
9. The engineered cell of claim 1, wherein the secreted polypeptide comprises an antibody specific for TIGIT, CAIX, GITR, PD-L1, PD-L2, PD-1, or CCR4.
10. The engineered cell of claim 9, wherein the antibody is specific for TIGIT.
11. The engineered cell of claim 1, wherein the secreted polypeptide comprises

a CDR1 of the VH region of having the sequence: GYTF....TSYG (SEQ ID NO:65); CDR2 of the VH region having the sequence: ISAY..NGNT (SEQ ID NO:66); CDR3 of the VH region having the sequence: ARDPGLWFG LTHDYFDY (SEQ ID NO:67); and

a CDR1 of the VL region having the sequence SSNI...GSNT (SEQ ID NO:68); CDR2 of the VL region having the sequence: RN.....N (SEQ ID NO:69); and CDR3 of the VL region having the sequence: AAWDDSRSGPV (SEQ ID NO:70);

a CDR1 of the VH region of having the sequence: GFTF....SDYS (SEQ ID NO:71); CDR2 of the VH region having the sequence: INSD..GSRT (SEQ ID NO: 72); CDR3 of the VH region having the sequence: ARGPGFFGFDI (SEQ ID NO: 73);

a CDR1 of the VL region having the sequence RSNI...GRNS (SEQ ID NO: 74); CDR2 of the VL region having the sequence: SN.....N (SEQ ID NO: 75); and CDR3 of the VL region having the sequence: AAWDARLTGPL (SEQ ID NO: 76);

a CDR1 of the VH region of having the sequence: GYSF....TNYW (SEQ ID NO:77); CDR2 of the VH region having the sequence: INPV..NSRT (SEQ ID NO: 78); CDR3 of the VH region having the sequence: ARYYYYAMEV (SEQ ID NO: 79);

a CDR1 of the VL region having the sequence SSNI....GSNT (SEQ ID NO: 80); CDR2 of the VL region having the sequence: RN.....N (SEQ ID NO: 81); and CDR3 of the VL region having the sequence: EAWDDSLNGPV (SEQ ID NO: 82);

a CDR1 of the VH region of having the sequence: GYTF....TNYG (SEQ ID NO:83); CDR2 of the VH region having the sequence: VDNN..NGNI (SEQ ID NO: 84); CDR3 of the VH region having the sequence: ARGFLSSRWYLFDFP (SEQ ID NO: 85); and

a CDR1 of the VL region having the sequence SSDVG...GYNY (SEQ ID NO: 86); CDR2 of the VL region having the sequence: EV.....T (SEQ ID NO: 87); and CDR3 of the VL region having the sequence: SSYTRSSTSYVV (SEQ ID NO: 88);

a CDR1 of the VH region of having the sequence: GGTF....SSYA (SEQ ID NO:89); CDR2 of the VH region having the sequence: ILPM..FGST (SEQ ID NO: 90); CDR3 of the VH region having the sequence: ARG RDIVAPNSGFDV (SEQ ID NO: 91);

a CDR1 of the VL region having the sequence SNNV....GNQG (SEQ ID NO: 92); CDR2 of the VL region having the sequence: RN.....D (SEQ ID NO: 93); and CDR3 of the VL region having the sequence: SAYDRSLNAWV (SEQ ID NO: 94);

or any combination thereof.

12. A nucleic acid encoding a chimeric antigen receptor and a polypeptide, wherein the chimeric antigen receptor is specific for two or more antigens on the surface of a cancer cell, and wherein the polypeptide comprises an antibody or fragment thereof that can be secreted from an engineered cell.

13. The nucleic acid of claim 12, wherein the two or more antigens comprise CXCR4 and claudin-4.
14. The nucleic acid of claim 12, wherein the polypeptide comprises an antibody specific for TIGIT, CAIX, GITR, PD-L1, PD-L2, PD-1, or CCR4.
15. A vector comprising the nucleic acid of claim 12.
16. A cell comprising the vector of claim 15.
17. A method for treating a subject afflicted with cancer, the method comprising administering the subject a therapeutically effective amount of the engineered cell of claim 1.
18. A method of reducing progression or promoting regression of a cancer in a subject, the method comprising administering the subject a therapeutically effective amount of the engineered cell of claim 1.
19. A method of reducing cellular proliferation of a cancer cell in a subject, the method comprising administering the subject a therapeutically effective amount of the engineered cell of claim 1.
20. A method for assessing the killing capability of engineered CAR T cells, the method comprising:
 - obtaining cells from one or more types of cancer;
 - admixing the cells with a dye, so as to stain the cells;
 - seeding the cells in a plate;
 - incubating the admixture for a period of time;
 - adding different T cell types to the admixture to create a second admixture;
 - co-culturing the second admixture for a period of time; and
 - assessing the killing capability of the engineered CAR T cells.
21. The method of claim 20, wherein assessing comprises scanning and analyzing the plate.

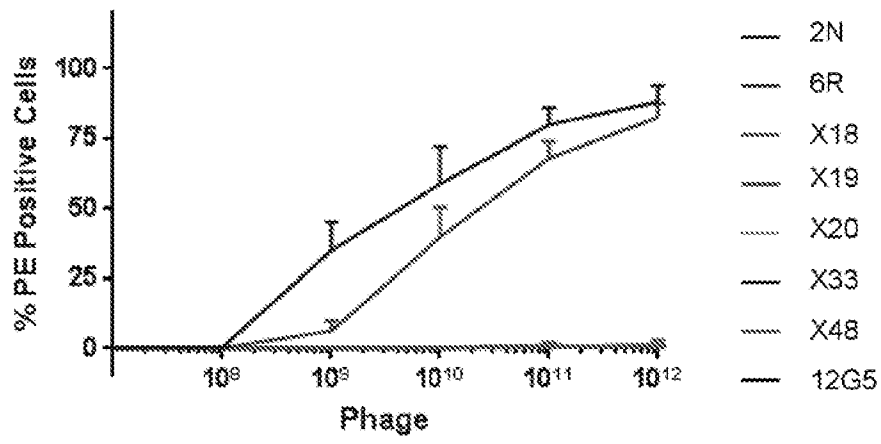
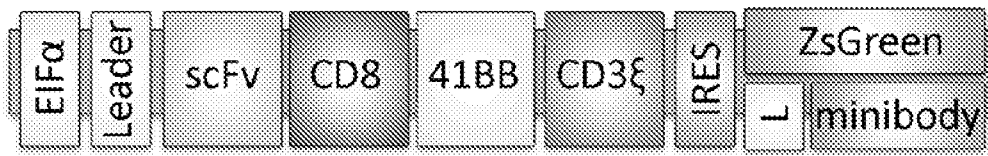


FIG. 1

A



B

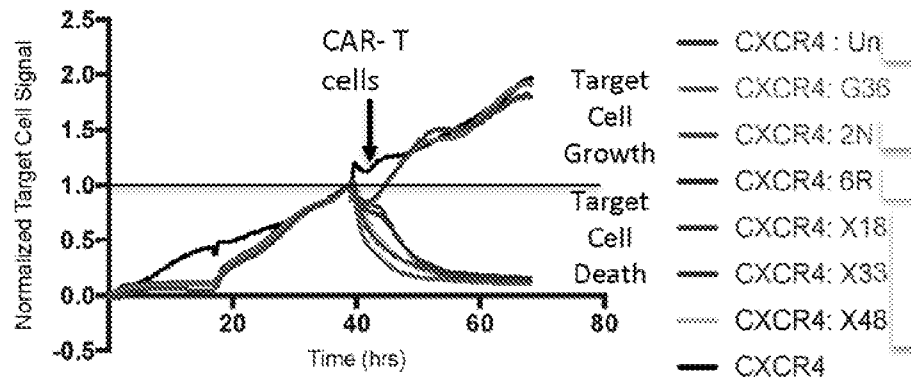


FIG. 2

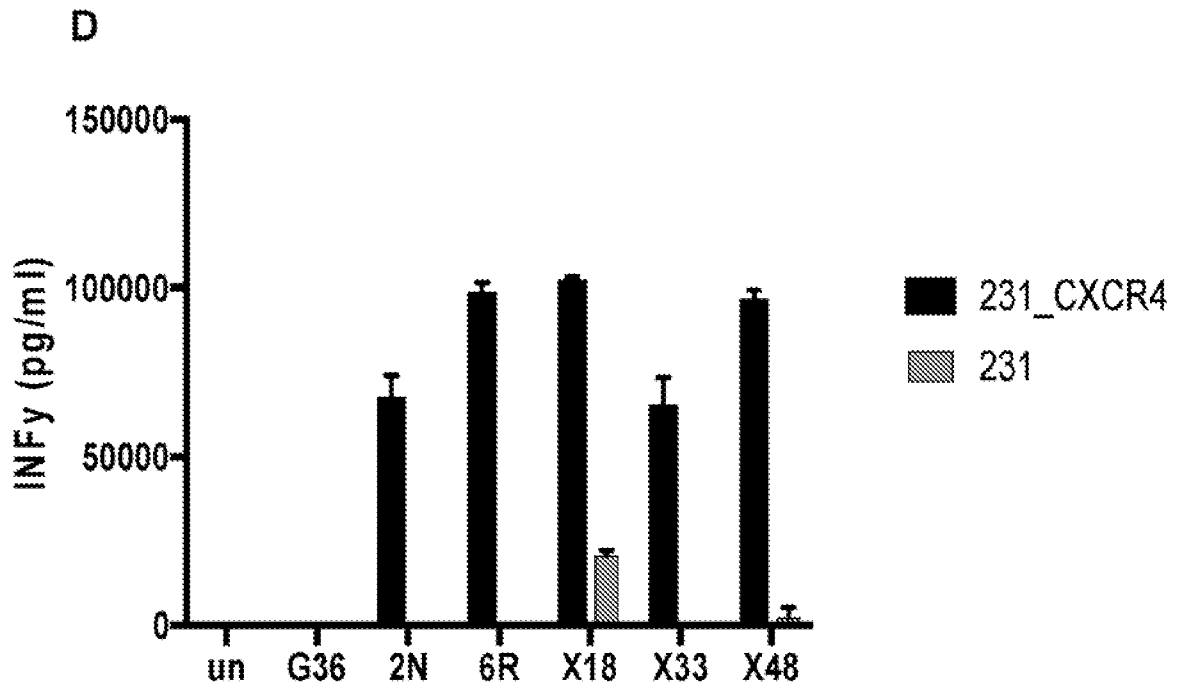
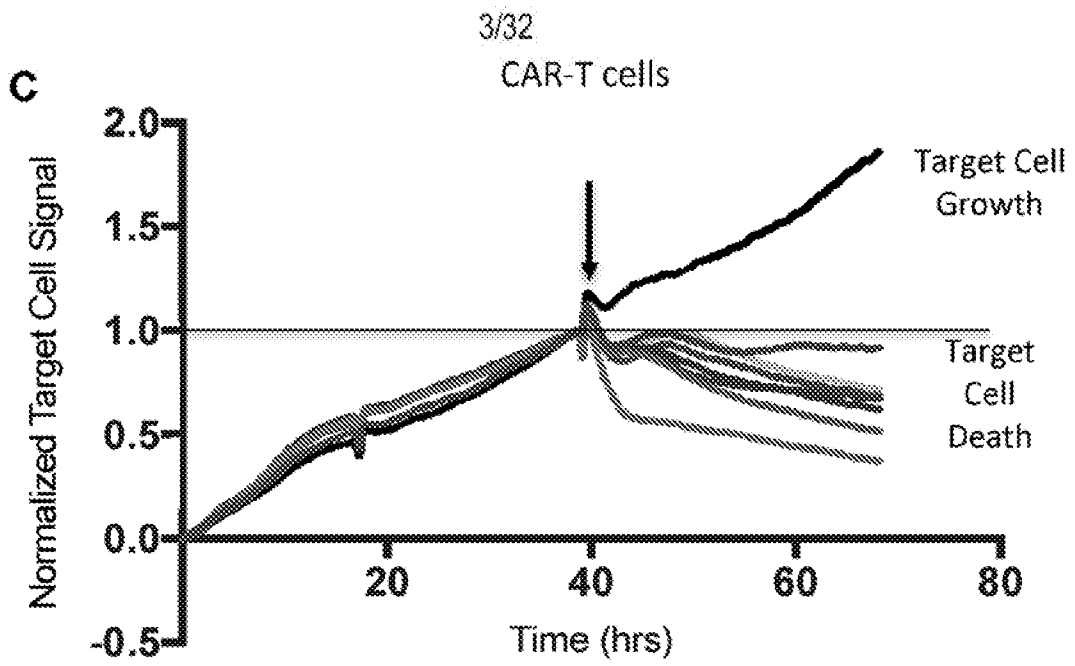
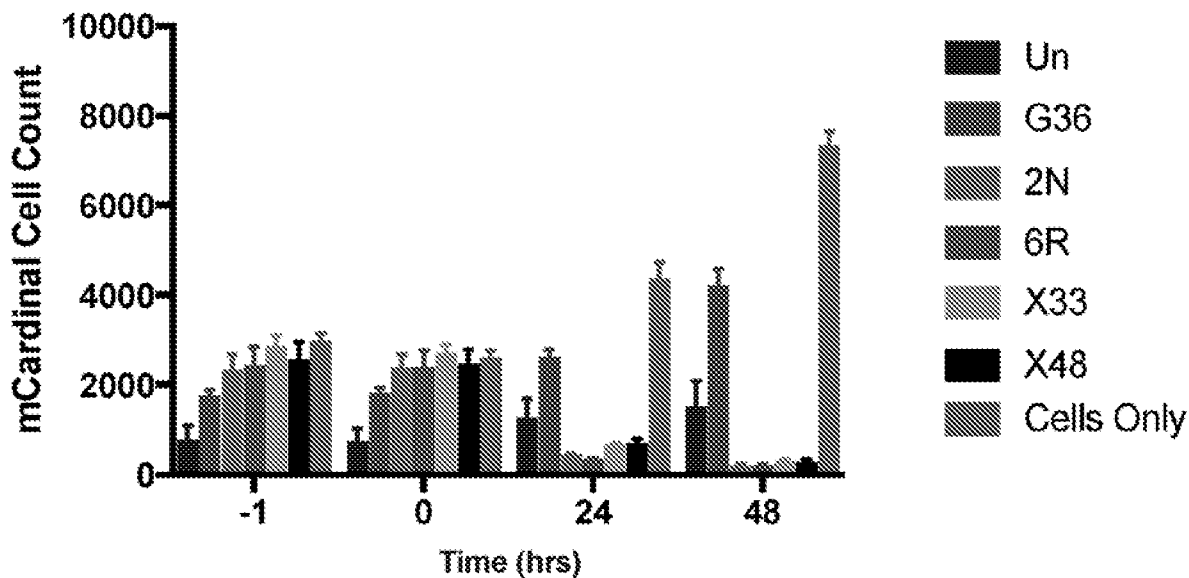


FIG. 2 CON'T
SUBSTITUTE SHEET (RULE 26)

231 CXCR4



231

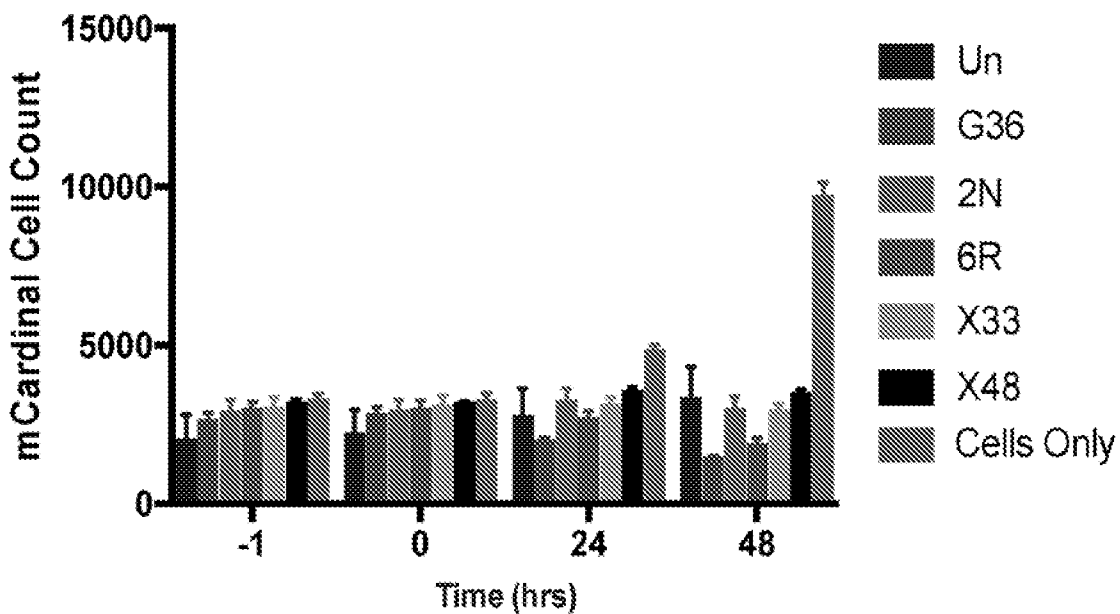


FIG. 3

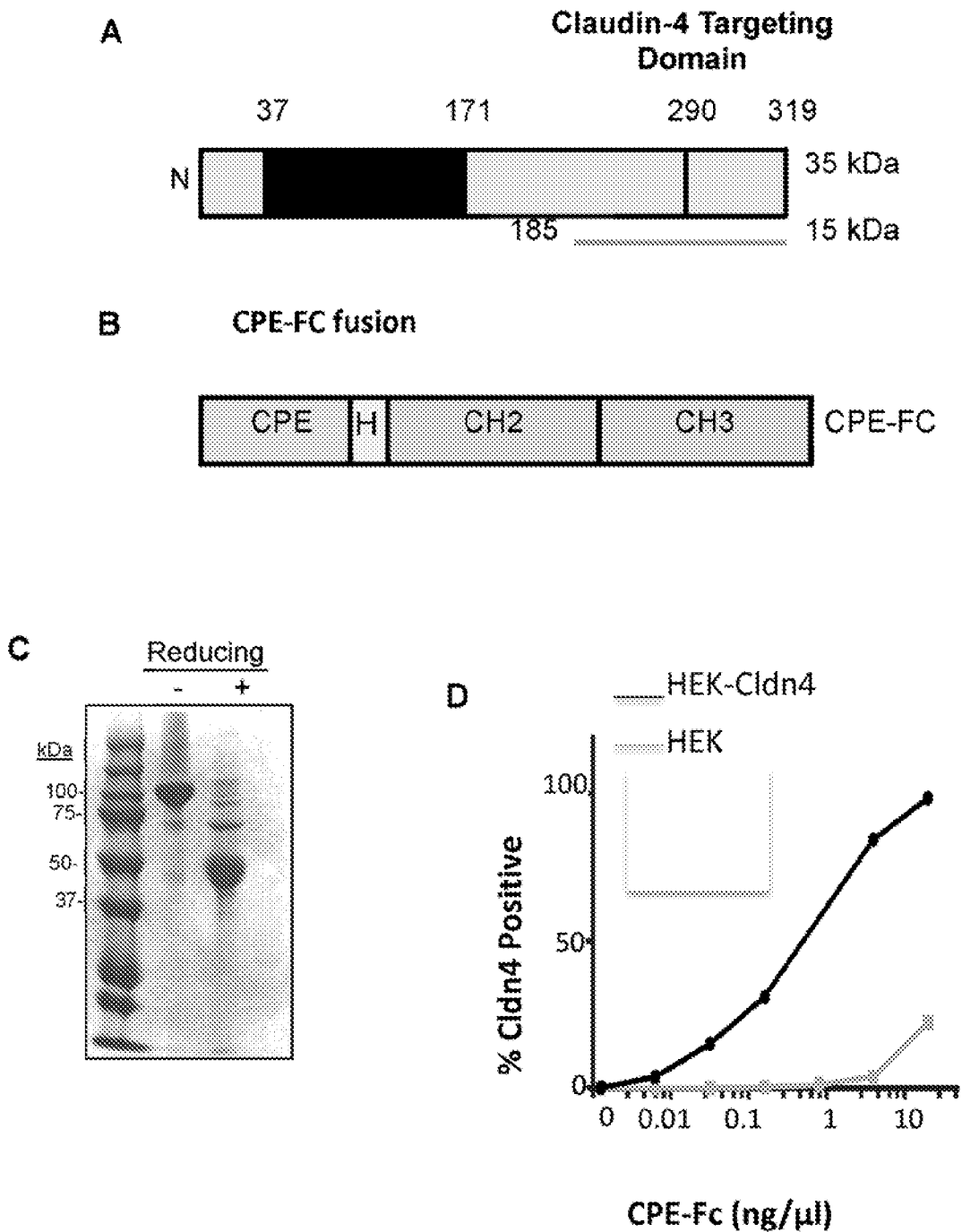
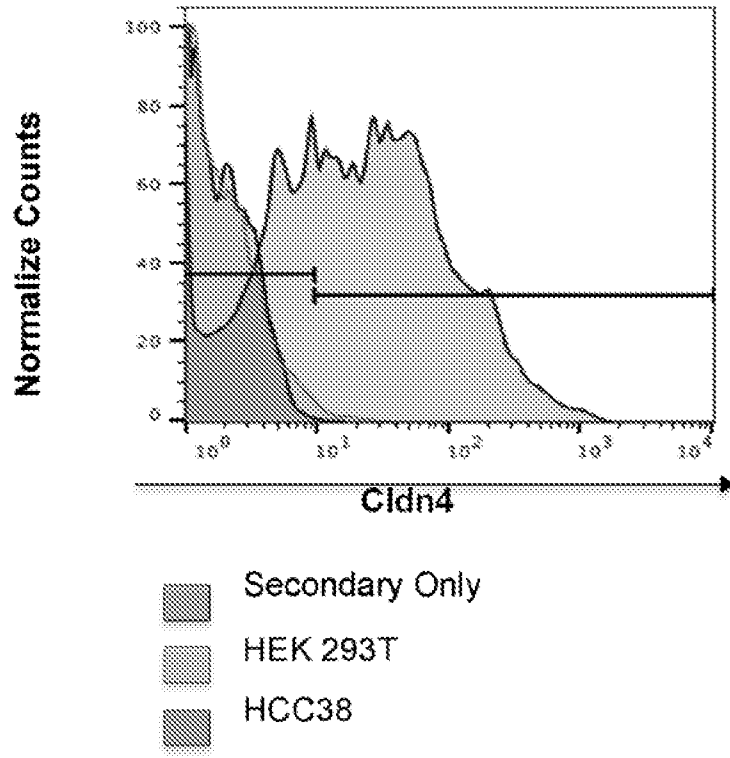


FIG. 4

SUBSTITUTE SHEET (RULE 26)

A



B

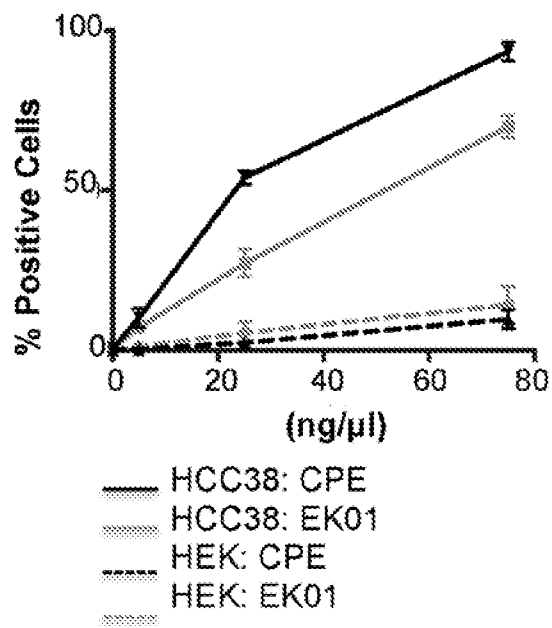


FIG. 5

SUBSTITUTE SHEET (RULE 26)

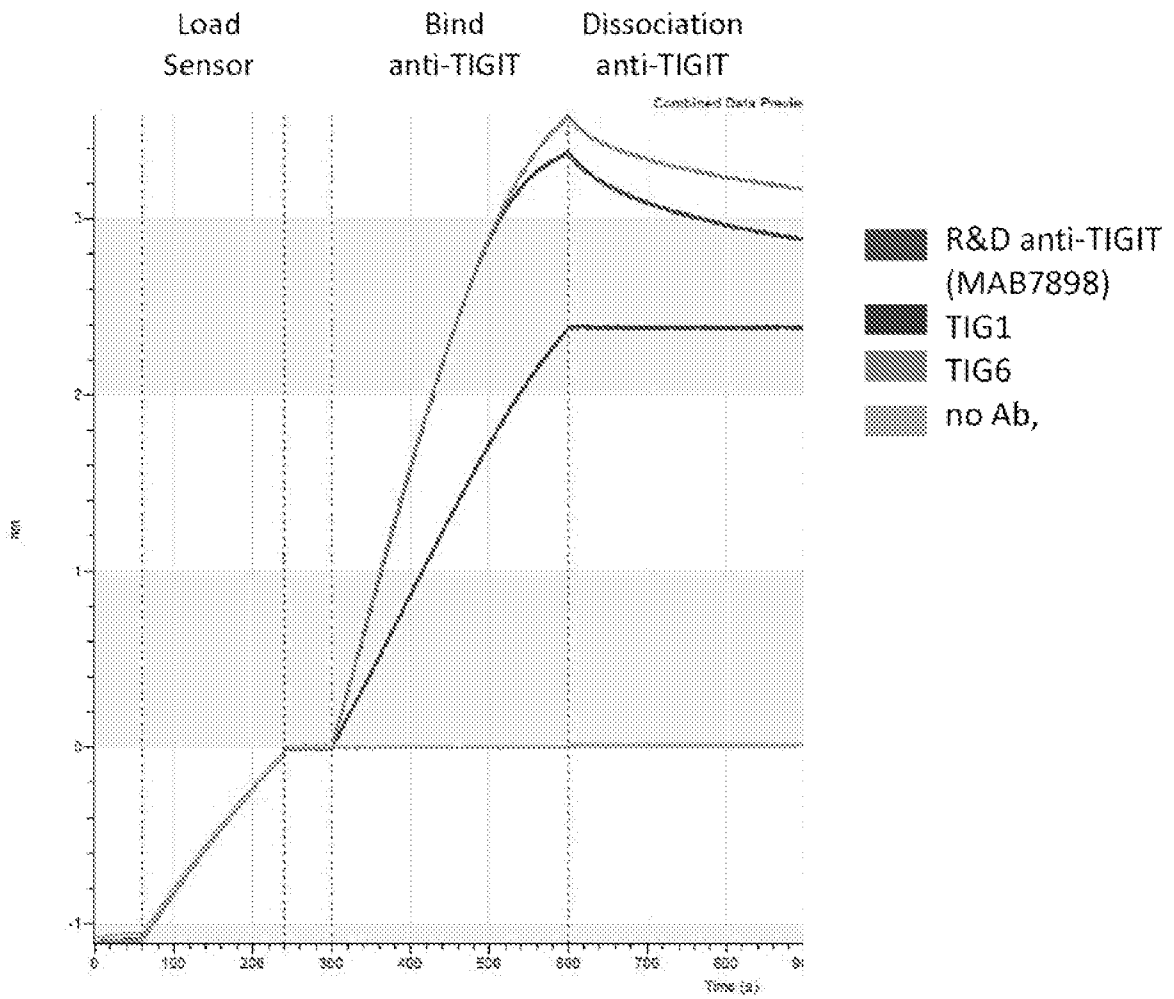


FIG. 6

24 hr post CX2 CAR-T

Incubation

MDA-231
CXCR4

MDA-231

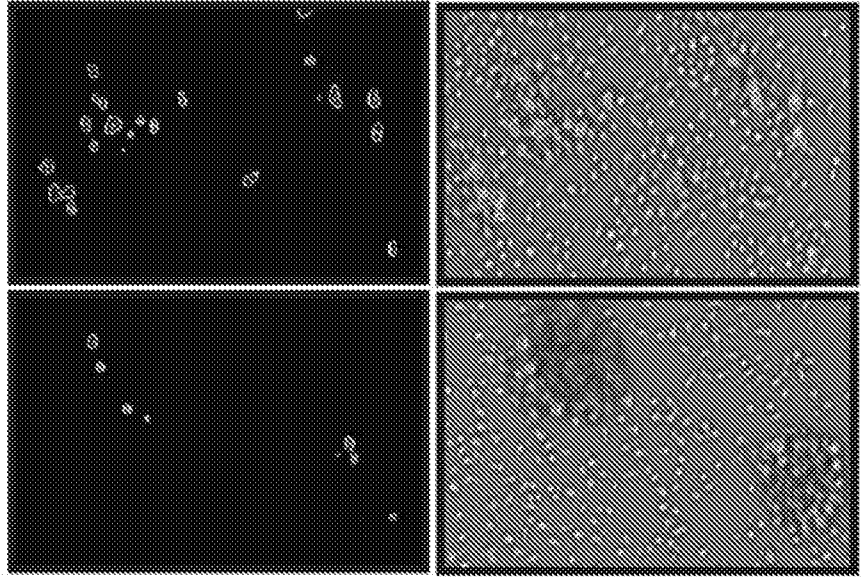


FIG. 7

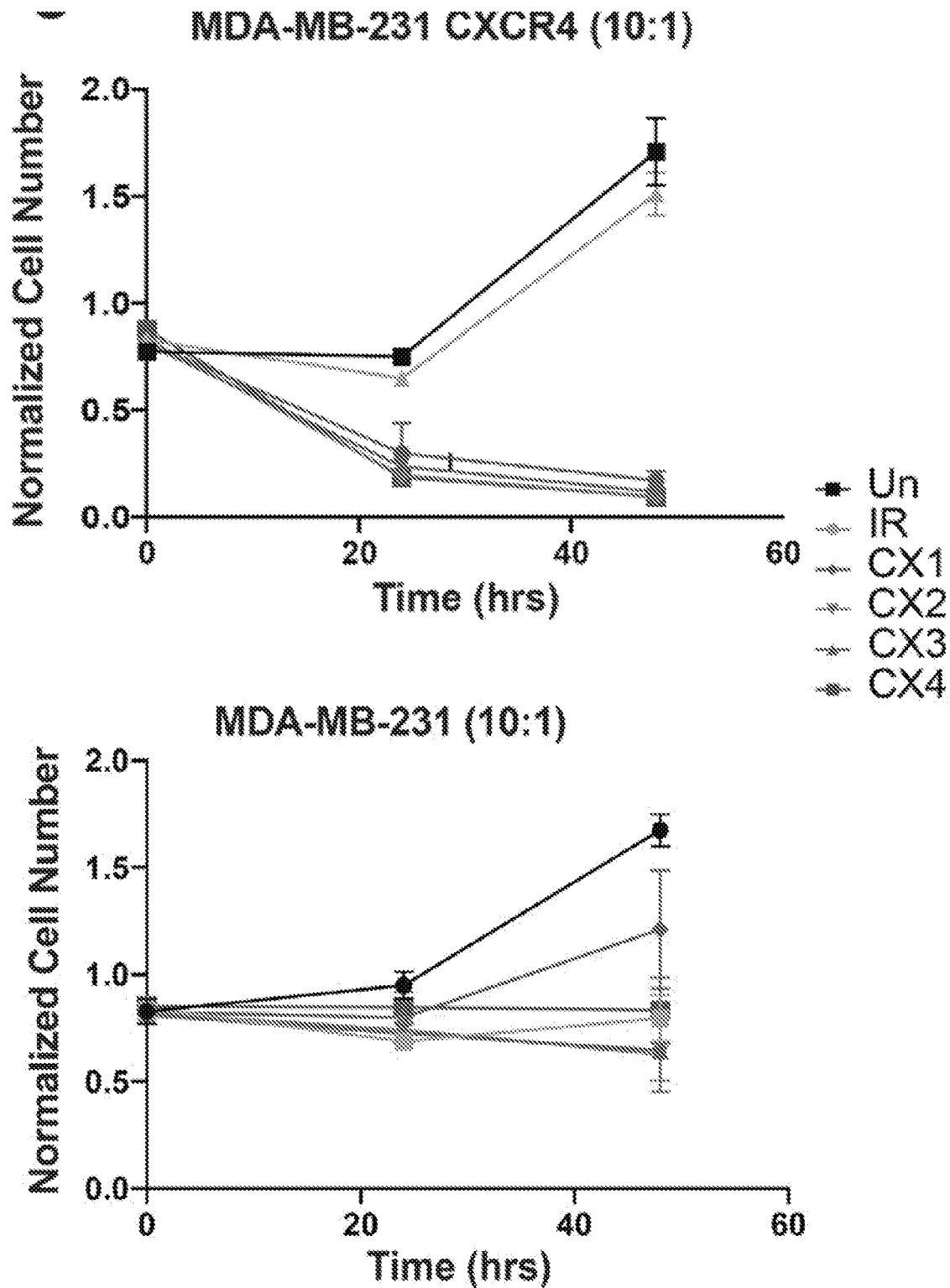


FIG. 8

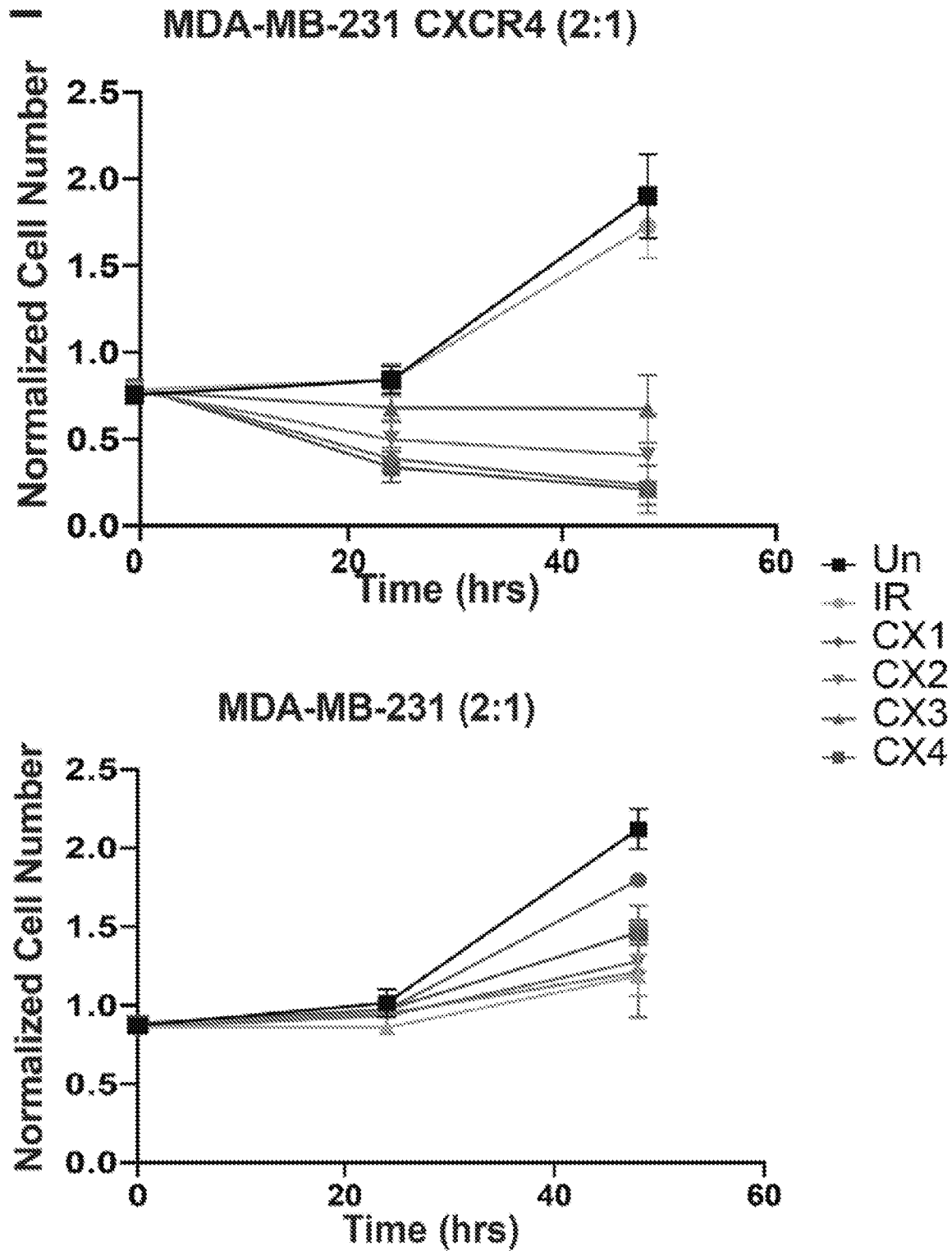


FIG. 9

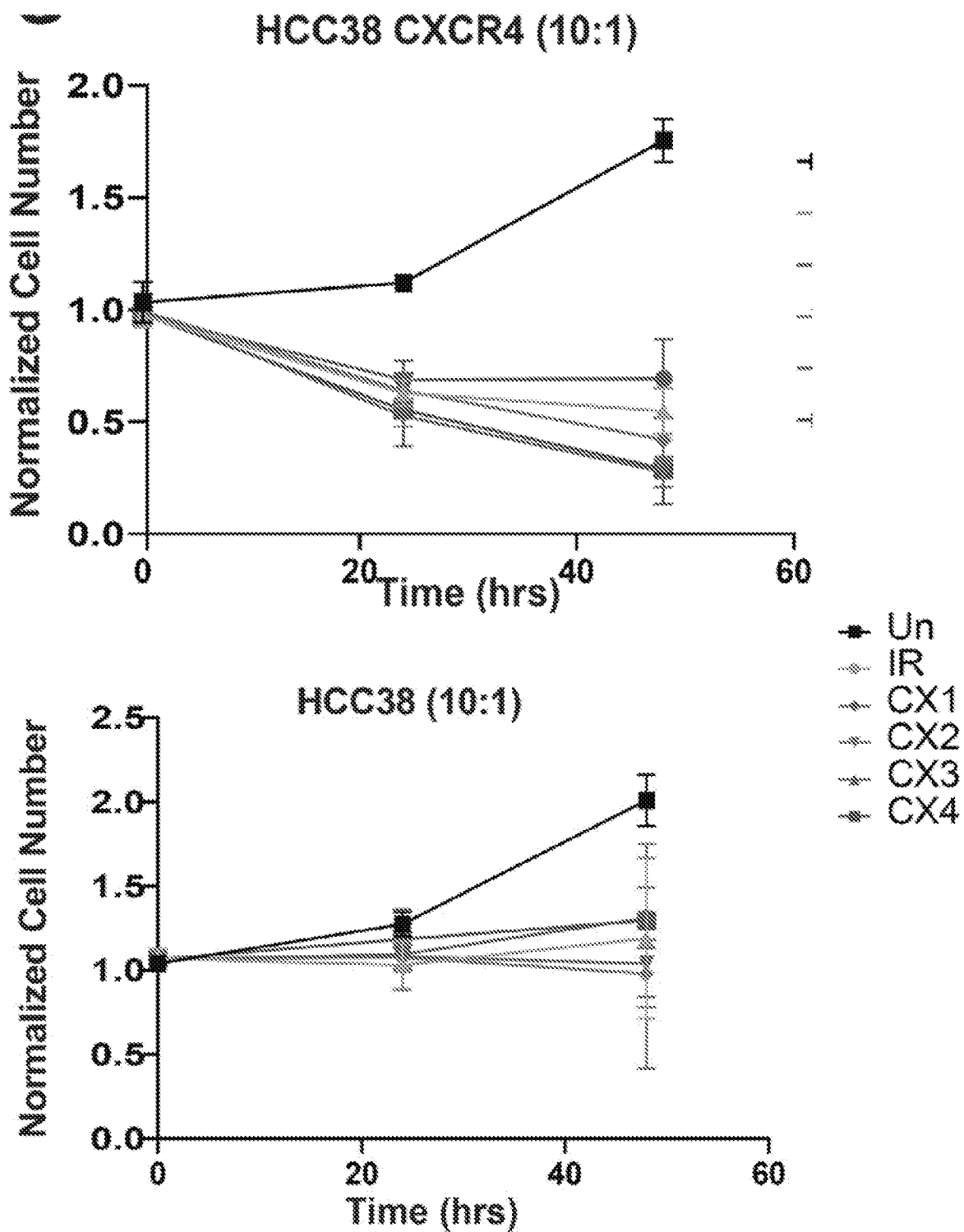


FIG. 10

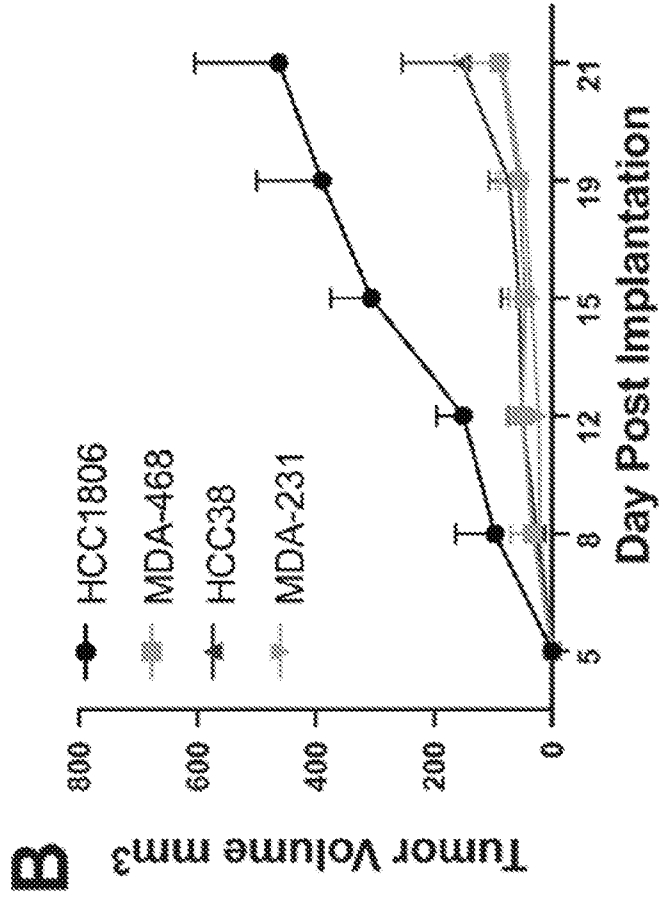
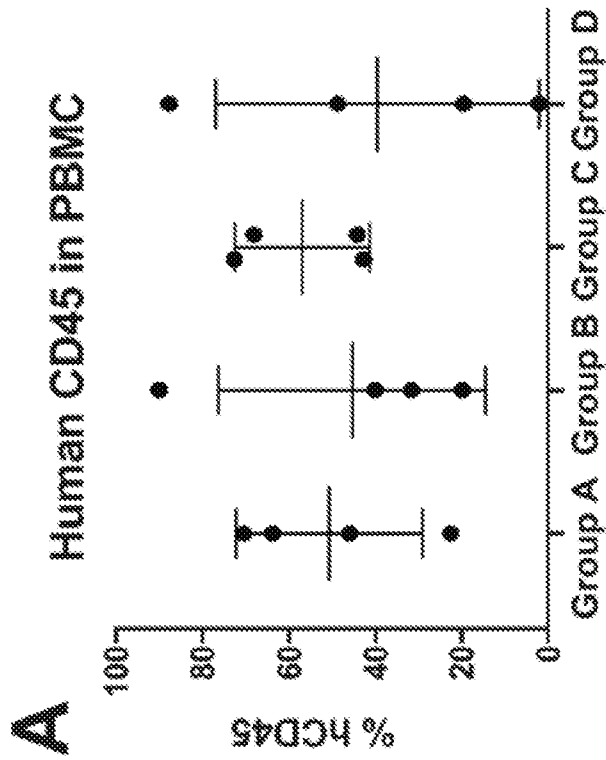


FIG. 11

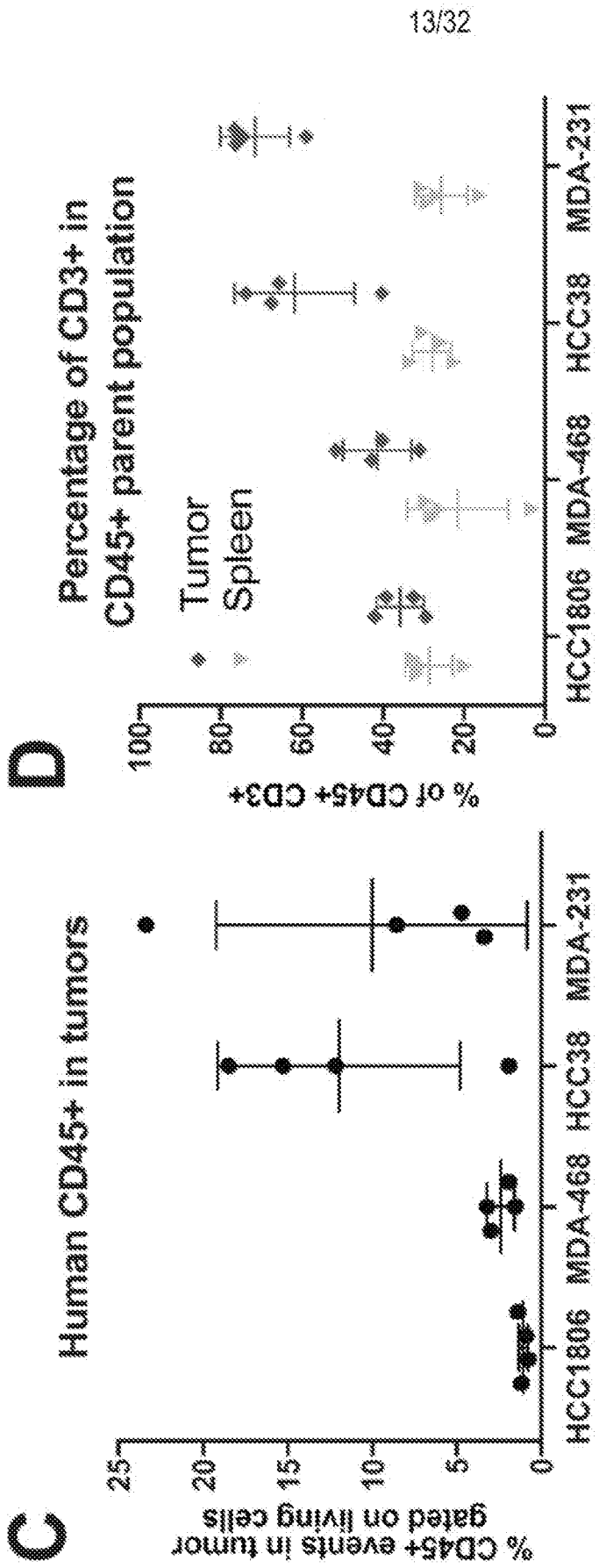


FIG. 11 - CONT.

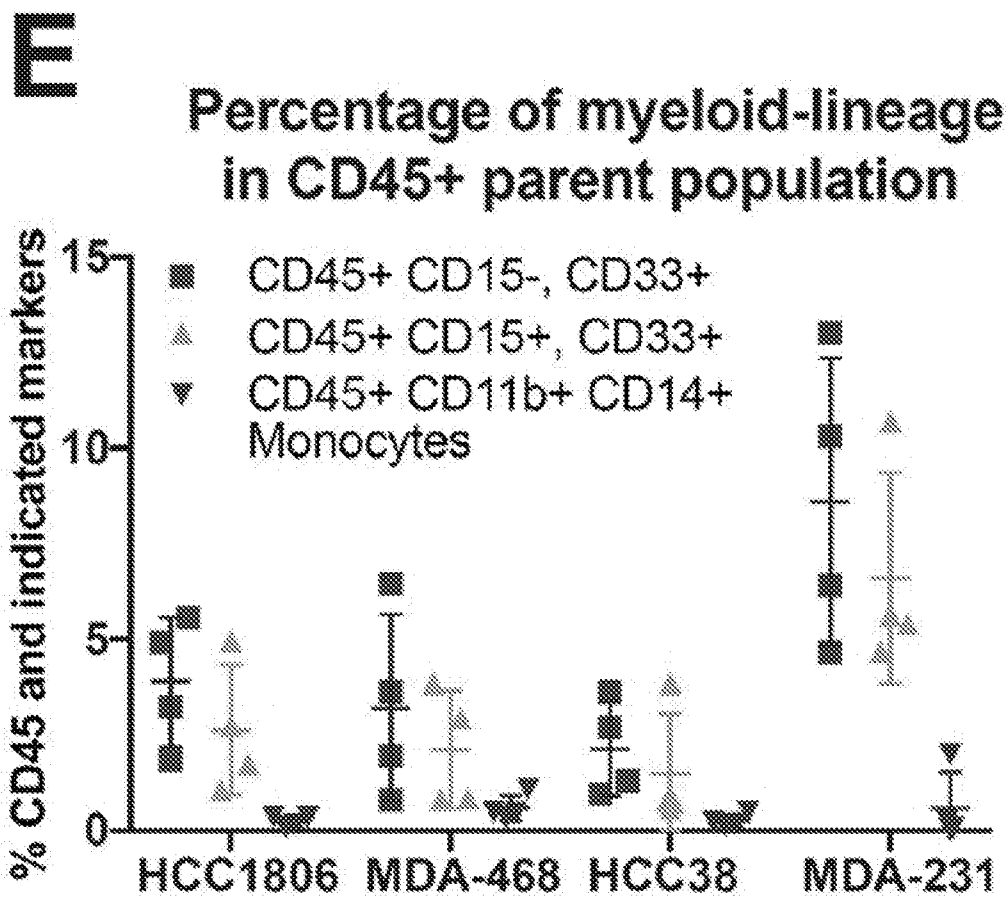


FIG. 11 - CONT.

**Table 1:
Tumor Weight**

(mg)	
HCC1806	402.5
MDA-468	40
HCC38	97.5
MDA-231	67.5

FIG. 12

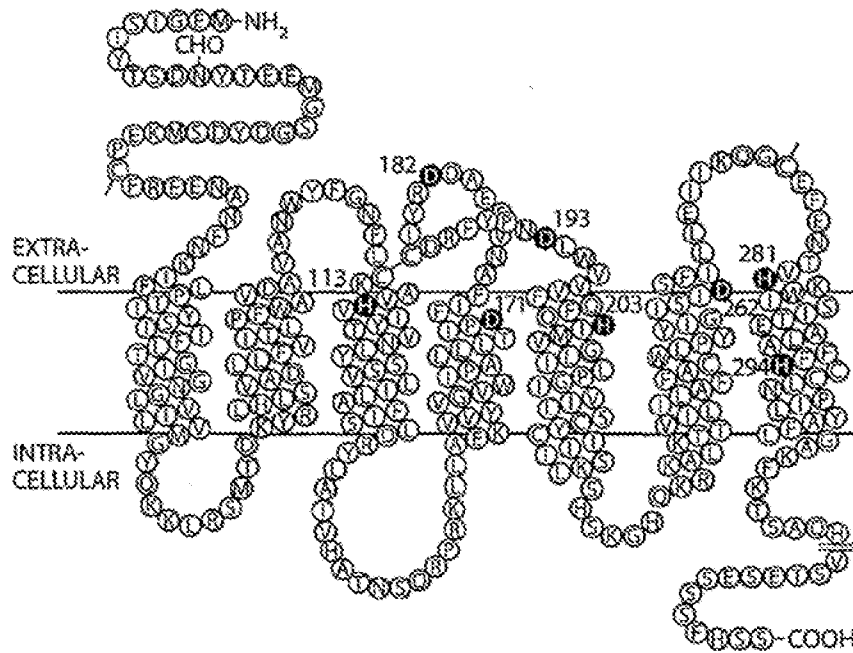
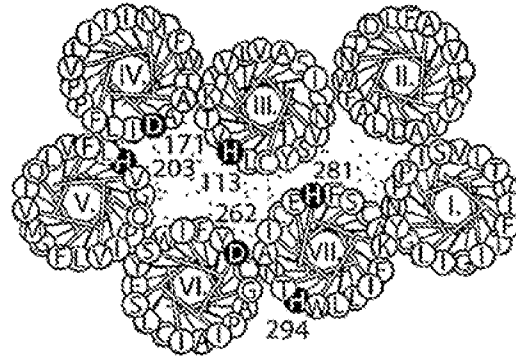


FIG. 14

SUBSTITUTE SHEET (RULE 26)

A.

Sequence number	Sequence ID	V-GENE and allele	J-GENE and allele	D-GENE and allele	FR1-IMGT	CDR1-IMGT	FR2-IMGT
1	EK01_heavy	IGHV3-53*01 F	IGHJ3*02 F	IGHD4-23*01 ORF	EVQLVQSGGGGLIQPGGSLRLSCAAS	GFTV...SSNY	MSWYRCAPGKGLEWWSV
2	EK01_light	IGKV1-5*03 F	IGKJ4*01 F		DIVMTQTPSTLSASVDRVTTCRAS	QSI.....NSW	LAWYQQKSGKAPKLLY

FIG. 15

CDR2-IMGT FR3-IMGT CDR3-IMGT FR4-IMGT
 IYSG...GST YYADSVKGRFTISRDNSKNTLYLQMINSRAEDTAVYIC ARDNPISAFDI WGQGLVTIVSL
 KA.....S SLGSGVPSRFSSG.SDIEFTLISSLOPDPFATYIC QQYQDPLI FGGGTIVEIK

B.

M99679 Homsap IGHV3-53*01 F
 EK01_heavy

FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3	FR4
1 10 20	30 40 50	60 70 80 90 100				
EVQLVESGGGLYFDSGKSLTCDASGEPY...SSWYINRWGQVGGLEWVSIVYISG...GSTYYADSVKGRFTISRDNSKNTLYLQMINSRAEDTAVYICAR						
EVQVQSGGGLIQPQSSLRSLCSASQETV...SSWYINRWGQVGGLEWVSIVYISG...GSTYYADSVKGRFTISRDNSKNTLYLQMINSRAEDTAVYICARDPISAFDIWGQGLVTIVSL						

X72813 Homsap IGKV1-5703 F
 EK01_light

FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3	FR4
1 10 20	30 40 50	60 70 80 90 100				
DIQMFDGDTLSASVQAVVITTCASQSI...SSWYINRWGQVGGLEWVSIVYISG...GSTYYADSVKGRFTISRDNSKNTLYLQMINSRAEDTAVYICARDPISAFDIWGQGLVTIVSL						
DIWDTFPSLSSASTGAVVITTCASQSI...SSWYINRWGQVGGLEWVSIVYISG...GSTYYADSVKGRFTISRDNSKNTLYLQMINSRAEDTAVYICARDPISAFDIWGQGLVTIVSL						

FIG. 15
CONTINUED

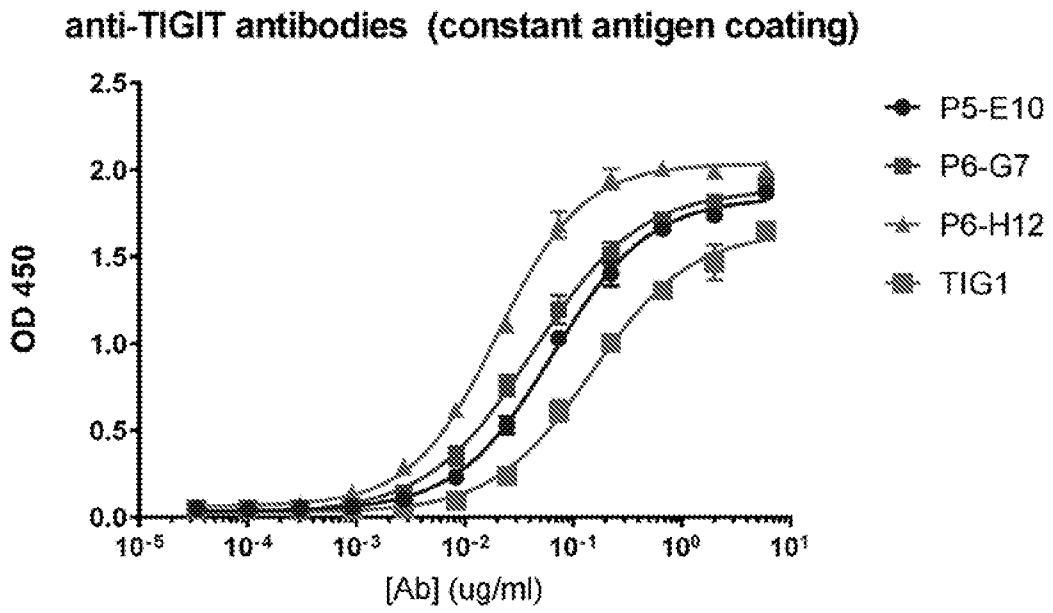


FIG. 16

SUBSTITUTE SHEET (RULE 26)

	FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3	FW4
M09668 HomSep IGHV5-51*01 F P5-E10	1 10 20 EVQLVDSKLVKPGASVKLSCKGSGS EVQLVDSKLVKPGASVKLSCKGSGS	30 40 50 ...TNR IGHVQPGQGLKEMGI ...TNR IGHVQPGQGLKEMGI	60 70 80 90 100 ...DNR KISPSRQ... ...DNR KISPSRQ...	CDR3	FW4		
Z79664 HomSep IGLV1-44*01 F P5-E10	1 10 20 QVQLVQPPS... LPVHQPES...	30 40 50 ...SSM VHWYQLP... ...GSRF VHWYQLP...	60 70 80 90 100 ...DRESSAP... ...DRESSAP...	CDR3	FW4	22/32	
M09641 HomSep IGHV1-18*01 F P6-H12	1 10 20 QVQLVQSG... EVQLVDSKLVKPGASVKLSCKGSGS	30 40 50 ...TSK IHWYQ... ...TNG IHWYQ...	60 70 80 90 100 ...DNR KISPSRQ... ...DNR KISPSRQ...	CDR3	FW4		

FIG. 17
CONTINUED

FR1-INGT (1-26)	CDR1-INGT (27-38)	FR2-INGT (39-55)	CDR2-INGT (56-65)	FR3-INGT (66-104)	CDR3	FW4
1 10 20	30	40	60	70 80 90 100		
Z73664 Homsap IGLV2-14*01 F PG-H12						

FR1-INGT (1-26)	CDR1-INGT (27-38)	FR2-INGT (39-55)	CDR2-INGT (56-65)	FR3-INGT (66-104)	CDR3	FW4
1 10 20	30	40	60	70 80 90 100		23/32
L22582 Homsap IGHV1-69*01 F PG-G7						

FR1-INGT (1-26)	CDR1-INGT (27-38)	FR2-INGT (39-55)	CDR2-INGT (56-65)	FR3-INGT (66-104)	CDR3	FW4
1 10 20	30	40	60	70 80 90 100		
Z73676 Homsap IGLV10-54*01 F PG-G7						

FIG. 17
CONTINUED

Sequence ID	V-GENE and allele	J-GENE and allele	D-GENE and allele	FR1-IMG1	CDR1-IMG1	FR2-IMG1
hTIGItHis-E1-C3 (TIG1)	HV1-18*01 F	HJ4*02 F	HD3-10*01 F	QVQLVQSGAEVKKPGASVKYSCKAS	GYTF...TSYG	ISWVROAPGGGLEWIMGW
hTIGItHis-E2-E7 (TIG6)	HV3-74*02 F	HJ3*02 F	HD3-9*01 F	EIVQLVQSGGGLVKPQGSRLSCEAS	GYTF...SDYS	MSWVROAPGGGLEWVSR
P5-E10	HV5-51*01 F	HJ6*02 F		EIVQLVQSGAEVKKPQESLKISCKGS	GYSF...TNYW	ICWVROMPGKGLEWIMGI
P6-H12	HV1-18*01 F	HJ5*02 F	HD6-13*01 F	EIVQLVQSGAEVKKPGASVKYSCKAS	GYTF...TNYG	ISWVROAPGGGLEWIMGW
P6-G7	HV1-69*01 F	HJ6*02 F	HD5-12*01 F	QVQLQDSGAEVKKPQSSVKYSCKAS	GGTF...SSVA	ISWVROAPGGGLEWIMGG

Sequence ID	V-GENE and allele	J-GENE and allele	D-GENE and allele	FR1-IMG1	CDR1-IMG1	FR2-IMG1
hTIGItHis-E1-C3 (TIG1)	LV1-44*01 F	LJ3*02 F		SYELTOPPS.ASGTPGORVTISCSGS	SSNIL...GSNT	VSWYQQLPGTAPKLLY
hTIGItHis-E2-E7 (TIG6)	LV1-44*01 F	LJ2*01 F		SYELTOPPS.ASGTPGORVTISCSGS	RSNIL...GRNS	VNWYQQLPGTAPKLLY
P5-E10	LV1-44*01 F	LJ3*02 F		LPVLTQPPS.ASGTPGORVTISCSGS	SSNIL...GSNT	VNWYQQLPGTAPKLLY
P6-H12	LV2-14*01 F	LJ2*01 F		QSALTQPPS.VSGSPGOSITISCTGT	SSDVG...GYNY	VSWYQQLPGTAPKLLMY
P6-G7	LV10-54*01 F	LJ3*02 F		SYELTOPPS.VSKGLRQTATLICTGN	SNWV...GNCG	AAWILQCHGHPKLLSY

FIG. 17
CONTINUED

CDR2-IMGT	FR3-IMGT	CDR3-IMGT	FR4-IMGT
ISAY..NGNT	NYACKLQ.GRVYMTTDTSTSTAYMELRSLRSDDTAVYYC	ARDPGLWFGLTHDYFDY	WGGGTLTVSS
INSD..GSRT	NYADSVK.GRFTISRDNKNTLYLQINSLRAEDTAMYYC	ARGPFFGFDI	WGGGTLTVSS
INPV..NSRT	IYSPSFQ.GCVTISDKSVTTAYLQWSSLKASDTAMYYC	ARYYYAMEV	WGRGTLTVSS
VDNI..NGNI	NYACKEL.GRVYMTTDTSTSTAYMELRSLRSDDTAVYYC	ARGLFSSRWYLVFDP	WGGGTLTVSS
ILPM..FGST	NYACKFQ.GRLTIADSTRIVYLEINSLTSEDATVYYC	ARGRDIVAPSNSGFDV	WGGGTLTVSS

CDR2-IMGT	FR3-IMGT	CDR3-IMGT	FR4-IMGT
RN.....N	QRPSGVP.DRFSGSK..SGTSASLAINGLQSEDEADYYC	AAWDDSRSGPV	FGGTRLTIVL
SN.....N	QRPSGVP.GRFSGSR..SGTSASLAISGLQSEDETDYYC	AAWDARLTGPL	FGGKLSVL
RN.....N	QRPSGVP.DRFSGST..SGTSASLAISGLQSEDEADYYC	EAWDLSNGPV	FGGKLTIVL
EV.....T	ERPSGVS.NRFSGSK..SGNTASLTISGLQAEDEGDDYYC	SSYTRSTSYW	FGGKIVTL
RN.....D	NRPSGIS.ERFSASR..SGNTASLTISGLQPEDEADYYC	SAYDRSLNANV	FGGKLTIVL

FIG. 17
CONTINUED

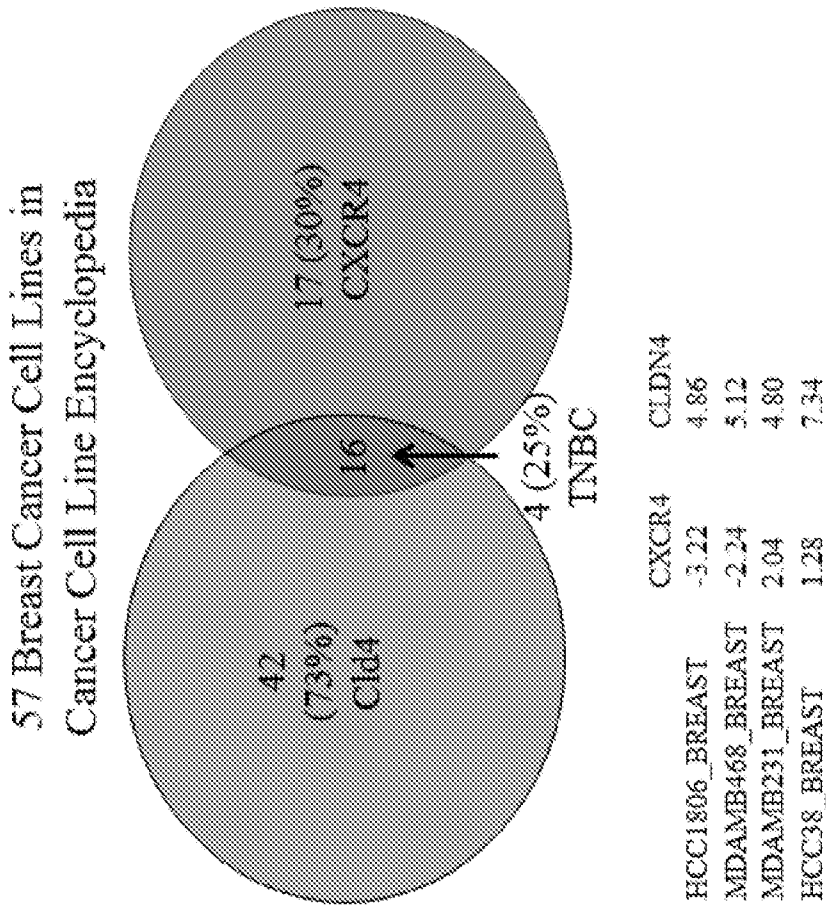


FIG. 18

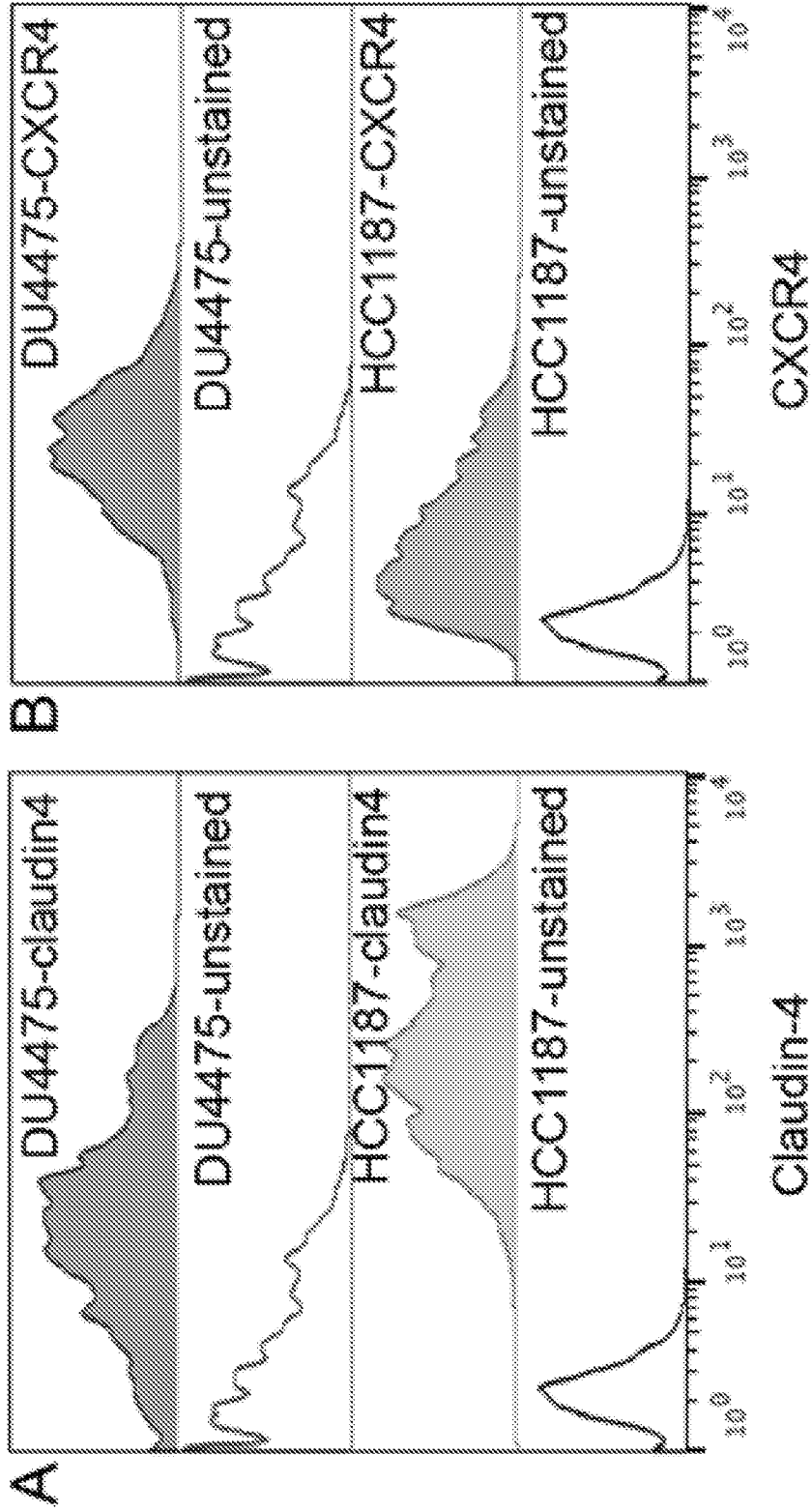
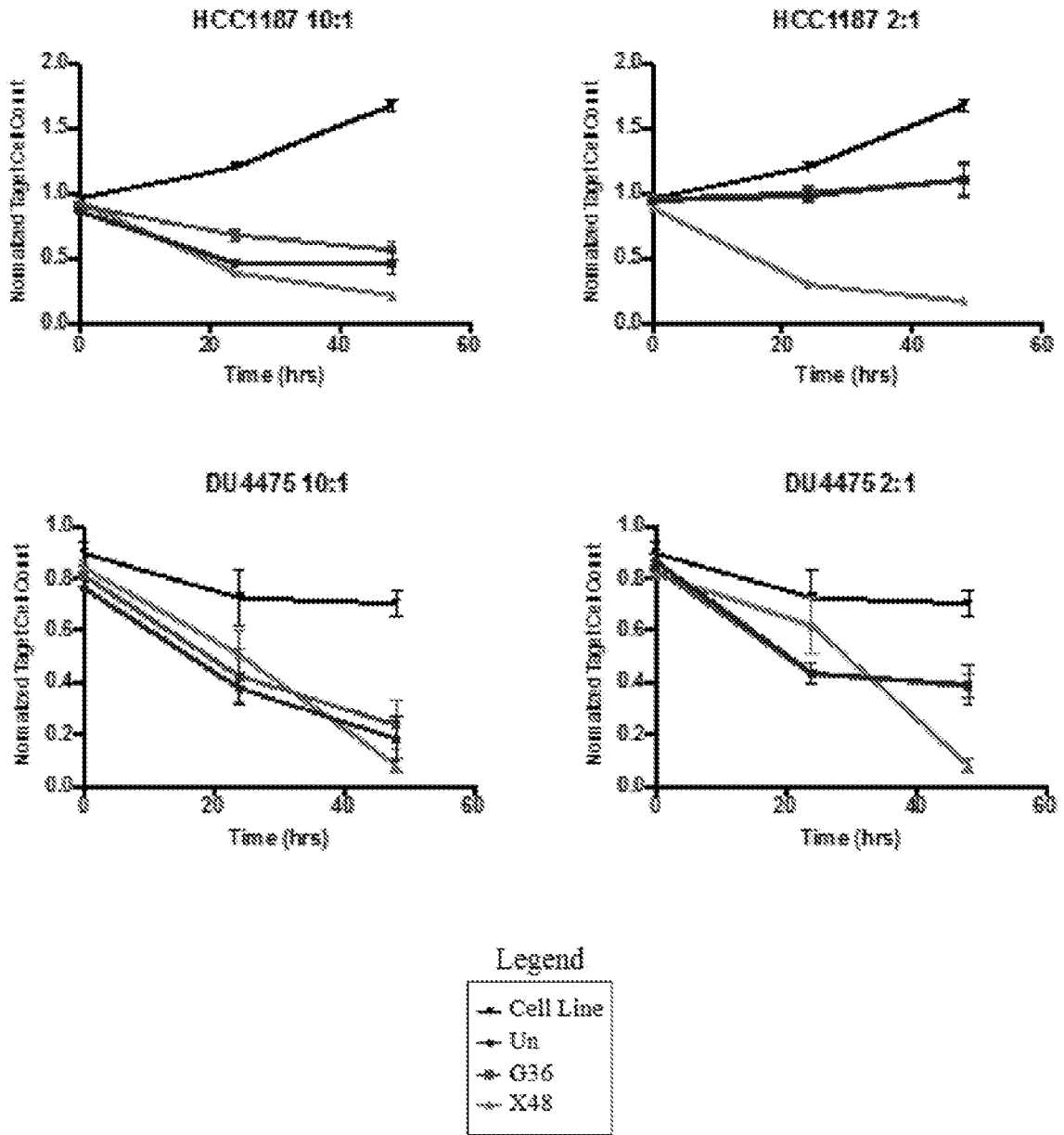


FIG. 19

FIG. 20

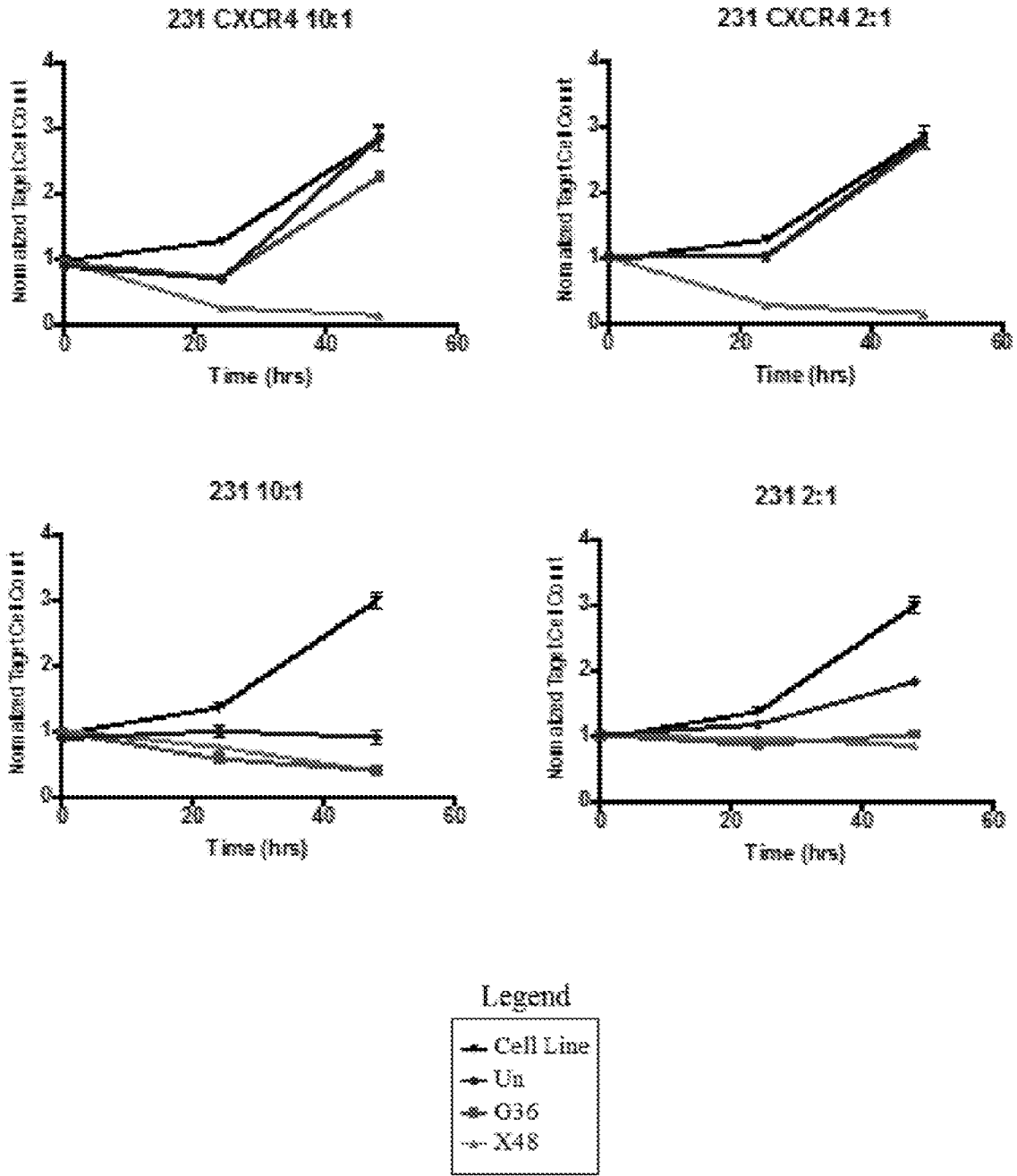


Legend

- Cell Line
- Un
- G36
- X48

N=1

FIG. 20 CON'T



N=1

30/32

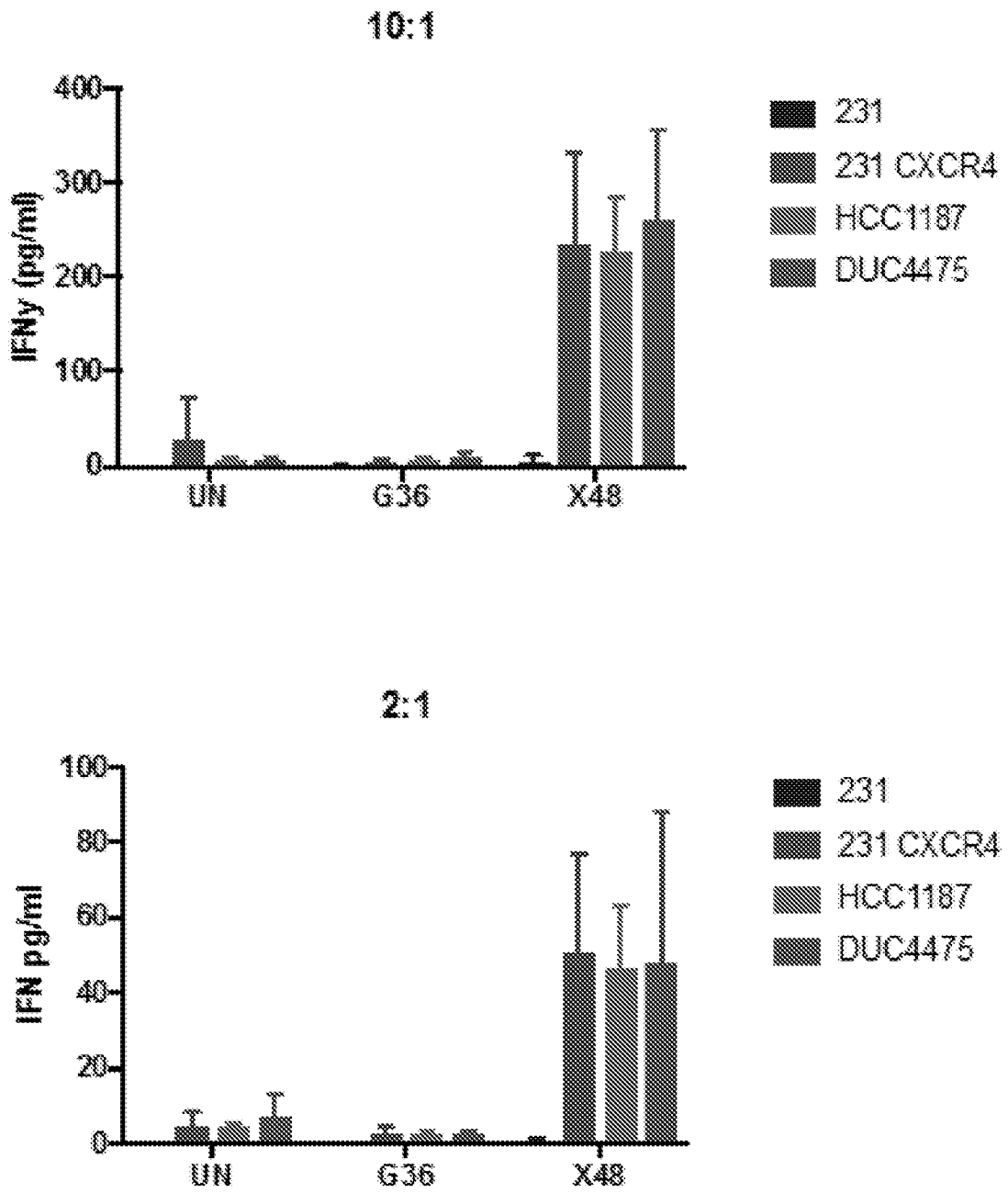
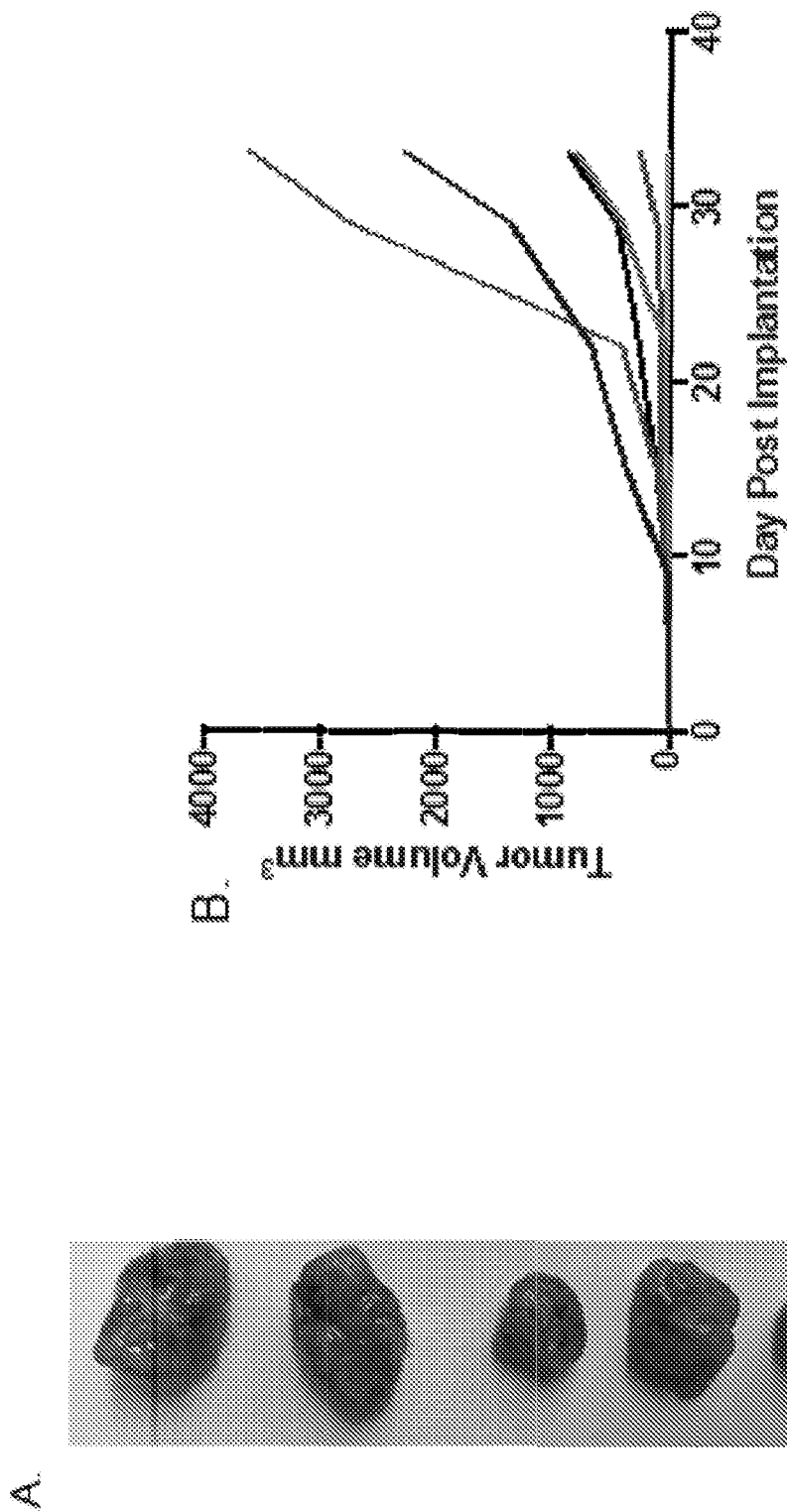


FIG. 21



Average weight: 0.974 g

FIG. 22

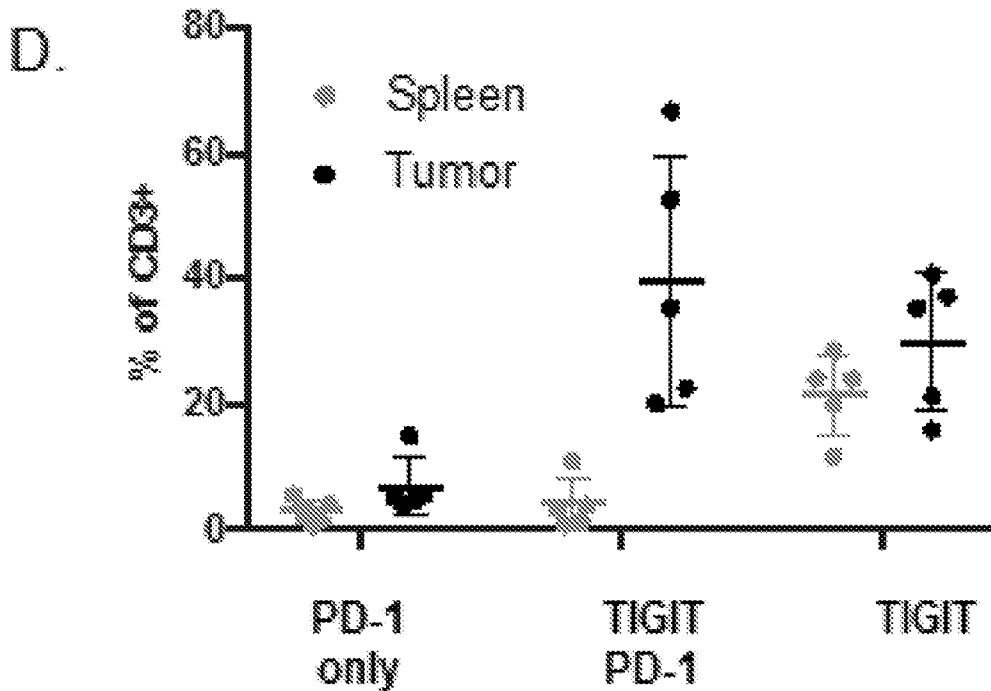
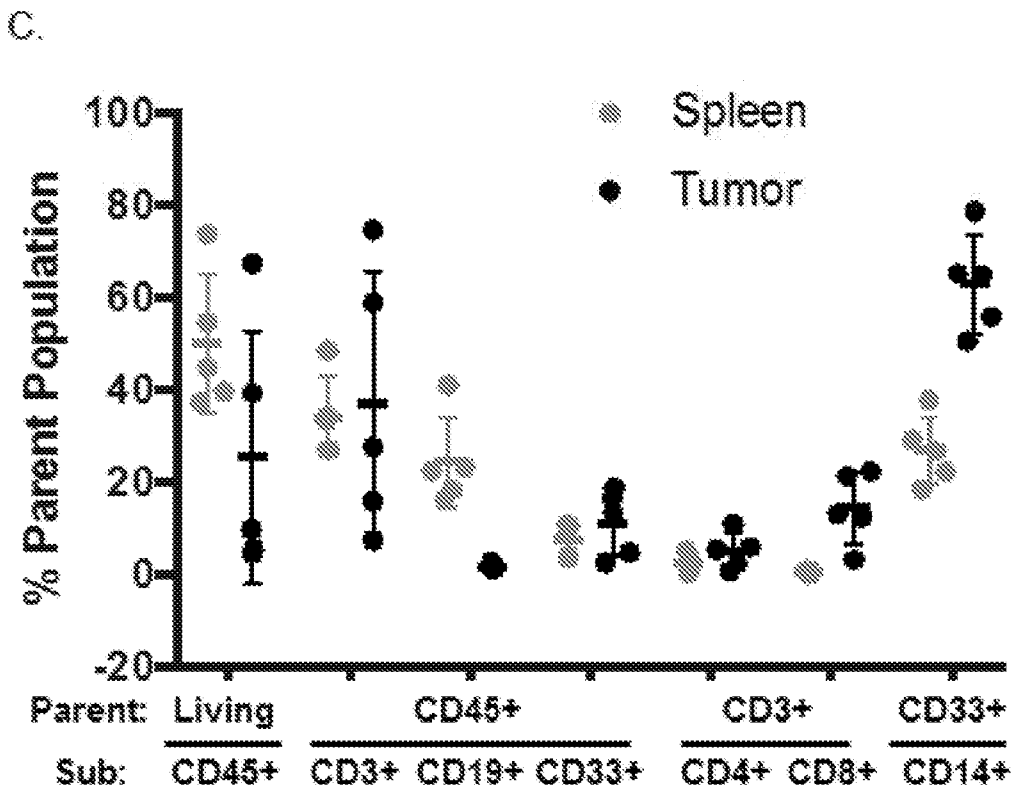


FIG. 22 CON'T

Sequence Listing

1	Sequence Listing Information	
1-1	File Name	P0023723AUD1.xml
1-2	DTD Version	V1_3
1-3	Software Name	WIPO Sequence
1-4	Software Version	2.3.0
1-5	Production Date	2026-03-09
1-6	Original free text language code	
1-7	Non English free text language code	
2	General Information	
2-1	Current application: IP Office	AU
2-2	Current application: Application number	
2-3	Current application: Filing date	
2-4	Current application: Applicant file reference	P0023723AUD1
2-5	Earliest priority application: IP Office	US
2-6	Earliest priority application: Application number	US62/643,040
2-7	Earliest priority application: Filing date	2018-03-14
2-8en	Applicant name	Dana-Farber Cancer Institute, Inc.
2-8	Applicant name: Name Latin	
2-9en	Inventor name	
2-9	Inventor name: Name Latin	
2-10en	Invention title	Engineered cells, T cell immune modulating antibodies and methods for using the same
2-11	Sequence Total Quantity	131

11 Mar 2026

2026201842

3-1	Sequences	
3-1-1	Sequence Number [ID]	1
3-1-2	Molecule Type	AA
3-1-3	Length	130
3-1-4	Features	REGION 1..130
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide MOD_RES 32..33 note=Any amino acid MOD_RES 59 note=Any amino acid MOD_RES 61 note=Any amino acid MOD_RES 103..115 note=Any amino acid MOD_RES 117 note=Any amino acid source 1..130 mol_type=protein organism=synthetic construct
3-1-5	NonEnglishQualifier Value Residues	QVQLVQSGGG VVQPGRSLRL SCAASGFIFS SXXYGMHWVR QAPGKLEWV AVISYDGSXK 60 XYYADSVKGR FTISRDN SKN TLYLQMNSLR AKDTAVYYCA RDXXXXXXXX XXXXXGXDVW 120 GQGT TVTVSS 130
3-2	Sequences	
3-2-1	Sequence Number [ID]	2
3-2-2	Molecule Type	AA
3-2-3	Length	121
3-2-4	Features	REGION 1..121
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..121 mol_type=protein organism=synthetic construct
3-2-5	NonEnglishQualifier Value Residues	EVQLVESGGG VVQPGRSLRL SCAASGFIFS SYGMHWVRQA PGKLEWVAV ISYDGSNKYY 60 ADSVKGRFTI SRDN SKNTLY LQVSSLRAED TAVYYCVRDL VAAAGTAFDI WQGT TVTVSS 120 S 121
3-3	Sequences	
3-3-1	Sequence Number [ID]	3
3-3-2	Molecule Type	AA
3-3-3	Length	120
3-3-4	Features	REGION 1..120
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..120 mol_type=protein organism=synthetic construct
3-3-5	NonEnglishQualifier Value Residues	QVQLQQSGPG LVKPSQTL SL TCAVSGDSVS SNFVAWNWIR QSPSRGLEWL GRYYRSRWY 60 NDYAVSVQSR IRVTPDTSKN QFSLHLDSVT PEDTAVYYCA RQHGSGDFW GQGT LVTVSS 120
3-4	Sequences	
3-4-1	Sequence Number [ID]	4
3-4-2	Molecule Type	AA
3-4-3	Length	126
3-4-4	Features	REGION 1..126
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..126 mol_type=protein organism=synthetic construct
3-4-5	NonEnglishQualifier Value Residues	QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYGISWVRQA PGQGLEWVGW ISAYNGNTNY 60 AQKLQGRVIM TTDSTSTAY MELRSLRSDD TAVYYCARDT PGIAARRYYY YGMDVWGQGT 120 TVTVSS 126
3-5	Sequences	
3-5-1	Sequence Number [ID]	5
3-5-2	Molecule Type	AA
3-5-3	Length	121
3-5-4	Features	REGION 1..121
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..121 mol_type=protein

3-5-5	NonEnglishQualifier Value Residues	organism=synthetic construct QVQLVQSGGG VVQPGRSLRL SCAASGFTFS SYPMHWRQA PGKGLEWMTV ISSDGRNKYY 60 PDSVKGRFTI SRDNSKNTLY LQMNSLRPED TAVYYCARGG YHDFWSPDY WGQGLVTVTS 120 S 121
3-6 3-6-1 3-6-2 3-6-3 3-6-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	6 AA 127 REGION 1..127 note=Description of Artificial Sequence: Synthetic polypeptide source 1..127 mol_type=protein organism=synthetic construct
3-6-5	NonEnglishQualifier Value Residues	EVQLVQSGGG LVQPGGSLRL SCAASGFTFS SYAMSWVRQA PGKGLEWVAN IKQDGSEKYY 60 VDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCARDQ VSGITIFGGK WRSPDVWGQG 120 TTVTVSS 127
3-7 3-7-1 3-7-2 3-7-3 3-7-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	7 AA 126 REGION 1..126 note=Description of Artificial Sequence: Synthetic polypeptide source 1..126 mol_type=protein organism=synthetic construct
3-7-5	NonEnglishQualifier Value Residues	QVTLKESGGG VVQPGRSLRL SCAASGFTFT NYGLHWVRQA PGKGLEWVAV ISHDGTKKYY 60 ADSVKGRFTI SRDSSENTLY LQMNSLRPED SALYYCARDG GYCSGGRCYS YGMDVWGQGT 120 TVTSS 126
3-8 3-8-1 3-8-2 3-8-3 3-8-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	8 AA 122 REGION 1..122 note=Description of Artificial Sequence: Synthetic polypeptide source 1..122 mol_type=protein organism=synthetic construct
3-8-5	NonEnglishQualifier Value Residues	QVQLVQSGGG VVLPGRSLRL SCVASGFTFR RYGMHWVRQA PGKGLEWVSL ISYDGSKTFY 60 GESVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCARAT VTIDGYYMD VWGKGTTVTV 120 SS 122
3-9 3-9-1 3-9-2 3-9-3 3-9-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	9 AA 114 REGION 1..114 note=Description of Artificial Sequence: Synthetic polypeptide MOD_RES 12..14 note=Any amino acid MOD_RES 24 note=Any amino acid MOD_RES 26..27 note=Any amino acid MOD_RES 29..30 note=Any amino acid MOD_RES 32..35 note=Any amino acid MOD_RES 42 note=Any amino acid MOD_RES 45 note=Any amino acid MOD_RES 53..54 note=Any amino acid MOD_RES 56 note=Any amino acid MOD_RES 92

3-9-5	NonEnglishQualifier Value Residues	<p>note=Any amino acid MOD_RES 96..102 note=Any amino acid source 1..114 mol_type=protein organism=synthetic construct</p> <p>QSVLTQPPSV SXXXPGQTVT ISCXGXXSXX GXXXXVSWYQ QXPGXAPKLL IYXXNXRPSG 60 IPDRFSGSKS GNTASLTITG LQAKDAKKYY CXSWDXXXXX XXVFGGGTKL TVLG 114</p>
3-10	Sequences	
3-10-1	Sequence Number [ID]	10
3-10-2	Molecule Type	AA
3-10-3	Length	111
3-10-4	Features	REGION 1..111
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..111 mol_type=protein organism=synthetic construct
3-10-5	NonEnglishQualifier Value Residues	<p>QPVLTPRSV SGSPGQSVTI SCTGTISDVG GHNFVSWYQQ NPGKAPKLII FEVTKRPAGV 60 PDRFSGSKSG NTASLTVSGL QAEDEGEYYC SSYGGSNDVI FGGGTKLSVL G 111</p>
3-11	Sequences	
3-11-1	Sequence Number [ID]	11
3-11-2	Molecule Type	AA
3-11-3	Length	111
3-11-4	Features	REGION 1..111
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..111 mol_type=protein organism=synthetic construct
3-11-5	NonEnglishQualifier Value Residues	<p>QSVLTQPPSV SKGLRQTATL TCTGNSNNVG NQGAAWLQQH QGHPPKLLSY RNNNRPSGIS 60 ERFSASRSGN TASLTITGLQ PEDEADYCS AMDNRLKTYV FGTGGKVTVL G 111</p>
3-12	Sequences	
3-12-1	Sequence Number [ID]	12
3-12-2	Molecule Type	AA
3-12-3	Length	109
3-12-4	Features	REGION 1..109
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..109 mol_type=protein organism=synthetic construct
3-12-5	NonEnglishQualifier Value Residues	<p>SSELTQDPAV SVALGQTVRI TCQGDLSLRF FASWYQQKPG QAPVLVIYVK NSRPSGIPDR 60 FSGSNSRNTA SLTITGAQAE DEGDIYCNSR DSRDNHQVFG AGTKVTVLS 109</p>
3-13	Sequences	
3-13-1	Sequence Number [ID]	13
3-13-2	Molecule Type	AA
3-13-3	Length	108
3-13-4	Features	REGION 1..108
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..108 mol_type=protein organism=synthetic construct
3-13-5	NonEnglishQualifier Value Residues	<p>EIVLTQSPAT LSVSPGKRAT LSCRASQSVN TNLAWYQQKP GQAPRLLIYG ASSRATGIPD 60 RFSGSGSGTD FTLTISRLEP EDFAVYYCQH YGSSPLTFGG GTKLEIKR 108</p>
3-14	Sequences	
3-14-1	Sequence Number [ID]	14
3-14-2	Molecule Type	AA
3-14-3	Length	107
3-14-4	Features	REGION 1..107
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..107 mol_type=protein organism=synthetic construct
3-14-5	NonEnglishQualifier Value Residues	<p>SSELTQDPAV SVALGQTVRI TCQGDLSLSY YASWYQQKPG QAPVLVIYVK NNRPSGIPDR 60 FSGSKSHNTA YLTITGAQAE DEADYFCNSR SGSQRVFGG TKLTVLG 107</p>

3-15	Sequences		
3-15-1	Sequence Number [ID]	15	
3-15-2	Molecule Type	AA	
3-15-3	Length	111	
3-15-4	Features	REGION 1..111	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..111 mol_type=protein organism=synthetic construct	
3-15-5	NonEnglishQualifier Value Residues	QSVLTQPPSA SGTGPGQRVTI SCSGSRSNIG SNTVNWYQQL PGTAPKLLIY TNNQRPSGVP 60 DRFSGSKSGT SASLAITGLQ AEDEADYYCL SFDSSLTSYV FGTGTKVTVL G 111	
3-16	Sequences		
3-16-1	Sequence Number [ID]	16	
3-16-2	Molecule Type	AA	
3-16-3	Length	112	
3-16-4	Features	REGION 1..112	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..112 mol_type=protein organism=synthetic construct	
3-16-5	NonEnglishQualifier Value Residues	QSVLTQPPSA SGTGPGQRVTI SCSGSRSNIG GNTVNWYQQL PGTAPKLLIY ANNQRPSGVP 60 DRFSGSKSGT SASLAISGLR SEDEADYYCA AWDDNLSGHV VFGGGTKLTV LR 112	
3-17	Sequences		
3-17-1	Sequence Number [ID]	17	
3-17-2	Molecule Type	AA	
3-17-3	Length	5	
3-17-4	Features	REGION 1..5	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..5 mol_type=protein organism=synthetic construct	
3-17-5	NonEnglishQualifier Value Residues	SYGMH 5	
3-18	Sequences		
3-18-1	Sequence Number [ID]	18	
3-18-2	Molecule Type	AA	
3-18-3	Length	17	
3-18-4	Features	REGION 1..17	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..17 mol_type=protein organism=synthetic construct	
3-18-5	NonEnglishQualifier Value Residues	VISYDGSNKY YADSVKG 17	
3-19	Sequences		
3-19-1	Sequence Number [ID]	19	
3-19-2	Molecule Type	AA	
3-19-3	Length	12	
3-19-4	Features	REGION 1..12	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..12 mol_type=protein organism=synthetic construct	
3-19-5	NonEnglishQualifier Value Residues	DLVAAAGTAF DI 12	
3-20	Sequences		
3-20-1	Sequence Number [ID]	20	
3-20-2	Molecule Type	AA	
3-20-3	Length	14	
3-20-4	Features	REGION 1..14	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..14 mol_type=protein organism=synthetic construct	
3-20-5	NonEnglishQualifier Value Residues	TGTISDVGGH NFVS 14	

3-21	Sequences		
3-21-1	Sequence Number [ID]	21	
3-21-2	Molecule Type	AA	
3-21-3	Length	7	
3-21-4	Features	REGION 1..7	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..7 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-21-5	Residues	EVTKRPA	7
3-22	Sequences		
3-22-1	Sequence Number [ID]	22	
3-22-2	Molecule Type	AA	
3-22-3	Length	10	
3-22-4	Features	REGION 1..10	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..10 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-22-5	Residues	SSYGGSNDVI	10
3-23	Sequences		
3-23-1	Sequence Number [ID]	23	
3-23-2	Molecule Type	AA	
3-23-3	Length	7	
3-23-4	Features	REGION 1..7	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..7 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-23-5	Residues	SNFVAWN	7
3-24	Sequences		
3-24-1	Sequence Number [ID]	24	
3-24-2	Molecule Type	AA	
3-24-3	Length	18	
3-24-4	Features	REGION 1..18	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..18 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-24-5	Residues	RTYYRSRWYN DYAVSVQS	18
3-25	Sequences		
3-25-1	Sequence Number [ID]	25	
3-25-2	Molecule Type	AA	
3-25-3	Length	8	
3-25-4	Features	REGION 1..8	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-25-5	Residues	GQHSGFDF	8
3-26	Sequences		
3-26-1	Sequence Number [ID]	26	
3-26-2	Molecule Type	AA	
3-26-3	Length	13	
3-26-4	Features	REGION 1..13	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..13 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-26-5	Residues	TGNSNNVGNQ GAA	13
3-27	Sequences		
3-27-1	Sequence Number [ID]	27	

3-27-2	Molecule Type	AA	
3-27-3	Length	7	
3-27-4	Features	REGION 1..7	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..7	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-27-5	Residues	RNNNRPS	7
3-28	Sequences		
3-28-1	Sequence Number [ID]	28	
3-28-2	Molecule Type	AA	
3-28-3	Length	11	
3-28-4	Features	REGION 1..11	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..11	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-28-5	Residues	SAWDNRLKTY V	11
3-29	Sequences		
3-29-1	Sequence Number [ID]	29	
3-29-2	Molecule Type	AA	
3-29-3	Length	5	
3-29-4	Features	REGION 1..5	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..5	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-29-5	Residues	SYGIS	5
3-30	Sequences		
3-30-1	Sequence Number [ID]	30	
3-30-2	Molecule Type	AA	
3-30-3	Length	17	
3-30-4	Features	REGION 1..17	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..17	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-30-5	Residues	WISAYNGNTN YAQKLQG	17
3-31	Sequences		
3-31-1	Sequence Number [ID]	31	
3-31-2	Molecule Type	AA	
3-31-3	Length	17	
3-31-4	Features	REGION 1..17	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..17	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-31-5	Residues	DTPGIAARRY YYYGMDV	17
3-32	Sequences		
3-32-1	Sequence Number [ID]	32	
3-32-2	Molecule Type	AA	
3-32-3	Length	11	
3-32-4	Features	REGION 1..11	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..11	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-32-5	Residues	QGDSLRLKFFA S	11
3-33	Sequences		
3-33-1	Sequence Number [ID]	33	
3-33-2	Molecule Type	AA	
3-33-3	Length	7	

3-33-4	Features Location/Qualifiers	REGION 1..7 note=Description of Artificial Sequence: Synthetic peptide source 1..7 mol_type=protein organism=synthetic construct	
3-33-5	NonEnglishQualifier Value Residues	GKNSRPS	7
3-34	Sequences		
3-34-1	Sequence Number [ID]	34	
3-34-2	Molecule Type	AA	
3-34-3	Length	11	
3-34-4	Features Location/Qualifiers	REGION 1..11 note=Description of Artificial Sequence: Synthetic peptide source 1..11 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-34-5	Residues	NSRDSRDNHQ V	11
3-35	Sequences		
3-35-1	Sequence Number [ID]	35	
3-35-2	Molecule Type	AA	
3-35-3	Length	5	
3-35-4	Features Location/Qualifiers	REGION 1..5 note=Description of Artificial Sequence: Synthetic peptide source 1..5 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-35-5	Residues	SYPMH	5
3-36	Sequences		
3-36-1	Sequence Number [ID]	36	
3-36-2	Molecule Type	AA	
3-36-3	Length	17	
3-36-4	Features Location/Qualifiers	REGION 1..17 note=Description of Artificial Sequence: Synthetic peptide source 1..17 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-36-5	Residues	VISSDGRNKY YPDSVKG	17
3-37	Sequences		
3-37-1	Sequence Number [ID]	37	
3-37-2	Molecule Type	AA	
3-37-3	Length	12	
3-37-4	Features Location/Qualifiers	REGION 1..12 note=Description of Artificial Sequence: Synthetic peptide source 1..12 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-37-5	Residues	GGYHDFWSGP DY	12
3-38	Sequences		
3-38-1	Sequence Number [ID]	38	
3-38-2	Molecule Type	AA	
3-38-3	Length	11	
3-38-4	Features Location/Qualifiers	REGION 1..11 note=Description of Artificial Sequence: Synthetic peptide source 1..11 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-38-5	Residues	RASQSVNTNL A	11
3-39	Sequences		
3-39-1	Sequence Number [ID]	39	
3-39-2	Molecule Type	AA	
3-39-3	Length	7	
3-39-4	Features Location/Qualifiers	REGION 1..7 note=Description of Artificial Sequence: Synthetic peptide	

3-39-5	NonEnglishQualifier Value Residues	source 1..7 mol_type=protein organism=synthetic construct GASSRAT	7
3-40 3-40-1 3-40-2 3-40-3 3-40-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	40 AA 9 REGION 1..9 note=Description of Artificial Sequence: Synthetic peptide source 1..9 mol_type=protein organism=synthetic construct	
3-40-5	NonEnglishQualifier Value Residues	QHYGSSPLT	9
3-41 3-41-1 3-41-2 3-41-3 3-41-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	41 AA 5 REGION 1..5 note=Description of Artificial Sequence: Synthetic peptide source 1..5 mol_type=protein organism=synthetic construct	
3-41-5	NonEnglishQualifier Value Residues	SYAMS	5
3-42 3-42-1 3-42-2 3-42-3 3-42-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	42 AA 17 REGION 1..17 note=Description of Artificial Sequence: Synthetic peptide source 1..17 mol_type=protein organism=synthetic construct	
3-42-5	NonEnglishQualifier Value Residues	NIKQDGSEKY YVDSVKG	17
3-43 3-43-1 3-43-2 3-43-3 3-43-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	43 AA 18 REGION 1..18 note=Description of Artificial Sequence: Synthetic peptide source 1..18 mol_type=protein organism=synthetic construct	
3-43-5	NonEnglishQualifier Value Residues	DQVSGITIFG GKWRSPDV	18
3-44 3-44-1 3-44-2 3-44-3 3-44-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	44 AA 11 REGION 1..11 note=Description of Artificial Sequence: Synthetic peptide source 1..11 mol_type=protein organism=synthetic construct	
3-44-5	NonEnglishQualifier Value Residues	QGDSLRSYYA S	11
3-45 3-45-1 3-45-2 3-45-3 3-45-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	45 AA 7 REGION 1..7 note=Description of Artificial Sequence: Synthetic peptide source 1..7 mol_type=protein	

3-45-5	NonEnglishQualifier Value Residues	organism=synthetic construct GKNNRPS	7
3-46	Sequences		
3-46-1	Sequence Number [ID]	46	
3-46-2	Molecule Type	AA	
3-46-3	Length	9	
3-46-4	Features Location/Qualifiers	REGION 1..9 note=Description of Artificial Sequence: Synthetic peptide source 1..9 mol_type=protein organism=synthetic construct	
3-46-5	NonEnglishQualifier Value Residues	NSRSGSQRV	9
3-47	Sequences		
3-47-1	Sequence Number [ID]	47	
3-47-2	Molecule Type	AA	
3-47-3	Length	5	
3-47-4	Features Location/Qualifiers	REGION 1..5 note=Description of Artificial Sequence: Synthetic peptide source 1..5 mol_type=protein organism=synthetic construct	
3-47-5	NonEnglishQualifier Value Residues	NYGLH	5
3-48	Sequences		
3-48-1	Sequence Number [ID]	48	
3-48-2	Molecule Type	AA	
3-48-3	Length	17	
3-48-4	Features Location/Qualifiers	REGION 1..17 note=Description of Artificial Sequence: Synthetic peptide source 1..17 mol_type=protein organism=synthetic construct	
3-48-5	NonEnglishQualifier Value Residues	VISHDGTKKY YADSVKG	17
3-49	Sequences		
3-49-1	Sequence Number [ID]	49	
3-49-2	Molecule Type	AA	
3-49-3	Length	17	
3-49-4	Features Location/Qualifiers	REGION 1..17 note=Description of Artificial Sequence: Synthetic peptide source 1..17 mol_type=protein organism=synthetic construct	
3-49-5	NonEnglishQualifier Value Residues	DGGYCSGGRC YSYGMDV	17
3-50	Sequences		
3-50-1	Sequence Number [ID]	50	
3-50-2	Molecule Type	AA	
3-50-3	Length	13	
3-50-4	Features Location/Qualifiers	REGION 1..13 note=Description of Artificial Sequence: Synthetic peptide source 1..13 mol_type=protein organism=synthetic construct	
3-50-5	NonEnglishQualifier Value Residues	SGSRSNIGSN TVN	13
3-51	Sequences		
3-51-1	Sequence Number [ID]	51	
3-51-2	Molecule Type	AA	
3-51-3	Length	7	
3-51-4	Features Location/Qualifiers	REGION 1..7 note=Description of Artificial Sequence: Synthetic peptide source 1..7 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		

3-51-5	Residues	TNNQRPS	7
3-52	Sequences		
3-52-1	Sequence Number [ID]	52	
3-52-2	Molecule Type	AA	
3-52-3	Length	11	
3-52-4	Features	REGION 1..11	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..11 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-52-5	Residues	LSFDSSLTSY V	11
3-53	Sequences		
3-53-1	Sequence Number [ID]	53	
3-53-2	Molecule Type	AA	
3-53-3	Length	5	
3-53-4	Features	REGION 1..5	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..5 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-53-5	Residues	RYGMH	5
3-54	Sequences		
3-54-1	Sequence Number [ID]	54	
3-54-2	Molecule Type	AA	
3-54-3	Length	17	
3-54-4	Features	REGION 1..17	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..17 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-54-5	Residues	LISYDGSKTF YGESVKG	17
3-55	Sequences		
3-55-1	Sequence Number [ID]	55	
3-55-2	Molecule Type	AA	
3-55-3	Length	13	
3-55-4	Features	REGION 1..13	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..13 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-55-5	Residues	ATVTTDGYYY MDV	13
3-56	Sequences		
3-56-1	Sequence Number [ID]	56	
3-56-2	Molecule Type	AA	
3-56-3	Length	13	
3-56-4	Features	REGION 1..13	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..13 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-56-5	Residues	SGSRSNIGGN TVN	13
3-57	Sequences		
3-57-1	Sequence Number [ID]	57	
3-57-2	Molecule Type	AA	
3-57-3	Length	7	
3-57-4	Features	REGION 1..7	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..7 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-57-5	Residues	ANNQRPS	7
3-58	Sequences		

3-58-1	Sequence Number [ID]	58	
3-58-2	Molecule Type	AA	
3-58-3	Length	12	
3-58-4	Features	REGION 1..12	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..12	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-58-5	Residues	AAWDDNLSGH VV	12
3-59	Sequences		
3-59-1	Sequence Number [ID]	59	
3-59-2	Molecule Type	AA	
3-59-3	Length	8	
3-59-4	Features	REGION 1..8	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..8	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-59-5	Residues	GFTVSSNY	8
3-60	Sequences		
3-60-1	Sequence Number [ID]	60	
3-60-2	Molecule Type	AA	
3-60-3	Length	7	
3-60-4	Features	REGION 1..7	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..7	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-60-5	Residues	IYSGGST	7
3-61	Sequences		
3-61-1	Sequence Number [ID]	61	
3-61-2	Molecule Type	AA	
3-61-3	Length	11	
3-61-4	Features	REGION 1..11	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..11	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-61-5	Residues	ARDNPLSAFD I	11
3-62	Sequences		
3-62-1	Sequence Number [ID]	62	
3-62-2	Molecule Type	AA	
3-62-3	Length	6	
3-62-4	Features	REGION 1..6	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..6	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-62-5	Residues	QSINSW	6
3-63	Sequences		
3-63-1	Sequence Number [ID]	63	
3-63-2	Molecule Type		
3-63-3	Length		
3-63-4	Features		
	Location/Qualifiers		
	NonEnglishQualifier Value		
3-63-5	Residues	000	3
3-64	Sequences		
3-64-1	Sequence Number [ID]	64	
3-64-2	Molecule Type	AA	
3-64-3	Length	9	
3-64-4	Features	REGION 1..9	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	

3-64-5	NonEnglishQualifier Value Residues	source 1..9 mol_type=protein organism=synthetic construct QQYDDLPLT	9
3-65 3-65-1 3-65-2 3-65-3 3-65-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	65 AA 8 REGION 1..8 note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
3-65-5	NonEnglishQualifier Value Residues	GYTFTSYG	8
3-66 3-66-1 3-66-2 3-66-3 3-66-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	66 AA 8 REGION 1..8 note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
3-66-5	NonEnglishQualifier Value Residues	ISAYNGNT	8
3-67 3-67-1 3-67-2 3-67-3 3-67-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	67 AA 18 REGION 1..18 note=Description of Artificial Sequence: Synthetic peptide source 1..18 mol_type=protein organism=synthetic construct	
3-67-5	NonEnglishQualifier Value Residues	ARDPGLWFGL THDYFDY	18
3-68 3-68-1 3-68-2 3-68-3 3-68-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	68 AA 8 REGION 1..8 note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
3-68-5	NonEnglishQualifier Value Residues	SSNIGSNT	8
3-69 3-69-1 3-69-2 3-69-3 3-69-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	69	
3-69-5	NonEnglishQualifier Value Residues	000	3
3-70 3-70-1 3-70-2 3-70-3 3-70-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	70 AA 11 REGION 1..11 note=Description of Artificial Sequence: Synthetic peptide source 1..11 mol_type=protein organism=synthetic construct	
3-70-5	NonEnglishQualifier Value Residues	AAWDDSRSGP V	11

3-71	Sequences		
3-71-1	Sequence Number [ID]	71	
3-71-2	Molecule Type	AA	
3-71-3	Length	8	
3-71-4	Features	REGION 1..8	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..8	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-71-5	Residues	GFTFSDYS	8
3-72	Sequences		
3-72-1	Sequence Number [ID]	72	
3-72-2	Molecule Type	AA	
3-72-3	Length	8	
3-72-4	Features	REGION 1..8	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..8	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-72-5	Residues	INSDGSRT	8
3-73	Sequences		
3-73-1	Sequence Number [ID]	73	
3-73-2	Molecule Type	AA	
3-73-3	Length	11	
3-73-4	Features	REGION 1..11	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..11	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-73-5	Residues	ARGPGFFGFD I	11
3-74	Sequences		
3-74-1	Sequence Number [ID]	74	
3-74-2	Molecule Type	AA	
3-74-3	Length	8	
3-74-4	Features	REGION 1..8	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..8	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-74-5	Residues	RSNIGRNS	8
3-75	Sequences		
3-75-1	Sequence Number [ID]	75	
3-75-2	Molecule Type		
3-75-3	Length		
3-75-4	Features		
	Location/Qualifiers		
	NonEnglishQualifier Value		
3-75-5	Residues	000	3
3-76	Sequences		
3-76-1	Sequence Number [ID]	76	
3-76-2	Molecule Type	AA	
3-76-3	Length	11	
3-76-4	Features	REGION 1..11	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide	
		source 1..11	
		mol_type=protein	
		organism=synthetic construct	
	NonEnglishQualifier Value		
3-76-5	Residues	AAWDARLTGP L	11
3-77	Sequences		
3-77-1	Sequence Number [ID]	77	
3-77-2	Molecule Type	AA	
3-77-3	Length	8	
3-77-4	Features	REGION 1..8	

	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
3-77-5	NonEnglishQualifier Value Residues	GYSFTNYW	8
3-78	Sequences		
3-78-1	Sequence Number [ID]	78	
3-78-2	Molecule Type	AA	
3-78-3	Length	8	
3-78-4	Features	REGION 1..8	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
3-78-5	NonEnglishQualifier Value Residues	INPVNSRT	8
3-79	Sequences		
3-79-1	Sequence Number [ID]	79	
3-79-2	Molecule Type	AA	
3-79-3	Length	10	
3-79-4	Features	REGION 1..10	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..10 mol_type=protein organism=synthetic construct	
3-79-5	NonEnglishQualifier Value Residues	ARYYYYAMEV	10
3-80	Sequences		
3-80-1	Sequence Number [ID]	80	
3-80-2	Molecule Type	AA	
3-80-3	Length	8	
3-80-4	Features	REGION 1..8	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
3-80-5	NonEnglishQualifier Value Residues	SSNIGSNT	8
3-81	Sequences		
3-81-1	Sequence Number [ID]	81	
3-81-2	Molecule Type		
3-81-3	Length		
3-81-4	Features		
	Location/Qualifiers		
3-81-5	NonEnglishQualifier Value Residues	000	3
3-82	Sequences		
3-82-1	Sequence Number [ID]	82	
3-82-2	Molecule Type	AA	
3-82-3	Length	11	
3-82-4	Features	REGION 1..11	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..11 mol_type=protein organism=synthetic construct	
3-82-5	NonEnglishQualifier Value Residues	EAWDDSLNGP V	11
3-83	Sequences		
3-83-1	Sequence Number [ID]	83	
3-83-2	Molecule Type	AA	
3-83-3	Length	8	
3-83-4	Features	REGION 1..8	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		

3-83-5	Residues	GYTFTNYG	8
3-84	Sequences		
3-84-1	Sequence Number [ID]	84	
3-84-2	Molecule Type	AA	
3-84-3	Length	8	
3-84-4	Features	REGION 1..8	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-84-5	Residues	VDNNGNI	8
3-85	Sequences		
3-85-1	Sequence Number [ID]	85	
3-85-2	Molecule Type	AA	
3-85-3	Length	15	
3-85-4	Features	REGION 1..15	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..15 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-85-5	Residues	ARGLFSSRWY LWFDP	15
3-86	Sequences		
3-86-1	Sequence Number [ID]	86	
3-86-2	Molecule Type	AA	
3-86-3	Length	9	
3-86-4	Features	REGION 1..9	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..9 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-86-5	Residues	SSDVGGYNY	9
3-87	Sequences		
3-87-1	Sequence Number [ID]	87	
3-87-2	Molecule Type		
3-87-3	Length		
3-87-4	Features		
	Location/Qualifiers		
	NonEnglishQualifier Value		
3-87-5	Residues	000	3
3-88	Sequences		
3-88-1	Sequence Number [ID]	88	
3-88-2	Molecule Type	AA	
3-88-3	Length	12	
3-88-4	Features	REGION 1..12	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..12 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-88-5	Residues	SSYTRSSTSY VV	12
3-89	Sequences		
3-89-1	Sequence Number [ID]	89	
3-89-2	Molecule Type	AA	
3-89-3	Length	8	
3-89-4	Features	REGION 1..8	
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
	NonEnglishQualifier Value		
3-89-5	Residues	GGTFSSYA	8
3-90	Sequences		
3-90-1	Sequence Number [ID]	90	
3-90-2	Molecule Type	AA	
3-90-3	Length	8	

3-90-4	Features Location/Qualifiers	REGION 1..8 note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
3-90-5	NonEnglishQualifier Value Residues	ILPMFGST	8
3-91	Sequences		
3-91-1	Sequence Number [ID]	91	
3-91-2	Molecule Type	AA	
3-91-3	Length	16	
3-91-4	Features Location/Qualifiers	REGION 1..16 note=Description of Artificial Sequence: Synthetic peptide source 1..16 mol_type=protein organism=synthetic construct	
3-91-5	NonEnglishQualifier Value Residues	ARGRDIVAPS NSGFV	16
3-92	Sequences		
3-92-1	Sequence Number [ID]	92	
3-92-2	Molecule Type	AA	
3-92-3	Length	8	
3-92-4	Features Location/Qualifiers	REGION 1..8 note=Description of Artificial Sequence: Synthetic peptide source 1..8 mol_type=protein organism=synthetic construct	
3-92-5	NonEnglishQualifier Value Residues	SNNVGNQG	8
3-93	Sequences		
3-93-1	Sequence Number [ID]	93	
3-93-2	Molecule Type		
3-93-3	Length		
3-93-4	Features Location/Qualifiers		
3-93-5	NonEnglishQualifier Value Residues	000	3
3-94	Sequences		
3-94-1	Sequence Number [ID]	94	
3-94-2	Molecule Type	AA	
3-94-3	Length	11	
3-94-4	Features Location/Qualifiers	REGION 1..11 note=Description of Artificial Sequence: Synthetic peptide source 1..11 mol_type=protein organism=synthetic construct	
3-94-5	NonEnglishQualifier Value Residues	SAYDRSLNAW V	11
3-95	Sequences		
3-95-1	Sequence Number [ID]	95	
3-95-2	Molecule Type	AA	
3-95-3	Length	328	
3-95-4	Features Location/Qualifiers	REGION 315..316 note=Non-consecutive residues source 1..328 mol_type=protein organism=Homo sapiens	
3-95-5	NonEnglishQualifier Value Residues	MEGISIYTS D NYTEEMGSGD YDSMKEPCFR EENANFNKIF LPTIYSIIFL TGIVGNGLVI 60 LVMGYQKCLR SMTDKYRLHL SVADLLFVIT LPFWAVDAVA NWFYFGNFLCK AVHVIYTVNL 120 YSSVLILAFI SLDRYLAI VH ATNSQRPRKL LAEKVVYVGV WIPALLLTIP DFIFANVSEA 180 DDRYICDRFY PNDLWVVVFQ FQHIMVGLIL PGIVILSCYC IIISKLSHSK GHQKRKALKT 240 TVILILAFFA CWLPYYIGIS IDSFILLEII KQGCEFENTV HKWISITEAL AFFHCCLNPI 300 LYAFLGAKFK TSAQHVSTES ESSSFHSS 328	
3-96	Sequences		
3-96-1	Sequence Number [ID]	96	
3-96-2	Molecule Type	AA	
3-96-3	Length	117	
3-96-4	Features	REGION 1..117	

3-96-5	Location/Qualifiers NonEnglishQualifier Value Residues	note=Description of Artificial Sequence: Synthetic polypeptide source 1..117 mol_type=protein organism=synthetic construct EVQLVQSGGG LIQPGGSLRL SCAASGFTVS SNYMSWVRQA PGKGLEWVSV IYSGGSTYYA 60 DSVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARDNP LSAFDIWGQG TLVTVSL 117
3-97 3-97-1 3-97-2 3-97-3 3-97-4 3-97-5	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers NonEnglishQualifier Value Residues	97 AA 107 REGION 1..107 note=Description of Artificial Sequence: Synthetic polypeptide source 1..107 mol_type=protein organism=synthetic construct DIVMTQTPST LSASVGDRVT ITCRASQSIN SWLAWYQQKS GKAPKLLIYK ASSLGSVPS 60 RFSGSGSDIE FTLTISSLQP DDFATYYCQQ YDDLPLTFGG GTKVEIK 107
3-98 3-98-1 3-98-2 3-98-3 3-98-4 3-98-5	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers NonEnglishQualifier Value Residues	98 AA 97 source 1..97 mol_type=protein organism=Homo sapiens EVQLVESGGG LIQPGGSLRL SCAASGFTVS SNYMSWVRQA PGKGLEWVSV IYSGGSTYYA 60 DSVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCAR 97
3-99 3-99-1 3-99-2 3-99-3 3-99-4 3-99-5	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers NonEnglishQualifier Value Residues	99 AA 117 REGION 1..117 note=Description of Artificial Sequence: Synthetic polypeptide source 1..117 mol_type=protein organism=synthetic construct EVQLVQSGGG LIQPGGSLRL SCAASGFTVS SNYMSWVRQA PGKGLEWVSV IYSGGSTYYA 60 DSVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCARDNP LSAFDIWGQG TLVTVSL 117
3-100 3-100-1 3-100-2 3-100-3 3-100-4 3-100-5	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers NonEnglishQualifier Value Residues	100 AA 95 source 1..95 mol_type=protein organism=Homo sapiens DIQMTQSPST LSASVGDRVT ITCRASQSI SWLAWYQQPK GKAPKLLIYK ASSLEGSVPS 60 RFSGSGSGTE FTLTISSLQP DDFATYYCQQ YNSYS 95
3-101 3-101-1 3-101-2 3-101-3 3-101-4 3-101-5	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers NonEnglishQualifier Value Residues	101 AA 107 REGION 1..107 note=Description of Artificial Sequence: Synthetic polypeptide source 1..107 mol_type=protein organism=synthetic construct DIVMTQTPST LSASVGDRVT ITCRASQSIN SWLAWYQQKS GKAPKLLIYK ASSLGSVPS 60 RFSGSGSDIE FTLTISSLQP DDFATYYCQQ YDDLPLTFGG GTKVEIK 107
3-102 3-102-1 3-102-2 3-102-3 3-102-4	Sequences Sequence Number [ID] Molecule Type Length Features Location/Qualifiers	102 AA 98 source 1..98 mol_type=protein

3-102-5	NonEnglishQualifier Value Residues	organism=Homo sapiens QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYGISWVRQA PGQGLEWMGW ISAYNGNTNY 60 AQLQGRVTM TTDSTSTAY MELRSLRSD TAVYYCAR 98
3-103	Sequences	
3-103-1	Sequence Number [ID]	103
3-103-2	Molecule Type	AA
3-103-3	Length	125
3-103-4	Features Location/Qualifiers	REGION 1..125 note=Description of Artificial Sequence: Synthetic polypeptide source 1..125 mol_type=protein organism=synthetic construct
3-103-5	NonEnglishQualifier Value Residues	QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYGISWVRQA PGQGLEWMGW ISAYNGNTNY 60 AQLQGRVTM TTDSTSTAY MELRSLRSD TAVYYCARDP GLWFGFLTHDY YFDYWGQGTL 120 VTVSS 125
3-104	Sequences	
3-104-1	Sequence Number [ID]	104
3-104-2	Molecule Type	AA
3-104-3	Length	98
3-104-4	Features Location/Qualifiers	source 1..98 mol_type=protein organism=Homo sapiens
3-104-5	NonEnglishQualifier Value Residues	QSVLTQPPSA SGTPGQRVTI SCSGSSSNIG SNTVNWYQQL PGTAPKLLIY SNNQRPSGVP 60 DRFSGSKSGT SASLAISGLQ SEDEADYYCA AWDDSLNG 98
3-105	Sequences	
3-105-1	Sequence Number [ID]	105
3-105-2	Molecule Type	AA
3-105-3	Length	110
3-105-4	Features Location/Qualifiers	REGION 1..110 note=Description of Artificial Sequence: Synthetic polypeptide source 1..110 mol_type=protein organism=synthetic construct
3-105-5	NonEnglishQualifier Value Residues	SYELTQPPSA SGTPGQRVTI SCSGSSSNIG SNTVSWYQQL PGTAPKLLIY RNNQRPSGVP 60 DRFSGSKSGT SASLAINGLQ SEDEADYYCA AWDDSRSGPV FGGGTRLTVL 110
3-106	Sequences	
3-106-1	Sequence Number [ID]	106
3-106-2	Molecule Type	AA
3-106-3	Length	98
3-106-4	Features Location/Qualifiers	source 1..98 mol_type=protein organism=Homo sapiens
3-106-5	NonEnglishQualifier Value Residues	EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYWMHWVRQA PGKGLVWVSR INSDGSSTSY 60 ADSVKGRFTI SRDPAKNTLY LQMNSLRAED TAVYYCAR 98
3-107	Sequences	
3-107-1	Sequence Number [ID]	107
3-107-2	Molecule Type	AA
3-107-3	Length	118
3-107-4	Features Location/Qualifiers	REGION 1..118 note=Description of Artificial Sequence: Synthetic polypeptide source 1..118 mol_type=protein organism=synthetic construct
3-107-5	NonEnglishQualifier Value Residues	EVQLVQSGGG LVKPGGSLRL SCEASGFTFS DYSMSWIRQA PGKGLEWVSR INSDGSRNTY 60 ADSVKGRFTI SRDPAKNTLY LQMNSLRAED TAMYYCARGP GFFGFDIWGQ GTLVTVSS 118
3-108	Sequences	
3-108-1	Sequence Number [ID]	108
3-108-2	Molecule Type	AA
3-108-3	Length	98
3-108-4	Features Location/Qualifiers	source 1..98 mol_type=protein organism=Homo sapiens
	NonEnglishQualifier Value	

3-108-5	Residues	QSVLTQPPSA SGTGQQRVTI SCSGSSSNIG SNTVNWYQQL PGTAPKLLIY SNNQRPSGVP 60 DRFSGSKSGT SASLAISGLQ SEDEADYYCA AWDDSLNG 98
3-109	Sequences	
3-109-1	Sequence Number [ID]	109
3-109-2	Molecule Type	AA
3-109-3	Length	110
3-109-4	Features	REGION 1..110
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..110 mol_type=protein organism=synthetic construct
3-109-5	Residues	SYELTQPPSA SGTGQQRVTI SCSGSRSNIG RNSVNWYQQL PGTAPKLLIY SNNQRPSGVP 60 GRFSGSRSGT SASLAISGLQ SEDETDYYCA AWDARLTGPL FGGGTKLSVL 110
3-110	Sequences	
3-110-1	Sequence Number [ID]	110
3-110-2	Molecule Type	AA
3-110-3	Length	98
3-110-4	Features	source 1..98
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
3-110-5	Residues	EVQLVQSGAE VKKPGESLKI SCKGSGYSFT SYWIGWVRQM PGKGLEWNGI IYPGDSDFRY 60 SPSFQGGVTI SADKSISTAY LQWSSLKASD TAMYYCAR 98
3-111	Sequences	
3-111-1	Sequence Number [ID]	111
3-111-2	Molecule Type	AA
3-111-3	Length	117
3-111-4	Features	REGION 1..117
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..117 mol_type=protein organism=synthetic construct
3-111-5	Residues	EVQLVQSGAE VKKPGESLKI SCKGSGYSFT NYWIGWVRQM PGKGLEWNGI INPVNSRTIY 60 SPSFQGGVTI SVDKSVTTAY LQWSSLKASD TAMYYCARY YAMEVWGRG TLVTVSS 117
3-112	Sequences	
3-112-1	Sequence Number [ID]	112
3-112-2	Molecule Type	AA
3-112-3	Length	98
3-112-4	Features	source 1..98
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
3-112-5	Residues	QSVLTQPPSA SGTGQQRVTI SCSGSSSNIG SNTVNWYQQL PGTAPKLLIY SNNQRPSGVP 60 DRFSGSKSGT SASLAISGLQ SEDEADYYCA AWDDSLNG 98
3-113	Sequences	
3-113-1	Sequence Number [ID]	113
3-113-2	Molecule Type	AA
3-113-3	Length	110
3-113-4	Features	REGION 1..110
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..110 mol_type=protein organism=synthetic construct
3-113-5	Residues	LPVLTQPPSA SGTGQQRVTI SCSGSSSNIG SNTVNWYQQL PGTAPKLLIY RNNQRPSGVP 60 DRFSGSTSGT SASLAISGLQ SEDEADYYCE AWDDSLNGPV FGGGTKLTVL 110
3-114	Sequences	
3-114-1	Sequence Number [ID]	114
3-114-2	Molecule Type	AA
3-114-3	Length	98
3-114-4	Features	source 1..98
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
3-114-5	Residues	QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYGISWVRQA PGQGLEWNGW ISAYNGNTNY 60 AQLKQGRVTM TTDSTSTAY MELRSLRSDD TAVYYCAR 98
3-115	Sequences	

3-115-1	Sequence Number [ID]	115
3-115-2	Molecule Type	AA
3-115-3	Length	122
3-115-4	Features	REGION 1..122
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..122 mol_type=protein organism=synthetic construct
3-115-5	NonEnglishQualifier Value Residues	EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGISWVRQA PGGGLEWMGW VDNNGNININ 60 AQKFLGRVTM TTDSTSTAY MELRSLRSD TAVYYCARGL FSSRWYLWFD PWGQGLTIVT 120 SS 122
3-116	Sequences	
3-116-1	Sequence Number [ID]	116
3-116-2	Molecule Type	AA
3-116-3	Length	99
3-116-4	Features	source 1..99
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
3-116-5	NonEnglishQualifier Value Residues	QSALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLM I YEVSNRPSGV 60 SNRFGSKSG NTASLTISGL QAEDEADYYC SSYTSSSTL 99
3-117	Sequences	
3-117-1	Sequence Number [ID]	117
3-117-2	Molecule Type	AA
3-117-3	Length	112
3-117-4	Features	REGION 1..112
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..112 mol_type=protein organism=synthetic construct
3-117-5	NonEnglishQualifier Value Residues	QSALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLM I YEVTERPSGV 60 SNRFGSKSG NTASLTISGL QAEDEGDYYC SSYTRSTSY VVFGGGTKVT VL 112
3-118	Sequences	
3-118-1	Sequence Number [ID]	118
3-118-2	Molecule Type	AA
3-118-3	Length	98
3-118-4	Features	source 1..98
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
3-118-5	NonEnglishQualifier Value Residues	QVQLVQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG IIPFIFTANY 60 AQKFQGRVTI TADESTSTAY MELSSLRSED TAVYYCAR 98
3-119	Sequences	
3-119-1	Sequence Number [ID]	119
3-119-2	Molecule Type	AA
3-119-3	Length	123
3-119-4	Features	REGION 1..123
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..123 mol_type=protein organism=synthetic construct
3-119-5	NonEnglishQualifier Value Residues	QVQLQQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVRQA PGQGLEWMGG ILPMFGSTNY 60 AQKFQGRVTL IADESTRVY LELNSLTSED TAVYYCARGR DIVAPNSNGF DVWGQGTIVT 120 VSS 123
3-120	Sequences	
3-120-1	Sequence Number [ID]	120
3-120-2	Molecule Type	AA
3-120-3	Length	98
3-120-4	Features	source 1..98
	Location/Qualifiers	mol_type=protein organism=Homo sapiens
3-120-5	NonEnglishQualifier Value Residues	QAGLTQPPSV SKGLRQTATL TCTGNSNNVG NQGAAWLQQH QGHPPKLLSY RNNNRPSGIS 60 ERLSASRSGN TASLTITGLQ PEDEADYYCS AWDSSLSA 98
3-121	Sequences	
3-121-1	Sequence Number [ID]	121

3-121-2	Molecule Type	AA
3-121-3	Length	110
3-121-4	Features	REGION 1..110
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..110 mol_type=protein organism=synthetic construct
3-121-5	NonEnglishQualifier Value Residues	SYELTQPPSV SKGLRQTATL TCTGNSNNVG NQGAAWLQQH QGHPPKLLSY RNDNRPSGIS 60 ERFSASRSGN TASLTISGLQ PEDEADYYCS AYDRSLNAWV FGGGTKLTVL 110
3-122	Sequences	
3-122-1	Sequence Number [ID]	122
3-122-2	Molecule Type	AA
3-122-3	Length	125
3-122-4	Features	REGION 1..125
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..125 mol_type=protein organism=synthetic construct
3-122-5	NonEnglishQualifier Value Residues	QVQLVQSGAE VKKPGASVKV SCKASGYTFT SYGISWVRQA PGQGLEWMGW ISAYNGNTNY 60 AQKLGQRVTM TTDSTSTAY MELRSLRSDD TAVYYCARDP GLWFGLETHDY YFDYWGQGTL 120 VTVSS 125
3-123	Sequences	
3-123-1	Sequence Number [ID]	123
3-123-2	Molecule Type	AA
3-123-3	Length	118
3-123-4	Features	REGION 1..118
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..118 mol_type=protein organism=synthetic construct
3-123-5	NonEnglishQualifier Value Residues	EVQLVQSGGG LVKPGGSLRL SCEASGFTFS DYSMSWIRQA PGKGLEWVSR INSDGSRITNY 60 ADSVKGRFTI SRDPAKNTLY LQMNSLRRAED TAMYYCARGP GFFGFDIWGQ GTLVTVSS 118
3-124	Sequences	
3-124-1	Sequence Number [ID]	124
3-124-2	Molecule Type	AA
3-124-3	Length	117
3-124-4	Features	REGION 1..117
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..117 mol_type=protein organism=synthetic construct
3-124-5	NonEnglishQualifier Value Residues	EVQLVQSGAE VKKPGESLKI SCKGSGYSFT NYWIGWVRQM PGKGLEWMGI INPVNSRTIY 60 SPSPQGGVTI SVDKSVTTAY LQWSSLKASD TAMYYCARYY YYAMEVWGRG TLVTVSS 117
3-125	Sequences	
3-125-1	Sequence Number [ID]	125
3-125-2	Molecule Type	AA
3-125-3	Length	122
3-125-4	Features	REGION 1..122
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..122 mol_type=protein organism=synthetic construct
3-125-5	NonEnglishQualifier Value Residues	EVQLVQSGAE VKKPGASVKV SCKASGYTFT NYGISWVRQA PGGGLEWMGW VDNNGNINNY 60 AQKFLGRVTM TTDSTSTAY MELRSLRSDD TAVYYCARGL FSSRWYLFWD PWGQGTLVTV 120 SS 122
3-126	Sequences	
3-126-1	Sequence Number [ID]	126
3-126-2	Molecule Type	AA
3-126-3	Length	123
3-126-4	Features	REGION 1..123
	Location/Qualifiers	note=Description of Artificial Sequence: Synthetic polypeptide source 1..123 mol_type=protein organism=synthetic construct

3-126-5	NonEnglishQualifier Value Residues	QVQLQQSGAE VKKPGSSVKV SCKASGGTFS SYAISWVROA PGQGLEWMGG ILPMFGSTNY 60 AOKFQGRLLT IADESTRTVY LELNSLTSED TAVYYCARGR DIVAPNSNGF DVWGQGTTVT 120 VSS 123
3-127	Sequences	
3-127-1	Sequence Number [ID]	127
3-127-2	Molecule Type	AA
3-127-3	Length	110
3-127-4	Features Location/Qualifiers	REGION 1..110 note=Description of Artificial Sequence: Synthetic polypeptide source 1..110 mol_type=protein organism=synthetic construct
3-127-5	NonEnglishQualifier Value Residues	SYELTQPPSA SGTGQQRVTI SCSGSSSNIG SNTVSWYQQL PGTAPKLLIY RNNQRPSGVP 60 DRFSGSKSGT SASLAINGLQ SEDEADYYCA AWDDSRSGPV FGGGTRRLTVL 110
3-128	Sequences	
3-128-1	Sequence Number [ID]	128
3-128-2	Molecule Type	AA
3-128-3	Length	110
3-128-4	Features Location/Qualifiers	REGION 1..110 note=Description of Artificial Sequence: Synthetic polypeptide source 1..110 mol_type=protein organism=synthetic construct
3-128-5	NonEnglishQualifier Value Residues	SYELTQPPSA SGTGQQRVTI SCSGSRSNIG RNSVNWYQQL PGTAPKLLIY SNNQRPSGVP 60 GRFSGRSRGT SASLAISGLQ SEDETYYCA AWDARLTGPL FGGGTKLSVL 110
3-129	Sequences	
3-129-1	Sequence Number [ID]	129
3-129-2	Molecule Type	AA
3-129-3	Length	110
3-129-4	Features Location/Qualifiers	REGION 1..110 note=Description of Artificial Sequence: Synthetic polypeptide source 1..110 mol_type=protein organism=synthetic construct
3-129-5	NonEnglishQualifier Value Residues	LPVLTQPPSA SGTGQQRVTI SCSGSSSNIG SNTVNWYQQL PGTAPKLLIY RNNQRPSGVP 60 DRFSGSTSGT SASLAISGLQ SEDEADYYCE AWDDSLNGPV FGGGTKLTVL 110
3-130	Sequences	
3-130-1	Sequence Number [ID]	130
3-130-2	Molecule Type	AA
3-130-3	Length	112
3-130-4	Features Location/Qualifiers	REGION 1..112 note=Description of Artificial Sequence: Synthetic polypeptide source 1..112 mol_type=protein organism=synthetic construct
3-130-5	NonEnglishQualifier Value Residues	QSALTQPASV SGSPGQSITI SCTGTSSDVG GYNYVSWYQQ HPGKAPKLM IYEVTERPSGV 60 SNRFSGSKSG NTASLTISGL QAEDGEYYC SSTRSSTSY VVFGGGTKVT VL 112
3-131	Sequences	
3-131-1	Sequence Number [ID]	131
3-131-2	Molecule Type	AA
3-131-3	Length	110
3-131-4	Features Location/Qualifiers	REGION 1..110 note=Description of Artificial Sequence: Synthetic polypeptide source 1..110 mol_type=protein organism=synthetic construct
3-131-5	NonEnglishQualifier Value Residues	SYELTQPPSV SKGLRQTATL TCTGNSNNGV NQGAAWLQQH QGHPPKLLSY RNDNRPSGIS 60 ERFSASRSGN TASLTISGLQ PEDEADYYCS AYDRSLNAWV FGGGTKLTVL 110