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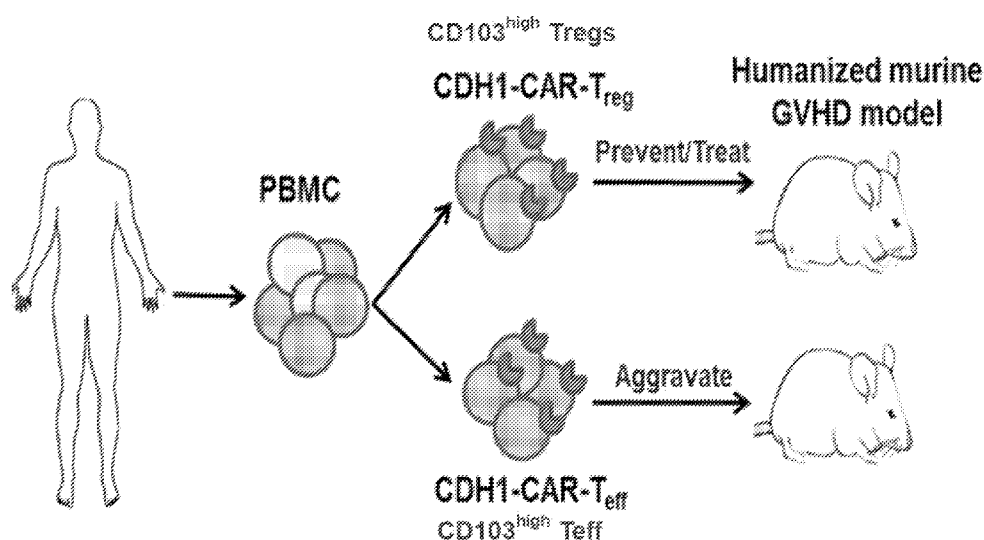


FIG. 1

(57) **Abstract:** This document provides methods and materials involved in treating a mammal (e.g., a human) having (or risk of developing) graft-versus-host disease (GVHD). For example, T cells (e.g., regulatory T cells) expressing one or more antigen receptors targeting one or more epithelial-specific antigens are provided. Also provided are methods for administering T-cells expressing one or more antigen receptors targeting one or more epithelial-specific antigens to a mammal having (or at risk of developing) GVHD to treat the GVHD.



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METHODS AND MATERIALS FOR TREATING GRAFT-VERSUS-HOST DISEASE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims benefit of priority from U.S. Provisional Application Serial No. 62/655,013, filed on April 9, 2018. The disclosure of the prior application is considered
5 part of (and is incorporated by reference in) the disclosure of this application.

BACKGROUND

1. Technical Field

This document relates to methods and materials involved in treating a mammal (e.g., a human) having or at risk of developing graft-versus-host disease (GVHD). For example,
10 one or more T cells (e.g., one or more regulatory T cells (Tregs)) expressing one or more antigen receptors targeting one or more epithelial-specific antigens can be administered to a mammal (e.g., a human) having GVHD (or at risk of developing GVHD) to treat the GVHD (or to reduce the risk of developing GVHD).

2. Background Information

15 Acute GVHD (aGVHD) is a major cause of morbidity and mortality after allogeneic hematopoietic cell transplantation (HCT) (Socie *et al.*, *Blood*, 114(20):4327-36 (2007); and Martin *et al.*, *Bone Marrow Transplant*, 21(8):1343-59 (2014)). There are currently no standard treatments beyond steroids; however, even with steroid treatment, mortality at 12 weeks for aGVHD patients is 63% (Yalniz *et al.*, *Biology of Blood and Marrow Transplant*,
20 23(9):1478-1484 (2017)).

SUMMARY

This document provides methods and materials involved in treating a mammal (e.g., a human) having or at risk of developing GVHD. For example, one or more T cells (e.g., one or more Tregs) expressing one or more antigen receptors targeting one or more epithelial-
25 specific antigens can be administered (e.g., by adoptive transfer) to a mammal having GVHD (or at risk of developing GVHD) to treat the GVHD (or to reduce the risk of developing GVHD). In some cases, a chimeric antigen receptor (CAR) targeting an epithelial-specific antigen (e.g., epithelial cadherin (E-cadherin, also referred to as CDH1)) can be expressed by

a Treg to target the Treg to epithelial tissues. In some cases, a cluster of differentiation 103 (CD103) polypeptide targeting an epithelial-specific antigen (e.g., CDH1) can be expressed on a Treg to target or redirect the Treg to epithelial tissues.

As demonstrated herein, donor-derived Tregs or autologous Tregs can be engineered
5 to target host epithelial tissues by engineering the Tregs to express CARs targeting CDH1 (e.g., CDH1-CAR). Also as discussed herein, donor-derived Tregs or autologous Tregs can be engineered to target host epithelial tissues by engineering the Tregs to express a CD103 polypeptide. Tregs directed to host epithelial tissues can release inhibitory cytokines and reduce inflammation caused by graft versus host disease. Thus, Tregs directed to epithelial
10 tissues can be incorporated into adoptive T cell therapies (e.g., CART cell therapies) to treat a mammal (e.g., a human) having GVHD (or at risk of developing GVHD) to treat the GVHD (or to reduce the risk of developing GVHD).

In general, one aspect of this document features a method for treating a mammal having GVHD. The method comprises (or consists essentially of or consists of)
15 administering to the mammal a composition comprising regulatory T cells (Tregs) comprising exogenous nucleic acid encoding an antigen receptor targeting an epithelial-specific antigen, wherein the Tregs express the antigen receptor. The mammal can be a human. The GVHD can be acute GVHD or chronic GVHD. The GVHD can be GVHD that occurred following allogeneic transplantation. The epithelial-specific antigen can be E-cadherin (CDH1). The antigen receptor can be a chimeric antigen receptor. The chimeric
20 antigen receptor can comprise a single chain variable fragment (scFv). The scFv can comprise a light chain and a heavy chain from an anti-CDH1 antibody. The anti-CDH1 antibody can be hSC10.17. The antigen receptor can comprise cluster of differentiation 103 (CD103). The Tregs, prior to the administration, can be engineered to express the antigen
25 receptor *ex vivo*. A symptom of the GVHD can be reduced at least 10 percent.

In another aspect, this document features a method for treating a mammal at risk of developing GVHD. The method comprises (or consists essentially of or consists of) administering to the mammal a composition comprising regulatory T cells (Tregs) comprising exogenous nucleic acid encoding an antigen receptor targeting an epithelial-
30 specific antigen, wherein the Tregs express the antigen receptor. The mammal can be a human. The GVHD can be acute GVHD or chronic GVHD. The mammal can be a mammal that received an allogeneic transplantation. The epithelial-specific antigen can be E-cadherin

(CDH1). The antigen receptor can be a chimeric antigen receptor. The chimeric antigen receptor can comprise a scFv. The scFv can comprise a light chain and a heavy chain from an anti-CDH1 antibody. The anti-CDH1 antibody can be hSC10.17. The antigen receptor can comprise CD103. The Tregs, prior to the administration, can be engineered to express the antigen receptor *ex vivo*.

In another aspect, this document features a nucleic acid construct encoding a chimeric antigen receptor targeting an epithelial-specific antigen. The epithelial-specific antigen can be E-cadherin (CDH1). The chimeric antigen receptor can comprise a scFv. The scFv can comprise a light chain and a heavy chain from an anti-CDH1 antibody. The anti-CDH1 antibody can be hSC10.17. A heavy chain of the hSC10.17 can be encoded by a nucleic acid sequence set forth in SEQ ID NO:2. A light chain of the hSC10.17 can be encoded by a nucleic acid sequence set forth in SEQ ID NO:4. The chimeric antigen receptor can comprise a CD8 hinge region. The CD8 hinge region can be encoded by a nucleic acid sequence set forth in SEQ ID NO:5. The chimeric antigen receptor can comprise a CD28 transmembrane domain. The CD28 transmembrane domain can be encoded by a nucleic acid sequence set forth in SEQ ID NO:7. The chimeric antigen receptor can comprise a CD3zeta signaling domain. The CD3zeta signaling domain can be encoded by a nucleic acid sequence set forth in SEQ ID NO:8.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. Although methods and materials similar or equivalent to those described herein can be used to practice the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 contains a schematic of an exemplary method for treating aGVHD. Tregs that express CDH1-CAR or overexpress CD103 prevent and treat GVHD in a humanized mouse model and in patients with acute GVHD, while effector T cells that express CDH1-CAR or overexpress CD103 exacerbate GVHD in humanized mouse models and in patients with acute GVHD.

Figure 2 contains a schematic of an exemplary method for expanding and/or engineering Tregs. T cells are isolated from healthy donors, $CD4^+CD25^+FOXP3^+$ cells are isolated using negative selection beads (STEM CELL). T cells were then stimulated with CD3/CD28 beads on day 0, cultured in the presence of IL-2, and transduced with lentivirus express the transgene of interest on day 1 (CDH1-CAR or CD103). T cell expansion can last for a period of 6-14 days or 7-21 days. At the end of expansion, flow cytometric analysis confirmed that Tregs maintained their phenotype and suppressive functions. Cryopreserved Tregs were used for *in vitro* and *in vivo* experiments. Prior to their use, cells were thawed and rested for 12 hours.

Figures 3A and 3B contain graphs showing that Tregs isolated from human peripheral blood mononuclear cells (PBMCs) were expanded *in vitro* (Figure 3A) with high purity (Figure 3B).

Figure 4 contains a graph showing that Jurkat cells transduced with lentivirus particles encoding a CDH1-CAR nucleic acid expressed a CAR containing hSC10.17 clone (CDH1-specific scFv) linked to a CD8 hinge (CD8 H), a CD28 transmembrane domain (CD28 TM), and a CD3zeta signaling domain (CD3z).

Figure 5 contains a schematic of an exemplary humanized mouse model for evaluating CDH1-CAR therapy.

Figure 6. Regulatory T cells suppress effector T cell proliferation. Effector T cells were labeled with CFSE and mixed with regulator t cells isolated from the same donor, in the presence of stimulatory beads (4:1 or 1:1 ratio). The 4:1 ratio is to expand effector T cells, and 1:1 ratio is to activate T cells. Under both conditions, regulatory T cells inhibit the expansion of effector T cells, which is dependent on the cell dose. The higher the ratio of regulatory T cells to effector T cells, the more profound is the inhibition of effector T cells.

Figure 7. Regulatory T cells suppress effector $CD8^+$ T cell proliferation. Effector T cells were labeled with CFSE and mixed with regulatory T cells isolated from the same

donor, in the presence of stimulatory beads (4:1 or 1:1 ratio). The 4:1 ratio is to expand effector T cells, and 1:1 ratio is to activate T cells. Under both conditions, regulatory T cells inhibit the expansion of effector T cells, which is dependent on the cell dose. The higher the ratio of regulator T cells to effector T cells, the more profound is the inhibition of effector CD8⁺ T cells.

Figure 8 shows a schematic of an exemplary method for treatment and/or prevention of GVHD through modulation of the interaction between CDH1 on epithelial cells and its ligand (CD103) on T-cells. This method involves a xenograft model of a luciferase positive human epithelial cell line (MCF7). Mice are injected with CDH1-CAR-Teff, which has a killing effect on the xenograft, and are randomized to receive one of the following treatments to suppress the killing effect: 1) CDH1-CAR-Treg, 2) CD103^{high}-Treg, 3) UTD-Treg, and 4) saline. The killing effect on the xenograft is measured and compared between groups using serial bioluminescence imaging on days 5, 10, 15, and 20.

Figure 9 shows a schematic of another exemplary method for treatment and/or prevention of GVHD through modulation of the interaction between CDH1 on epithelial cells and its ligand (CD103) on T-cells. This method involves a xenograft model of human hematopoietic cells, where non-irradiated NSG (NOD-SCID- γ chain^{-/-}) mice are injected with 10 million human PBMC. These mice will normally develop xenogeneic GVHD after approximately 4-5 weeks. To prevent GVHD, the mice are also randomized to receive one of the following treatments: 1) anti-CD103-CAR-Teff, 2) CD103^{high}-Treg, 3) UTD-Treg, and 4) saline. The GVHD in the mice is assessed through serial measurement of the mice every 2-3 days.

Figure 10A-10D are flow cytometry plots showing isolation of Tregs (Figures 10C and 10D) from PBMCs (Figures 10A and 10B) as indicated by various markers. Tregs make up about 5% of peripheral T-cells. Typical phenotypic markers of Tregs are CD4⁺, CD25⁺, and FoxP3⁺, but since FoxP3 is an intracellular marker, CD127 low expression is used instead.

Figures 11A and 11B are flow cytometry plots showing isolation of natural Tregs. The stability of Tregs after *in vitro* expansion is a known issue (Hoffmann et al., *Blood*, 108(13):4260-4267 (2006)). To overcome this issue, the specific subpopulation of natural Tregs that are characterized by CD45RA⁺/CD4⁺/CD25⁺/CD127 low expression was isolated.

Figure 12 is a graph plotting *in vitro* Treg expansion, which was achieved by stimulating Tregs with anti-CD3/CD28 beads at a 3:1 bead to cell ratio supplemented with IL2 (400 IU/ml) for one week, followed by resting condition (no beads and low dose IL2 at 100 IU/ml), followed by a second stimulation with anti-CD3/CD28 beads at a 1:1 bead to cell ratio supplemented with IL2 (400 IU/ml) for another week. Using this technique, a 30- to 300- fold expansion was achieved at the end of 21 days.

Figure 13A is a schematic showing the structure of a second generation anti-CDH1 scFv CAR construct with a CD28 co-stimulatory signal. Figures 13B-13E are flow cytometry plots showing production of CDH1-CAR-T cells. Teffs (Figure 13B and 13C) and Tregs (Figures 13D and 13E) were transduced with the construct shown in Figure 13A, using lentivirus.

Figures 14A-14D are flow cytometry plots showing CD103^{high} T-cell production. Lentivirus technology similar to a first generation CAR was used to generate Teffs (Figures 14A and 14B) and Tregs (Figures 14C and 14D) with high expression of CD103, without the co-stimulatory signals.

Figures 15A and 15B are flow cytometry plots showing Treg purity at day 0 (Figure 15A) and after *in vitro* expansion for 9 days (Figure 15B). These studies demonstrated that Tregs maintained their regulatory phenotype at the end of expansion.

Figure 16A and 16B are graphs plotting Treg suppressive function *in vitro*. Effector cells were stained with CFSE, stimulated with anti-CD3/CD28 beads, and co-cultured with varying ratios of Tregs, and the number of proliferating cells was measured after four days. This was tested on day 0 (Figure 16A) with freshly isolated Tregs and at day 9 (Figure 16B) at the end of the first expansion. As shown in both graphs, proliferation of effector cells was decreased with higher ratios of Tregs. Notably, the suppressive function was more pronounced with the use of *in vitro* expanded Tregs.

Figure 17 is a graph plotting CDH-1-CAR-Treg function *in vitro*. To demonstrate CDH-1 specific suppressive function of Tregs, a proliferation assay was conducted in which CAR19 (used as effector cells) was co-cultured with different ratios of CDH1-CAR-Treg. This allowed the use of a different stimulus for CAR19 than for Tregs. As shown in the graph, CDH1-CAR-Tregs exerted their suppressive function on CAR19 in the presence of MCF7 cells but not in the absence of MCF7 cells.

Figure 18 is a graph plotting CD103^{high} *in vitro* function. The CD103^{high} Tregs had more potent suppressive function than transduced Tregs when they were co-cultured with effector T cells.

Figure 19 is an image showing CDH1-CAR-teff and CD103^{high}-Teff function *in vitro*. In particular, the *in vitro* function of CDH-1-CAR-Teffs and CD103^{high} Teffs was assessed in a killing assay, in which luciferase+ human epithelial cells (in this case MCF7) were mixed with different ratios of CDH1-CAR-Teffs or CD103^{high} Teffs. After 48 hours, a significant killing effect was observed from CDH1-CAR-Teff, but not from UTD or CD103^{high} Teffs.

Figure 20A shows bioluminescent imaging of NSG mice, demonstrating successful engraftment of MCF7 tumor cells one week after subcutaneous injection (left image) and eradication of MCF7 tumors 7 days after treatment with CDH1-CAR-Teffs (right image). Figure 20B is a graph plotting of tumor burden of MCF7 xenografts treated with untransduced T cells, CDH-1-CAR Teff, or CD103^{high} Teff cells, measured by bioluminescence imaging.

Figure 21 is a graph plotting data from a xenograft model of GVHD, in which non-irradiated NSG mice were injected with 10 million PBMC and monitored for development of GVHD. Mice began to develop signs of acute GVHD around day 28, as demonstrated by their rapid weight loss after day 28 in the graph.

Figure 22 depicts the general structure of an anti-CD103 CAR construct in which an anti-CD103 scFv was built into a second generation CAR with a CD28 co-stimulatory signal. The sequences encoding the light and heavy chains (SEQ ID NOS:9 and 10, respectively) are provided below the structure.

Figure 23 is a flow cytometry plot showing anti-CD103-CAR transduction; effector T- cells were successfully transduced with anti-CD103-CAR using lentivirus technology.

DETAILED DESCRIPTION

This document provides methods and materials involved in treating a mammal (e.g., a human) having or at risk of developing GVHD. For example, one or more T cells (e.g., one or more Tregs) expressing one or more antigen receptors targeting one or more epithelial-specific antigens can be administered (e.g., by adoptive transfer) to a mammal having GVHD (or at risk of developing GVHD) to treat the GVHD (or to reduce the risk of developing GVHD). In some cases, a CAR targeting an epithelial-specific antigen (e.g., CDH1) can be

expressed on a Treg to target the Treg to epithelial tissues. For example, one or more T cells expressing one or more CARs targeting CDH1 (e.g., CDH1-CARs) can be administered (e.g., by adoptive transfer) to a mammal having GVHD (or at risk of developing GVHD) to treat the GVHD (or to reduce the risk of developing GVHD). In some cases, a CD103

5 polypeptide targeting an epithelial-specific antigen (e.g., CDH1) can be expressed on a Treg to target the Treg to epithelial tissues. For example, one or more T cells expressing one or more CD103 polypeptides can be administered (e.g., by adoptive transfer) to a mammal having GVHD (or at risk of developing GVHD) to treat the GVHD (or to reduce the risk of developing GVHD). In some cases, a CD103 polypeptide targeting an epithelial-specific
10 antigen (e.g., CDH1) can be expressed on a Treg to target the Treg to epithelial tissues to treat an epithelial disease and/or an autoimmune disease (e.g., an autoimmune disease that involves epithelial inflammation in, for example, skin, gut tissue, and/or liver tissue).

A T cell described herein (e.g., a T cell expressing epithelial-specific antigen receptors) can be any appropriate T cell. In some cases, a T cell can be a naïve T cell. In
15 some cases, a T cell can be an immunosuppressive T cell. An example of T cells that can be used as described herein includes, without limitation, Tregs. For example, a T cell expressing one or more antigen receptors targeting one or more epithelial-specific antigens can be a Treg. In some cases, the methods and materials described herein can be used to redirect CAR T cells with an inhibitory signal or to redirect stem cells (e.g., mesenchymal
20 stem cells).

A T cell described herein can express (e.g., can be engineered to express) any appropriate antigen receptor that binds an epithelial-specific antigen (e.g., an antigen present on epithelial cells with minimal, or no, expression on other cell types). For example, a T cell can be engineered to express an antigen receptor that targets an antigen (e.g., a cell surface
25 antigen) expressed by epithelial cells in a mammal having or at risk of developing GVHD. In some cases, an antigen receptor can be a heterologous antigen receptor. In some cases, an antigen receptor can be a CAR. In some cases, an antigen receptor can be a recombinant antigen receptor. In some cases, an antigen receptor can include an antibody or a fragment thereof. For example, an antigen receptor can include an antigen-binding (Fab) fragment
30 from an antibody that targets an epithelial-specific antigen. In some cases, an antigen receptor can include one or more variable regions of the heavy (VH) chains and one or more variable regions of the light (VL) chains (e.g., as a recombinant protein or a fusion protein)

from an antibody that targets an epithelial-specific antigen. For example, an antigen receptor can be a single chain variable fragment (scFv) that targets an epithelial-specific antigen. Examples of antigen receptors that bind an epithelial antigen include, without limitation, CD103, hSC10.17, CD234, EPCAM, EMA, MUC1, cytokeratin, CA125, ALCAM, HLA, Desmin, Epithelial Antigen antibody, CD227, ESA, Galactin 3, GGT, HLA-DR, Lectin, LAMP-1, MMR, MOC-31, p16, p63, p-Cadherin, PSA, surfactant, Transthyretin, VAT-1, and Vimentin. For example, a T cell engineered to target epithelial tissues can express a CD103 polypeptide. In such cases, the engineered T cell can include exogenous nucleic acid that encodes and expresses the CD103 polypeptide. As another example, a T cell engineered to target epithelial tissues can express an antibody or antibody fragment of an anti-CDH1 antibody (e.g., an hSC10.17 scFv). In such cases, the engineered T cell can include exogenous nucleic acid that encodes and expresses the hSC10.17 scFv. In some cases, a T cell engineered to target epithelial tissues can express both a CD103 polypeptide and an antibody or antibody fragment of an anti-CDH1 antibody (e.g., an hSC10.17 scFv).

An epithelial antigen can be any appropriate epithelial antigen. An epithelial antigen can be expressed on any appropriate type of epithelial cell (e.g., skin cells, gastrointestinal tract cells, lung cells, liver cells, brain cells, kidney cells, ovarian cells, uterus cells, bladder cells, and pancreatic cells). In some cases, an epithelial antigen can be a cell adhesion molecule (CAM). Examples of epithelial antigens include, without limitation, CDH1, CD103, hSC10.17, CD234, EPCAM, EMA, MUC1, cytokeratin, CA125, ALCAM, HLA, Desmin, Epithelial Antigen antibody, CD227, ESA, Galactin 3, GGT, HLA-DR, Lectin, LAMP-1, MMR, MOC-31, p16, p63, p-Cadherin, PSA, surfactant, Transthyretin, VAT-1, and Vimentin. For example, a T cell engineered to target epithelial tissues can bind to CDH1. In some cases, a T cell can be engineered to express a CDH1-CAR to target CDH1 expressed by epithelial cells in a mammal having or at risk of developing GVHD. In some cases, a T cell can be engineered to express a CD103 polypeptide to target CDH1 expressed by epithelial cells in a mammal having or at risk of developing GVHD.

Any appropriate method can be used to express an antigen receptor described herein (e.g., an antigen receptor targeting one or more epithelial-specific antigens) on a T cell described herein. For example, a nucleic acid encoding an antigen receptor can be introduced into a T cell. In some cases, a nucleic acid encoding an antigen receptor can be introduced into a T cell by transduction (e.g., viral transduction) or transfection. In some

cases, a nucleic acid encoding an antigen receptor can be introduced *ex vivo* into one or more T cells. For example, *ex vivo* engineering of T cells to express an antigen receptor can include transducing isolated T cells with a lentiviral vector encoding an antigen receptor. In cases where T cells are engineered *ex vivo* to express an antigen receptor, the T cells can be
5 obtained from any appropriate source (e.g., a mammal such as the mammal to be treated or a donor mammal, or a cell line). In some cases, CAR T cells can be prepared as described herein (see, e.g., Figure 2 and Example 1). For example, a CDH1-CAR can be expressed on a Treg to direct the Treg to epithelial tissues by introducing one or more constructs containing a nucleic acid encoding the CAR (e.g., a CAR targeting CDH1) into the Treg. In
10 some cases, CAR T cells can be prepared as described elsewhere (see, e.g., Blat *et al.*, *Mol. Ther.*, 22(5):1018-28 (2014); MacDonald *et al.*, *J. Clin. Invest.*, 126(4):1413-24 (2016); and Yoon *et al.*, *Blood*, 129(2):238-245 (2017)).

Also provided herein are CARs and constructs (e.g., nucleic acid constructs) encoding CARs described herein (e.g., CARs targeting an epithelial-specific antigen (e.g., CDH1)).

15 For example, a construct encoding a CAR targeting CDH1 (e.g., a CDH1-CAR) can include a nucleic acid sequence encoding one or more molecules that bind CDH1 described herein. In some cases, a CDH1-CAR can include an anti-CDH1 antibody (e.g., hSC10.17) heavy chain and an anti-CDH1 antibody (e.g., hSC10.17) light chain. A CAR described herein (e.g., a CDH1-CAR) also can include one or more additional components. Examples of
20 additional components that can be included in a CAR include, without limitation, a leader sequence (e.g., a CD8 leader sequence), a hinge (e.g., a CD8 hinge), a transmembrane domain (e.g., a CD8 transmembrane domain or a CD28 transmembrane domain), a signaling domain such as a CD3zeta signaling domain or a signaling domain used to generate a third generation CART or CAR Treg that increases immunosuppression that may increase
25 secretion of immunosuppressive cytokines, such as PD1, CTLA4, TIM3, or other inhibitory molecules. In some cases, nucleic acid encoding a component of a construct encoding a CAR can be separated from nucleic acid encoding another component using one or more linkers. Nucleic acids in a construct encoding a CAR can be present in any order. For example, constructs encoding a CHD1-CAR can be generated in a light to heavy orientation
30 of the scFv or in a heavy to light orientation of the scFv. Exemplary nucleic acid sequences for some additional components that can be included in a construct described herein are as follows.

CD8 leader sequence (SEQ ID NO:1)

ATGGCCTTACCAGTGACCGCCTTGCTCCTGCCGCTGGCCTTGCTGCTCCACGCCGC
CAGGCCG

5

hSC10.17 heavy chain (HC) (SEQ ID NO:2)

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTG
AGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGTAGCTATGGCATGCACTGGGT
CCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGCATACATTACTACTAGAAGT
10 AGTACCATATACTACGCAGACTCTGTGAAGGGCCGATTACCATCTCCAGAGACAA
TGCCAAGAACTCACTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGC
TGTGTATTACTGTACTAGAGAACCCCTAACTGGATACTATGCTATGGACTACTGGGG
TCAAGGAACCTCAGTCACCGTCTCCTCAG

15 Linker (SEQ ID NO:3)

GGAGGTGGCGGATCAGGCGGAGGAGGCAGCGGCGGAGGTGGATCAGGAGGCGG
AGGGTCA

hSC10.17 light chain (LC) (SEQ ID NO:4)

20 GATGTTGTGATGACTCAGTCTCCACTCTCCCTGCCCGTCACCCTTGGACAGCCGG
CCTCCATCTCCTGCAGGTCTAGTCAAAGCATCGTACACAGTGATGGAAACACCTA
CTTGGAATGGTATCAGCAGAGGCCAGGCCAATCTCCAAGGCGCCTAATTTATAAG
GTTTCTAACCGGTTCTCTGGGGTCCCAGACAGATTACAGCGGCAGTGGGGTCAGGCA
CTGATTTACACTGAAAATCAGCAGGGTGGAGGCTGAGGATGTTGGGGTTATTAC
25 TGCTTTCAAGGTTACATGCTCCGTGGACGTTTCGGTGGAGGCACCAAGGTGGAAA
TCAAAC

CD8 hinge (SEQ ID NO:5)

ACCACGACGCCAGCGCCGCGACCACCAACACCGGCGCCCACCATCGCGTCGCAG
30 CCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGGGCGCAGTGCAC
ACGAGGGGGCTGGACTTCGCCTGTGAT

CD8 transmembrane domain (SEQ ID NO:6)

ATCTACATCTGGGCGCCCTTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGT
TATCACCCCTTTACTGC

5 CD28 signaling domain (SEQ ID NO:7)

AGGAGTAAGAGGAGCAGGCTCCTGCACAGTGACTACATGAACATGACTCCCCGC
CGCCCCGGGCCCACCCGCAAGCATTACCAGCCCTATGCCCCACCACGCGACTTCG
CAGCCTATCGCTCC

10 CD3zeta signaling domain (SEQ ID NO:8)

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACAAGCAGGGCCAGAA
CCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGGAC
AAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAACCC
TCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCTACAG
15 TGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGGCCTTTA
CCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACATGCAGGCC
CTGCCCCCTCGC

In some cases, a nucleic acid construct encoding a CDH1-CAR can include a CD8
20 leader sequence, a hSC10.17 heavy chain, a linker, a hSC10.17 light chain, a CD8 hinge, a
CD28 transmembrane domain, and a CD3zeta signaling domain. For example, a nucleic acid
construct encoding a CHD1-CAR can include a CD8 leader including the sequence set forth
in SEQ ID NO:1, a hSC10.17 heavy chain including the sequence set forth in SEQ ID NO:2,
a linker including the sequence set forth in SEQ ID NO:3, a hSC10.17 light chain including
25 the sequence set forth in SEQ ID NO:4, a CD8 hinge including the sequence set forth in SEQ
ID NO:5, a CD28 transmembrane domain including the sequence set forth in SEQ ID NO:7,
and a CD3zeta signaling domain including the sequence set forth in SEQ ID NO:8.

This document also provides materials and methods for treating mammals (e.g.,
humans) having (or at risk of developing) GVHD. For example, T cells described herein
30 (e.g., T cells expressing epithelial-specific antigen receptors) can be administered (e.g., by
adoptive transfer) to a mammal having or at risk of developing GVHD to reduce the severity
of GVHD within the mammal. In some cases, reducing the severity of GVHD in a mammal

can include reducing or eliminating one or more symptoms of GVHD (e.g., skin rashes, immune-mediated pneumonitis, intestinal inflammation, sloughing of the intestinal mucosal membrane, severe diarrhea, abdominal pain, nausea, vomiting, and/or elevated bilirubin levels). In some cases, reducing the severity of GVHD in a mammal can include reducing the stage of GVHD. The stage of GVHD can be evaluated as described elsewhere (see, e.g., Jacobsohn *et al.*, *Orphanet. J. Rare Dis.*, 2:35 (2007)).

Any appropriate mammal having (or at risk of developing) GVHD can be treated as described herein. For example, T cells described herein (e.g., T cells expressing epithelial-specific antigen receptors) can be administered (e.g., by adoptive transfer) to humans and other primates such as monkeys having (or at risk of developing) GVHD to treat the GVHD. In some cases, dogs, cats, horses, cows, pigs, sheep, mice, and rats can be treated as described herein.

Any appropriate GVHD can be treated as described herein. GVHD can be acute GVHD (aGVHD) or chronic GVHD. GVHD can be allogeneic GVHD (allo-GVHD) or autologous GVHD (auto-GVHD). GVHD can be any stage of GVHD. In some cases, GVHD can be associated with (e.g., following) a transplant. A transplant can be an allogeneic transplant such as HCT. A transplant can be autologous such as autologous hematopoietic progenitor cell transplantation (HPCT).

In some cases, a mammal can be identified as having (or as being at risk of developing) GVHD. Any appropriate method for identifying a mammal as having (or as being at risk of developing) GVHD can be used. Once identified as having (or as being at risk of developing) GVHD, the mammal can be administered (e.g., by adoptive transfer) or instructed to self-administer one or more T cells described herein (e.g., T cells expressing epithelial-specific antigen receptors) to treat the GVHD within the mammal.

Any appropriate method can be used to administer T cells described herein (e.g., T cells expressing epithelial-specific antigen receptors) to a mammal (e.g., a mammal having, or at risk of developing, GVHD). Examples of methods of administering T cells described herein to a mammal can include, without limitation, injection (e.g., IV, ID, IM, or subcutaneous injection). For example, T cells expressing epithelial-specific antigen receptors can be administered to a human by intravenous injection.

In some cases, a mammal having (or at risk of developing) GVHD being treated as described herein (e.g., by administration of one or more T cells described herein (e.g., T cells

expressing an epithelial-specific antigen receptors)) also can be treated with one or more therapeutic agents. A therapeutic agent used in combination with T cells described herein can be any appropriate therapeutic agent. In some cases, a therapeutic agent can be a GVHD agent. In some cases, a therapeutic can be an immunosuppressive agent. Examples of
5 therapeutic agents that can be used in combination with T cells described herein include, without limitation, systemic steroids (e.g., corticosteroids), topical steroids, infliximab, tocilizumab, natalizumab, ibrutinib, ruxolitinib, immunoglobulins (e.g., anti-thymocyte globulin (ATG)), ECP (extracorporeal photopheresis), TNF- α blocking agents, alemtuzumab, IVIG, calcineurin inhibitors (e.g., tacrolimus and/or cyclosporine), sirolimus, IL-2 blocking
10 agents, low dose IL-2, mycophenolae mofetil, pentostatin, T cell depleting chemotherapy, rituximab, brentuximab, and mesenchymal stem cells.

This document also provides kits containing one or more materials described herein. For example, materials provided in kits described herein can be used for treating mammals (e.g., humans) having (or at risk of developing) GVHD as described herein.

15 In some cases, one or more T cells described herein (e.g., T cells expressing an epithelial-specific antigen receptors) can be combined with packaging material and sold as a kit. The packaging material included in such a kit typically contains instructions or a label describing how the composition can be used, for example, in an adoptive transfer to treat GVHD as described herein. In some cases, one or more constructs (e.g., nucleic acid
20 constructs) described herein (e.g., encoding CARs that bind one or more epithelial-specific antigens) can be combined with packaging material and sold as a kit. The packaging material included in such a kit typically contains instructions or a label describing how the composition can be used, for example, to express one or more CARs in T cells (e.g., Tregs) to engineer the T cells to express CARs that bind one or more epithelial-specific antigens
25 (e.g., CDHa-CARs). A kit also can include instructions or a label describing how the engineered T cells can be used, for example, in adoptive transfer to treat GVHD as described herein. A kit also can include materials for use in an adoptive transfer procedure.

The invention will be further described in the following examples, which do not limit the scope of the invention described in the claims.

EXAMPLES

Example 1: Targeting E-Cadherin (CDH1) with Chimeric Antigen Receptor Regulatory T-cells for the Treatment of Acute Graft-Versus-Host Disease

Methods

5 Cell lines and primary samples

Cell lines were obtained from ATCC (K-562, MCF-7, and NALM6). All cell lines were tested for sterility before experiments. For some experiments, MCF-7 and NALM-6 cells were transduced with zsGreen/GFP lentivirus and then sorted to obtain >99% positive population. Cell lines MOLM-14 and K562 were used as controls as indicated in the relevant
10 figures. The cell lines were maintained in culture with RPMI1640 (Gibco, 11875–085, LifeTechnologies) supplemented with 10% FBS (Gemini, 100–106) and 50 U/mL penicillin/streptomycin (Gibco, Life Technologies, 15070–063). For all functional studies, primary cells were thawed at least 12 hours before experiment and rested at 37°C.

Generation of CAR constructs and CAR T cells

15 The anti-CDH1 chimeric antigen receptor (single chain variable fragment derived from clone hSC10.17, CD8 hinge, 4-1BB costimulatory domain and CD3 zeta signaling domain) was generated de novo and cloned into a third generation lentivirus. Normal donor T regulatory cells were selected using negative selection Kit (Stem Cell), and expanded *in vitro* using anti-CD3/CD28 Dynabeads (Invitrogen, Life Technologies, Grand Island, NY,
20 USA, added on the first day of culture) and IL-2 at different concentrations (100, 500 and 1000 IU/mL). T cells were transduced with lentiviral supernatant one day following stimulation at a multiplicity of infection of 3. The anti-CD3/CD28 Dynabeads were removed on day 6, and T cells were grown in T-cell media (X-vivo 15 media, human serum 5%, penicillin, streptomycin and glutamax). CART cells were then cryopreserved on day 8 for
25 future experiments. Prior to all experiments, T cells were thawed and rested overnight at 37°C.

Multiparametric flow cytometry

Anti-human antibodies were purchased from Biolegend (San Diego, CA, USA), eBioscience (San Diego, CA, USA), or BD Biosciences (San Jose, CA, USA). Cells were

isolated from *in vitro* culture or from animals, washed once in phosphate-buffered saline supplemented with 2% fetal calf serum, and stained at 4 °C after blockade of Fc receptors. For cell number quantitation, Countbright beads (Invitrogen) were used according to the manufacturer's instructions (Invitrogen). In all analyses, the population of interest was gated
5 based on time gating, followed by forward vs side scatter characteristics, followed by singlet gating, and live cells were gated using Live Dead Aqua (Invitrogen). Surface expression of anti-CDH1 CAR was detected by staining with an Alexa Fluor 647-conjugated goat anti-mouse F(ab')₂ antibody from Jackson ImmunoResearch (West Grove, PA, USA) or with protein L. Flow cytometry was performed on a four-laser analyzer (BD Canto-II). All
10 analyses were performed using FlowJo X10.0.7r2.

T-cell function assays

T-cell degranulation and intracellular cytokine assays

Briefly, T cells were incubated with target cells at a 1:5 ratio. After staining for CAR expression, antibodies against CD107a, CD28, CD49d and monensin were added at the time
15 of incubation. After 4 hours, cells were harvested and stained for CD3 and Live Dead staining (Invitrogen). Cells were fixed and permeabilized (FIX & PERM Cell Fixation & Cell Permeabilization Kit, Life Technologies), and intracellular cytokine staining was then performed as indicated in the specific experiments.

Proliferation assays

20 T cells were washed and re-suspended at 1×10^7 /mL in 100 μ L of phosphate-buffered saline and labeled with 100 μ L of carboxyfluorescein succinimidyl ester (CFSE) 2.5 μ M (Life Technologies) for 5 minutes at 37 °C. The reaction was then quenched with cold R10, and the cells were washed three times. Targets were irradiated at a dose of 100 Gy. T cells were incubated at a 1:1 ratio with irradiated target cells for 120 hours. Cells
25 were then harvested, stained for CD3, CAR and Live Dead aqua (Invitrogen), and Countbright beads (Invitrogen) were added prior to flow cytometric analysis.

Treg suppressive assays

Regulatory T cells were co-cultured with CFSE labeled effector T cells, in the presence of low concentrations of CD3/CD28 beads, for 5 days. Proliferation of CFSE

labeled T effector cells was calculated by flow cytometry using CountBright beads for absolute counting.

The suppressive effect of regulatory T cells on CART cell functions was measured through the incubation of regulatory T cells with CFSE labeled CART19 cells, in the presence of their targets. Proliferation of CART cells was analyzed by flow cytometry using CountBright beads for absolute counts.

Cytotoxicity assays

MCF-7 cells were used for cytotoxicity assay. In brief, targets were incubated at the indicated ratios with CDH1-CAR T effectors or CDH1-CART regulatory for 4 or 16 hours or 24 to 48 hours. Killing was calculated either by bioluminescence imaging on a Xenogen IVIS-200 Spectrum camera (PerkinElmer, Hopkinton, MA, USA)

Secreted cytokine measurement

Effector and target cells were incubated at a 1:1 ratio in T-cell media for 24 or 72 hours as indicated. Supernatant was harvested and analyzed by 30-plex Luminex array according to the manufacturer's protocol (Millipore).

In vivo experiments

NOD-SCID- γ chain^{-/-} (NSG) originally obtained from Jackson Laboratories were maintained in our laboratory under an IACUC approved breeding protocol. Schemas of the utilized xenograft models are discussed in detail in Figure 1, Figure 2, Figure 8, and Figure 9. Cells were injected in 200 μ L of phosphate-buffered saline at the indicated concentration into the tail veins of mice. Bioluminescent imaging was performed using a Xenogen IVIS-200 Spectrum camera. Images were acquired and analyzed using Living Image version 4.4 (Caliper LifeSciences, Inc., PerkinElmer).

Humanized NSG mice were purchased from the Jackson laboratories. In brief, these mice were injected with CD34⁺ cells as neonates. They develop full human hematopoiesis. After 8 weeks, engraftment was confirmed through bleeding of these mice. They were then treated with CDH1-CAR T effectors or CDH1-CAR T regulatory cells.

Statistical analysis

All statistical analyses were performed as indicated using GraphPad Prism 6 for Windows, version 6.04. Student *t* test was used to compare two groups; in analysis where multiple groups were compared, one-way ANOVA was performed with Holm–Sidak
5 correction for multiple comparisons. When multiple groups at multiple time points/ratios were compared, the Student *t* test or ANOVA for each time points/ratios was used. Survival curves were compared using the log-rank test. In the figures, asterisks represent *P* values (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$) and “ns” means “not significant” ($P > 0.05$). Further details of the statistics for each experiment are listed in
10 figure legends.

Results

Tregs

Tregs were isolated from human peripheral blood mononuclear cells (PBMCs), and expanded in vitro with high purity (96%) at day 7. Tregs were expanded using CD3/CD28
15 and IL-2 for a period of 6-8 days. At the end of expansion, Tregs maintained their phenotype (CD4⁺CD25⁺FOXP3⁺). See, e.g., Figures 3A-3B, Figures 10A-10D, Figures 11A-11B, and Figure 12.

CARTregs

A second generation CDH1-CAR was constructed and Jurkat cells were transduced
20 with lentivirus particles encoding the construct.

CDH1 CAR was generated *de novo* and was composed of a hSC10.17 clone linked to a CD8 hinge, a CD28 transmembrane domain, and a CD3zeta signaling domain (see, Figure 13A). Two constructs were generated using light to heavy and heavy to light orientation of the scFv. T cells were transduced with this construct to generate CDH1-CART cells. See,
25 e.g., Figure 4.

Additional results are presented in Figure 6, Figure 7, Figures 13A-13E, and Figures 14A-14D.

Example 2: Immunomodulatory Function of CDH1-CAR-Tregs

CDH1-CAR-Treg cells continue to exhibit inhibitory and immunomodulatory functions to treat GVHD.

To generate CDH1-CAR-Tregs, *ex vivo* expanded Tregs are transduced with
 5 lentivirus particles encoding CDH1-CAR to generate CDH1-CAR-Tregs, and the purity and immune-phenotype of CDH1-CAR-Tregs before and after expansion were analyzed. CDH1-CAR- T effector (Teff) cells were generated as positive controls. Results are presented in Figures 15A-15B.

To evaluate the suppressive and immunomodulatory function of *ex vivo* expanded
 10 CDH1-CAR-Treg cells, the proliferation of CDH1-CAR Teff cells in the presence or absence of CDH1-CAR-Treg cells were analyzed. Results are presented in Figures 16A-16B, Figure 17, and Figure 18. CDH1-CAR Tregs and CDH1-CAR Teff cells were co-cultured with the CDH1⁺ luciferin⁺ cell line MCF7 and cytokine production and the killing of target cells in the co-culture was assessed. Results are presented in Figure 19. In further studies, cytokine
 15 production is measured to evaluate the suppressive and immunomodulatory function of *ex vivo* expanded CDH1-CAR-Treg cells.

Example 3: Engineering Tregs to Overexpress CD103

Treg cells overexpressing CD103 polypeptides treat aGVHD.

To investigate the ability of CDH1-CAR Tregs to treat aGVHD, humanized mouse
 20 models were randomized to receive CDH1-CAR Treg, control Treg, CDH-1-CAR Teff, control Teff, CD103^{high} Tregs, or CD103^{high} Teff (Figure 5). CDh1-CAR Tregs ameliorated GVHD, and CDh1-CAR-Teff cells exacerbated GVHD. Results are presented in Figures 20A-20B and Figure 21.

Example 4: transduced effector T-cells with anti-CD103-CAR

25 An anti-CD103 CAR construct was generated in which an CD103 scF was built into a second generation CAR with a CD28 co-stimulatory signal (Figure 22). Using lentivirus technology, effector T- cells were successfully transduced with the anti-CD103-CAR. *See*, Figure 23.

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other
5 aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A method for treating a mammal having graft versus host disease (GVHD), wherein said method comprises administering to said mammal a composition comprising regulatory T cells (Tregs) comprising exogenous nucleic acid encoding an antigen receptor targeting an epithelial-specific antigen, wherein said Tregs express said antigen receptor.
2. The method of claim 1, wherein said mammal is a human.
3. The method of any one of claims 1-2, wherein said GVHD is acute GVHD.
4. The method of any one of claims 1-3, wherein said GVHD occurred following allogeneic transplantation.
5. The method of any one of claims 1-4, wherein said epithelial-specific antigen is E-cadherin (CDH1).
6. The method of any one of claim 1-5, wherein said antigen receptor is a chimeric antigen receptor.
7. The method of claim 6, wherein said chimeric antigen receptor comprises a single chain variable fragment (scFv).
8. The method of claim 7, wherein said scFv comprises a light chain and a heavy chain from an anti-CDH1 antibody.
9. The method of claim 8, wherein said anti-CDH1 antibody is hSC10.17.
10. The method of any one of claim 1-5, wherein said antigen receptor comprises cluster of differentiation 103 (CD103).

11. The method of any one of claims 1-10, wherein said Tregs, prior to said administration, are engineered to express said antigen receptor *ex vivo*.
12. The method of any one of claims 1-11, wherein a symptom of said GVHD is reduced at least 10 percent.
13. A method for treating a mammal at risk of developing graft versus host disease (GVHD), wherein said method comprises administering to said mammal a composition comprising regulatory T cells (Tregs) comprising exogenous nucleic acid encoding an antigen receptor targeting an epithelial-specific antigen, wherein said Tregs express said antigen receptor.
14. The method of claim 13, wherein said mammal is a human.
15. The method of any one of claims 13-14, wherein said GVHD is acute GVHD.
16. The method of any one of claims 13-15, wherein said mammal received an allogeneic transplantation.
17. The method of any one of claims 13-16, wherein said epithelial-specific antigen is E-cadherin (CDH1).
18. The method of any one of claim 13-17, wherein said antigen receptor is a chimeric antigen receptor.
19. The method of claim 18, wherein said chimeric antigen receptor comprises a single chain variable fragment (scFv).
20. The method of claim 19, wherein said scFv comprises a light chain and a heavy chain from an anti-CDH1 antibody.

21. The method of claim 20, wherein said anti-CDH1 antibody is hSC10.17.
22. The method of any one of claim 13-17, wherein said antigen receptor comprises cluster of differentiation 103 (CD103).
23. The method of any one of claims 13-22, wherein said Tregs, prior to said administration, are engineered to express said antigen receptor *ex vivo*.
24. A nucleic acid construct encoding a chimeric antigen receptor targeting an epithelial-specific antigen.
25. The nucleic acid construct of claim 24, wherein said epithelial-specific antigen is E-cadherin (CDH1).
26. The nucleic acid construct of any one of claims 24-25, wherein said chimeric antigen receptor comprises a single chain variable fragment (scFv).
27. The nucleic acid construct of claim 26, wherein said scFv comprises a light chain and a heavy chain from an anti-CDH1 antibody.
28. The nucleic acid construct of claim 27, wherein said anti-CDH1 antibody is hSC10.17.
29. The nucleic acid construct of claim 28, wherein a heavy chain of said hSC10.17 is encoded by a nucleic acid sequence set forth in SEQ ID NO:2.
30. The nucleic acid construct of any one of claims 28-29, wherein a light chain of said hSC10.17 is encoded by a nucleic acid sequence set forth in SEQ ID NO:4.
31. The nucleic acid construct of any one of claims 24-30, wherein said chimeric antigen receptor comprises a CD8 hinge region.

32. The nucleic acid construct of claim 31, wherein said CD8 hinge region is encoded by a nucleic acid sequence set forth in SEQ ID NO:5.
33. The nucleic acid construct of any one of claims 24-32, wherein said chimeric antigen receptor comprises a CD28 transmembrane domain.
34. The nucleic acid construct of claim 33, wherein said CD28 transmembrane domain is encoded by a nucleic acid sequence set forth in SEQ ID NO:7.
35. The nucleic acid construct of any one of claims 24-34, wherein said chimeric antigen receptor comprises a CD3zeta signaling domain.
36. The nucleic acid construct of claim 35, wherein said CD3zeta signaling domain is encoded by a nucleic acid sequence set forth in SEQ ID NO:8.

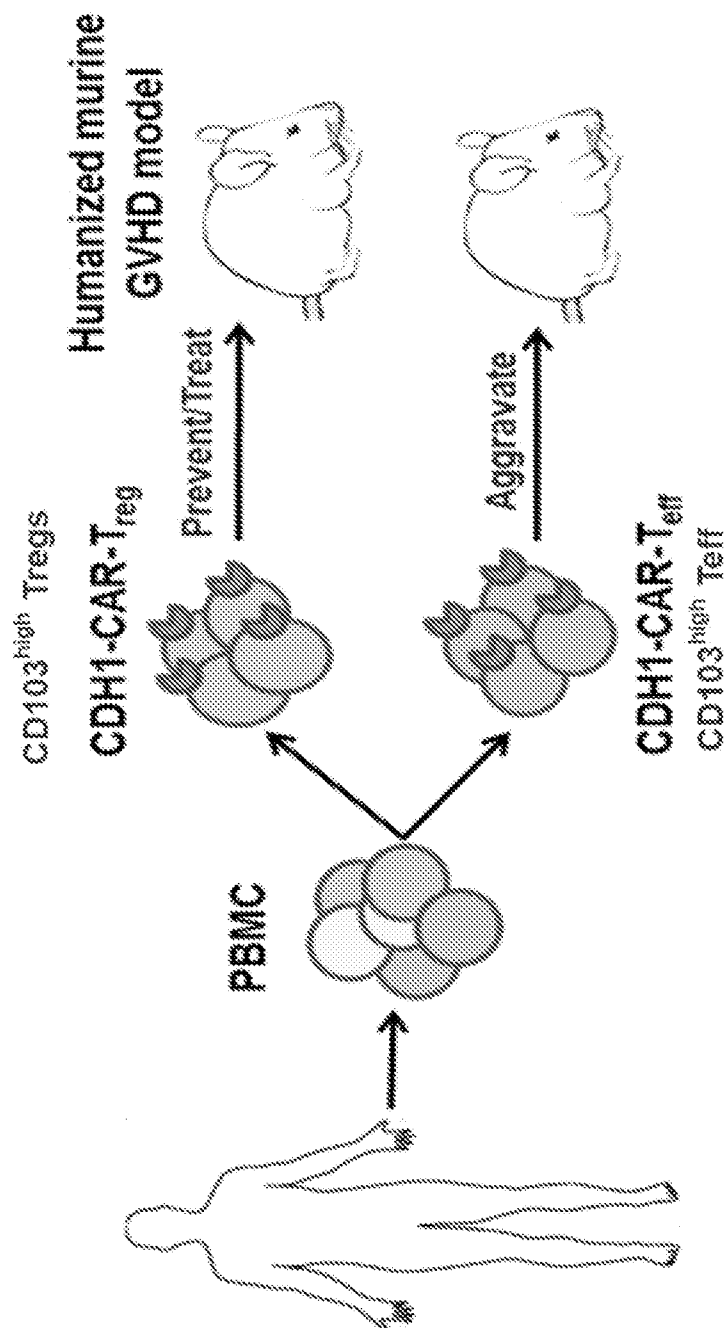


FIG. 1

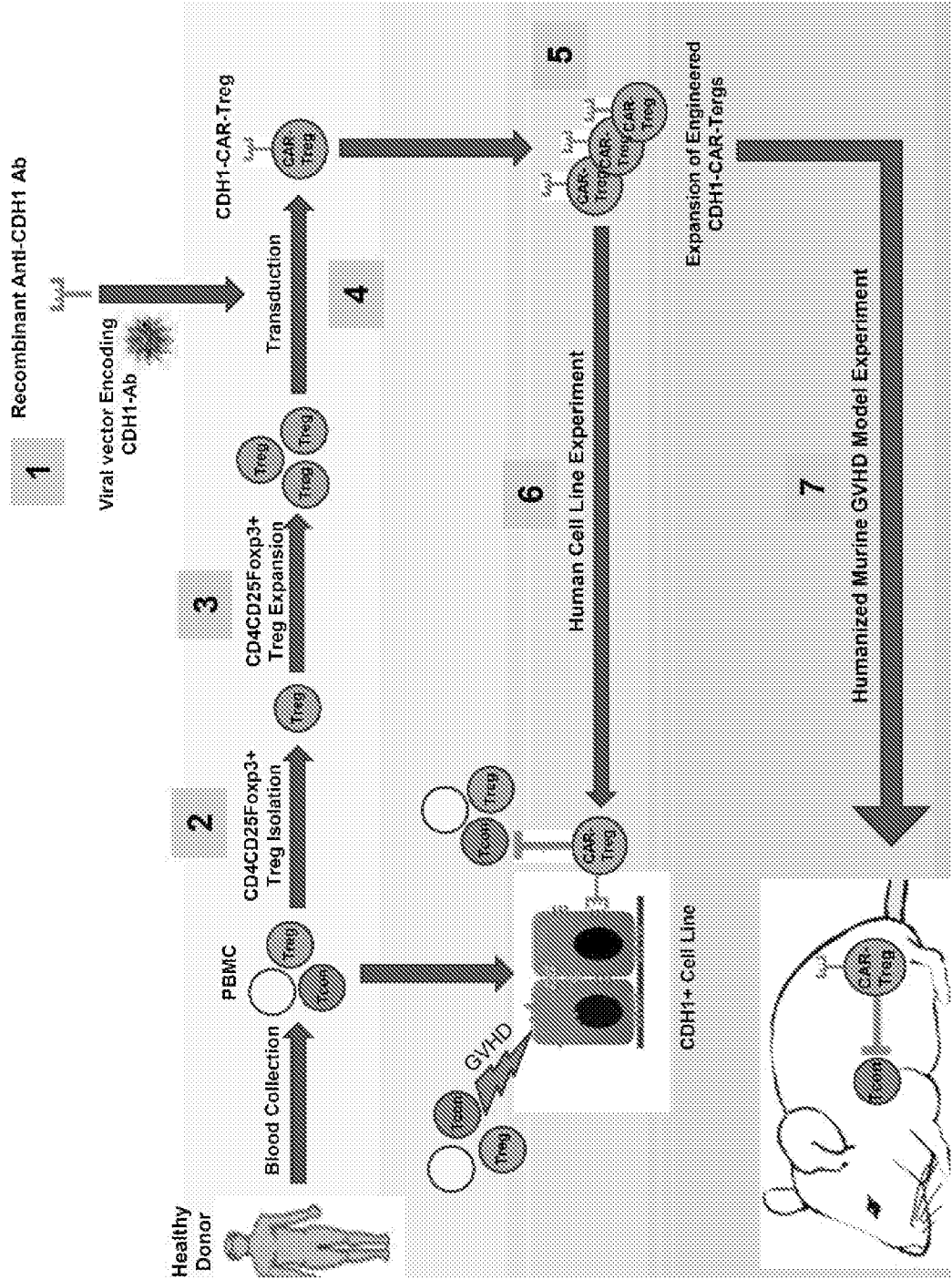


FIG. 2

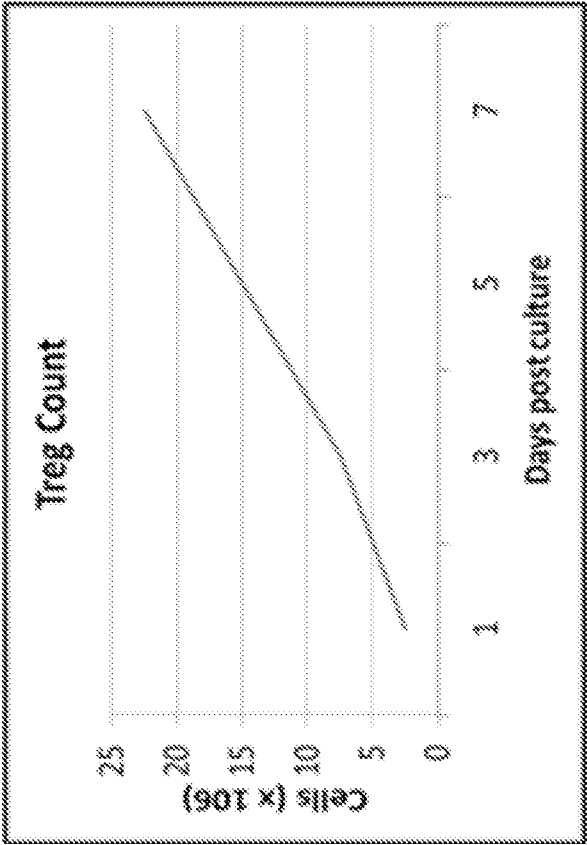


FIG. 3A

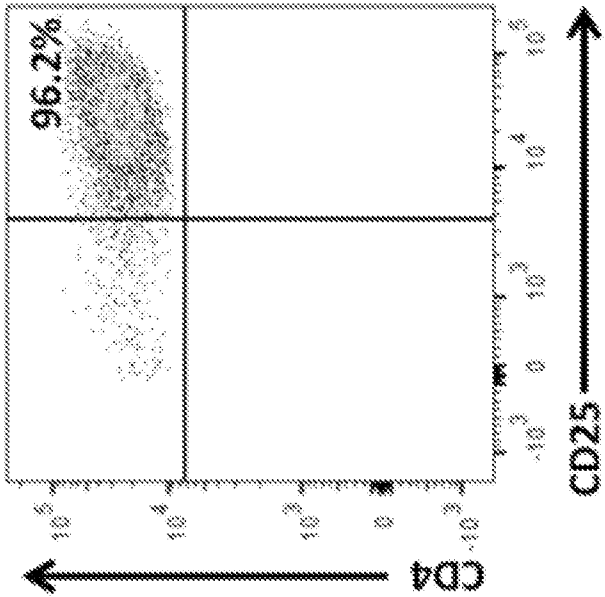


FIG. 3B

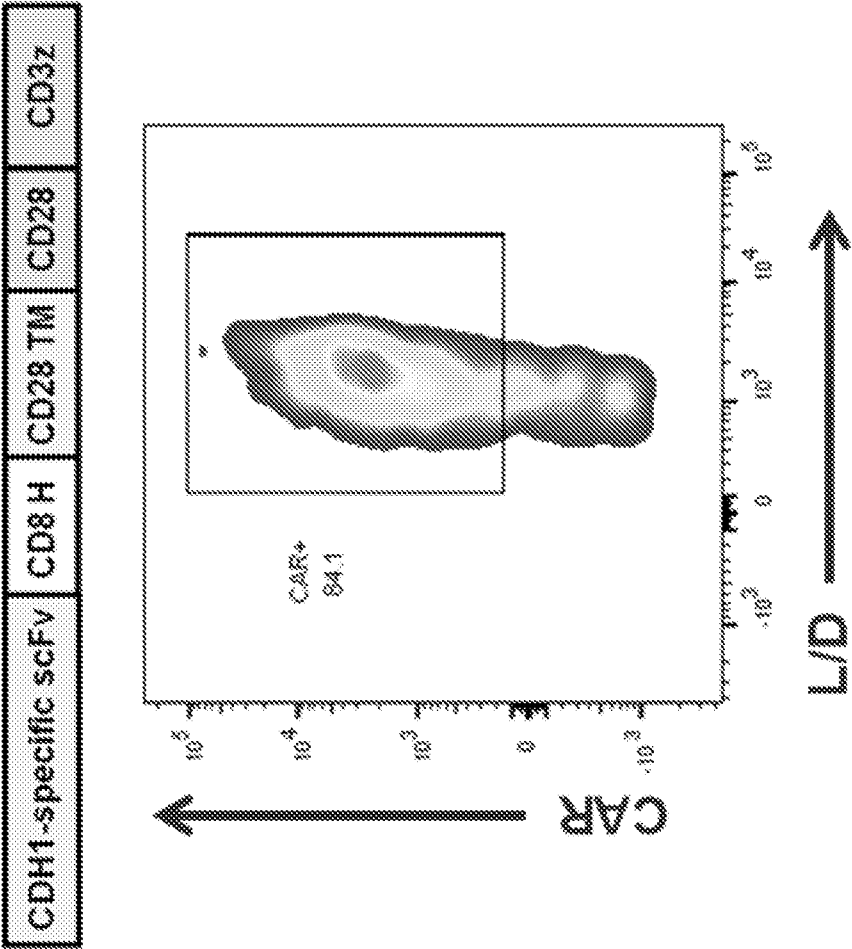


FIG. 4

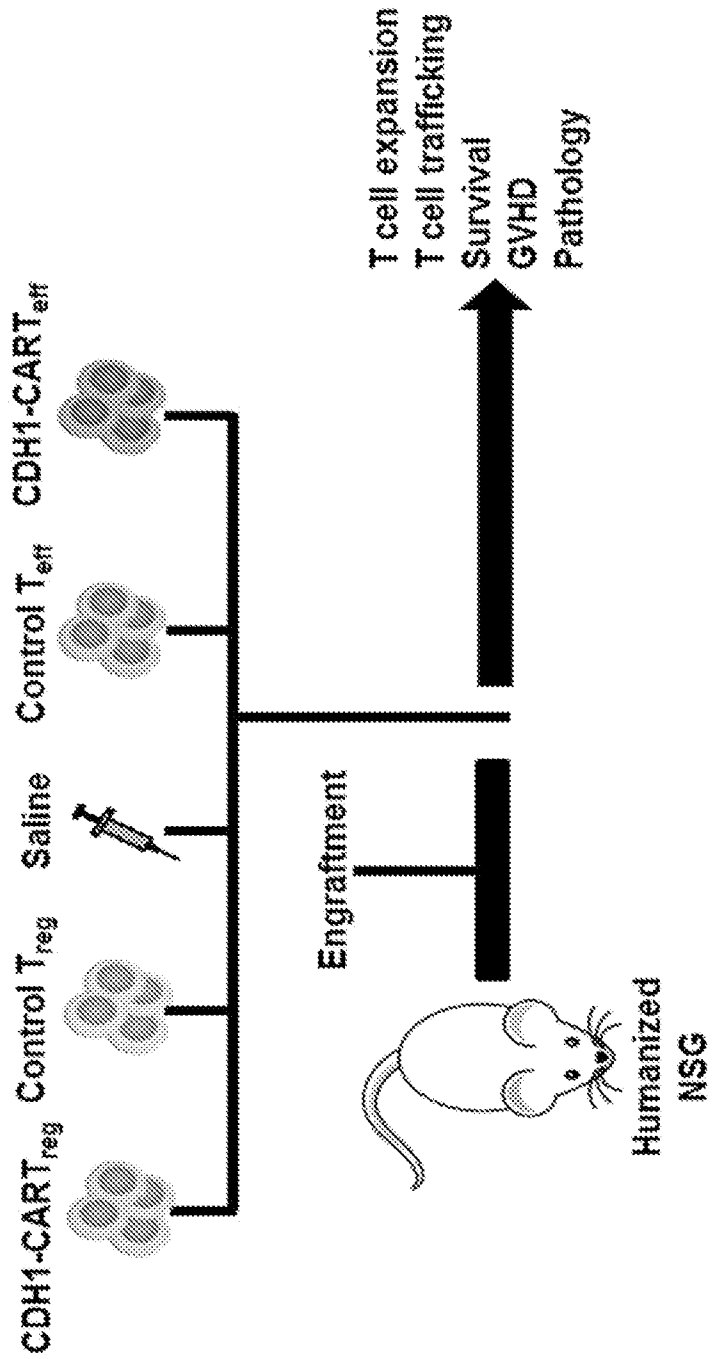


FIG. 5

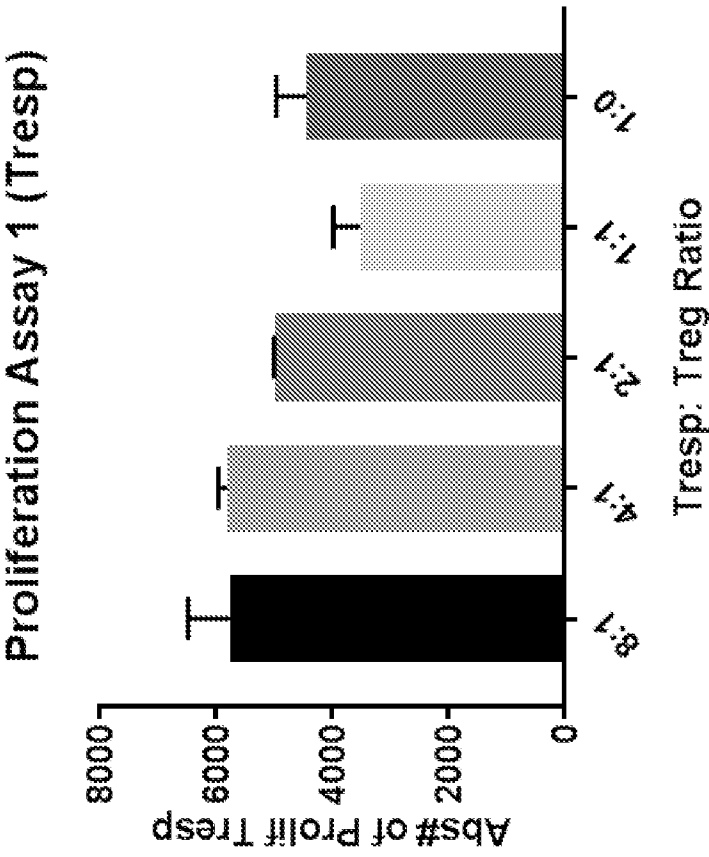


FIG. 6

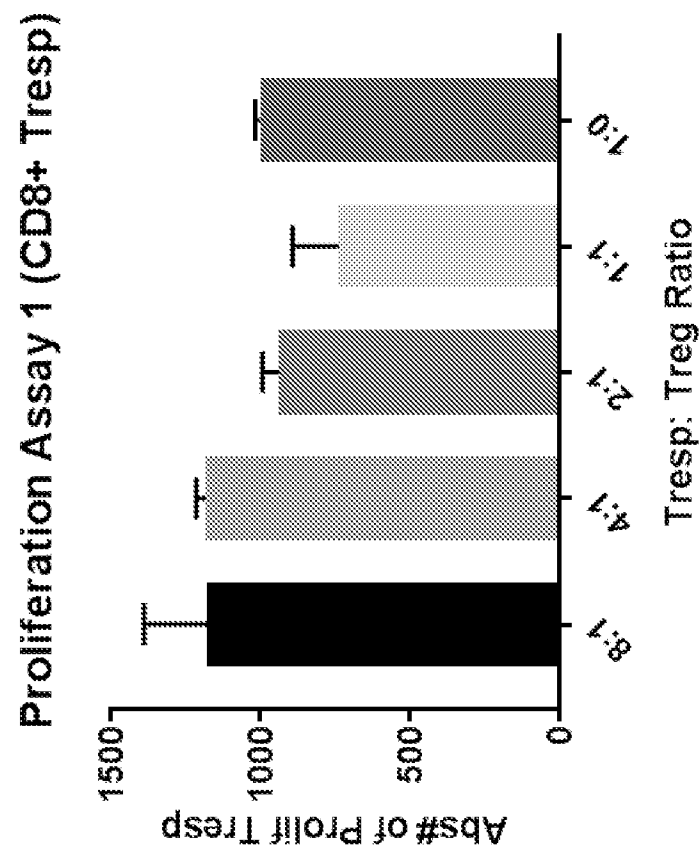


FIG. 7

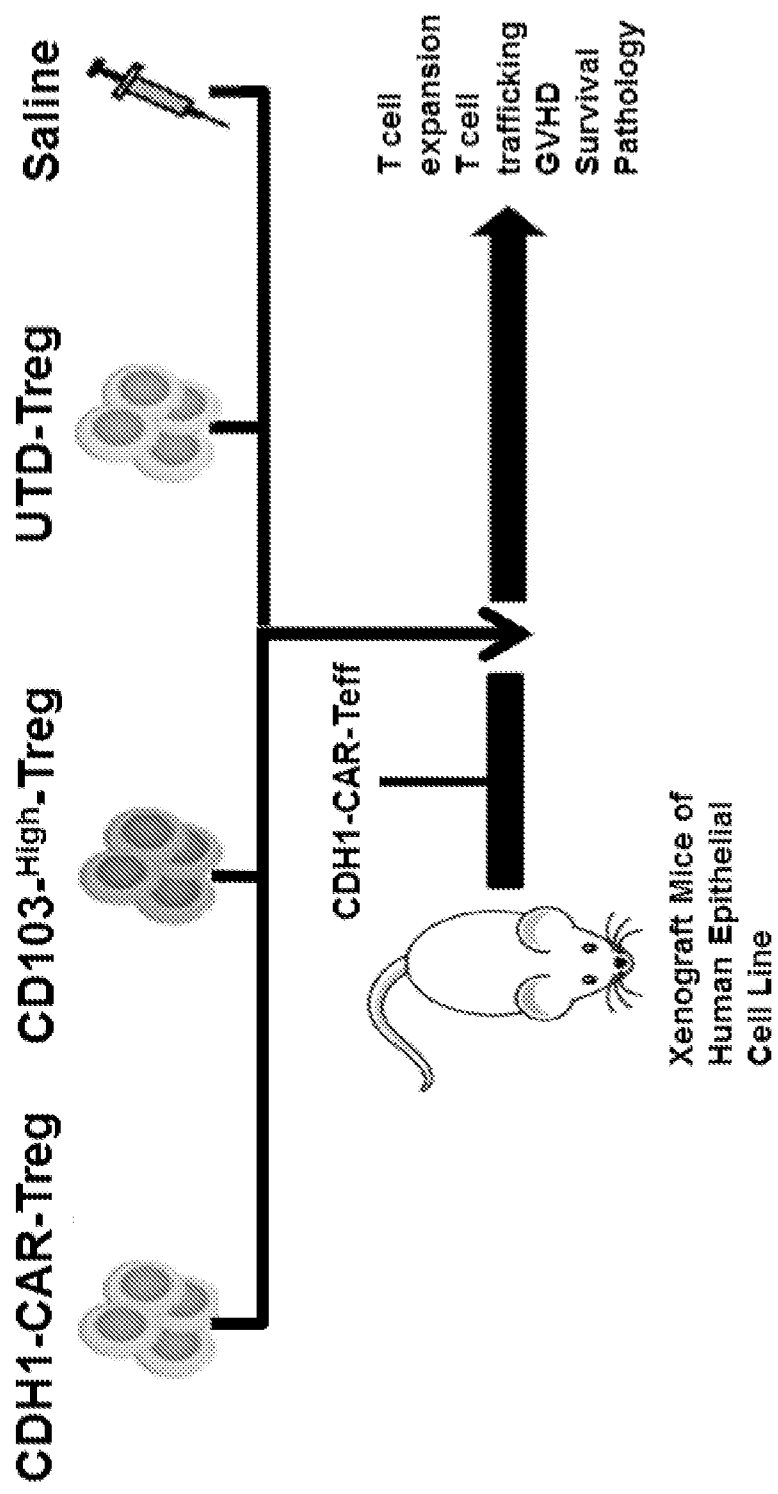


FIG. 8

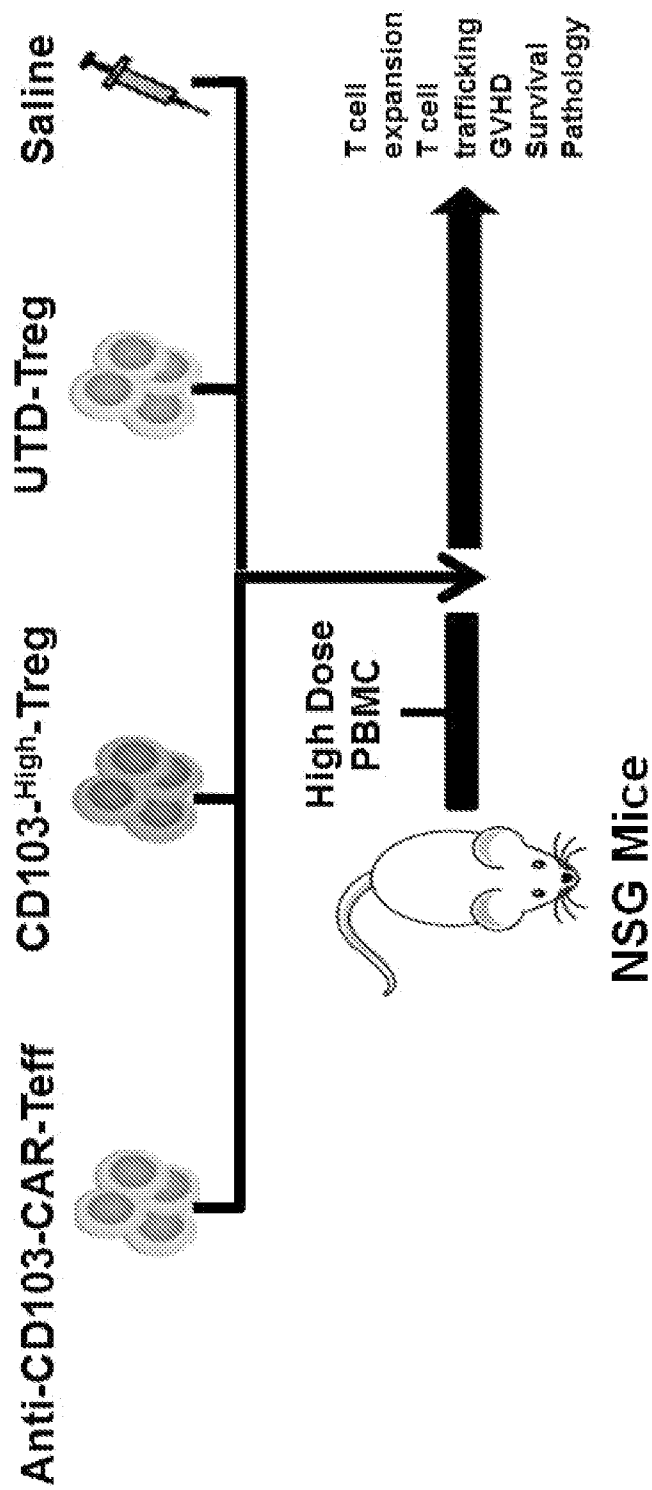


FIG. 9

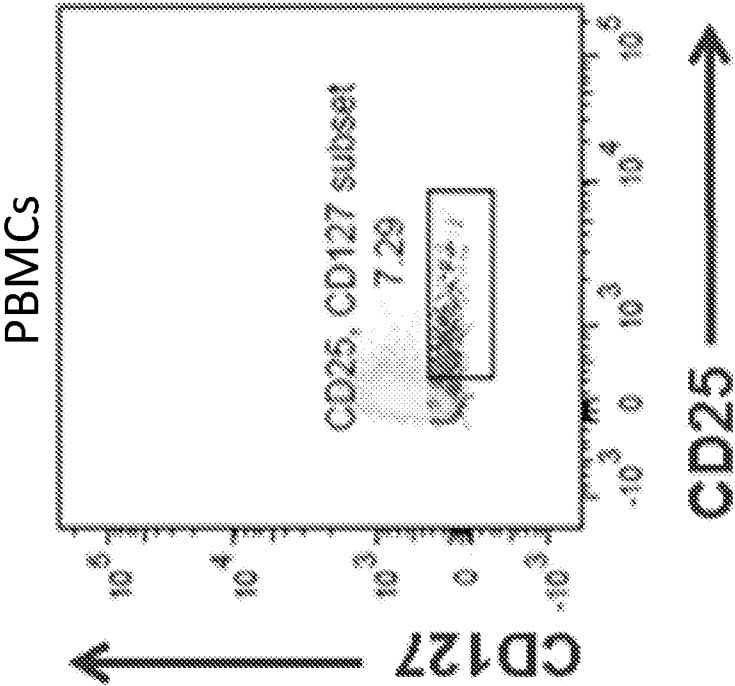


FIG. 10B

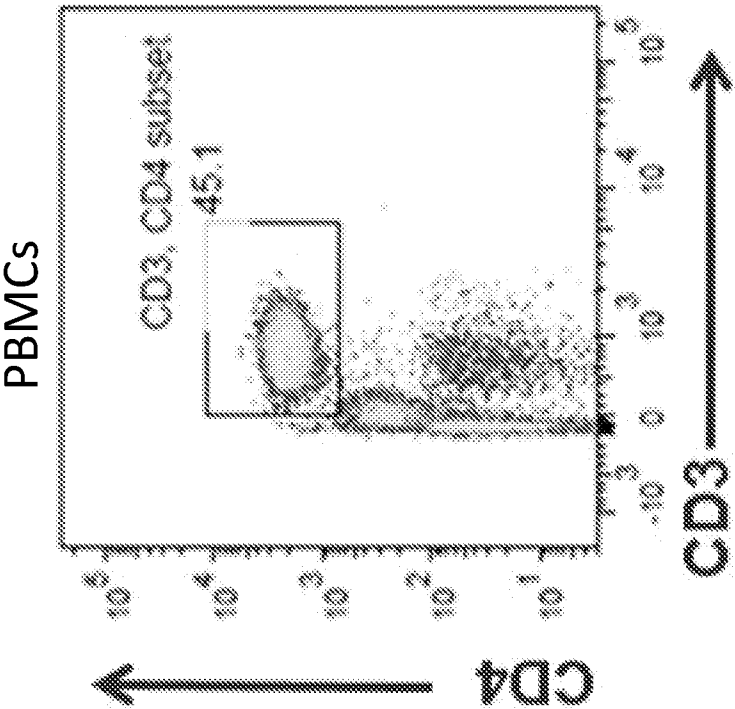


FIG. 10A

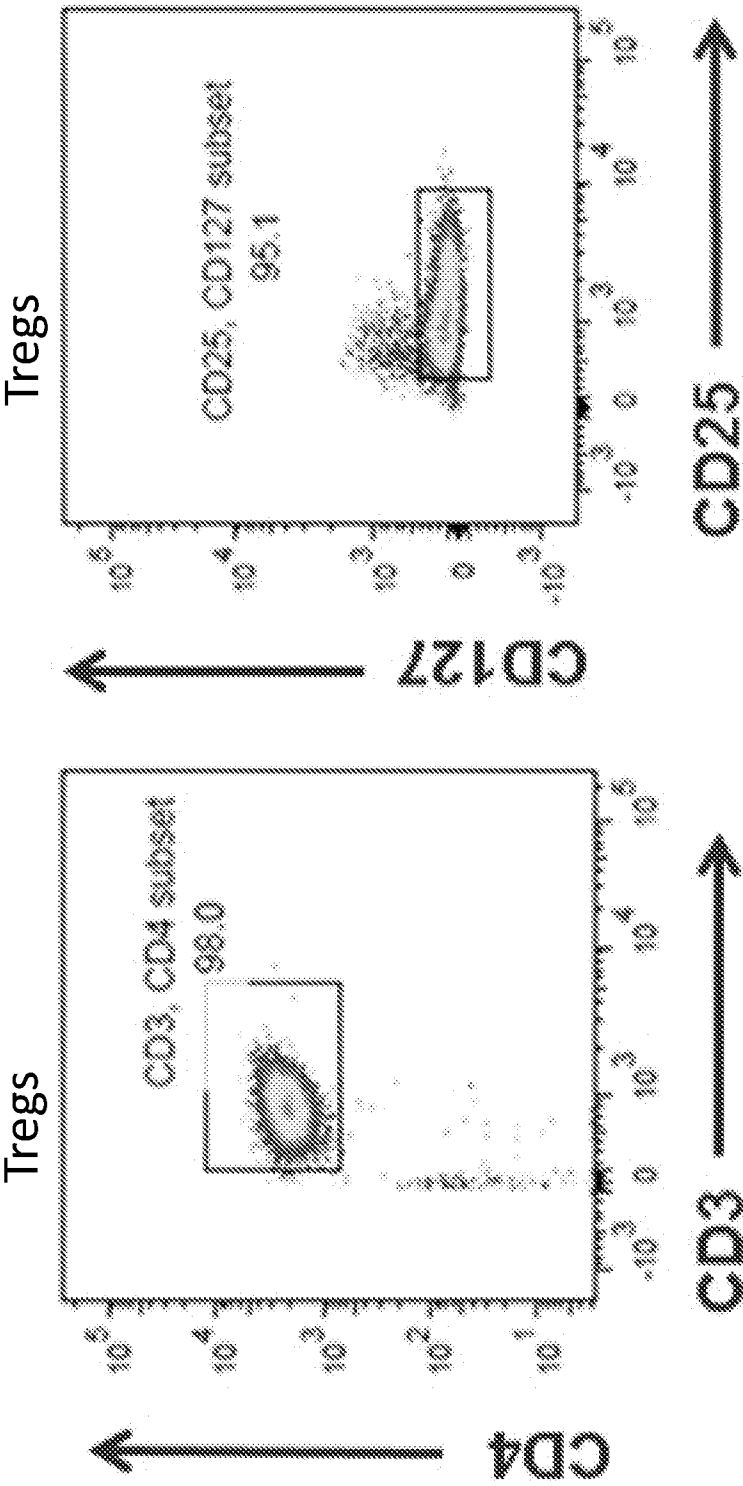


FIG. 10C

FIG. 10D

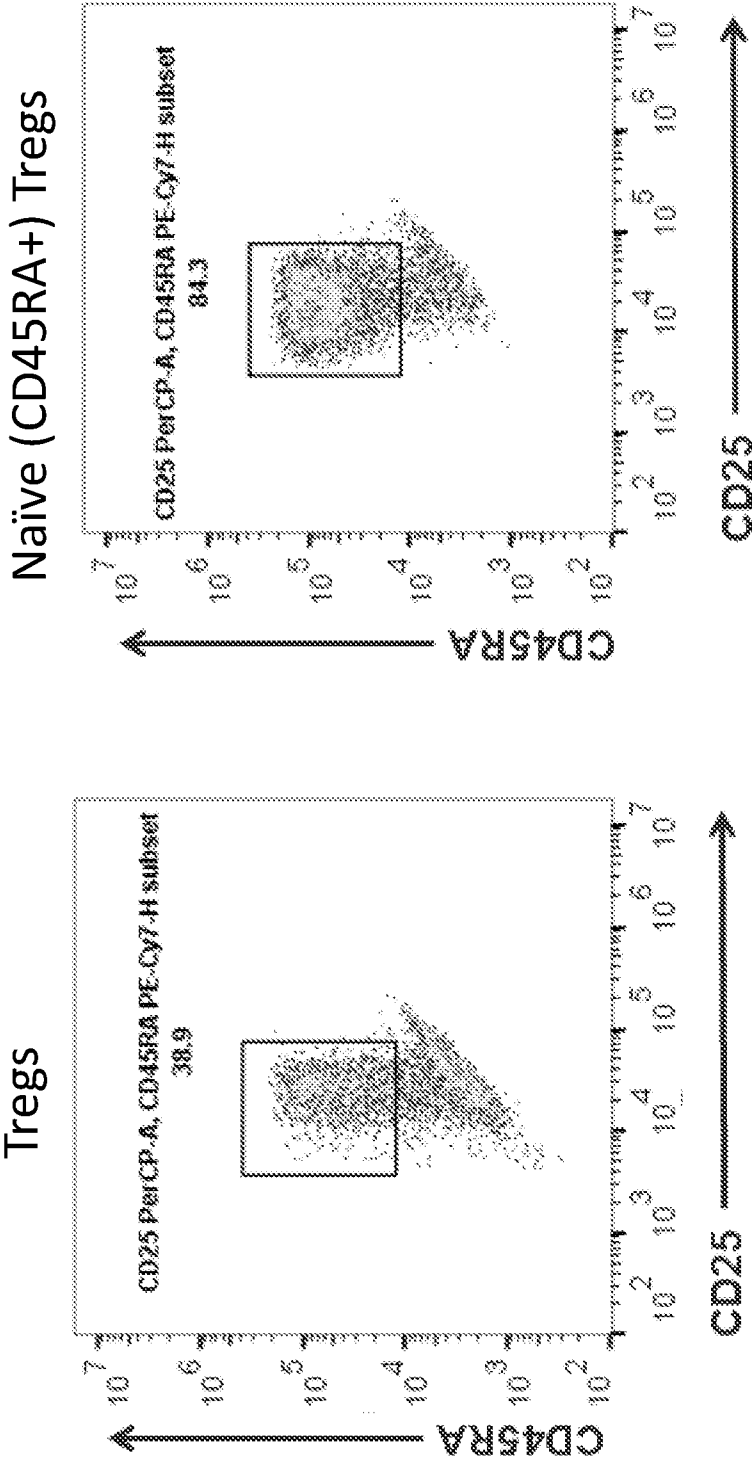


FIG. 11A

FIG. 11B

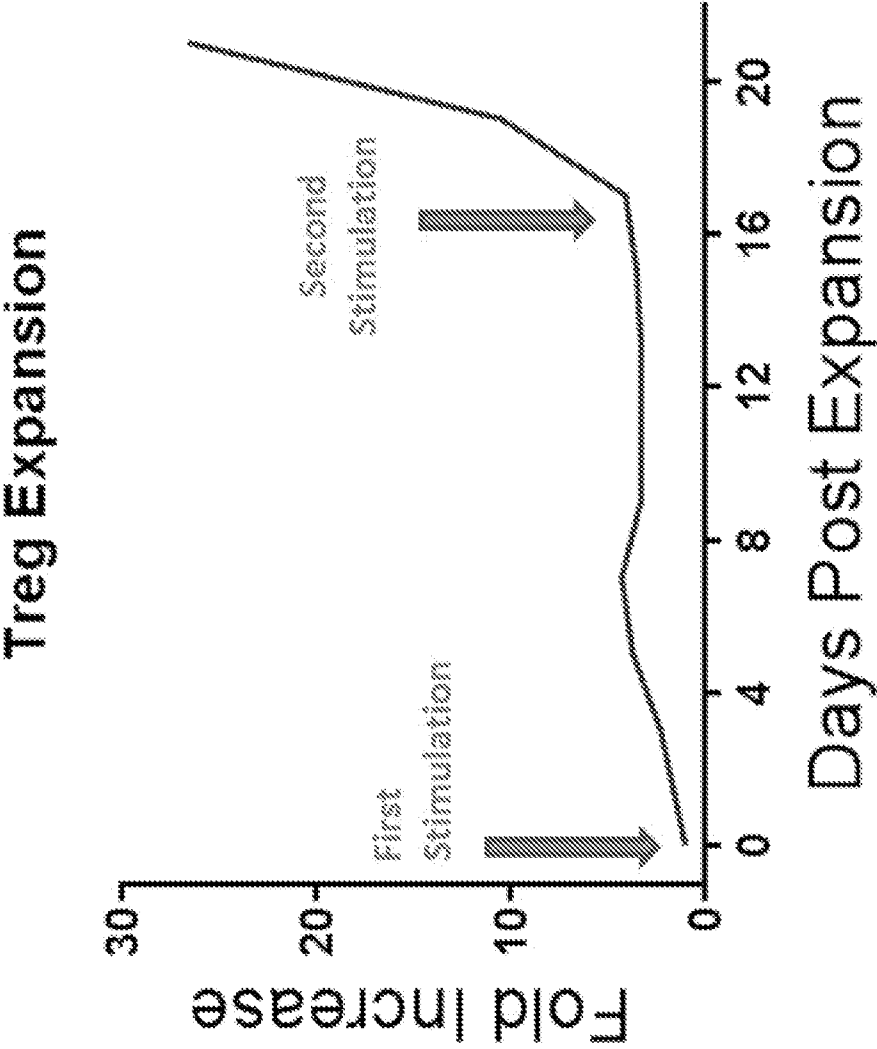


FIG. 12



FIG. 13A

FIG. 13B

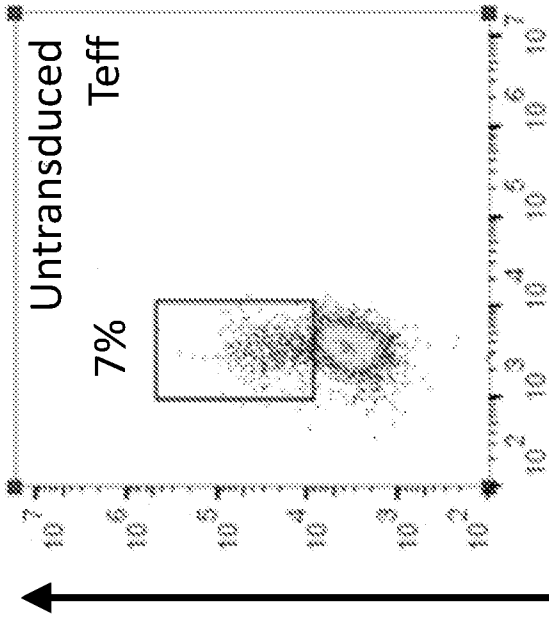


FIG. 13C

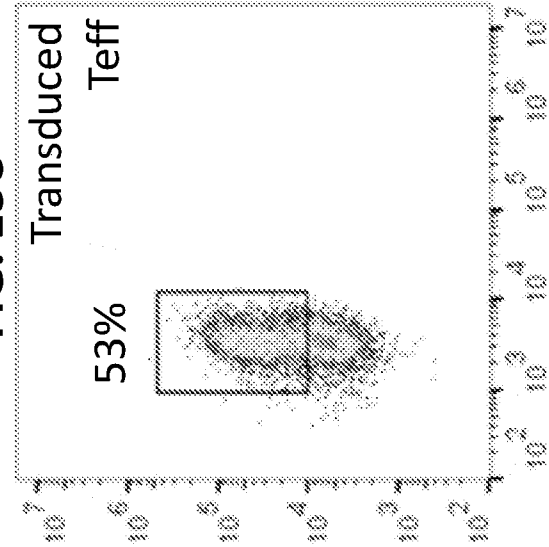


FIG. 13D

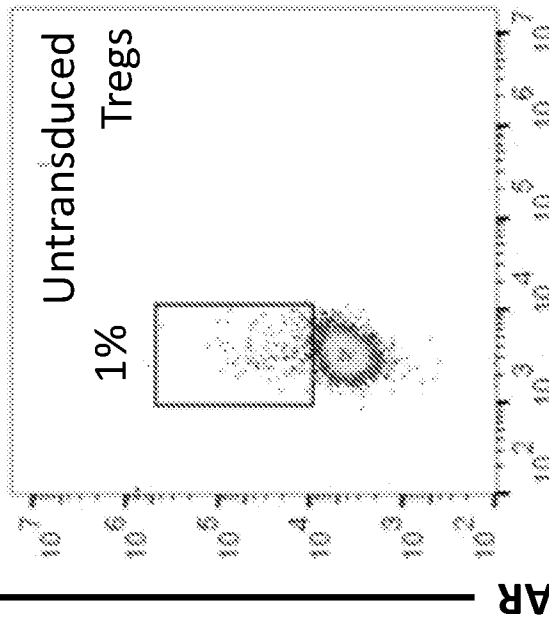
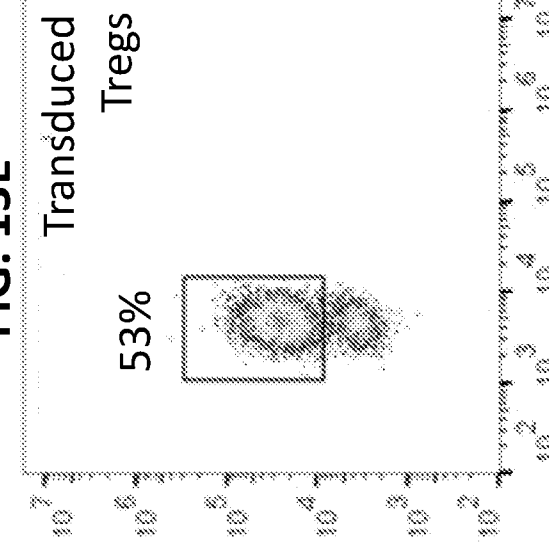


FIG. 13E



CAR

L/D

FIG. 14A

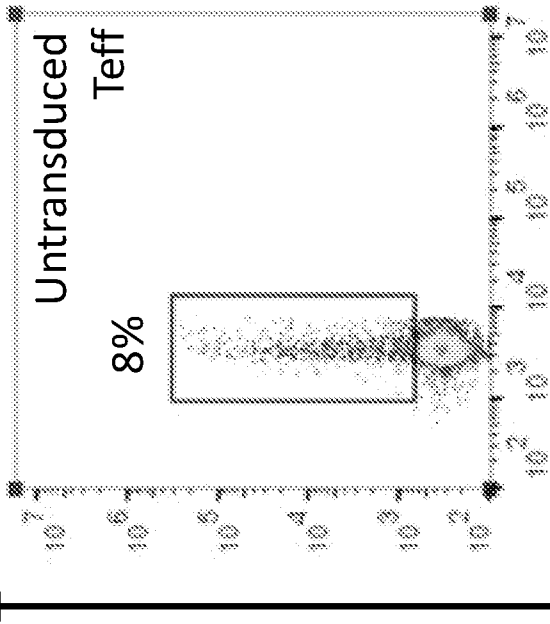


FIG. 14B

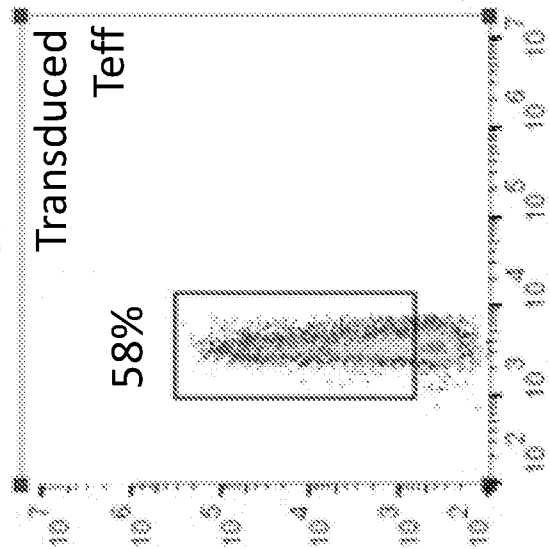


FIG. 14C

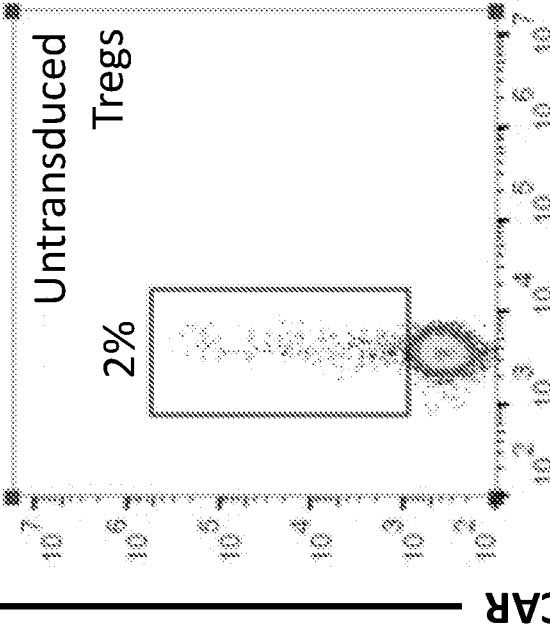
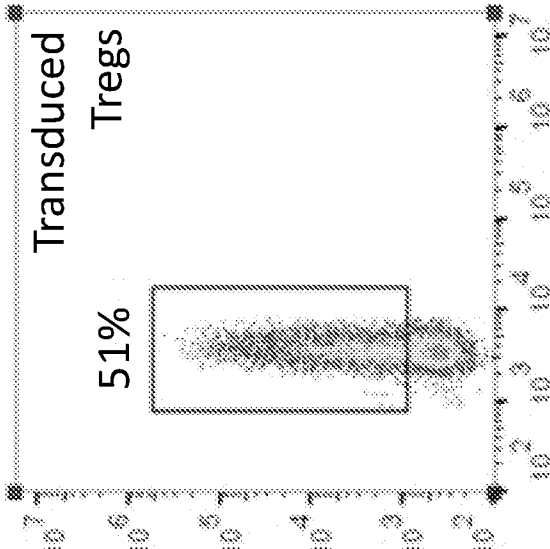


FIG. 14D



L/D

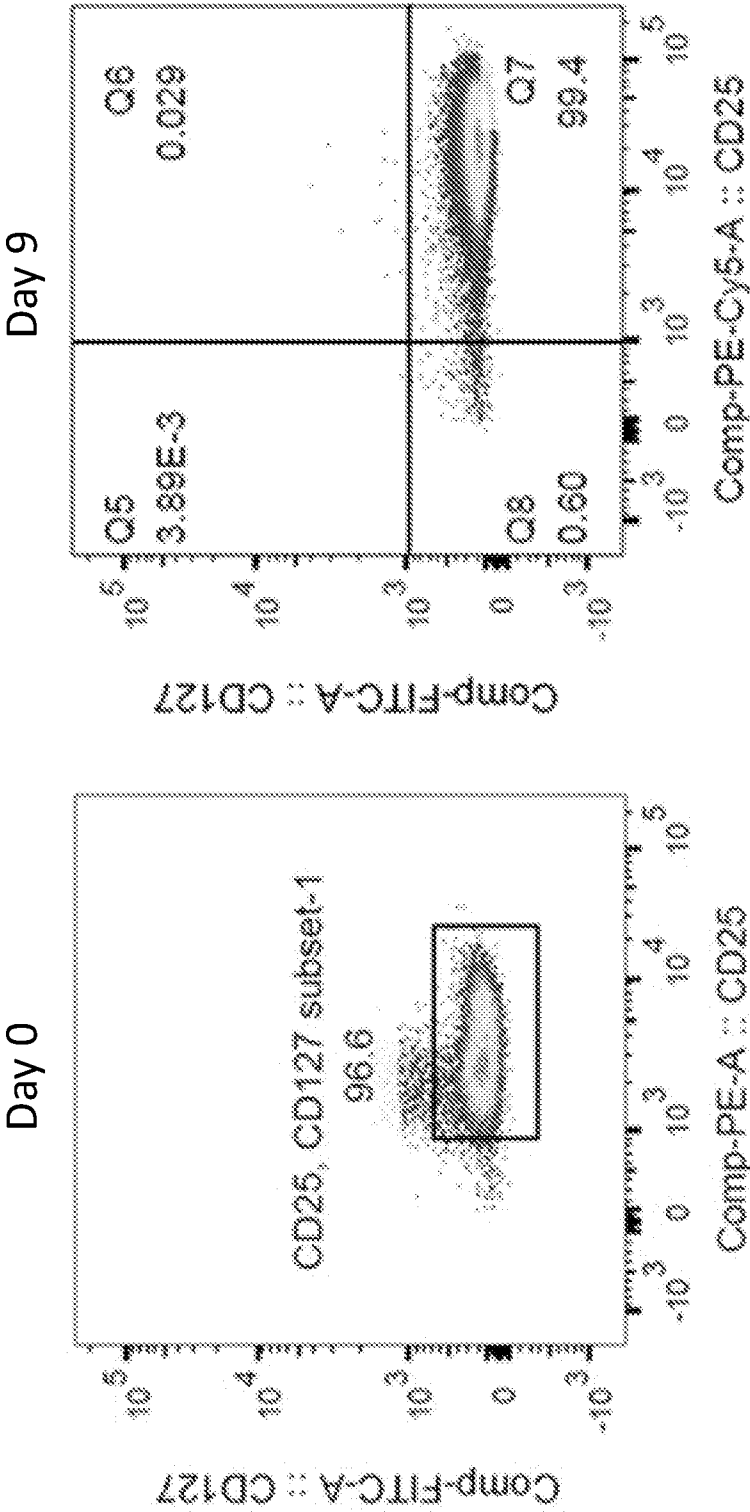
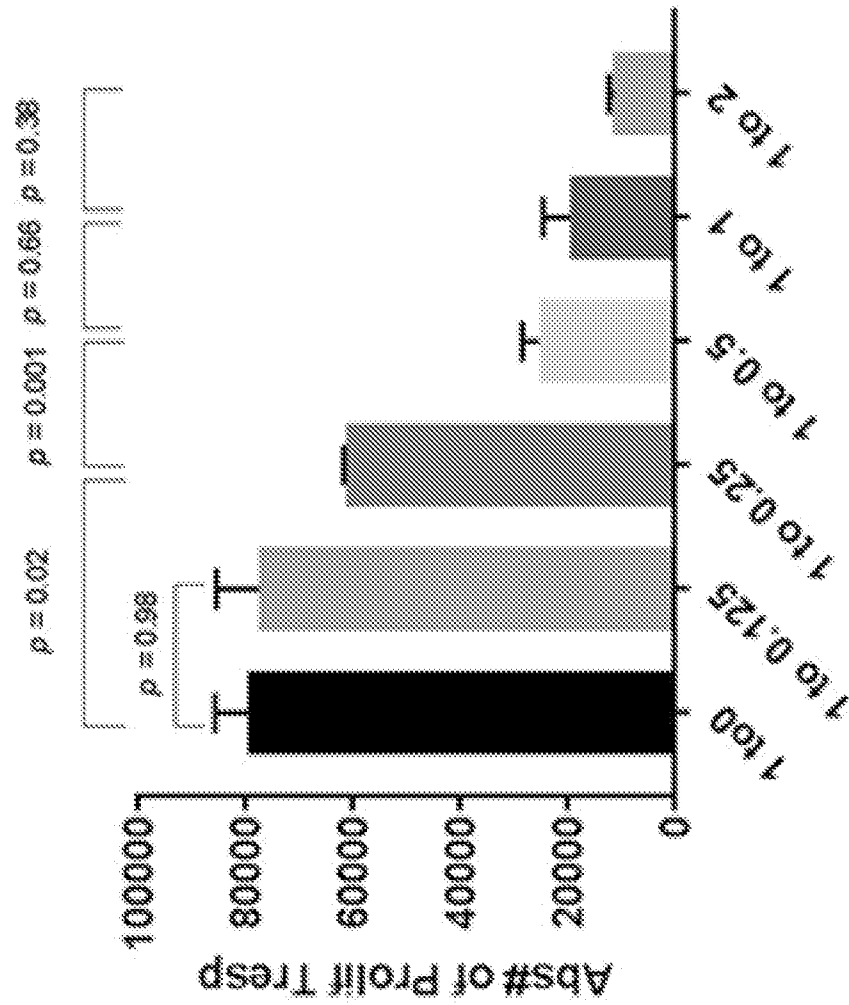


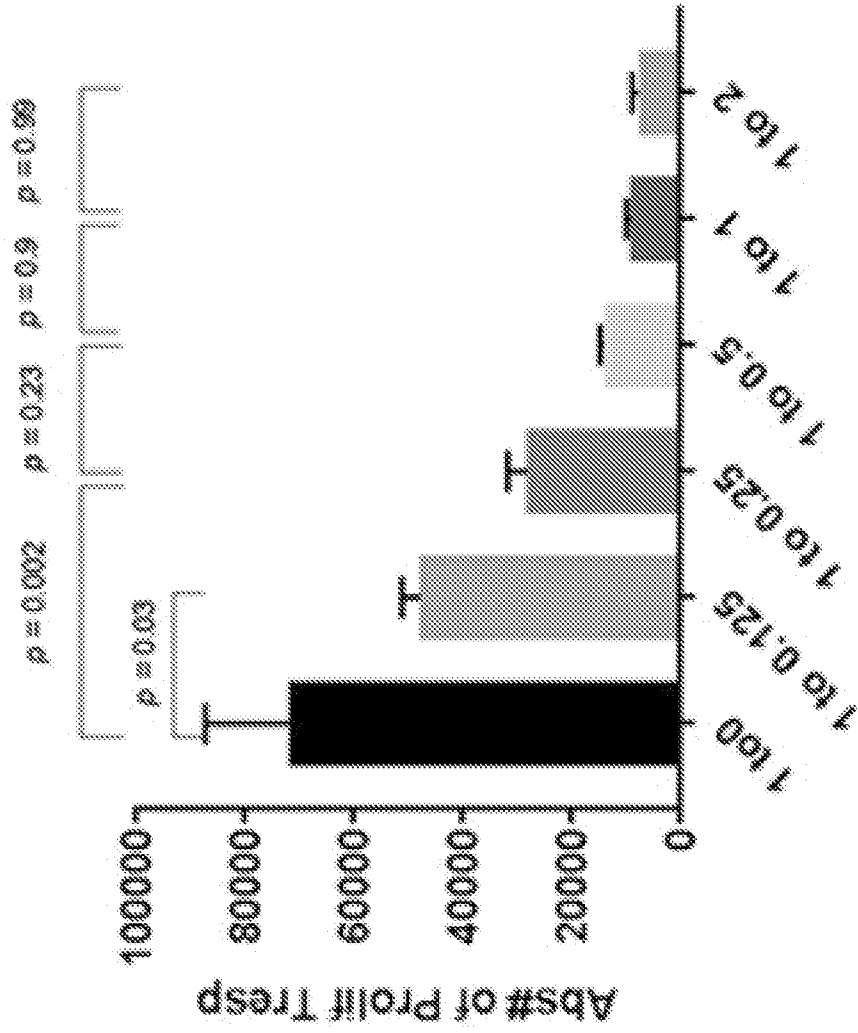
FIG. 15A

FIG. 15B



Tresp to Treg Ratio (Day 0)

FIG. 16A



Tresp to Treg Ratio (Day 9)

FIG. 16B

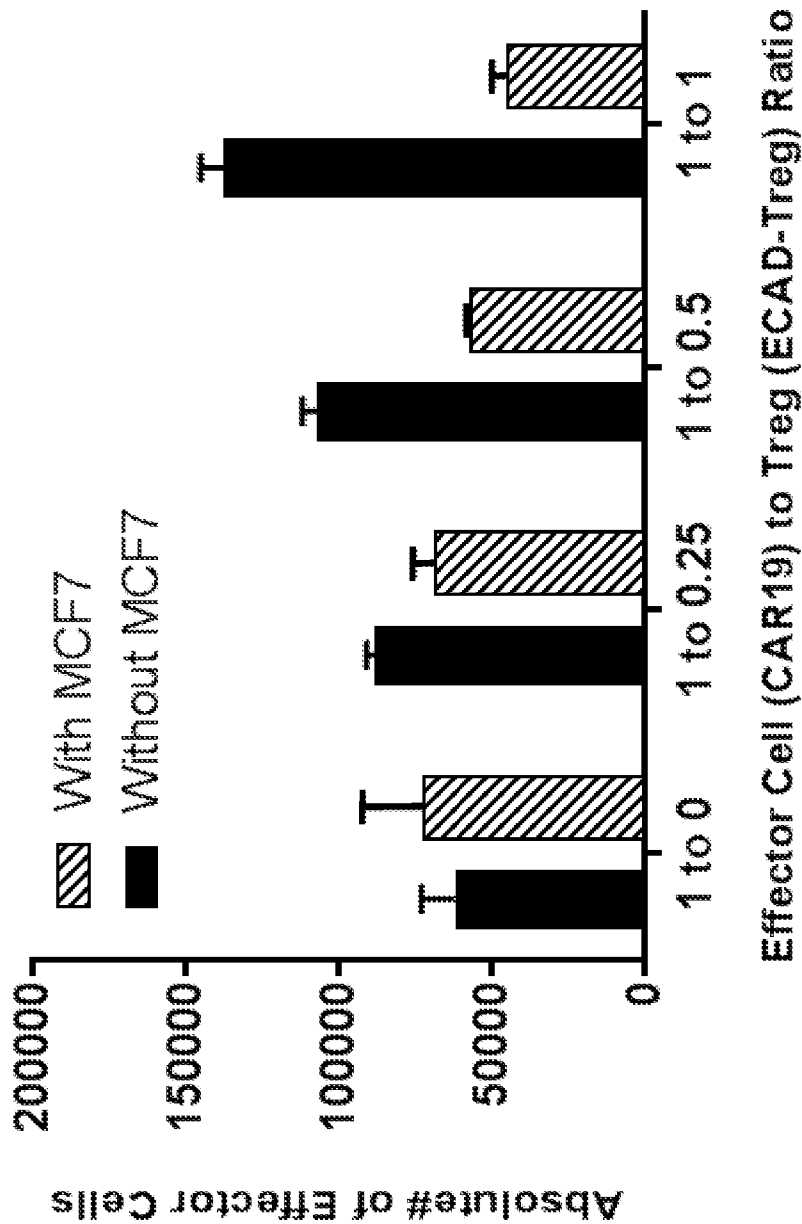


FIG. 17

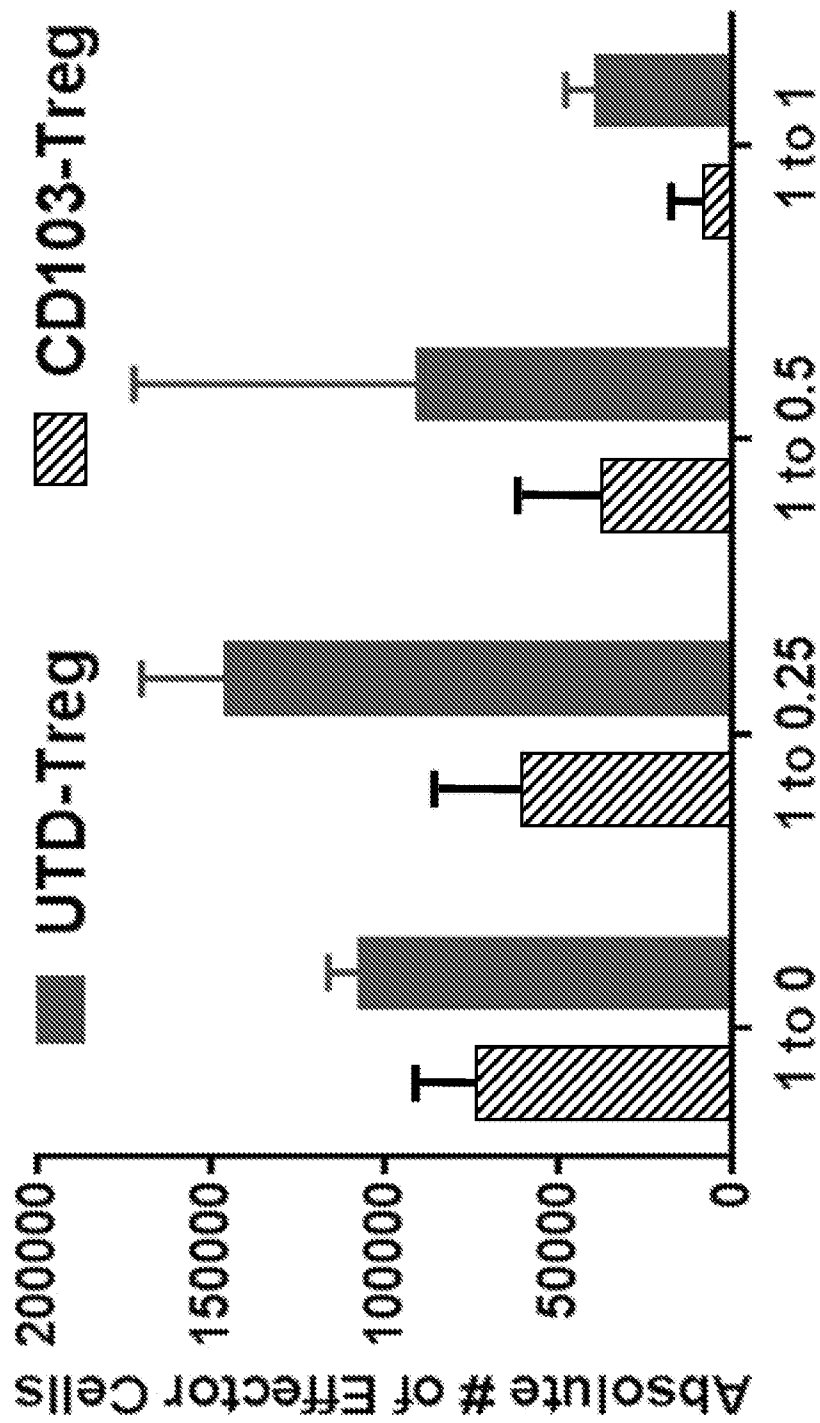
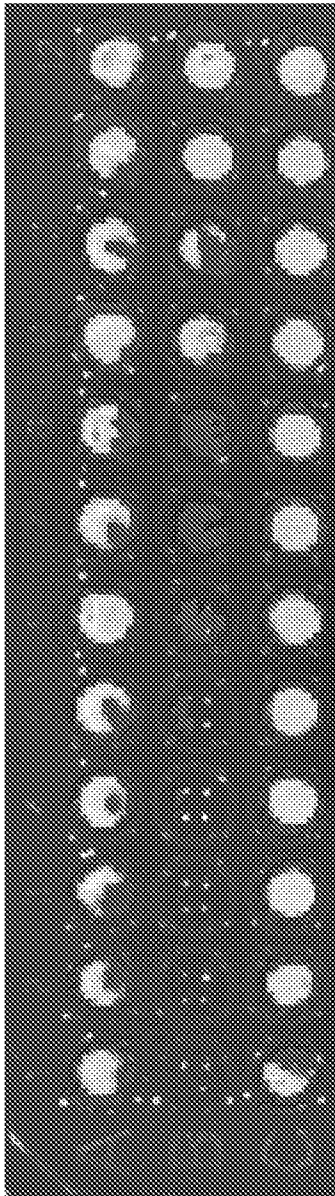


FIG. 18



UTD-Teff
CDH1-Teff
CD103-Teff

FIG. 19

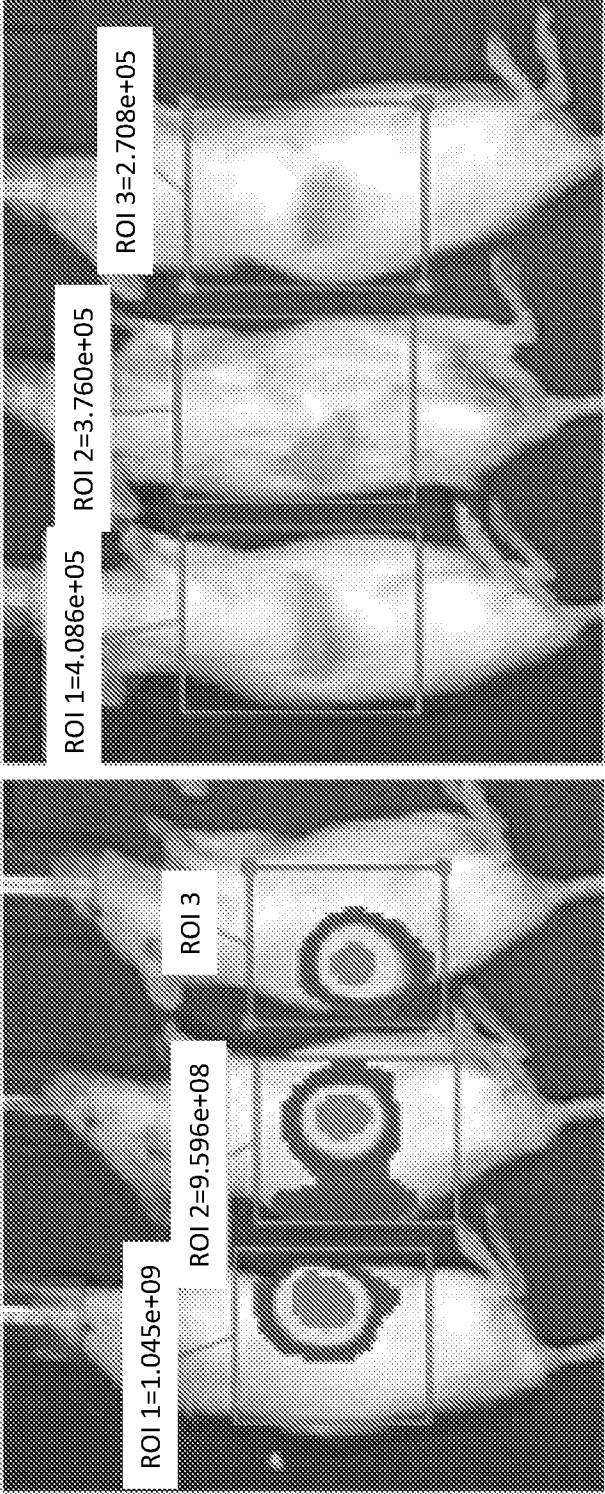


FIG. 20A

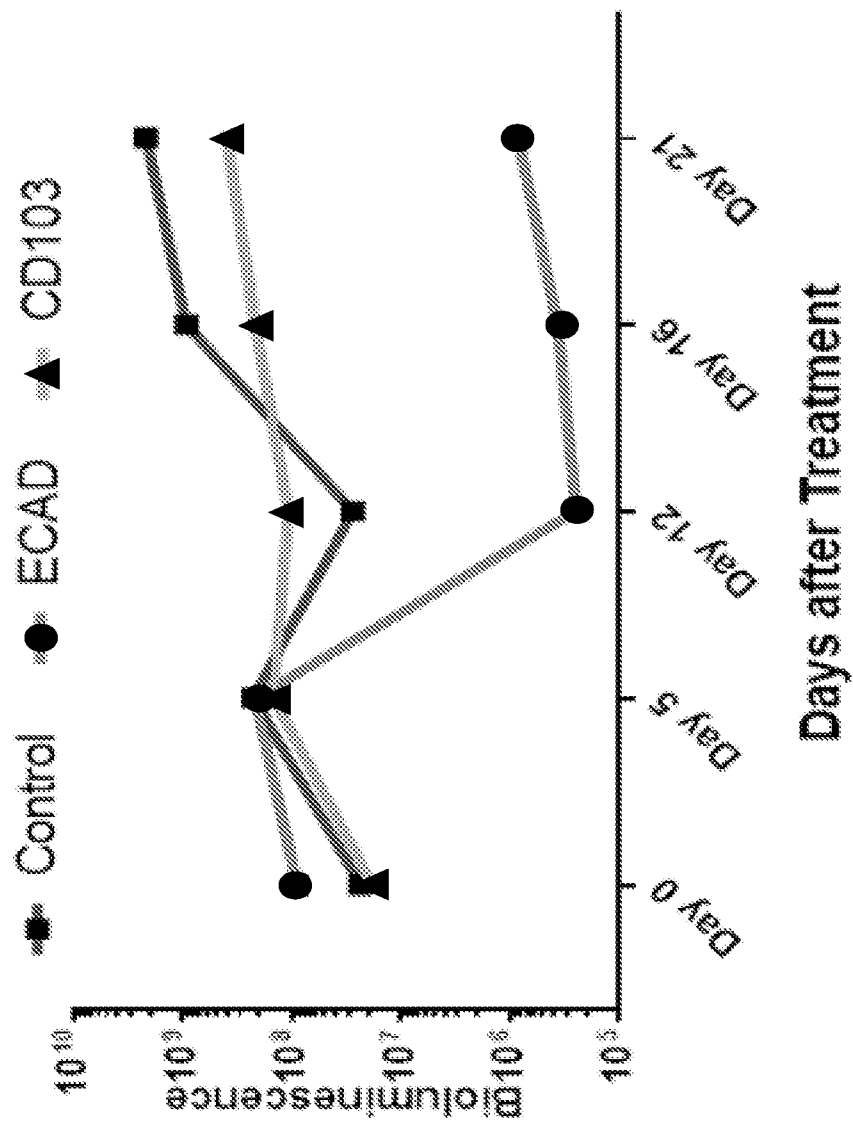
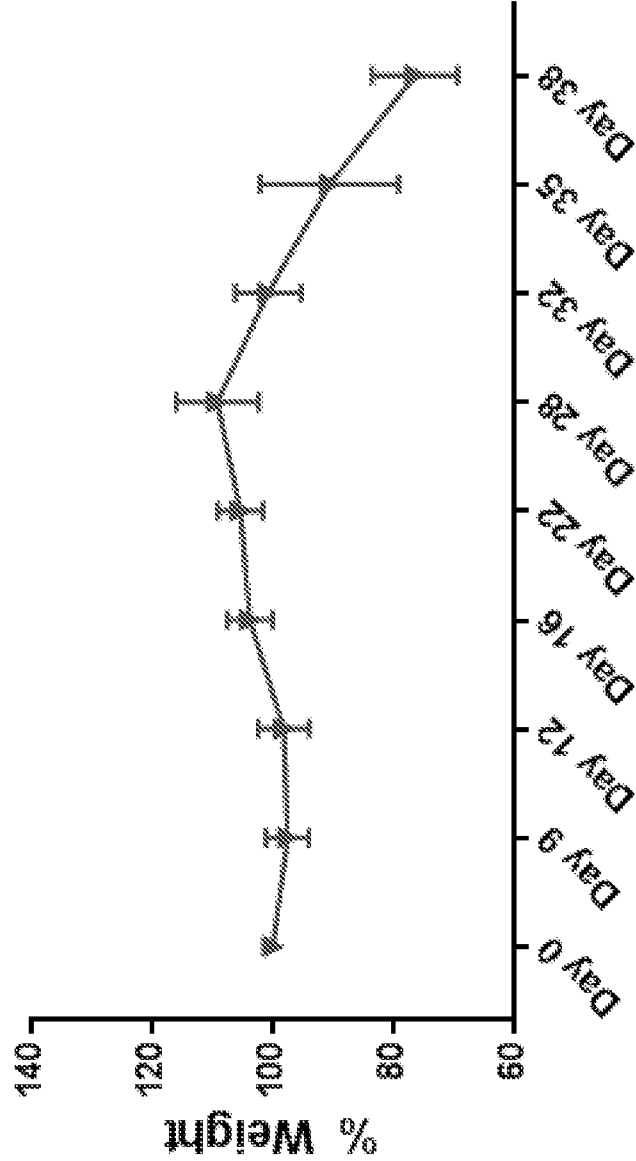


FIG. 20B



Days after injection with 10 million PBMC

FIG. 21



Light Chain Sequence

aaaattgttgacacagtctccagccaccctgtcttgtctccaggggaaagagccaccctctctgcaggcca
gtcagagtgttagcagctacttagcctggtaccaacagaaaacctggccaggctccaggctcctcatctatgatgc
atccaacagggccactggcatccagccaggctcagtggtcagtggtctgggacagacttcactctcaccatcag
cagcctagagcctgaagattttgcagtttattactgtcagcagcgtagcaactggcctcggggacgttcggccaa
gggaccaaggtggaaatcaaa (SEQ ID NO:9)

Heavy Chain Sequence

caggtgcagctggtgcagtctggagcagaggtgaaaaagcccgaggagtctctgaagatctcctgaagggttc
tggatacagctttaccagctattggatcggtgggtgcgccagatgccgggaaagccctggagtggatgggga
tcattatcctggtgactctggtcccagatacagcccgtcttccaaggccaggtcaccatctcagccgacaagtc
catcacccgcctacctgagtgagcagcctgaaggcctcggaaccccatgtattactgtgcgcgactgtc
gtataccagcacctggtaccctactactttgactactggggccagggaaccctgggtcacccgtctctca (SEQ
ID NO:10)

FIG. 22

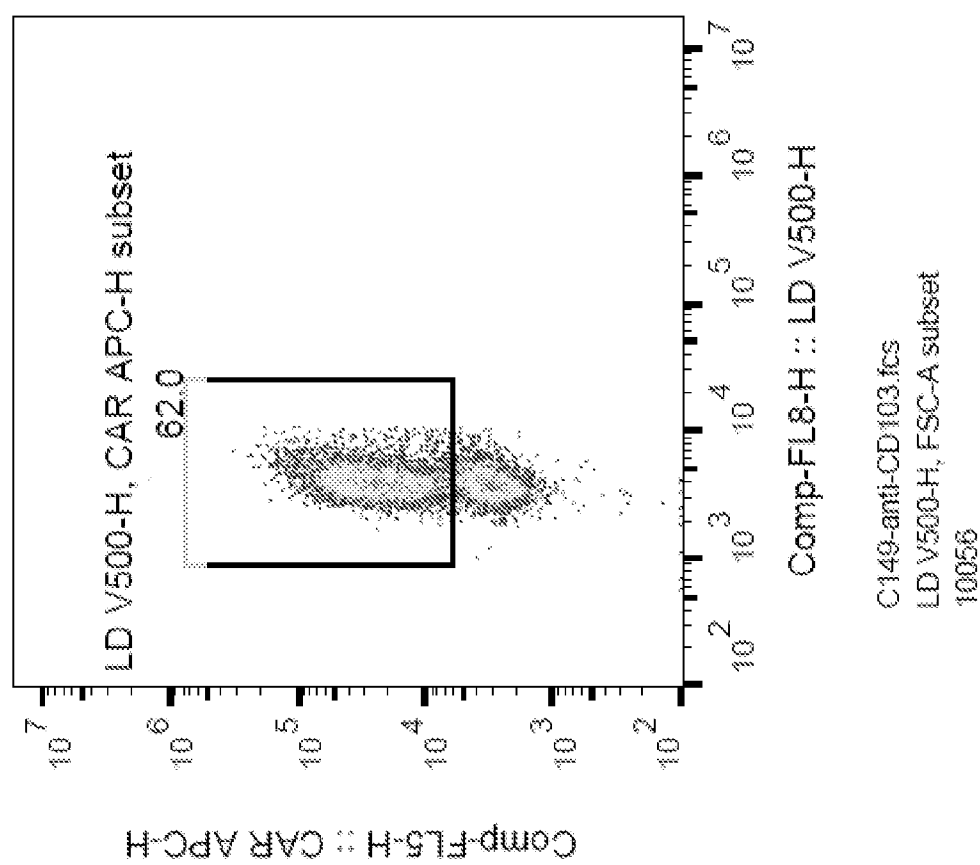


FIG. 23

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/026544

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/5025; A61K 31/506; A61K 31/713; A61K 35/17 (2019.01)

CPC - A61K 48/005; C07K 14/5412; C07K 2319/33; C12N 2740/16043 (2019.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 424/93.7; 424/93.71; 435/328; 435/455; 435/320.1; 536/23.4 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	WO 2017/143094 A1 (DANA-FARBER CANCER INSTITUTE, INC.) 24 August 2017 (24.08.2017) entire document	1, 2, 13, 14 ----- 3, 15
X -- Y	WO 2017/172981 A2 (UNIVERSITY OF SOUTHERN CALIFORNIA) 05 October 2017 (05.10.2017) entire document	24-28 ----- 29
Y	US 9,730,988 B2 (INCE et al) 15 August 2017 (15.08.2017) entire document	3, 15
Y	US 9,534,058 B2 (STULL et al) 03 January 2017 (03.01.2017) entire document	29
A	WO 2017/222593 A1 (ICELL GENE THERAPEUTICS LLC et al) 28 December 2017 (28.12.2017) entire document	1-3, 13-15, 24-30
A	US 2017/0274095 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 28 September 2017 (28.09.2017) entire document	1-3, 13-15, 24-30

☐

Further documents are listed in the continuation of Box C.

☐

See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

20 June 2019

Date of mailing of the international search report

27 JUN 2019

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Blaine R. Copenheaver

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2019/026544

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-12, 16-23, 31-36
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.