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(71) Applicant: **TAKEDA PHARMACEUTICAL COMPANY LIMITED** [JP/JP]; 1-1, Doshomachi 4-chome, Chuo-ku, Osaka-shi, Osaka 5410045 (JP).

(72) Inventors: **SHAPIRO, Gary**; c/o Millennium Pharmaceuticals, Inc., 40 Landsdowne Street, Cambridge, MA 02139 (US). **CHOUITAR, Johara**; c/o Millennium Pharmaceuticals, Inc., 40 Landsdowne Street, Cambridge, MA 02139 (US). **HE, Xingyue**; c/o Millennium Pharmaceuticals, Inc., 40 Landsdowne Street, Cambridge, MA 02139 (US). **NG, Mei, Rosa**; c/o Millennium Pharmaceuticals, Inc., 40 Landsdowne Street, Cambridge, MA 02139 (US).

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(54) Title: COMPOSITIONS OF GUANYLYL CYCLASE C (GCC) ANTIGEN BINDING AGENTS AND METHODS OF USE THEREOF



FIG. 1A

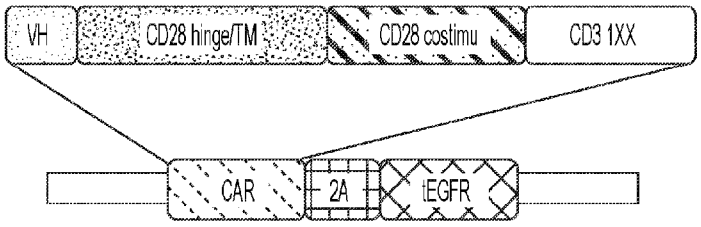


FIG. 1B

(57) Abstract: Antigen binding agents (e.g., single domain antibodies) that bind guanylyl cyclase C (GCC) and chimeric antigen receptors comprising GCC antigen binding domains are disclosed. Nucleic acids, recombinant expression vectors, host cells, antigen binding fragments, and pharmaceutical compositions comprising these antigen binding agents and fragments thereof are also disclosed. The invention also provides therapeutic methods for utilizing the antibodies and antigen-binding molecules are provided herein.

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Compositions of Guanylyl Cyclase C (GCC) Antigen Binding Agents and Methods of Use Thereof

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States Provisional Patent Application Ser. No. 63/123,331, filed on December 9, 2020, which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 19, 2021, is named MIL-005WO_SL.txt and is 140,478 bytes in size.

BACKGROUND

[0003] Guanylyl cyclase C (GCC) is a transmembrane cell surface receptor that functions in the maintenance of intestinal fluid, electrolyte homeostasis and cell proliferation, see, e.g., Carrithers et al., Proc. Natl. Acad. Sci. USA 100:3018-3020 (2003). GCC is expressed at the mucosal cells lining the small intestine, large intestine and rectum (Carrithers et al., Dis Colon Rectum 39: 171-181 (1996)). GCC expression is maintained upon neoplastic transformation of intestinal epithelial cells, with expression in all primary and metastatic colorectal tumors (Carrithers et al., Dis Colon Rectum 39: 171-181 (1996); Buc et al. Eur J Cancer 41: 1618-1627 (2005); Carrithers et al., Gastroenterology 107: 1653-1661 (1994)). There is a need for novel and improved methods for targeting GCC.

SUMMARY

[0004] Disruptions in GCC signaling pathways have been linked to numerous gastrointestinal disorders including colorectal cancer. The present invention provides, among

other things, novel anti-GCC antigen binding molecules (e.g., single domain antibodies (sdAb)) and chimeric antigen receptors (CARs) that include anti-GCC antigen binding domains (e.g., sdAb). In addition, the present invention provides host cells (e.g., T cells) expressing the CARs, and nucleic acid molecules encoding the CAR or anti-GCC antigen binding molecule. The CAR cells comprise an anti-GCC CAR molecules expressed on the cell surface. In some embodiments, the CARs exhibit a high surface expression on transduced T cells, with a high degree of cytolysis and transduced T cell expansion and persistence *in vivo*. Methods of using the disclosed CARs, host cells, and nucleic acid molecules are also provided, for example, to treat a cancer in a subject.

[0005] In one aspect, the present invention provides an anti-guanylyl cyclase C (GCC) chimeric antigen receptor (CAR) comprising an extracellular antigen binding domain that binds to guanylyl cyclase C (GCC), a transmembrane domain, and at least one intracellular signaling domain.

[0006] In some embodiments, the anti-GCC CAR comprises an antigen binding domain comprising: a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of HYYWS (HCDR1) (SEQ ID NO: 8), RIYPSGSTSYNPSLKS (HCDR2) (SEQ ID NO: 11) and DRSTGWSEWNSDL (HCDR3) (SEQ ID NO: 16); a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMS (HCDR1) (SEQ ID NO: 9), KIRHDGGEKYYVDSVKG (HCDR2) (SEQ ID NO: 12) and DYTRDV (HCDR3) (SEQ ID NO: 17); a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIKYDGSEKYYADSVKG (HCDR2) (SEQ ID NO: 13) and DYNKDY (HCDR3) (SEQ ID NO: 18); a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIRHDGGEKYYPDSVKG (HCDR2) (SEQ ID NO: 14) and DYNKDL (HCDR3) (SEQ ID NO: 19) or a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIRHDGGEKYYADSVKG (HCDR2) (SEQ ID NO: 15) and DYNKDY (HCDR3) (SEQ ID NO: 18).

[0007] In some embodiments, the antigen binding domain of the anti-GCC CAR comprises a heavy chain variable region (VH) that is at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 20.

[0008] In some embodiments, the antigen binding domain of the anti-GCC CAR comprises an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 21; an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 26; an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 27; or an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 28.

[0009] In some embodiments, the antigen binding domain of the anti-GCC CAR comprises an immunoglobulin variable heavy chain only anti-GCC antigen binding domain.

[0010] In some embodiments, the extracellular anti-GCC antigen binding domain of the anti-GCC CAR is preceded by a leader nucleotide sequence encoding a leader peptide.

[0011] In some embodiments, the leader peptide comprises SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 42.

[0012] In some embodiments, the anti-GCC CAR, further comprising a hinge domain.

[0013] In some embodiments, the hinge domain is comprises a hinge domain of CD28.

[0014] In some embodiments, the CD28 hinge domain comprises SEQ ID NO: 29.

[0015] In some embodiments, the anti-GCC CAR comprises a hinge domain fused to the transmembrane domain.

[0016] In some embodiments, the transmembrane domain comprises a transmembrane domain of a protein selected from the alpha, beta or zeta chain of the T-cell receptor, CD8, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD83, CD86, CD134, CD137, CD154, and TNFRSF19, and any combination thereof.

[0017] In some embodiments, the transmembrane domain comprises a CD28 transmembrane domain.

[0018] In some embodiments, the CD28 transmembrane domain comprises SEQ ID NO: 30.

[0019] In some embodiments, the at least one intracellular signaling domain comprises a costimulatory domain and a primary signaling domain.

[0020] In some embodiments, the costimulatory domain comprises a functional signaling domain of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, and 4-1BB (CD137), or any combination thereof.

[0021] In some embodiments, the costimulatory domain comprises a functional signaling domain of CD28.

[0022] In some embodiments, the CD28 costimulatory domain comprises SEQ ID NO: 32.

[0023] In some embodiments, the primary signaling domain comprises a CD3zeta signaling domain.

[0024] In some embodiments, the CD3 zeta signaling domain comprises SEQ ID NO: 33.

[0025] In one aspect, the anti-GCC CAR comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 47-52.

[0026] In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 47. In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 48. In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 49. In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 50. In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 51. In some

embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 52.

[0027] In one aspect, the present invention provides an isolated polynucleotide encoding an anti-GCC CAR described herein.

[0028] In some embodiments, the isolated polynucleotide encoding an anti-GCC CAR described herein, further comprising a truncated sequence of epidermal growth factor receptor (tEGFR).

[0029] In some embodiments, the tEGFR comprises a nucleic acid sequence that encodes an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 43.

[0030] In some embodiments, the tEGFR comprises a nucleic acid sequence that encodes an amino acid sequence that is identical to SEQ ID NO: 43.

[0031] In some embodiments, the isolated polynucleotide encoding an anti-GCC CAR described herein, further comprises a furin recognition site and downstream 2A self-cleaving peptide sequence, designed for simultaneous bicistronic expression of the tag sequence and the CAR sequence.

[0032] In some embodiments, the 2A self-cleaving peptide is selected from F2A, P2A, E2A and T2A.

[0033] In some embodiments, the 2A self-cleaving peptide is P2A.

[0034] In one aspect, the present invention provides a vector comprising the isolated polynucleotide encoding an anti-GCC CAR described herein.

[0035] In some embodiments, the vector is an adenoviral vector, an adenovirus-associated vector, a DNA vector, a lentiviral vector, a plasmid, a retroviral vector, or an RNA vector. In some embodiments, the vector is a retroviral vector.

[0036] In one aspect, the present invention provides a cell the isolated polynucleotide encoding an anti-GCC CAR described herein.

[0037] In some embodiments, the cell expresses an anti-GCC CAR described herein

[0038] In some embodiments, the cell is a T cell, an allogeneic T cell, an autologous T cell, or a tumor-infiltrating lymphocyte (TIL).

[0039] In one aspect, the present invention provides a pharmaceutical composition comprising a population of the cells expressing or capable of expressing an anti-GCC CAR described herein. In some embodiments, the pharmaceutical composition comprises a population of cells, wherein greater than 70%, 80%, 90%, or 95% of the cells in the population express the anti-GCC CAR.

[0040] In one aspect, the present invention provides a method of treating a cancer comprising administering a pharmaceutical composition comprising an anti-GCC CAR described herein to a subject in need of treatment.

[0041] In some embodiments, the cancer is selected from gastrointestinal cancer, colorectal cancer, colorectal adenocarcinoma, colorectal leiomyosarcoma, colorectal lymphoma, colorectal melanoma, a colorectal neuroendocrine tumor, metastatic colon cancer, stomach cancer, gastric adenocarcinoma, gastric lymphoma, gastric sarcoma, esophageal cancer, squamous cell carcinoma, adenocarcinoma of the esophagus, or pancreatic cancer.

[0042] In some embodiments, the cancer is a gastrointestinal cancer.

[0043] In some embodiments, the gastrointestinal cancer is colon cancer, colorectal cancer, stomach cancer, or esophageal cancer.

[0044] In one aspect, the present invention provides a method of reducing tumor growth or tumor size by administering a pharmaceutical composition comprising an anti-GCC CAR described herein.

[0045] In one aspect, the present invention provides a guanylyl cyclase C (GCC) binding agent comprising a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of HYYWS (HCDR1) (SEQ ID NO: 8), RIYPSGSTSYNPSLKS (HCDR2) (SEQ ID NO: 11) and DRSTGWSEWNSDL (HCDR3) (SEQ ID NO: 16).

[0046] In one aspect, the present invention provides a guanylyl cyclase C (GCC) binding agent comprising a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMS (HCDR1) (SEQ ID NO: 9),

KIRHDGGEKYYVDSVKG (HCDR2) (SEQ ID NO: 12) and DYTRDV (HCDR3) (SEQ ID NO: 17).

[0047] In one aspect, the present invention provides a guanylyl cyclase C (GCC) binding agent comprising a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIKYDGSEKYYADSVKG (HCDR2) (SEQ ID NO: 13) and DYNKDY (HCDR3) (SEQ ID NO: 18).

[0048] In one aspect, the present invention provides a guanylyl cyclase C (GCC) binding agent comprising a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIRHDGGEKYYPDSVKG (HCDR2) (SEQ ID NO: 14) and DYNKDL (HCDR3) (SEQ ID NO: 19)

[0049] In one aspect, the present invention provides a guanylyl cyclase C (GCC) binding agent comprising a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIRHDGGEKYYADSVKG (HCDR2) (SEQ ID NO: 15) and DYNKDY (HCDR3) (SEQ ID NO: 18).

[0050] In some embodiments, the GCC binding agent comprises an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 20.

[0051] In some embodiments, the GCC binding agent comprises an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 21.

[0052] In some embodiments, the GCC binding agent comprises an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 26.

[0053] In some embodiments, the GCC binding agent comprises an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 27.

[0054] In some embodiments, the GCC binding agent comprises an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 28.

[0055] In one aspect, the present invention provides a guanylyl cyclase C (GCC) binding agent comprising an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 20.

[0056] In one aspect, the present invention provides a guanylyl cyclase C (GCC) binding agent comprising an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 21.

[0057] In one aspect, the present invention provides a guanylyl cyclase C (GCC) binding agent comprising an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 26.

[0058] In one aspect, the present invention provides a guanylyl cyclase C (GCC) binding agent comprising an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 27.

[0059] In one aspect, the present invention provides a guanylyl cyclase C (GCC) binding agent comprising an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 28.

[0060] In some embodiments, the GCC binding agent comprises a VH region comprising an amino acid sequence that is at least 95% identical to any one of SEQ ID Nos: 1, 20, 21, 26, 27, or 28.

[0061] In some embodiments, the GCC binding agent comprises a VH region comprises an amino acid sequence that is identical to any one of SEQ ID NOs:1, 20, 21, 26, 27, or 28.

[0062] In some embodiments, the GCC binding agent is selected from the group consisting of an IgA antibody, IgG antibody, IgE antibody, IgM antibody, bi- or multi-specific antibody, Fab fragment, Fab' fragment, F(ab')2 fragment, Fd' fragment, Fd fragment, isolated CDRs or sets thereof; single-chain variable fragment (scFv), polypeptide-Fc fusion, single domain antibody (sdAb), VH, camelid antibody; masked antibody, Small Modular

ImmunoPharmaceuticals ("SMIPsTM"), single chain, Tandem diabody, VHHs, Anticalin, Nanobody, humabody, minibodies, BiTE, ankyrin repeat protein, DARPIN, Avimer, DART, TCR-like antibody, Adnectin, Affilin, Trans-body; Affibody, TrimerX, MicroProtein, Fynomer, Centyrin; and KALBITOR.

[0063] In some embodiments, the GCC binding agent is a single domain antibody (sdAb).

[0064] In some embodiments, the GCC binding agent is a heavy chain only antibody (VH).

[0065] In some embodiments, the GCC binding agent binds GCC with a KD between about 0.3 nanomolar (nM) and about 10 nM.

[0066] In some embodiments, the GCC binding agent binds GCC on target cells with an EC50 between about 0.5 nM and about 8 nM.

[0067] In one aspect, the present invention provides a method of treating a cancer comprising administering the GCC binding agent described herein to a subject in need of treatment.

[0068] In some embodiments, the cancer is selected from gastrointestinal cancer, colorectal cancer, colorectal adenocarcinoma, colorectal leiomyosarcoma, colorectal lymphoma, colorectal melanoma, a colorectal neuroendocrine tumor, metastatic colon cancer, stomach cancer, gastric adenocarcinoma, gastric lymphoma, gastric sarcoma, esophageal cancer, squamous cell carcinoma, adenocarcinoma of the esophagus, or pancreatic cancer.

[0069] In some embodiments, the cancer is a gastrointestinal cancer.

[0070] In some embodiments, the gastrointestinal cancer is colon cancer, colorectal cancer, stomach cancer, or esophageal cancer.

[0071] In one aspect, the present invention provides a pharmaceutical composition comprising a GCC binding agent and a pharmaceutically acceptable carrier, wherein the GCC binding agent comprises: a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of HYYWS (HCDR1) (SEQ ID NO: 8), RIYPSGSTSYNPSLKS (HCDR2) (SEQ ID NO: 11) and DRSTGWSEWNSDL (HCDR3) (SEQ ID NO: 16); a heavy chain variable region (VH) with complementarity determining

region (CDR) sequences of RYWMS (HCDR1) (SEQ ID NO: 9), KIRHDGGEKYYVDSVKG (HCDR2) (SEQ ID NO: 12) and DYTRDV (HCDR3) (SEQ ID NO: 17); a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIKYDGSEKYYADSVKG (HCDR2) (SEQ ID NO: 13) and DYNKDY (HCDR3) (SEQ ID NO: 18); a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIRHDGGEKYYPDSVKG (HCDR2) (SEQ ID NO: 14) and DYNKDL (HCDR3) (SEQ ID NO: 19) or a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIRHDGGEKYYADSVKG (HCDR2) (SEQ ID NO: 15) and DYNKDY (HCDR3) (SEQ ID NO: 18).

[0072] In one aspect, the present invention provides a method of treating a cancer comprising administering an GCC binding agent to a subject in need of treatment, wherein the GCC binding agent comprises: a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of HYYWS (HCDR1) (SEQ ID NO: 8), RIYPSGSTSYNPSLKS (HCDR2) (SEQ ID NO: 11) and DRSTGWSEWNSDL (HCDR3) (SEQ ID NO: 16); a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMS (HCDR1) (SEQ ID NO: 9), KIRHDGGEKYYVDSVKG (HCDR2) (SEQ ID NO: 12) and DYTRDV (HCDR3) (SEQ ID NO: 17); a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIKYDGSEKYYADSVKG (HCDR2) (SEQ ID NO: 13) and DYNKDY (HCDR3) (SEQ ID NO: 18); a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIRHDGGEKYYPDSVKG (HCDR2) (SEQ ID NO: 14) and DYNKDL (HCDR3) (SEQ ID NO: 19) or a heavy chain variable region (VH) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIRHDGGEKYYADSVKG (HCDR2) (SEQ ID NO: 15) and DYNKDY (HCDR3) (SEQ ID NO: 18).

[0073] In one aspect, the present invention provides a nucleic acid encoding a VH amino acid sequence that is identical to any one of SEQ ID Nos: 1, 20, 21, 26, 27, or 28.

[0074] In one aspect, the present invention provides a vector comprising a nucleic acid encoding a VH amino acid sequence that is identical to any one of SEQ ID Nos: 1, 20, 21, 26, 27, or 28.

[0075] In one aspect, the present invention provides an isolated cell comprising the vector comprising a nucleic acid encoding a VH amino acid sequence that is identical to any one of SEQ ID Nos: 1, 20, 21, 26, 27, or 28.

[0076] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

BRIEF DESCRIPTION OF THE DRAWING

[0077] The Drawing included herein, which is comprised of the following Figures, is for illustration purposes only not for limitation.

[0078] ***Figures 1A and 1B*** depict exemplary chimeric antigen receptor (CAR) constructs.

[0079] ***Figures 2A-2D*** show exemplary anti-GCC CAR-T *in vitro* cytotoxicity performed using four tumor cell lines. HT29-GCC cells, a human colorectal cancer cell line HT29 engineered to stably express GCC) (Fig 2A); HT29-VEC (vector control GCC-negative cell line) (Fig 2B); and two tumor cell lines endogenously expressing GCC: GSU (Fig 2C) and LS1034 (Fig 2D). Bars represent mean+SD values from three technical replicates. Data are representative of >3 independent experiments performed with anti-GCC CAR T cells from >3 donors. CAR T cytotoxicity was determined in the absence of truncated EGFR (tEGFR).

[0080] ***Figures 3A-3D*** show exemplary anti-GCC CAR-T cells *in vitro* cytotoxicity performed using four tumor cell lines. HT29-GCC cells, a human colorectal cancer cell line HT29 engineered to stably express GCC) (Fig. 3A); HT29-VEC (vector control GCC-negative cell line) (Fig. 3B; and two tumor cell lines endogenously expressing GCC: GSU (Fig. 3C) and LS1034 (Fig. 3D) Bars represent mean+SD values from three technical replicates. Data are representative of >3 independent experiments performed with anti-GCC

CAR T cells from >3 donors. CAR T cytotoxicity was determined in the presence of truncated EGFR (tEGFR).

[0081] ***Figures 4A-4D*** show exemplary IFN-g cytokine secretion by anti-GCC CAR-T cells co-cultured with GCC-expressing (HT29-GCC) (Fig. 4A), GSU (Fig. 4C), LS1034 (Fig. 4D) and GCC-negative (HT29-VEC, Fig. 4B) tumor cells *in vitro*. Secreted IFNg in the supernatant was detected using the Intellicyt QBeads Human PlexScreen kit (Sartorius, 90702). Bars represent mean +SD values from three technical replicates. Data are representative of >3 independent experiments performed with anti-GCC CAR T cells from >3 donors. Cytokine secretion was determined in the absence of truncated EGFR (tEGFR).

[0082] **Figures 5A-5D** show exemplary IFN-g cytokine secretion by anti-GCC CAR-T co-culture with GCC-expressing (HT29-GCC (Fig. 5A), GSU (Fig. 5C), LS1034 (Fig. 5D) and GCC-negative (HT29-VEC, Fig. 5B) tumor cells *in vitro*. Secreted IFNg in the supernatant was detected using the Intellicyt QBeads Human PlexScreen kit (Sartorius, 90702). Bars represent mean +SD values from three technical replicates. Data are representative of >3 independent experiments performed with anti-GCC CAR T cells from >3 donors. Cytokine secretion was determined in the presence of truncated EGFR (tEGFR).

[0083] ***Figures 6A-6D*** show exemplary IL-2 cytokine secretion by ant-GCC CAR-T co-culture with GCC-expressing (HT29-GCC (Fig. 6A), GSU (Fig. 6C), LS1034 (Fig. 6D) and GCC-negative (HT29-VEC, Fig. 6B) tumor cells *in vitro*. Secreted IFL-2 in the supernatant was detected using the Intellicyt QBeads Human PlexScreen kit (Sartorius, 90702). Bars represent mean +SD values from three technical replicates. Data are representative of >3 independent experiments performed with anti-GCC CAR T cells from >3 donors. Cytokine secretion was determined in the absence of truncated EGFR (tEGFR).

[0084] ***Figures 7A-7D*** show exemplary IL-2 cytokine secretion by anti-GCC CAR-T co-culture with GCC-(HT29-GCC (Fig. 7A), GSU (Fig. 7C), LS1034 (Fig. 7D) and GCC-negative (HT29-VEC, Fig. 7B) tumor cells *in vitro*. Secreted IFL-2 in the supernatant was detected using the Intellicyt QBeads Human PlexScreen kit (Sartorius, 90702). Bars represent mean +SD values from three technical replicates. Data are representative of >3 independent experiments performed with anti-GCC CAR T cells from >3 donors. Cytokine secretion was determined in the presence of truncated EGFR (tEGFR).

[0085] ***Figures 8A and 8B*** show exemplary anti-tumor effect results of change in average tumor volume (mm^3) over time (40 days) in a HT55 model (endogenously expressing GCC expressing colorectal cancer, GCC H-score=300/300) treated with anti-GCC CAR-T cells manufactured from two different healthy donors D393 (Fig. 8A) and D686 (Fig. 8B). Anti-tumor effect was determined in the absence of truncated EGFR (tEGFR).

[0086] ***Figure 9A-9C*** show exemplary anti-tumor effect results of change in average tumor volume (mm^3) over time (42 days) in a HT55 model (endogenously GCC expressing colorectal cancer, GCC H-score=300/300) treated with anti-GCC CAR-T cells manufactured from three different healthy donors D393 (Fig. 9A), D797 (Fig. 9B) and D954 (Fig. 9C). Anti-tumor effect was determined in the absence of truncated EGFR (tEGFR).

[0087] ***Figures 10A-10C*** show exemplary anti-tumor effect results of change in average tumor volume (mm^3) over time (42 days) in a HT55 model (endogenously GCC expressing colorectal cancer, GCC H-score=300/300) treated with anti-GCC CAR-T cells manufactured from three different healthy donors D393 (Fig. 10A), D797 (Fig. 10B) and D954 (Fig. 10C). Anti-tumor effect was determined in the presence of truncated EGFR (tEGFR).

[0088] ***Figures 11A-11C*** show exemplary anti-tumor effect results of change in average tumor volume (mm^3) over time (42 days) in a HT55 model (endogenously GCC expressing colorectal cancer, GCC H-score=300/300) treated with anti-GCC CAR-T cells manufactured from three different healthy donors D393 (Fig. 11A), D954 (Fig. 11B) and D686 (Fig. 11C). Anti-tumor effect was determined in the absence of truncated EGFR (tEGFR).

[0089] ***Figures 12A-12C*** show exemplary anti-tumor effect results of change in average tumor volume (mm^3) over time (42 days) in a HT55 model (endogenously GCC expressing colorectal cancer, GCC H-score=300/300) treated with anti-GCC CAR-T cells manufactured from three different healthy donors D393 (Fig. 12A), D954 (Fig. 12B) and D686 (Fig. 12C). Anti-tumor effect was determined in the presence of truncated EGFR (tEGFR).

[0090] ***Figures 13A and 13B*** show exemplary anti-tumor effect results of change in average tumor volume (mm3) over time (42 days) in a GSU model (endogenously GCC

expressing gastric cancer, GCC H-score=170/300) treated with anti-GCC CAR-T cells manufactured from two different healthy donors D393 (Fig. 13A) and D954 (Fig. 13B). Anti-tumor effect was determined in the absence of truncated EGFR (tEGFR)

[0091] ***Figures 14A and 14B*** show exemplary anti-tumor effect results of change in average tumor volume (mm3) over time (42 days) in a GSU model (endogenously GCC expressing gastric cancer, GCC H-score=170/300) treated with anti-GCC CAR-T cells manufactured from two different healthy donors D393 (Fig. 14A) and D954 (Fig. 14B). Anti-tumor effect was determined in the presence of truncated EGFR (tEGFR).

[0092] ***Figures 15A-C*** show exemplary anti-tumor effect results of change in average tumor volume (mm3) over time (42 days) in a GSU model (endogenously GCC expressing gastric cancer, GCC H-score=170/300) treated with anti-GCC CAR-T cells manufactured from three different healthy donors D393 (Fig. 15A), D954 (Fig. 15B) and D686 (Fig. 15C). Anti-tumor effect was determined in the absence of truncated EGFR (tEGFR).

[0093] ***Figure 16A-C*** show exemplary anti-tumor effect results of change in average tumor volume (mm3) over time (42 days) in a GSU model (endogenously GCC expressing gastric cancer, GCC H-score=170/300) treated with anti-GCC CAR-T cells manufactured from three different healthy donors D393 (Fig. 16A), D954 (Fig. 16B) and D686 (Fig. 16C). Anti-tumor effect was determined in the absence of truncated EGFR (tEGFR).

[0094] **Figure 17** shows exemplary anti-tumor efficacy of change in average tumor volume (mm3) over time (42 days) in a LS1034 model (endogenously GCC expressing colorectal cancer, GCC H-score=300/300) treated with anti-GCC CAR-T cells manufactured from a healthy donor. Anti-tumor effect was determined in the absence of truncated EGFR (tEGFR).

[0095] **Figure 18** shows exemplary anti-tumor effect results of change in average tumor volume (mm3) over time (42 days) in a LS1034 model (endogenously GCC expressing colorectal cancer, GCC H-score=300/300) treated with anti-GCC CAR-T cells manufactured from a healthy donor. Anti-tumor effect was determined in the presence of truncated EGFR (tEGFR).

DEFINITIONS

[0096] In order for the present invention to be more readily understood, certain terms are first defined below. Additional definitions for the following terms and other terms are set forth through the specification.

[0097] As used in this specification and the appended claims, the singular forms “a”, "an", and "the" include plural references unless the context clearly dictates otherwise. Thus for example, a reference to "a method" includes one or more methods, and/or steps of the type described herein and/or which will become apparent to those persons skilled in the art upon reading this disclosure and so forth.

[0098] ***Administer***: As used herein, “administering” a composition to a subject means to give, apply or bring the composition into contact with the subject. Administration can be accomplished by any of a number of routes, such as, for example, topical, oral, subcutaneous, intramuscular, intraperitoneal, intravenous, intrathecal, and intradermal.

[0099] ***Affinity***: As used herein, the term “affinity” refers to the characteristics of a binding interaction between a binding moiety (e.g., an antigen binding agent (e.g., variable domain described herein) and a target (e.g., an antigen (e.g., GCC) and that indicates the strength of the binding interaction. In some embodiments, the measure of affinity is expressed as a dissociation constant (K_D). In some embodiments, a binding moiety has a high affinity for a target (e.g., a K_D of less than about 10^{-7} M, less than about 10^{-8} M, or less than about 10^{-9} M). In some embodiments, a binding moiety has a low affinity for a target (e.g., a K_D of higher than about 10^{-7} M, higher than about 10^{-6} M, higher than about 10^{-5} M, or higher than about 10^{-4} M).

[0100] ***Animal***: As used herein, the term “animal” refers to any member of the animal kingdom. In some embodiments, “animal” refers to humans, at any stage of development. In some embodiments, “animal” refers to non-human animals, at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, and/or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, insects, and/or worms. In some embodiments, an animal may be a transgenic animal, genetically-engineered animal, and/or a clone.

[0101] ***Autologous***: As used herein, the term "autologous" is refers to any material derived from the same individual to whom it is later to be re-introduced into the individual.

[0102] ***Allogeneic***: "Allogeneic" refers to material derived from one individual administered to a different individual or individuals.

[0103] ***Antibody or Antigen Binding Agent:*** As used herein, the term "antibody" or "antigen binding agent" refers to a polypeptide that includes canonical immunoglobulin sequence elements sufficient to confer specific binding to a particular target antigen. Those skilled in the art will appreciate that the terms may be used herein interchangeably. In some embodiments, as used herein, the term "antibody" or "antigen binding agent" also refers to an "antibody fragment" or "antibody fragments" or "antigen binding portion", which includes a portion of an intact antibody, such as, for example, the antigen-binding or variable region of an antibody. Examples of "antibody fragments" include Fab, Fab', F(ab')2, and Fv fragments; triabodies; tetrabodies; linear antibodies; single-chain antibody molecules; and CDR-containing moieties included in multi-specific antibodies formed from antibody fragments. Those skilled in the art will appreciate that the term "antibody fragment" does not imply and is not restricted to any particular mode of generation. An antibody fragment may be produced through use of any appropriate methodology, including but not limited to cleavage of an intact antibody, chemical synthesis, recombinant production, etc. As is known in the art, intact antibodies as produced in nature are approximately 150 kD tetrameric agents comprised of two identical heavy chain polypeptides (about 50 kD each) and two identical light chain polypeptides (about 25 kD each) that associate with each other into what is commonly referred to as a "Y-shaped" structure. Each heavy chain is comprised of at least four domains (each about 110 amino acids long)--an amino-terminal variable (V_H) domain (located at the tips of the Y structure), followed by three constant domains: C_H1, C_H2, and the carboxy-terminal C_H3 (located at the base of the Y's stem). A short region, known as the "switch", connects the heavy chain variable and constant regions. The "hinge" connects C_H2 and C_H3 domains to the rest of the antibody. Two disulfide bonds in this hinge region connect the two heavy chain polypeptides to one another in an intact antibody. Each light chain is comprised of two domains -- an amino-terminal variable (V_L) domain, followed by a carboxy-terminal constant (C_L) domain, separated from one another by another "switch". Intact antibody tetramers are comprised of two heavy chain-light chain dimers in which the

heavy and light chains are linked to one another by a single disulfide bond; two other disulfide bonds connect the heavy chain hinge regions to one another, so that the dimers are connected to one another and the tetramer is formed. Naturally-produced antibodies are also glycosylated, typically on the C_H2 domain. Each domain in a natural antibody has a structure characterized by an "immunoglobulin fold" formed from two beta sheets (e.g., 3-, 4-, or 5-stranded sheets) packed against each other in a compressed antiparallel beta barrel. Each variable domain contains three hypervariable loops known as "complement determining regions" (CDR1, CDR2, and CDR3) and four somewhat invariant "framework" regions (FR1, FR2, FR3, and FR4). When natural antibodies fold, the FR regions form the beta sheets that provide the structural framework for the domains, and the CDR loop regions from both the heavy and light chains are brought together in three-dimensional space so that they create a single hypervariable antigen binding site located at the tip of the Y structure. Amino acid sequence comparisons among antibody polypeptide chains have defined two light chain (κ and λ) classes, several heavy chain (e.g., μ, γ, α, ε, δ) classes, and certain heavy chain subclasses ($\alpha1$, $\alpha2$, $\gamma1$, $\gamma2$, $\gamma3$, and $\gamma4$). Antibody classes (IgA [including IgA1, IgA2], IgD, IgE, IgG [including IgG1, IgG2, IgG3, and IgG4], and IgM) are defined based on the class of the utilized heavy chain sequences.

[0104] For purposes of the present invention, in certain embodiments, any polypeptide or complex of polypeptides that includes sufficient immunoglobulin domain sequences as found in natural antibodies can be referred to and/or used as an "antibody" or "antigen binding agent", whether such polypeptide is naturally produced (e.g., generated by an organism reacting to an antigen), or produced by recombinant engineering, chemical synthesis, or other artificial system or methodology. In some embodiments, an antibody is monoclonal; in some embodiments, an antibody is polyclonal. In some embodiments, an antibody has constant region sequences that are characteristic of mouse, rabbit, primate, or human antibodies. In some embodiments, an antibody sequence elements are humanized, primatized, chimeric, etc., as is known in the art. Moreover, the term "antibody" or "antigen binding agent" as used herein, will be understood to encompass (unless otherwise stated or clear from context) can refer in appropriate embodiments to any of the art-known or developed constructs or formats for capturing antibody structural and functional features in alternative presentation. For example, in some embodiments, the terms can refer to bi- or

other multi-specific (e.g., zybodies, etc.) antibodies, Small Modular ImmunoPharmaceuticals ("SMIPs™"), single chain antibodies, camelid antibodies, and/or antibody fragments. In some embodiments, an antibody may lack a covalent modification (e.g., attachment of a glycan) that it would have if produced naturally. In some embodiments, an antibody may contain a covalent modification (e.g., attachment of a glycan, a payload [e.g., a detectable moiety, a therapeutic moiety, a catalytic moiety, etc.], or other pendant group [e.g., poly-ethylene glycol, etc.]).

[0105] ***Approximately or about:*** As used herein, the term "approximately" or "about," as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term "approximately" or "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0106] ***Complementarity Determining Region (CDR)***: A "CDR" of a variable domain are amino acid residues within the variable region that are identified in accordance with the definitions of the Kabat, Chothia, the accumulation of both Kabat and Chothia, AbM, contact, and/or conformational definitions or any method of CDR determination well known in the art. Antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. See, e.g., Kabat et al., 1992, Sequences of Proteins of Immunological Interest, 5th ed., Public Health Service, NIH, Washington D.C. The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others. See, e.g., Chothia et al., Nature 342:877-883, 1989. Other approaches to CDR identification include the "AbM definition," which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software (now Accelrys®), or the "contact definition" of CDRs based on observed antigen contacts, set forth in MacCallum et al., J. Mol. Biol., 262:732-745, 1996. In another approach, referred to herein as the "conformational definition" of CDRs, the positions of the CDRs may be identified as the residues that make enthalpic contributions to antigen binding. See, e.g., Makabe et al., Journal of Biological Chemistry, 283: 1 156-1166, 2008. Still other CDR boundary definitions may not strictly follow one of the above approaches, but will nonetheless overlap with at least a

portion of the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. Unless stated otherwise, as used herein, CDR definitions are according to Kabat CDRs.

[0107] ***Effector functions:*** As used herein, the term "effector functions" refers to those biological activities attributable to an antigen binding agent described herein. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity; Fc receptor binding; antibody—dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptors; and B cell activation. "Reduced or minimized" antibody effector function means that which is reduced by at least 50% (alternatively 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99%) from the wild type or unmodified antibody. The determination of antibody effector function is readily determinable and measurable by one of ordinary skill in the art. In some embodiments, the antibody effector functions of complement binding, complement dependent cytotoxicity and antibody dependent cytotoxicity are affected. In some embodiments, effector function is eliminated through a mutation in the constant region that eliminated glycosylation, e.g., "effector-less mutation." In one aspect, the effector-less mutation is an N297A or DANA mutation (D265A+N297A) in the CH2 region. Shields et al., J. Biol. Chem. 276(9): 6591-6604 (2001). Alternatively, additional mutations resulting in reduced or eliminated effector function include: K322A and L234A/L235A (LALA). Alternatively, effector function can be reduced or eliminated through production techniques, such as expression in host cells that do not glycosylate (e.g., E. coli) or in which result in an altered glycosylation pattern that is ineffective or less effective at promoting effector function (e.g., Shinkawa et al., J. Biol. Chem. 278 (5):3466-3473 (2003).

[0108] ***Antibody-dependent cell-mediated cytotoxicity*** or ADCC refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., natural killer (NK) cells, neutrophils and macrophages) enable these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell with cytotoxins. The antibodies "arm" the cytotoxic cells and are required for killing of the target cell by this mechanism. The primary cells for mediating ADCC, NK cells, express FcγRIII only, whereas monocytes express FcγRI, FcγRII and FcγRIII. Fc expression

on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9: 457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed *in vivo*, e.g., in an animal model such as that disclosed in Clynes et al., PNAS USA 95: 652-656 (1998).

[0109] ***Antigen:*** As used herein, the term "antigen", refers to an agent that elicits an immune response; and/or an agent that is bound by a T cell receptor (e.g., when presented by an MHC molecule) or to an antibody (e.g., produced by a B cell) when exposed or administered to an organism. In some embodiments, an antigen elicits a humoral response (e.g., including production of antigen-specific antibodies) in an organism; alternatively or additionally, in some embodiments, an antigen elicits a cellular response (e.g., involving T-cells whose receptors specifically interact with the antigen) in an organism. It will be appreciated by those skilled in the art that a particular antigen may elicit an immune response in one or several members of a target organism (e.g., mice, rabbits, primates, humans), but not in all members of the target organism species. In some embodiments, an antigen elicits an immune response in at least about 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% of the members of a target organism species. In some embodiments, an antigen binds to an antibody and/or T cell receptor, and may or may not induce a particular physiological response in an organism. In some embodiments, for example, an antigen may bind to an antibody and/or to a T cell receptor in vitro, whether or not such an interaction occurs in vivo. In some embodiments, an antigen reacts with the products of specific humoral or cellular immunity, including those induced by heterologous immunogens. In some embodiments of the disclosed compositions and methods, GCC protein is an antigen.

[0110] ***Associated with:*** Two events or entities are "associated" with one another, as that term is used herein, if the presence, level and/or form of one is correlated with that of the other. For example, a particular entity (e.g., polypeptide) is considered to be associated with a particular disease, disorder, or condition, if its presence, level and/or form correlates with incidence of and/or susceptibility of the disease, disorder, or condition (e.g., across a relevant

population). In some embodiments, two or more entities are physically "associated" with one another if they interact, directly or indirectly, so that they are and remain in physical proximity with one another. In some embodiments, two or more entities that are physically associated with one another are covalently linked to one another; in some embodiments, two or more entities that are physically associated with one another are not covalently linked to one another but are non-covalently associated, for example by means of hydrogen bonds, van der Waals interaction, hydrophobic interactions, magnetism, and combinations thereof.

[0111] ***Binding***: It will be understood that the term "binding", as used herein, typically refers to a non-covalent association between or among two or more entities. "Direct" binding involves physical contact between entities or moieties; indirect binding involves physical interaction by way of physical contact with one or more intermediate entities. Binding between two or more entities can be assessed in any of a variety of contexts – including where interacting entities or moieties are studied in isolation or in the context of more complex systems (e.g., while covalently or otherwise associated with a carrier entity and/or in a biological system or cell). As used herein, "K_a" refers to an association rate of a particular binding moiety and a target to form a binding moiety/target complex. As used herein, "K_d" refers to a dissociation rate of a particular binding moiety/target complex. As used herein, "K_D" refers to a dissociation constant, which is obtained from the ratio of K_d to K_a (i.e., K_d/K_a) and is expressed as a molar concentration (M). K_D values can be determined using methods well established in the art, e.g., by using surface plasmon resonance, or using a biosensor system such as a Biacore® system.

[0112] ***Carrier:*** As used herein, the term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which a composition is administered. In some exemplary embodiments, carriers can include sterile liquids, such as, for example, water and oils, including oils of petroleum, animal, vegetable or synthetic origin, such as, for example, peanut oil, soybean oil, mineral oil, sesame oil and the like. In some embodiments, carriers are or include one or more solid components.

[0113] ***Characteristic Portion:*** As used herein, the term "characteristic portion" is used, in the broadest sense, to refer to a portion of a substance whose presence (or absence) correlates with presence (or absence) of a particular feature, attribute, or activity of the

substance. In some embodiments, a characteristic portion of a substance is a portion that is found in the substance and in related substances that share the particular feature, attribute or activity, but not in those that do not share the particular feature, attribute or activity.

[0114] ***Codon-optimized:*** As used herein, a "codon-optimized" nucleic acid sequence refers to a nucleic acid sequence that has been altered such that translation of the nucleic acid sequence and expression of the resulting protein is improved optimized for a particular expression system. A "codon-optimized" nucleic acid sequence encodes the same protein as a non-optimized parental sequence upon which the "codon-optimized" nucleic acid sequence is based. For example, a nucleic acid sequence may be "codon-optimized" for expression in mammalian cells (e.g., CHO cells, human cells, mouse cells etc.), bacterial cells (e.g., E.coli), insect cells, yeast cells or plant cells.

[0115] ***Comparable:*** The term "comparable", as used herein, refers to two or more agents, entities, situations, sets of conditions, etc. that may not be identical to one another but that are sufficiently similar to permit comparison there between so that conclusions may reasonably be drawn based on differences or similarities observed. Those of ordinary skill in the art will understand, in context, what degree of identity is required in any given circumstance for two or more such agents, entities, situations, sets of conditions, etc. to be considered comparable.

[0116] ***Corresponding to:*** As used herein, the term "corresponding to" is often used to designate the position/identity of an amino acid residue in a polypeptide of interest. Those of ordinary skill will appreciate that, for purposes of simplicity, residues in a polypeptide are often designated using a canonical numbering system based on a reference related polypeptide, so that an amino acid "corresponding to" a residue at position 190, for example, need not actually be the 190th amino acid in a particular amino acid chain but rather corresponds to the residue found at 190 in the reference polypeptide; those of ordinary skill in the art readily appreciate how to identify "corresponding" amino acids.

[0117] ***Derived from:*** As used herein the phrase, a sequence "derived from" or "specific for a designated sequence" refers to a sequence that comprises a contiguous sequence of approximately at least 6 nucleotides or at least 2 amino acids, at least about 9 nucleotides or at least 3 amino acids, at least about 10-12 nucleotides or 4 amino acids, or at

least about 15-21 nucleotides or 5-7 amino acids corresponding, i.e., identical or complementary to, e.g., a contiguous region of the designated sequence. In certain embodiments, the sequence comprises all of a designated nucleotide or amino acid sequence. The sequence may be complementary (in the case of a polynucleotide sequence) or identical to a sequence region that is unique to a particular sequence as determined by techniques known in the art. Regions from which sequences may be derived, include but are not limited to, regions encoding specific epitopes, regions encoding CDRs, regions encoding framework sequences, regions encoding constant domain regions, regions encoding variable domain regions, as well as non-translated and/or non-transcribed regions. The derived sequence will not necessarily be derived physically from the sequence of interest under study, but may be generated in any manner, including, but not limited to, chemical synthesis, replication, reverse transcription or transcription, that is based on the information provided by the sequence of bases in the region(s) from which the polynucleotide is derived. As such, it may represent either a sense or an antisense orientation of the original polynucleotide. In addition, combinations of regions corresponding to that of the designated sequence may be modified or combined in ways known in the art to be consistent with the intended use. For example, a sequence may comprise two or more contiguous sequences which each comprise part of a designated sequence, and are interrupted with a region which is not identical to the designated sequence but is intended to represent a sequence derived from the designated sequence. With regard to antibody molecules, "derived therefrom" includes an antibody molecule which is functionally or structurally related to a comparison antibody, e.g., "derived therefrom" includes an antibody molecule having similar or substantially the same sequence or structure, e.g., having the same or similar CDRs, framework or variable regions. "Derived therefrom" for an antibody also includes residues, e.g., one or more, e.g., 2, 3, 4, 5, 6 or more residues, which may or may not be contiguous, but are defined or identified according to a numbering scheme or homology to general antibody structure or three-dimensional proximity, i.e., within a CDR or a framework region, of a comparison sequence. The term "derived therefrom" is not limited to physically derived therefrom but includes generation by any manner, e.g., by use of sequence information from a comparison antibody to design another antibody.

[0118] ***Determine:*** Many methodologies described herein include a step of "determining". Those of ordinary skill in the art, reading the present specification, will

appreciate that such "determining" can utilize any of a variety of techniques available to those skilled in the art, including for example specific techniques explicitly referred to herein. In some embodiments, a determination involves manipulation of a physical sample. In some embodiments, a determination involves consideration and/or manipulation of data or information, for example utilizing a computer or other processing unit adapted to perform a relevant analysis. In some embodiments, a determination involves receiving relevant information and/or materials from a source. In some embodiments, determining involves comparing one or more features of a sample or entity to a comparable reference.

[0119] ***Engineered:*** The term "engineered", as used herein, describes a polynucleotide, polypeptide or a cell that has been designed or modified by man and/or whose existence and production require human intervention and/or activity. For example, an engineered cell that is intentionally designed to elicit a particular effect and that differs from the effect of naturally occurring cells of the same type. In some embodiments, an engineered cell expresses a chimeric antigen receptor described herein. Exemplary engineering methods are described in the detailed description and examples sections.

[0120] ***Epitope:*** As used herein, the term "epitope" includes any moiety that is specifically recognized by an immunoglobulin (e.g., antibody or receptor) binding component in whole or in part. In some embodiments, an epitope is comprised of a plurality of amino acids in an antigen. In some embodiments, such amino acid residues are surface-exposed when the antigen adopts a relevant three-dimensional conformation. In some embodiments, the amino acid residues are physically near to or contour with each other in space when the antigen adopts such a conformation. In some embodiments, at least some of the amino acids are physically separated from one another when the antigen adopts an alternative conformation (e.g., is linearized; e.g., a non-linear epitope).

[0121] ***Excipient***: As used herein, the term "excipient" refers to a non-therapeutic agent that may be included in a pharmaceutical composition, for example to provide or contribute to a desired consistency or stabilizing effect. Suitable pharmaceutical excipients include, for example, starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like.

[0122] ***Expression:*** The term "expression" or "expressed", when used in reference to a nucleic acid herein, refers to one or more of the following events: (1) production of an RNA transcript of a DNA template (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end formation); (3) translation of an RNA into a polypeptide; and/or (4) post-translational modification of a polypeptide.

[0123] ***Ex vivo:*** As used herein, the term "*ex vivo*" refers to events that occur in an external environment, *e.g.*, outside a multi-cellular organism. In some embodiments, a cell or population of cells is modified outside of the body of a multi-cellular organism (e.g., a mammal such as a non-human primate or human being) to express a anti-GCC molecule described herein, prior to administration of such a cell or population of cells to a subject in need thereof.

[0124] ***Fusion protein:*** As used herein, the term "fusion protein" refers to a protein encoded by a nucleic acid sequence engineered from nucleic acid sequences encoding at least a portion of two different (e.g., heterologous) proteins. As persons of skill are no doubt aware, to create a fusion protein nucleic acid sequences are joined such that the resulting reading frame does not contain an internal stop codon. In some embodiments, fusion proteins as described herein include an influenza HA polypeptide or fragment thereof.

[0125] ***Guanylyl cyclase C (GCC)***: As used herein, "GCC," also known as "STAR", "GUC2C", "GUCY2C" or "ST receptor" protein refers to mammalian GCC, preferably human GCC protein. Human GCC refers to the protein described in GenBank accession no.: NM—004963 and naturally occurring allelic protein variants thereof. Other variants are known in the art. See, e.g., accession number Ensp00002611 70, Ensembl Database, European Bioinformatics Institute and Wellcome Trust Sanger Institute, US patent application number 20060035852; or GenBank accession number AAB 19934. Typically, a naturally occurring allelic variant has an amino acid sequence at least 95%, 97% or 99% identical to the GCC sequence of SEQ ID NO: 41. The transcript encodes a protein product of 1073 amino acids, and is described in GenBank accession no.: NM—004963. GCC protein is characterized as a transmembrane cell surface receptor protein, and is believed to play a critical role in the maintenance of intestinal fluid, electrolyte homeostasis and cell proliferation.

[0126] ***Host:*** The term "host" is used herein to refer to a system (e.g., a cell, organism, etc.) in which a polypeptide of interest is present. In some embodiments, a host is a system that expresses a particular polypeptide of interest.

[0127] ***Host cell:*** As used herein, the phrase "host cell" refers to a cell into which exogenous DNA (recombinant or otherwise) has been introduced. For example, host cells may be used to produce the polypeptides described herein by standard recombinant techniques. Persons of skill upon reading this disclosure will understand that such terms refer not only to the particular subject cell, but, to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "*host cell*" as used herein. In some embodiments, host cells include any prokaryotic and eukaryotic cells suitable for expressing an exogenous DNA (e.g., a recombinant nucleic acid sequence). Exemplary cells include those of prokaryotes and eukaryotes (single-cell or multiple-cell), bacterial cells (e.g., strains of E. coli, Bacillus spp., Streptomyces spp., etc.), mycobacteria cells, fungal cells, yeast cells (e.g., S. cerevisiae, S. pombe, P. pastoris, P. methanolica, etc.), plant cells, insect cells (e.g., SF-9, SF-21, baculovirus-infected insect cells, Trichoplusia ni, etc.), non-human animal cells, human cells, or cell fusions such as, for example, hybridomas or quadromas. In some embodiments, the cell is a human, monkey, ape, hamster, rat, or mouse cell. In some embodiments, the cell is eukaryotic and is selected from the following cells: CHO (e.g., CHO K1, DXB-11 CHO, Veggie-CHO), COS (e.g., COS-7), retinal cell, Vero, CV1, kidney (e.g., HEK293, HEK293T, 293 EBNA, MSR 293, MDCK, HaK, BHK), HeLa, HepG2, WI38, MRC 5, Colo205, HB 8065, HL-60, (e.g., BHK21), Jurkat, Daudi, A431 (epidermal), CV-1, U937, 3T3, L cell, C127 cell, SP2/0, NS-0, MMT 060562, Sertoli cell, BRL 3A cell, HT1080 cell, myeloma cell, tumor cell, and a cell line derived from an aforementioned cell. In some embodiments, the cell comprises one or more viral genes, e.g., a retinal cell that expresses a viral gene (e.g., a PER.C6™ cell).

[0128] ***Immune response***: As used herein, the term "immune response" refers to a response of a cell of the immune system, such as a B cell, T cell, dendritic cell, macrophage or polymorphonucleocyte, to a stimulus such as an antigen or vaccine. An immune response can include any cell of the body involved in a host defense response, including for example,

an epithelial cell that secretes an interferon or a cytokine. An immune response includes, but is not limited to, an innate and/or adaptive immune response. As used herein, a protective immune response refers to an immune response that protects a subject from infection (prevents infection or prevents the development of disease associated with infection). Methods of measuring immune responses are well known in the art and include, for example, measuring proliferation and/or activity of lymphocytes (such as B or T cells), secretion of cytokines or chemokines, inflammation, antibody production and the like.

[0129] ***In vitro***: As used herein, the term "*in vitro*" refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, *etc.*, rather than within a multi-cellular organism.

[0130] ***In vivo***: As used herein, the term "*in vivo*" refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, *in vitro* or *ex vivo* systems).

[0131] ***Isolated***: As used herein, the term "isolated" refers to a substance and/or entity that has been (1) separated from at least some of the components with which it was associated when initially produced (whether in nature and/or in an experimental setting), and/or (2) designed, produced, prepared, and/or manufactured with human intervention. Isolated substances and/or entities may be separated from about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% of the other components with which they were initially associated. In some embodiments, isolated agents are about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is "pure" if it is substantially free of other components. In some embodiments, as will be understood by those skilled in the art, a substance may still be considered "isolated" or even "pure", after having been combined with certain other components such as, for example, one or more carriers or excipients (*e.g.*, buffer, solvent, water, etc.); in such embodiments, percent isolation or purity of the substance is calculated without including such carriers or excipients. To give but one example, in some

embodiments, a biological polymer such as a polypeptide or polynucleotide that occurs in nature is considered to be "isolated" when, a) by virtue of its origin or source of derivation is not associated with some or all of the components that accompany it in its native state in nature; b) it is substantially free of other polypeptides or nucleic acids of the same species from the species that produces it in nature; c) is expressed by or is otherwise in association with components from a cell or other expression system that is not of the species that produces it in nature. Thus, for instance, in some embodiments, a polypeptide that is chemically synthesized or is synthesized in a cellular system different from that which produces it in nature is considered to be an "isolated" polypeptide. Alternatively or additionally, in some embodiments, a polypeptide that has been subjected to one or more purification techniques may be considered to be an "isolated" polypeptide to the extent that it has been separated from other components a) with which it is associated in nature; and/or b) with which it was associated when initially produced. In some embodiments, cells can be "isolated" (e.g., purified or separated) from olther cells. For example, in some embodiments, genetically modified cells engineered to express a CAR described herein can be isolated from unmodified cells.

[0132] ***Nucleic acid:*** As used herein, the phrase "nucleic acid", in its broadest sense, refers to any compound and/or substance that is or can be incorporated into an oligonucleotide chain. In some embodiments, a nucleic acid is a compound and/or substance that is or can be incorporated into an oligonucleotide chain via a phosphodiester linkage. As will be clear from context, in some embodiments, "nucleic acid" refers to individual nucleic acid residues (*e.g.*, nucleotides and/or nucleosides); in some embodiments, "nucleic acid" refers to an oligonucleotide chain comprising individual nucleic acid residues. In some embodiments, a "nucleic acid" is or comprises RNA; in some embodiments, a "nucleic acid" is or comprises DNA. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleic acid residues. In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleic acid analogs. In some embodiments, a nucleic acid analog differs from a nucleic acid in that it does not utilize a phosphodiester backbone. For example, in some embodiments, a nucleic acid is, comprises, or consists of one or more "peptide nucleic acids", which are known in the art and have peptide bonds instead of phosphodiester bonds in the backbone, are considered within the scope of the present invention. Alternatively or

additionally, in some embodiments, a nucleic acid has one or more phosphorothioate and/or 5'-N-phosphoramidite linkages rather than phosphodiester bonds. In some embodiments, a nucleic acid is, comprises, or consists of one or more natural nucleosides (e.g., adenosine, thymidine, guanosine, cytidine, uridine, deoxyadenosine, deoxythymidine, deoxyguanosine, and deoxycytidine). In some embodiments, a nucleic acid is, comprises, or consists of one or more nucleoside analogs (e.g., 2-aminoadenosine, 2-thiothymidine, inosine, pyrrolo-pyrimidine, 3-methyl adenosine, 5-methylcytidine, C-5 propynyl-cytidine, C-5 propynyl-uridine, 2-aminoadenosine, C5-bromouridine, C5-fluorouridine, C5-iodouridine, C5-propynyl-uridine, C5-propynyl-cytidine, C5-methylcytidine, 2-aminoadenosine, 7-deazaadenosine, 7-deazaguanosine, 8-oxoadenosine, 8-oxoguanosine, O(6)-methylguanine, 2-thiocytidine, methylated bases, intercalated bases, and combinations thereof). In some embodiments, a nucleic acid comprises one or more modified sugars (e.g., 2'-fluororibose, ribose, 2'-deoxyribose, arabinose, and hexose) as compared with those in natural nucleic acids. In some embodiments, a nucleic acid has a nucleotide sequence that encodes a functional gene product such as an RNA or protein. In some embodiments, a nucleic acid includes one or more introns. In some embodiments, nucleic acids are prepared by one or more of isolation from a natural source, enzymatic synthesis by polymerization based on a complementary template (*in vivo* or *in vitro*), reproduction in a recombinant cell or system, and chemical synthesis. In some embodiments, a nucleic acid is at least 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 20, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 600, 700, 800, 900, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000 or more residues long. In some embodiments, a nucleic acid is single stranded; in some embodiments, a nucleic acid is double stranded. In some embodiments a nucleic acid has a nucleotide sequence comprising at least one element that encodes, or is the complement of a sequence that encodes, a polypeptide. In some embodiments, a nucleic acid has enzymatic activity.

[0133] ***Pharmaceutically acceptable vehicles***: The pharmaceutically acceptable carriers (vehicles) useful in this disclosure are conventional. Remington's Pharmaceutical Sciences, by E. W. Martin, Mack Publishing Co., Easton, PA, 15th Edition (1975), describes compositions and formulations suitable for pharmaceutical delivery of one or more therapeutic compositions, (e.g., a composition comprising a chimeric antigen receptor

described herein), and additional pharmaceutical agents. In general, the nature of the carrier will depend on the particular mode of administration being employed. For instance, parenteral formulations usually comprise injectable fluids that include pharmaceutically and physiologically acceptable fluids such as water, physiological saline, balanced salt solutions, aqueous dextrose, glycerol or the like as a vehicle. For solid compositions (for example, powder, pill, tablet, or capsule forms), conventional non-toxic solid carriers can include, for example, pharmaceutical grades of mannitol, lactose, starch, or magnesium stearate. In addition to biologically-neutral carriers, pharmaceutical compositions to be administered can contain minor amounts of non-toxic auxiliary substances, such as wetting or emulsifying agents, preservatives, and pH buffering agents and the like, for example sodium acetate or sorbitan monolaurate.

[0134] ***Polypeptide:*** A "polypeptide", generally speaking, is a string of at least two amino acids attached to one another by a peptide bond. In some embodiments, a polypeptide may include at least 3-5 amino acids, each of which is attached to others by way of at least one peptide bond. Those of ordinary skill in the art will appreciate that polypeptides sometimes include "non-natural" amino acids or other entities that nonetheless are capable of integrating into a polypeptide chain, optionally. In some embodiments, the term "polypeptide" is used to refer to specific functional classes of polypeptides, such as, an antibody, chimeric antigen receptor, or costimulatory domain polypeptides, etc. For each such class, the present specification provides and/or the art is aware of several examples of amino acid sequences of known exemplary polypeptides within the class; in some embodiments, one or more such known polypeptides is/are reference polypeptides for the class. In such embodiments, the term "polypeptide" refers to any member of the class that shows sufficient sequence homology or identity with a relevant reference polypeptide that one skilled in the art would appreciate that it should be included in the class. In many embodiments, a member of the representative class also shares significant activity with the reference polypeptide. For example, in some embodiments, a member polypeptide shows an overall degree of sequence homology or identity with a reference polypeptide that is at least about 30-40%, and is often greater than about 50%, 60%, 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more and/or includes at least one region (i.e., a conserved region, often including a characteristic sequence element) that shows very high

sequence identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99%. Such a conserved region usually encompasses at least 3-4 and often up to 20 or more amino acids; in some embodiments, a conserved region encompasses at least one stretch of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more contiguous amino acids.

[0135] It is understood that the antibodies and antigen binding agents of the invention may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on the polypeptide functions. Whether or not a particular substitution will be tolerated, i.e., will not adversely affect desired biological properties, such as binding activity, can be determined as described in Bowie, J U et al. Science 247:1306-1310 (1990) or Padlan et al. FASEB J. 9:133-139 (1995). A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine).

[0136] ***Prevention:*** The term "prevention", as used herein, refers to prophylaxis, avoidance of disease manifestation, a delay of onset, and/or reduction in frequency and/or severity of one or more symptoms of a particular disease, disorder or condition (e.g., infection for example with influenza virus). In some embodiments, prevention is assessed on a population basis such that an agent is considered to "prevent" a particular disease, disorder or condition if a statistically significant decrease in the development, frequency, and/or intensity of one or more symptoms of the disease, disorder or condition is observed in a population susceptible to the disease, disorder, or condition.

[0137] ***Pure:*** As used herein, an agent or entity is "pure" if it is substantially free of other components. For example, a preparation that contains more than about 90% of a particular agent or entity is typically considered to be a pure preparation. In some

embodiments, an agent or entity is at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% pure.

[0138] ***Recombinant:*** As used herein, the term "recombinant" is intended to refer to polypeptides (e.g., polypeptides as described herein) that are designed, engineered, prepared, expressed, created or isolated by recombinant means, such as polypeptides expressed using a recombinant expression vector transfected into a host cell, polypeptides isolated from a recombinant, combinatorial polypeptide library or polypeptides prepared, expressed, created or isolated by any other means that involves splicing selected sequence elements to one another. In some embodiments, one or more of such selected sequence elements is found in nature. In some embodiments, one or more of such selected sequence elements and/or combinations thereof is designed *in silico*. In some embodiments, one or more such selected sequence elements results from the combination of multiple (e.g., two or more) known sequence elements that are not naturally present in the same polypeptide (e.g., two epitopes from two separate HA polypeptides).

[0139] ***Reference:*** The term "reference" is often used herein to describe a standard or control agent, individual, population, sample, sequence or value against which an agent, individual, population, sample, sequence or value of interest is compared. In some embodiments, a reference agent, individual, population, sample, sequence or value is tested and/or determined substantially simultaneously with the testing or determination of the agent, individual, population, sample, sequence or value of interest. In some embodiments, a reference agent, individual, population, sample, sequence or value is a historical reference, optionally embodied in a tangible medium. Typically, as would be understood by those skilled in the art, a reference agent, individual, population, sample, sequence or value is determined or characterized under conditions comparable to those utilized to determine or characterize the agent, individual, population, sample, sequence or value of interest.

[0140] ***Single domain antibody:*** as used herein, the terms "single domain antibody (sdAb)", "variable single domain" or "immunoglobulin single variable domain (ISV)" "single heavy chain variable domain (VH) antibody" refer to the single variable fragment of an antibody that binds to a target antigen. These terms are used interchangeably herein. A sdAb is a single antigen-binding polypeptide having three complementary determining regions

(CDRs). The sdAb alone is capable of binding to the antigen without pairing with a corresponding CDR-containing polypeptide. A VH single domain antibody refers to a single domain antibody that has a human heavy chain variable domain or a domain that is derived from a human heavy chain variable domain. In some cases, single-domain antibodies are engineered from camelid HCAbs, and their heavy chain variable domains are referred to as "VHHs". Some VHHs may also be known as Nanobodies. Camelid sdAb is one of the smallest known antigen-binding antibody fragments (see, e.g., Hamers-Casterman et al., Nature 363: 446-8 (1993); Greenberg et al., Nature 374: 168-73 (1995); Hassanzadeh-Ghassabeh et al., Nanomedicine (Lond), 8:1013-26 (2013)). A basic VHH has the following structure from the N-terminus to the C-terminus: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4, in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3. As explained below, some embodiments of the various aspects of the invention relate to a binding agent comprising a single heavy chain variable domain antibodies/immunoglobulin heavy chain single variable domain which bind a GCC antigen in the absence of light chain.

[0141] ***Subject***: As used herein, the term "subject" means any mammal, including humans. In certain embodiments of the present invention the subject is an adult, an adolescent or an infant. In some embodiments, terms "individual" or "patient" are used and are intended to be interchangeable with "subject". Also contemplated by the present invention are the administration of the pharmaceutical compositions and/or performance of the methods of treatment in-utero. For example, a subject can be a patient (e.g., a human patient or a veterinary patient), having a cancer, (e.g., of gastrointestinal origin), a symptom of a cancer, in which at least some of the cells express GCC, or a predisposition toward a cancer, in which at least some of the cells express GCC. The term "non-human animals" of the invention includes all non-human vertebrates, e.g., non-human mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, reptiles, etc, unless otherwise noted.

[0142] ***Substantially***: As used herein, the term "substantially" refers to the qualitative condition of exhibiting total or near-total extent or degree of a characteristic or property of interest. One of ordinary skill in the biological arts will understand that biological and chemical phenomena rarely, if ever, go to completion and/or proceed to completeness or

achieve or avoid an absolute result. The term "substantially" is therefore used herein to capture the potential lack of completeness inherent in many biological and chemical phenomena.

[0143] ***Therapeutic agent:*** As used herein, the term "therapeutic agent" refers to an agent (e.g., an antigen binding agent) that has biological activity. The term is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. In some embodiments, the therapeutic agent may be an anti-cancer agent or a chemotherapeutic agent. As used herein, the terms "anti-cancer agent" or "chemotherapeutic agent" refer to agents that have the functional property of inhibiting a development or progression of a neoplasm in a human, particularly a malignant (cancerous) lesion, such as a carcinoma, sarcoma, lymphoma, or leukemia. Inhibition of metastasis or angiogenesis is frequently a property of anti-cancer or chemotherapeutic agents. A chemotherapeutic agent may be a cytotoxic or cytostatic agent. The term "cytostatic agent" refers to an agent which inhibits or suppresses cell growth and/or multiplication of cells. In some embodiments, the therapeutic agent is a genetically modified cell or antibody. In some embodiments, the therapeutic agent is an anti-GCC CAR. In some embodiments, the therapeutic agent is a cell (e.g., a population of cells) expressing a GCC CAR described herein.

[0144] ***Transformation:*** As used herein, refers to any process by which exogenous DNA is introduced into a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. In some embodiments, a particular transformation methodology is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, mating, transfection, lipofection. In some embodiments, a "*transformed*" cell is stably transformed in that the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome. In some embodiments, a transformed cell transiently expresses introduced nucleic acid for limited periods of time.

[0145] ***Treat or treatment***: As used herein, the term "treat" or "treatment" is defined as the administration of an anti-GCC antigen binding agent (e.g., an anti-GCC antibody or fragment thereof, an anti-GCC CAR, cells comprising an anti-GCC CAR, etc.) to a subject, e.g., a patient, or administration, e.g., by application, to an isolated tissue or cell from a subject which is returned to the subject. The anti-GCC antigen binding agent can be administered alone or in combination with a second agent. The treatment can be to cure, heal, alleviate, relieve, alter, remedy, ameliorate, palliate, improve or affect the disorder, the symptoms of the disorder or the predisposition toward the disorder, e.g., a cancer. While not wishing to be bound by theory, treating is believed to cause the inhibition, ablation, or killing of a cell in vitro or in vivo, or otherwise reducing capacity of a cell, e.g., an aberrant cell, to mediate a disorder, e.g., a disorder as described herein (e.g., a cancer).

[0146] ***Variable region or domain***: As used herein, the terms "variable region" or "variable domain" of an antibody refers to the amino-terminal domains of the heavy or light chain of an antibody. The variable domains of the heavy chain and light chain may be referred to as "VH" and "VL", respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites. Heavy-chain only antibodies have a single heavy chain variable region.

[0147] ***Vector:*** As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "expression vectors".

DETAILED DESCRIPTION OF CERTAIN EMBODIMENTS

[0148] The present invention is based on the discovery of novel antigen binding agents that specifically bind guanylyl cyclase C (GCC) and their use in therapeutic methods. The present application provides anti-GCC single-domain antibodies (sdAb) and chimeric antigen receptors (CARs) comprising an extracellular antigen binding domain comprising one or more GCC binding moieties (such as anti-GCC sdAbs).

[0149] The present invention is not limited to particular methods, and experimental conditions described, as such methods and conditions may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting unless indicated, since the scope of the present invention will be limited only by the appended claims.

Guanylyl Cyclase C

[0150] Guanylyl cyclase C (GCC) (also known as STAR, ST Receptor, GUC2C, and GUCY2C) is a transmembrane cell surface receptor that functions in the maintenance of intestinal fluid, electrolyte homeostasis and cell proliferation (Carrithers et al., *Proc Natl Acad Sci USA* 100: 3018-3020 (2003); Mann et al., *Biochem Biophys Res Commun* 239: 463-466 (1997); Pitari et al., *Proc Natl Acad Sci USA* 100: 2695-2699 (2003)); GenBank Accession No. NM_004963, each of which is incorporated herein by reference). This function is mediated through binding of guanylin (Wiegand et al. FEBS Lett. 311:150-154 (1992)). GCC also is a receptor for heat-stable enterotoxin (ST, e.g., having an amino acid sequence of NTFYCCELCCNPACAGCY, SEQ ID NO: 40) which is a peptide produced by *E. coli*, as well as other infectious organisms (Rao, M. C. *Ciba Found. Symp.* 112:74-93 (1985); Knoop F. C. and Owens, M. J. *Pharmacol. Toxicol. Methods* 28:67-72 (1992)). Binding of ST to GCC activates a signal cascade that results in enteric disease, e.g., diarrhea. Nucleotide sequence for human GCC (GenBank Accession No. NM_004963). The amino acid sequence for human GCC (GenPept Accession No. NP_004954):

MKTLLLDLALWSLLFQPGWLSFSSQVSQNCHNGSYEISVLMMGNSAFAEPLK
NLEDAVNEGLEIVRGRLQNAGLNVTVNATFMYSDGLIHNSGDCRSSTCEGLD
LLRKISNAQRMGCVLIGPSCTYSTFQMYLDTELSYPMISAGSFGLSCDYKETLT
RLMSPARKLMYFLVNFWKTNDLPFKTYSWSTSYVYKNGTETEDCFWYLNAL
EASVSYFSHELGFKVVLRQDKEFQDILMDHNRKSNVIIMCGGPEFLYKLKGDR

AVAEDIVIILVDLFNDQYFEDNVTAPDYMKNVLVLTLSPGNSLLNSSFSRNLSP
TKRDFALAYLNGILLFGHMLKIFLENGENITTPKFAHAFRNLTFEGYDGPVTLD
DWGDVDSTMVLLYTSVDTKKYKVLLTYDTHVNKTYPVDMSPTFTWKNSKL
PNDITGRGPQILMIAVFTLTGAVVLLLLVALLMLRKYRKDYELRQKKWSHIPP
ENIFPLETNETNHVSLKIDDDKRRDTIQRLRQCKYDKKRVILKDLKHNDGNFT
EKQKIELNKLLQIDYYNLTKFYGTVKLDTMIFGVIEYCERGSLREVLNDTISYP
DGTFMDWEFKISVLYDIAKGMSYLHSSKTEVHGRLKSTNCVVDSRMVVKITD
FGCNSILPPKKDLWTAPEHLRQANISQKGDVYSYGIIAQEIILRKETFYTLSCRD
RNEKIFRVENSNGMKPFRPDLFLETAEEKELEVYLLVKNCWEEDPEKRPDFKK
IETTLAKIFGLFHDQKNESYMDTLIRRLQLYSRNLEHLVEERTQLYKAERDRA
DRLNFMLLPRLVVKSLKEKGFVEPELYEEVTIYFSDIVGFTTICKYSTPMEVVD
MLNDIYKSFDHIVDHHDVYKVETIGDAYMVASGLPKRNGNRHAIDIAKMALE
ILSFMGTFELEHLPGLPIWIRIGVHSGPCAAGVVGIKMPRYCLFGDTVNTASRM
ESTGLPLRIHVSGSTIAILKRTECQFLYEVRGETYLKGRGNETTYWLTGMKDQ
KFNLPTPPTVENQQRLQAEFSDMIANSLQKRQAAGIRSQKPRRVASYKKGTLE
YLQLNTTDKESTYF (SEQ ID NO: 41)

[0151] The GCC protein has some generally accepted domains each of which contributes to the function of the GCC molecule. GCC functions include a signaling for directing the protein to the cell surface, an extracellular ligand binding, tyrosine kinase activity, and a guanylyl cyclase catalytic activity. In normal human tissues, GCC is expressed at the mucosal cells, e.g., at the apical brush border membranes, lining the small intestine, large intestine and rectum (Carrithers et al., *Dis Colon Rectum* 39: 171-181 (1996)). GCC expression is maintained upon neoplastic transformation of intestinal epithelial cells, with expression in all primary and metastatic colorectal tumors (Carrithers et al., *Dis Colon Rectum* 39: 171-181 (1996); Buc et al. *Eur J Cancer* 41: 1618-1627 (2005); Carrithers et al., *Gastroenterology* 107: 1653-1661 (1994)). Neoplastic cells from the stomach, esophagus and the gastroesophageal junction also express GCC (see, e.g., U.S. Pat. No. 6,767,704; Debruyne et al. *Gastroenterology* 130:1191-1206 (2006)). The tissue-specific expression and association with cancer, e.g., of gastrointestinal origin, (e.g., colon cancer, stomach cancer, or esophageal cancer), can be exploited for the use of GCC as a diagnostic marker for this

disease (Carrithers et al., *Dis Colon Rectum* 39: 171-181 (1996); Buc et al. *Eur J Cancer* 41: 1618-1627 (2005)).

[0152] As a cell surface protein, GCC can also serve as a therapeutic target for receptor binding proteins such as antibodies or ligands. In normal intestinal tissue, GCC is expressed on the apical side of epithelial cell tight junctions that form an impermeable barrier between the luminal environment and vascular compartment (Almenoff et al., *Mol Microbiol* 8: 865-873); Guarino et al., *Dig Dis Sci* 32: 1017-1026 (1987)). As such, systemic intravenous administration of a GCC-binding protein therapeutic will have minimal effect on intestinal GCC receptors, while having access to neoplastic cells of the gastrointestinal system, including invasive or metastatic colon cancer cells, extraintestinal or metastatic colon tumors, esophageal tumors or stomach tumors, adenocarcinoma at the gastroesophageal junction. Additionally, GCC internalizes through receptor mediated endocytosis upon ligand binding (Buc et al. *Eur J Cancer* 41: 1618-1627 (2005); Urbanski et al., *Biochem Biophys Acta* 1245: 29-36 (1995)).

[0153] Polyclonal antibodies raised against the extracellular domain of GCC (Nandi et al. *Protein Expr. Purif.* 8:151-159 (1996)) were able to inhibit the ST peptide binding to human and rat GCC and inhibit ST-mediated cGMP production by human GCC.

[0154] GCC has been characterized as a protein involved in cancers, including colon cancers. See also, Carrithers et al., *Dis Colon Rectum* 39: 171-181 (1996); Buc et al. *Eur J Cancer* 41: 1618-1627 (2005); Carrithers et al., *Gastroenterology* 107: 1653-1661 (1994); Urbanski et al., *Biochem Biophys Acta* 1245: 29-36 (1995).

[0155] Antigen binding molecule therapeutics directed to GCC described herein can be used to inhibit GCC-expressing cancerous cells. Anti-GCC antigen binding molecules of the invention can bind human GCC. In some embodiments, an anti-GCC antigen binding molecule of the invention can inhibit the binding of a ligand, e.g., guanylin or heat-stable enterotoxin to GCC.

Antigen Binding Molecules

[0156] The present invention relates to anti-GCC antigen binding molecules. In some embodiments, anti-GCC molecules of the present inventions cause a cellular reaction upon

binding to GCC on a GCC expressing cell to which it binds. In some embodiments, an anti-GCC antigen binding agent of the invention can block ligand binding to GCC.

[0157] The naturally occurring mammalian antibody structural unit is typified by a tetramer. Each tetramer is composed of two pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function. Human light chains can be classified as kappa and lambda light chains. Heavy chains can be classified as mu, delta, gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 more amino acids. See generally, *Fundamental Immunology* Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). The variable regions of each light/heavy chain pair form the antibody binding site. Preferred isotypes for the anti-GCC antibody molecules are IgG immunoglobulins, which can be classified into four subclasses, IgG1, IgG2, IgG3 and IgG4, having different gamma heavy chains. Most therapeutic antibodies are human, chimeric, or humanized antibodies of the IgG1 type. In a particular embodiment, the anti-GCC antibody molecule has the IgG1 isotype.

[0158] The variable regions of each heavy and light chain pair form the antigen binding site. Thus, an intact IgG antibody has two binding sites which are the same. However, bifunctional or bispecific antibodies are artificial hybrid constructs which have two different heavy/light chain pairs, resulting in two different binding sites.

[0159] The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat Sequences of Proteins of Immunological Interest (National Institutes of Health,

Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J. Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989). As used herein, CDRs are referred according to Kabat for each of the heavy (HCDR1, HCDR2, HCDR3) and light (LCDR1, LCDR2, LCDR3) chains.

[0160] An anti-GCC antibody molecule can comprise all, or an antigen binding subset of the CDRs or the heavy chain, of the antibodies described herein. Amino acid sequences of anti-GCC antigen binding agents described herein, including variable regions and CDRs, can be found in Tables 1-3.

[0161] Thus, in an embodiment the antibody molecule includes one or both of:

[0162] (a) one, two, three, or an antigen binding number of, light chain CDRs (LCDR1, LCDR2 and/or LCDR3) of a human antibody such as an antibody derived from a human hybridoma or a murine antibody (e.g., a light chain of an anti-GCC antibody described in US20180355062A1, which is incorporated by reference in it's entirety). In embodiments the CDR(s) may comprise an amino acid sequence of one or more or all of LCDR1-3 as follows: LCDR1, or modified LCDR1 wherein one to seven amino acids are conservatively substituted) LCDR2, or modified LCDR2 wherein one or two amino acids are conservatively substituted); or LCDR3, or modified LCDR3 wherein one or two amino acids are conservatively substituted; and

[0163] (b) one, two, three, or an antigen binding number of, heavy chain CDRs (HCDR1, HCDR2 and/or HCDR3) as described herein. In embodiments the CDR(s) may comprise an amino acid sequence of one or more or all of HCDR1-3 as follows: HCDR1, or modified HCDR1 wherein one or two amino acids are conservatively substituted; HCDR2, or modified HCDR2 wherein one to four amino acids are conservatively substituted; or HCDR3, or modified HCDR3 wherein one or two amino acids are conservatively substituted.

[0164] In some embodiments, an anti-GCC antibody molecule of the invention can draw antibody-dependent cellular cytotoxicity (ADCC) to a cell expressing GCC, e.g., a tumor cell. Antibodies with the IgG1 and IgG3 isotypes are useful for eliciting effector function in an antibody-dependent cytotoxic capacity, due to their ability to bind the Fc receptor. Antibodies with the IgG2 and IgG4 isotypes are useful to minimize an ADCC response because of their low ability to bind the Fc receptor. In related embodiments

substitutions in the Fc region or changes in the glycosylation composition of an antibody, e.g., by growth in a modified eukaryotic cell line, can be made to enhance the ability of Fc receptors to recognize, bind, and/or mediate cytotoxicity of cells to which anti-GCC antibodies bind (see, e.g., U.S. Pat. Nos. 7,317,091, 5,624,821 and publications including WO 00/42072, Shields, et al. *J. Biol. Chem.* 276:6591-6604 (2001), Lazar et al. *Proc. Natl. Acad. Sci. U.S.A.* 103:4005-4010 (2006), Satoh et al. *Expert Opin Biol. Ther.* 6:1161-1173 (2006)). In certain embodiments, the antibody or antigen-binding fragment (e.g., antibody of human origin, human antibody) can include amino acid substitutions or replacements that alter or tailor function (e.g., effector function). For example, a constant region of human origin (e.g., γ1 constant region, γ2 constant region) can be designed to reduce complement activation and/or Fc receptor binding. (See, for example, U.S. Pat. No. 5,648,260 (Winter et al.), U.S. Pat. No. 5,624,821 (Winter et al.) and U.S. Pat. No. 5,834,597 (Tso et al.), the entire teachings of which are incorporated herein by reference.) Preferably, the amino acid sequence of a constant region of human origin that contains such amino acid substitutions or replacements is at least about 95% identical over the full length to the amino acid sequence of the unaltered constant region of human origin, more preferably at least about 99% identical over the full length to the amino acid sequence of the unaltered constant region of human origin. Additional anti-GCC antigen binding molecules are further described in U.S. Pat. No. 8,785,600 (Nam et al.), the entire teachings of which are incorporated herein by reference.

[0165] In still another embodiment, effector functions can also be altered by modulating the glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody. For example, antibodies with enhanced ADCC activities with a mature carbohydrate structure that lacks fucose attached to an Fc region of the antibody are described in U.S. Patent Application Publication No. 2003/0157108 (Presta). See also U.S. Patent Application Publication No. 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Glycofi has also developed yeast cell lines capable of producing specific glycoforms of antibodies.

[0166] Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNac structures. Such altered

glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which are engineered to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hang et al. describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation. PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields, R. L. et al., 2002 *J. Biol. Chem.* 277:26733-26740). PCT Publication WO 99/54342 by Umana et al. describes cell lines engineered to express glycoprotein-modifying glycosyl transferases (e.g., beta(1,4)-N acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana et al., 1999 *Nat. Biotech.* 17:176-180).

[0167] Humanized antibodies can also be made using a CDR-grafted approach. Techniques of generation of such humanized antibodies are known in the art. Generally, humanized antibodies are produced by obtaining nucleic acid sequences that encode the variable heavy and variable light sequences of an antibody that binds to GCC, identifying the complementary determining region or "CDR" in the variable heavy and variable light sequences and grafting the CDR nucleic acid sequences on to human framework nucleic acid sequences. (See, for example, U.S. Pat. Nos. 4,816,567 and 5,225,539). The location of the CDRs and framework residues can be determined (see, Kabat, E. A., et al. (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, and Chothia, C. et al. *J. Mol. Biol.* 196:901-917 (1987)).

[0168] Anti-GCC antibody molecules described herein have the CDR amino acid sequences and nucleic acid sequences encoding CDRs listed in Tables 5 and 6. In some embodiments sequences from Tables 5 and 6 can be incorporated into molecules which recognize GCC for use in the therapeutic or diagnostic methods described herein. The human

framework that is selected is one that is suitable for in vivo administration, meaning that it does not exhibit immunogenicity. For example, such a determination can be made by prior experience with in vivo usage of such antibodies and studies of amino acid similarities. A suitable framework region can be selected from an antibody of human origin having at least about 65% amino acid sequence identity, and preferably at least about 70%, 80%, 90% or 95% amino acid sequence identity over the length of the framework region within the amino acid sequence of the equivalent portion (e.g., framework region) of the donor antibody, e.g., an anti-GCC antibody molecule (e.g., 3G1). Amino acid sequence identity can be determined using a suitable amino acid sequence alignment algorithm, such as CLUSTAL W, using the default parameters. (Thompson J. D. et al., *Nucleic Acids Res.* 22:4673-4680 (1994).)

[0169] Once the CDRs and FRs of the cloned antibody that are to be humanized are identified, the amino acid sequences encoding the CDRs are identified and the corresponding nucleic acid sequences grafted on to selected human FRs. This can be done using known primers and linkers, the selection of which are known in the art. All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen. After the CDRs are grafted onto selected human FRs, the resulting "humanized" variable heavy and variable light sequences are expressed to produce a humanized Fv or humanized antibody that binds to GCC. Preferably, the CDR-grafted (e.g., humanized) antibody binds a GCC protein with an affinity similar to, substantially the same as, or better than that of the donor antibody. Typically, the humanized variable heavy and light sequences are expressed as a fusion protein with human constant domain sequences so an intact antibody that binds to GCC is obtained. However, a humanized Fv antibody can be produced that does not contain the constant sequences.

[0170] Also within the scope of the invention are humanized antibodies in which specific amino acids have been substituted, deleted or added. In particular, humanized antibodies can have amino acid substitutions in the framework region, such as to improve binding to the antigen. For example, a selected, small number of acceptor framework residues of the humanized immunoglobulin chain can be replaced by the corresponding donor amino acids. Locations of the substitutions include amino acid residues adjacent to the CDR, or

which are capable of interacting with a CDR (see e.g., U.S. Pat. Nos. 5,585,089 or 5,859,205). The acceptor framework can be a mature human antibody framework sequence or a consensus sequence. As used herein, the term "consensus sequence" refers to the sequence found most frequently, or devised from the most common residues at each position in a sequence in a region among related family members. A number of human antibody consensus sequences are available, including consensus sequences for the different subgroups of human variable regions (see, Kabat, E. A., et al., *Sequences of Proteins of Immunological Interest*, Fifth Edition, U.S. Department of Health and Human Services, U.S. Government Printing Office (1991)). The Kabat database and its applications are freely available on line, e.g. via IgBLAST at the National Center for Biotechnology Information, Bethesda, Md. (also see, Johnson, G. and Wu, T. T., *Nucleic Acids Research* 29:205-206 (2001)).

[0171] In certain embodiments, the GCC antibody molecule is a human anti-GCC IgG1 antibody. Since such antibodies possess desired binding to the GCC molecule, any one of such antibodies can be readily isotype-switched to generate a human IgG4 isotype, for example, while still possessing the same variable region (which defines the antibody's specificity and affinity, to a certain extent). Accordingly, as antibody candidates are generated that meet desired "structural" attributes as discussed above, they can generally be provided with at least certain additional "functional" attributes that are desired through isotype switching.

[0172] In some aspects, the portion of a CAR composition of the invention that comprises an antibody fragment is humanized with retention of high affinity for the target antigen and other favorable biological properties. According to one aspect of the invention, humanized antibodies and antibody fragments are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, e.g., the analysis of residues that influence the ability of the candidate immunoglobulin to bind the target antigen.

[0173] In this way, FR residues can be selected and combined from the recipient and import sequences that the desired antibody or antibody fragment characteristic, such as increased affinity for the target antigen, is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

[0174] A humanized antibody or antibody fragment may retain a similar antigenic specificity as the original antibody, e.g., in the present invention, the ability to bind human GCC. In some embodiments, a humanized antibody or antibody fragment may have improved affinity and/or specificity of binding to human GCC.

[0175] In some embodiments, the anti-GCC antigen binding agent comprises one or more CDR sequences provided in Table 1. In some embodiments, the anti-GCC antigen binding agent comprises a heavy chain variable region with a CDR 1 provided in Table 1. In some embodiments, the anti-GCC antigen binding agent comprises a heavy chain variable region with a CDR 2 provided in Table 1. In some embodiments, the anti-GCC antigen binding agent comprises a heavy chain variable region with a CDR 3 provided in Table 1. In some embodiments, the anti-GCC antigen binding agent comprises a heavy chain variable region with a CDR1, CDR2, and CDR3 provided in Table 1. In some embodiments, the anti-GCC antigen binding agent comprises one or more CDR sequences provided in Table 1 wherein said CDR comprises 1, 2, or 3 amino acid substitutions. In one embodiment, said substitution does not adversely affect the binding of the binding agent to its target.

Table 1. Exemplary Anti-GCC CDR sequences

	HCDR 1	**HCDR 2**	**HCDR 3**
V1	HYYWS (SEQ ID NO: 8)	RIYPSGSTSYNPSLKS (SEQ ID NO: 11)	DRSTGWSEWNSDL (SEQ ID NO: 16)
V1-01	HYYWS (SEQ ID NO: 8)	RIYPSGSTSYNPSLKS (SEQ ID NO: 11)	DRSTGWSEWNSDL (SEQ ID NO: 16)
V5	RYWMS	KIRHDGGEKYYVDSVKG	DYTRDV

	(SEQ ID NO: 9)	(SEQ ID NO: 12)	(SEQ ID NO: 17)
V36	RYWMT (SEQ ID NO: 10)	KIKYDGSEKYYADSVKG (SEQ ID NO: 13)	DYNKDY (SEQ ID NO: 18)
V48	RYWMT (SEQ ID NO: 10)	KIRHDGGEKYYPDSVKG (SEQ ID NO: 14)	DYNKDL (SEQ ID NO: 19)
V51	RYWMT (SEQ ID NO: 10)	KIRHDGGEKYYADSVKG (SEQ ID NO: 15)	DYNKDY (SEQ ID NO: 18)

[0176] Anti-GCC antibodies that are not intact antibodies are also useful in this invention. Such antibodies may be derived from any of the antibodies described above. Useful antibody molecules of this type include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., *Nature* 341:544-546 (1989)), which consists of a VH domain; (vii) a single domain functional heavy chain antibody, which consists of a VHH domain (known as a nanobody) see e.g., Cortez-Retamozo, et al., *Cancer Res.* 64: 2853-2857 (2004), and references cited therein; and (vii) an isolated CDR, e.g., one or more isolated CDRs together with sufficient framework to provide an antigen binding fragment. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. *Science* 242:423-426 (1988); and Huston et al. *Proc. Natl. Acad. Sci. USA* 85:5879-5883 (1988). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding fragment" of an antibody. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies. Antibody fragments, such as Fv,

$F(ab')_2$ and Fab may be prepared by cleavage of the intact protein, e.g. by protease or chemical cleavage.

Single-domain Antibodies

[0177] Single-domain antibodies (sdAbs) are different from conventional 4-chain antibodies by having a single monomeric antibody variable domain. For example, camelids and sharks produce sdAbs named heavy chain-only antibodies (HcAbs), which naturally lack light chains. The antigen-binding fragment in each arm of the camelid heavy-chain only antibodies has a single heavy chain variable domain (VHH), which can have high affinity to an antigen without the aid of a light chain. Camelid VHH is known as the smallest functional antigen-binding fragment with a molecular weight of approximately 15 kD. In some embodiments, the antigen binding agents are single human heavy chain variable domain (VH) antibodies. Such binding molecules are also termed Humabody® and may be used interchangeably herein. Humabody® is a registered trademark of Crescendo Biologics Ltd.

[0178] One aspect of the present application provides isolated single-domain antibodies (referred herein as "anti-GCC sdAbs") that specifically bind to GCC, such as human GCC. In some embodiments, the anti- GCC sdAb modulates GCC activity. In some embodiments, the anti- GCC sdAb is an antagonist antibody. Further provided are antigen-binding fragments derived from any one of the anti- GCC sdAbs described herein, and antigen binding proteins comprising any one of the anti-GCC sdAbs described herein. In some embodiments, the anti-GCC sdAb comprise one, two and/or three CDR sequences provided in Table 1. Exemplary anti-GCC sdAbs are listed in Table 2 and 3. In some embodiments, the anti-GCC sdAb comprises a variable heavy chain provided in Table 2 or Table 3.

[0179] In some embodiments, some or all of the CDRs sequences, the heavy chain, can be used in another antigen binding agent, e.g., in a CDR-grafted, humanized, or chimeric antibody molecule. Embodiments include an antibody molecule that comprises sufficient CDRs, e.g., all three CDRs from one of the above-referenced heavy chain variable region, to allow binding to cell surface GCC.

[0180] Insome embodiments the CDRs, e.g., all of the HCDRs, are embedded in human or human derived framework region(s). Examples of human framework regions include human germline framework sequences, human germline sequences that have been affinity matured (either *in vivo* or *in vitro*), or synthetic human sequences, e.g., consensus sequences. In an embodiment the heavy chain framework is an IgG1 or IgG2 framework.

[0181] In some embodiments, the anti-GCC antigen binding agents of the present invention comprise a heavy chain variable region amino acid sequence provided in Table 2. In some embodiments, the anti-GCC antigen binding agents are single domain heavy chain only antibodies (e.g., antigen binding agents that do not comprise an immunoglobulin light chain).

Table 2. Exemplary Heavy Chain Variable Region (VH) Amino Acid Sequences

Description	VH Amino Acid Sequence
V1	QVQLQESGPGLVKPSETLSLTCTVSGASISHYYWSWFRQPA GKGLEWIGRIYPSGSTSYNPSLKSRVAMSVDTPKNQFSLNL SSVTAADTAVYYCARDRSTGWSEWNSDLWGRGTLVTVSS (SEQ ID NO: 1)
V1-01	EVQLQESGPGLVKPSETLSLTCTVSGASISHYYWSWFRQPA GKGLEWIGRIYPSGSTSYNPSLKSRVAMSVDTPKNQFSLKL SSVTAADTAVYYCARDRSTGWSEWNSDLWGRGTLVTVSS (SEQ ID NO: 20)
V5	QVQLVESGGGLVQPGGSLRLSCTASGFTFSRYWMSWVRQ APGKGLEWVAKIRHDGGEKYYVDSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYYCATDYTRDVWGQGTAVTVSS (SEQ ID NO: 21)
V36	EVQLVESGGGLAQPGGSLRLSCAASGFTFSRYWMTWVRQ APGGRLEWVAKIKYDGSEKYYADSVKGRFTISRDNAKNSL YLQMDSLRAEDTAVYYCTRDYNKDYWGQGTLVTVSS (SEQ ID NO: 26)
V48	EVQLVESGGGLVQPGGSLRLTCAASGFTFSRYWMTWVRQ APGKGLEWVAKIRHDGGEKYYPDSVKGRFTVSRDNAKNS

	LYLQMDNLRAEDTAMYYCTRDYNKDLWGQGTLVTVSS (SEQ ID NO: 27)
V51	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMTWVRQ APGKGLEWVAKIRHDGGEKYYADSVKGRFTISRDNAKNS LYLQMNSLRAEDTAVYYCTRDYNKDYWGQGTLVTVSS (SEQ ID NO: 28)

[0182] In some embodiments, the anti-GCC antigen binding agents of the present invention comprise a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a VH sequence provided in Table 2. In some embodiments, the VH anti-GCC antigen binding agent (e.g., single domain antibody) comprises a leader sequence. In some embodiments, the VH anti-GCC antigen binding agent comprises a leader sequence comprising MKHLWFFLLLVAAPRWVLS (SEQ ID NO: 6), MELGLSWVFLVAILEGVQC (SEQ ID NO: 7) or MEFGLSWVFLVAIIKGVQC (SEQ ID NO: 42). In some embodiments, the VH anti-GCC antigen binding agent comprises a leader sequence comprising MALPVTALLLPLALLLHAARP (SEQ ID NO: 45)

[0183] In some embodiments, the anti-GCC antigen binding agents of the present invention comprise a heavy chain variable region amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to a VH sequence provided in Table 3. In some embodiments, the anti-GCC antigen binding agents of the present invention comprise a heavy chain variable region amino acid sequence that is identical to a VHsequence provided in Table 3.

[0184] In some embodiments, the VH anti-GCC antigen binding agent (e.g., single domain antibody) comprises a leader sequence provided that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% in Table 3. In some embodiments, the VH anti-GCC antigen binding agent (e.g., single domain antibody) comprises a leader sequence provided in Table 3.

Table 3. Exemplary Heavy Chain Variable Region (VH) Amino Acid Sequences

	Leader Sequence	VH sequence

V1	MKHLWFFLLLVAAPRWVLS (SEQ ID NO: 6)	QVQLQESGPGLVKPSETLSLTCTVSGASISHYYWSWFRQPAGKGLEWIGRIYPSGSTSYNPSLKSRVAMSVDTPKNQFSLNLSSVTAADTAVYYCARDRSTGWSEWNSDLWGRGTLVTVSS (SEQ ID NO: 1)
V1-01	MKHLWFFLLLVAAPRWVLS (SEQ ID NO: 6)	EVQLQESGPGLVKPSETLSLTCTVSGASISHYYWSWFRQPAGKGLEWIGRIYPSGSTSYNPSLKSRVAMSVDTPKNQFSLKLSSVTAADTAVYYCARDRSTGWSEWNSDLWGRGTLVTVSS (SEQ ID NO: 20)
V5	MELGLSWVFLVAILEGVQC (SEQ ID NO: 7)	QVQLVESGGGLVQPGGSLRLSCTASGFTFSRYWMSWVRQAPGKGLEWVAKIRHDGGEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCATDYTRDVWGQGTAVTVSS (SEQ ID NO: 21)
V8	MELGLSWVFLVAILEGVQC (SEQ ID NO: 7)	QVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGLEWVAKIKYDGSEKYYVDSVKGRFTISRDNAKNSVYLQMNSLRAEDTGVYYCATDFTRDVWGQGTTVTVSS (SEQ ID NO: 22)
V9	MELGLSWVFLVAILEGVQC (SEQ ID NO: 7)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMTWVRQAPGRGLEWVAKIRYDGGEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCATDFTRDVWGQGTTVTVSS (SEQ ID NO: 23)
V30	MELGLSWVFLVAILEGVQC (SEQ ID NO: 7)	QVQLVESGGGLVQPGGSLRLSCAASGFNFGRYWMSWVRQAPGKGREWVAKIKYDGSEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCATDFTRDVWGQGTTVTVSS (SEQ ID NO: 24)
V31	MEFGLSWVFLVAIIKGVQC (SEQ ID NO: 42)	QVQLVESGGGVVRPGGSLRLSCAASGFTFSRYWMSWVRQAPGKGREWVAKIKYDGSEKYYADSVKGRFTISRDNAKNSLYLQMNSLRADDTAVYYCATDFTRDVWGQGTTVTVSS (SEQ ID NO: 25)
V36	MELGLSWVFLVAILEGVQC (SEQ ID NO: 7)	EVQLVESGGGLAQPGGSLRLSCAASGFTFSRYWMTWVRQAPGGRLEWVAKIKYDGSEKYYADSVKGRFTISRDNAK

		NSLYLQMDSLRAEDTAVYYCTRDYNKDYWGQGTLVTV SS (SEQ ID NO: 26)
V48	MELGLSWVF LVAILEGVQC (SEQ ID NO: 7)	EVQLVESGGGLVQPGGSLRLTCAASGFTFSRYWMTWVR QAPGKGLEWVAKIRHDGGEKYYPDSVKGRFTVSRDNAK NSLYLQMDNLRAEDTAMYYCTRDYNKDLWGQGTLVTV SS (SEQ ID NO: 27)
V51	MELGLSWVF LVAILEGVQC (SEQ ID NO: 7)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMTWVR QAPGKGLEWVAKIRHDGGEKYYADSVKGRFTISRDNAK NSLYLQMNSLRAEDTAVYYCTRDYNKDYWGQGTLVTV SS (SEQ ID NO: 28)

[0185] Antibody fragments for in vivo therapeutic or diagnostic use can benefit from modifications which improve their serum half-lives. Suitable organic moieties intended to increase the in vivo serum half-life of the antibody can include one, two or more linear or branched moiety selected from a hydrophilic polymeric group (e.g., a linear or a branched polymer (e.g., a polyalkane glycol such as polyethylene glycol, monomethoxy-polyethylene glycol and the like), a carbohydrate (e.g., a dextran, a cellulose, a polysaccharide and the like), a polymer of a hydrophilic amino acid (e.g., polylysine, polyaspartate and the like), a polyalkane oxide and polyvinyl pyrrolidone), a fatty acid group (e.g., a mono-carboxylic acid or a di-carboxylic acid), a fatty acid ester group, a lipid group (e.g., diacylglycerol group, sphingolipid group (e.g., ceramidyl)) or a phospholipid group (e.g., phosphatidyl ethanolamine group). Preferably, the organic moiety is bound to a predetermined site where the organic moiety does not impair the function (e.g., decrease the antigen binding affinity) of the resulting immunoconjugate compared to the non-conjugated antibody moiety. The organic moiety can have a molecular weight of about 500 Da to about 50,000 Da, preferably about 2000, 5000, 10,000 or 20,000 Da. Examples and methods for modifying polypeptides, e.g., antibodies, with organic moieties can be found, for example, in U.S. Pat. Nos. 4,179,337 and 5,612,460, PCT Publication Nos. WO 95/06058 and WO 00/26256, and U.S. Patent Application Publication No. 20030026805.

Chimeric Antigen Receptors

[0186] Chimeric Antigen Receptors (CARs) are hybrid molecules comprising three essential units: (1) an extracellular antigen-binding motif, (2) linking/transmembrane motifs, and (3) intracellular T-cell signaling motifs (Long A H, Haso W M, Orentas R J. Lessons learned from a highly-active CD22-specific chimeric antigen receptor. Oncoimmunology. 2013; 2 (4):e23621). In the various embodiments of the GCC-specific CARs disclosed herein, the general scheme is set forth in Fig. 1. In some embodiments, the anti-GCC CARs comprise from the N-terminus to the C-terminus, a signal or leader peptide, an antigen binding domain, a transmembrane and/or hinge domain, a costimulatory domain, and an intracellular domain.

[0187] The present invention provides a CAR (e.g., a CAR polypeptide) that comprises an anti-GCC binding domain (e.g., a GCC binding domain as described herein), a transmembrane domain, and an intracellular signaling domain, and wherein said anti-GCC binding domain comprises a heavy chain complementary determining region 1 (HC CDR1), a heavy chain complementary determining region 2 (HC CDR2), and a heavy chain complementary determining region 3 (HC CDR3) of any anti-GCC heavy chain binding domain amino acid sequences listed in Table 1 or 8. In some embodiments, the anti-GCC CARs comprise from the N-terminus to the C-terminus, a signal or leader peptide, anti-GCC vH, CD28 transmembrane and hinge, CD28 costimulatory domain, and CD3 zeta intracellular domain.

[0188] The antigen binding domain can be any protein that binds to the antigen including but not limited to a monoclonal antibody, a polyclonal antibody, a recombinant antibody, a human antibody, a humanized antibody, and a functional fragment thereof, including but not limited to a single-domain antibody such as a heavy chain variable domain (VH), a light chain variable domain (VL) and a variable domain (VHH) of camelid derived nanobody, and to an alternative scaffold known in the art to function as antigen binding domain, such as a recombinant fibronectin domain, and the like. In some embodiments, the antigen binding domain

[0189] The antigen-binding motif of a CAR is commonly fashioned after a single chain Fragment variable (ScFv), the minimal binding domain of an immunoglobulin (Ig) molecule or single domain antibody (For example, WO2018/028647A1). Alternate antigen-

binding motifs, such as receptor ligands (i.e., IL-13 has been engineered to bind tumor expressed IL-13 receptor), intact immune receptors, library-derived peptides, and innate immune system effector molecules (such as NKG2D) also have been engineered. There remains significant work with regard to defining the most active T-cell population to transduce with CAR vectors, determining the optimal culture and expansion techniques, and defining the molecular details of the CAR protein structure itself.

[0190] The linking motifs of a CAR can be a relatively stable structural domain, such as the constant domain of IgG, or designed to be an extended flexible linker. In some embodiments, the anti-GCC binding domain (e.g., a polypeptide comprising a sequence provided in Table 1 or Table 7), is attached to the transmembrane domain via a linker, e.g., a linker described herein. In some embodiments, the anti-GCC CAR includes a (Gly4-Ser)n linker, wherein n is 1, 2, 3, 4, 5, or 6 (SEQ ID NO: 72). In some embodiments, the linker comprises the amino acid sequence RAAA (SEQ ID NO: 53).

[0191] Structural motifs, such as those derived from IgG constant domains, can be used to extend the ScFv binding domain away from the T-cell plasma membrane surface. This may be important for some tumor targets where the binding domain is particularly close to the tumor cell surface membrane (such as for the disialoganglioside GD2; Orentas et al., unpublished observations). To date, the signaling motifs used in CARs always include the CD3-ζ chain because this core motif is the key signal for T cell activation. The first reported second-generation CARs featured CD28 signaling domains and the CD28 transmembrane sequence. This motif was used in third-generation CARs containing CD137 (4-1BB) signaling motifs as well (Zhao Y et al. J Immunol. 2009; 183 (9): 5563-74). With the advent of new technology, the activation of T cells with beads linked to anti-CD3 and anti-CD28 antibody, and the presence of the canonical "signal 2" from CD28 was no longer required to be encoded by the CAR itself. Using bead activation, third-generation vectors were found to be not superior to second-generation vectors in in vitro assays, and they provided no clear benefit over second-generation vectors in mouse models of leukemia (Haso W, Lee D W, Shah N N, Stetler-Stevenson M, Yuan C M, Pastan I H, Dimitrov D S, Morgan R A, FitzGerald D J, Barrett D M, Wayne A S, Mackall C L, Orentas R J. Anti-CD22-chimeric antigen receptors targeting B cell precursor acute lymphoblastic leukemia, Blood. 2013; 121 (7):1165-74; Kochenderfer J N et al. Blood. 2012; 119 (12):2709-20). This is borne out by the clinical

success of CD19-specific CARs that are in a second generation CD28/CD3-ζ (Lee D W et al. American Society of Hematology Annual Meeting. New Orleans, La.; Dec. 7-10, 2013) and a CD137/CD3-ζ signaling format (Porter D L et al. N Engl J Med. 2011; 365 (8): 725-33). In addition to CD137, other tumor necrosis factor receptor superfamily members such as OX40 also are able to provide important persistence signals in CAR-transduced T cells (Yvon E et al. Clin Cancer Res. 2009; 15(18):5852-60). Equally important are the culture conditions under which the CAR T-cell populations were cultured.

[0192] T-cell-based immunotherapy has become a new frontier in synthetic biology; multiple promoters and gene products are envisioned to steer these highly potent cells to the tumor microenvironment, where T cells can both evade negative regulatory signals and mediate effective tumor killing. The elimination of unwanted T cells through the drug-induced dimerization of inducible caspase 9 constructs with AP1903 demonstrates one way in which a powerful switch that can control T-cell populations can be initiated pharmacologically (Di Stasi A et al. N Engl J Med. 2011; 365(18):1673-83). Thus, while it appears that CARs can trigger T-cell activation in a manner similar to an endogenous T-cell receptor, a major impediment to the clinical application of this technology to date has been limited *in vivo* expansion of CAR+ T cells, rapid disappearance of the cells after infusion, and disappointing clinical activity. Accordingly, there is an urgent and long felt need in the art for discovering novel compositions and methods for treatment of cancer using an approach that can exhibit specific and efficacious anti-tumor effect without the aforementioned shortcomings (i.e. high toxicity, insufficient efficacy).

[0193] The present invention addresses these needs by providing CAR compositions and therapeutic methods that can be used to treat cancers and other diseases and/or conditions. In particular, the present invention as disclosed and described herein provides CARs that may be used the treatment of diseases, disorders or conditions associated with dysregulated expression of GCC and which CARs contain GCC antigen binding domains that exhibit a high surface expression on transduced T cells, exhibit a high degree of cytolysis and transduced T cell in vivo expansion and persistence.

[0194] In some embodiments, the anti-GCC antigen binding agents are chimeric antigen receptors (CAR). Characteristics of CARs include their ability to redirect T-cell

specificity and reactivity toward a selected target in a non-MHC-restricted manner, and exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T cells expressing CARs the ability to recognize antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape. Moreover, when expressed in T-cells, CARs advantageously do not dimerize with endogenous T cell receptor (TCR) alpha and beta chains.

Extracellular Domain

[0195] As described herein, the CAR comprises a target-specific binding element (e.g., at least a portion of an anti-GCC antigen binding agent) otherwise referred to as an antigen binding domain or moiety. The choice of domain depends upon the type and number of ligands that define the surface of a target cell. For example, the antigen binding domain may be chosen to recognize a ligand (e.g., GCC) that acts as a cell surface marker on target cells associated with a particular disease state (e.g., cancer). Thus examples of cell surface markers that may act as ligands for the antigen binding domain in the CAR include those associated with viral, bacterial and parasitic infections, autoimmune disease and cancer cells.

[0196] In some embodiments, the extracellular domain of the anti-GCC CAR comprises an antigen binding agent that comprises at least one CDR provided in Table 1. In some embodiments, the extracellular domain of the anti-GCC CAR comprises a variable heavy chain that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97% 98% or 99% identical to a VH amino acid sequence provided in Table 2 or Table 3. In some embodiments, the extracellular domain of the anti-GCC CAR comprises a variable heavy chain provided in Table 2. In some embodiments, the extracellular domain of the anti-GCC CAR comprises a variable heavy chain provided in Table 3.

Transmembrane Domain

[0197] With respect to the transmembrane domain, in various embodiments, a CAR can be designed to comprise a transmembrane domain that is attached to the extracellular domain of the CAR (e.g., the anti-GCC antigen binding domain. A transmembrane domain can include one or more additional amino acids adjacent to the transmembrane region, e.g., one or more amino acid associated with the extracellular region of the protein from which the transmembrane was derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the

extracellular region) and/or one or more additional amino acids associated with the intracellular region of the protein from which the transmembrane protein is derived (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 up to 15 amino acids of the intracellular region).

[0198] In one aspect, the transmembrane domain is one that is associated with one of the other domains of the CAR is used. In some instances, the transmembrane domain can be selected or modified by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same or different surface membrane proteins, e.g., to minimize interactions with other members of the receptor complex. In one aspect, the transmembrane domain is capable of homodimerization with another CAR on the CAR-expressing cell, e.g., CART cell, surface. In a different aspect the amino acid sequence of the transmembrane domain may be modified or substituted so as to minimize interactions with the binding domains of the native binding partner present in the same CAR-expressing cell, e.g., CART.

[0199] As described herein, the CAR comprises a transmembrane domain. With respect to the transmembrane domain, the CAR comprises one or more transmembrane domains fused to the extracellular GCC antigen binding domain of the CAR. The transmembrane domain may be derived either from a natural or from a synthetic source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein.

[0200] Transmembrane regions of particular use in the CARs described herein may be derived from (i.e. comprise at least the transmembrane region(s) of) the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, mesothelin, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154.

[0201] The transmembrane domain may be derived either from a natural or from a recombinant source. Where the source is natural, the domain may be derived from any membrane-bound or transmembrane protein. In one aspect the transmembrane domain is capable of signaling to the intracellular domain(s) whenever the CAR has bound to a target. A transmembrane domain of particular use in this invention may include at least the transmembrane region(s) of e.g., the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8 (e.g., CD8 alpha, CD8 beta), CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137, CD154. In some embodiments, a

transmembrane domain may include at least the transmembrane region(s) of a costimulatory molecule, e.g., MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CDlla/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLA1, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDlld, ITGAE, CD103, ITGAL, CDlla, LFA-1, ITGAM, CDllb, ITGAX, CDllc, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83.

[0202] In some embodiments, the CAR molecule comprises a transmembrane domain of a protein selected from the group consisting of the alpha, beta or zeta chain of the T-cell receptor, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD86, CD134, CD137 and CD154.

[0203] Alternatively the transmembrane domain may be synthetic, in which case it will comprise predominantly hydrophobic residues such as leucine and valine. In some embodiments, a triplet of phenylalanine, tryptophan and valine will be found at each end of a synthetic transmembrane domain. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage between the transmembrane domain and the cytoplasmic signaling domain of the CAR. In some embodiments, the linker is a glycine-serine doublet or a triple alanine linker.

[0204] In some embodiments, the transmembrane domain that naturally is associated with one of the domains in the CAR is used in addition to the transmembrane domains described supra. In some embodiments, the transmembrane domain can be selected by amino acid substitution to avoid binding of such domains to the transmembrane domains of the same

or different surface membrane proteins to minimize interactions with other members of the receptor complex.

[0205] In some embodiments, the transmembrane domain in the CAR of the invention is a CD28 transmembrane domain. In some embodiments, the CD28 transmembrane domain comprises the nucleic acid sequence of FWVLVVVGGVLACYSLLVTVAFIIFWV SEQ ID NO: 30. In some embodiments, the CD28 transmembrane domain comprises a nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 30. In some embodiments, the transmembrane domain comprises a sequence having at least one, two or three modifications (e.g., substitutions) but not more than 20, 10 or 5 modifications (e.g., substitutions) of an amino acid sequence of SEQ ID NO: 30 or a sequence with at least 95%, 96%, 97%, 98% or 99% identity to an amino acid sequence of SEQ ID NO: 30.

[0206] In some embodiments, the transmembrane domain can be attached to the extracellular region of the CAR, e.g., the antigen binding domain of the CAR, via a hinge, e.g., a hinge from a human protein. For example, in one embodiment, the hinge can be a human Ig (immunoglobulin) hinge, e.g., an IgG4 hinge, or a CD8a hinge.

[0207] In the CAR, a spacer domain, also termed hinge domain, can be arranged between the extracellular domain and the transmembrane domain, or between the intracellular domain and the transmembrane domain. The spacer domain means any oligopeptide or polypeptide that serves to link the transmembrane domain with the extracellular domain and/or the transmembrane domain with the intracellular domain. The spacer domain comprises up to 300 amino acids, preferably 10 to 100 amino acids, and most preferably 25 to 50 amino acids.

[0208] In several embodiments, the linker can include a spacer element, which, when present, increases the size of the linker such that the distance between the effector molecule or the detectable marker and the antibody or antigen binding fragment is increased. Exemplary spacers are known to the person of ordinary skill, and include those listed in U.S. Pat. Nos. 7,964,566 7,498,298, 6,884,869, 6,323,315, 6,239,104, 6,034,065, 5,780,588, 5,665,860, 5,663,149, 5,635,483, 5,599,902, 5,554,725, 5,530,097, 5,521,284, 5,504,191, 5,410,024, 5,138,036, 5,076,973, 4,986,988, 4,978,744, 4,879,278, 4,816,444, and 4,486,414, as well as

U.S. Pat. Pub. Nos. 20110212088 and 20110070248, each of which is incorporated by reference herein in its entirety.

[0209] The spacer domain preferably has a sequence that promotes binding of a CAR with an antigen and enhances signaling into a cell. Examples of an amino acid that is expected to promote the binding include cysteine, a charged amino acid, and serine and threonine in a potential glycosylation site, and these amino acids can be used as an amino acid constituting the spacer domain.

[0210] In some embodiments, the CAR comprises a hinge domain. In some embodiments, the hinge domain comprises the nucleic acid sequence of IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKP (SEQ ID NO: 29). In some embodiments, the hinge domain comprises a nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 29. In some embodiments, the hinge domain comprises a sequence having at least one, two or three modifications (e.g., substitutions) but not more than 20, 10 or 5 modifications (e.g., substitutions) of an amino acid sequence of SEQ ID NO: 29 or a sequence with 95-99% identity to an amino acid sequence of SEQ ID NO: 29.

[0211] In some embodiments, the CAR comprises a CD8 hinge domain. In some embodiments the hinge domain comprises the amino acid sequence TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACD (SEQ ID NO: 5). In some embnodiments the CAR comprises a CD8 transmembrane domain. In some embodiments the transmembrane domain comprises the amino acid sequence IYIWAPLAGTCGVLLLSLVITLYC (SEQ ID NO: 2).In some embodiments, the hinge and transmembrane domains are derived from the same molecule. In other embodiments, the hinge and transmembrane domains are derived from different molecules (e.g., CD8 fused to CD28). In some embodiments, the CAR comprises a hinge domain. In some embodiments, the hinge domain comprises the nucleic acid sequence of IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVT VAFIIFWV (SEQ ID NO: 31). In some embodiments, the hinge domain comprises a nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 31. In some embodiments, the hinge domain comprises a sequence having at least one, two or three

modifications (e.g., substitutions) but not more than 20, 10 or 5 modifications (e.g., substitutions) of an amino acid sequence of SEQ ID NO: 31.

[0212] In one embodiment, the CAR comprises a tag sequence encoding a truncated sequence of epidermal growth factor receptor (tEGFR). In some embodiments, the tEGFR tag comprises the amino acid sequence of SEQ ID NO: 43, or a sequence with at least 95%, 96%, 97%, 98% or 99% identity thereof.

Intracellular Domain

[0213] The cytoplasmic domain or otherwise the intracellular signaling domain of the CAR is responsible for activation of at least one of the normal effector functions of the immune cell in which the CAR has been placed in. The term "effector function" refers to a specialized function of a cell. Effector function of a T cell, for example, may be cytolytic activity or helper activity including the secretion of cytokines. Thus the term "intracellular signaling domain" refers to the portion of a protein which transduces the effector function signal and directs the cell to perform a specialized function. While usually the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

[0214] Examples of intracellular signaling domains for use in the CAR include the cytoplasmic sequences of the T cell receptor (TCR) and co-receptors that act in concert to initiate signal transduction following antigen receptor engagement, as well as any derivative or variant of these sequences and any synthetic sequence that has the same functional capability. Signals generated through the TCR alone are insufficient for full activation of the T cell and that a secondary or co-stimulatory signal is also required. Thus, T cell activation can be said to be mediated by two distinct classes of cytoplasmic signaling sequence: those that initiate antigen-dependent primary activation through the TCR (primary cytoplasmic

signaling sequences) and those that act in an antigen-independent manner to provide a secondary or co-stimulatory signal (secondary cytoplasmic signaling sequences).

[0215] Primary cytoplasmic signaling sequences regulate primary activation of the TCR complex either in a stimulatory way, or in an inhibitory way. Primary cytoplasmic signaling sequences that act in a stimulatory manner may contain signaling motifs which are known as immunoreceptor tyrosine-based activation motifs or ITAMs. In some embodiments, the ITAM-containing domain within the CAR recapitulates the signaling of the primary TCR independently of endogenous TCR complexes. In one aspect, the primary signal is initiated by, for instance, binding of a TCR/CD3 complex with an MHC molecule loaded with peptide, and which leads to mediation of a T cell response, including, but not limited to, proliferation, activation, differentiation, and the like. A primary cytoplasmic signaling sequence (also referred to as a "primary signaling domain") that acts in a stimulatory manner may contain a signaling motif which is known as immunoreceptor tyrosine-based activation motif or ITAM.

[0216] Examples of ITAM containing primary cytoplasmic signaling sequences that are of particular use in the CARs disclosed herein include those derived from TCR zeta (CD3 Zeta), FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, and CD66d. Specific, non-limiting examples, of the ITAM include peptides having sequences of amino acid numbers 51 to 164 of CD3.zeta. (NCBI RefSeq: NP.sub.--932170.1), amino acid numbers 45 to 86 of Fc.epsilon.RI.gamma. (NCBI RefSeq: NP.sub.--004097.1), amino acid numbers 201 to 244 of Fc.epsilon.RI.beta. (NCBI RefSeq: NP.sub.--000130.1), amino acid numbers 139 to 182 of CD3.gamma. (NCBI RefSeq: NP.sub.--000064.1), amino acid numbers 128 to 171 of CD3.delta. (NCBI RefSeq: NP.sub.--000723.1), amino acid numbers 153 to 207 of CD3.epsilon. (NCBI RefSeq: NP.sub.--000724.1), amino acid numbers 402 to 495 of CD5 (NCBI RefSeq: NP.sub.--055022.2), amino acid numbers 707 to 847 of 0022 (NCBI RefSeq: NP.sub.--001762.2), amino acid numbers 166 to 226 of CD79a (NCBI RefSeq: NP.sub.--001774.1), amino acid numbers 182 to 229 of CD79b (NCBI RefSeq: NP.sub.--000617.1), and amino acid numbers 177 to 252 of CD66d (NCBI RefSeq: NP.sub.--001806.2), and their variants having the same function as these peptides have. The amino acid number based on amino acid sequence information of NCBI RefSeq ID or GenBank described herein is numbered based on the full length of the precursor (comprising a signal peptide sequence etc.) of each protein.

[0217] In embodiments, the intracellular signal domain transduces the effector function signal and directs the cell to perform a specialized function. While the entire intracellular signaling domain can be employed, in many cases it is not necessary to use the entire chain. To the extent that a truncated portion of the intracellular signaling domain is used, such truncated portion may be used in place of the intact chain as long as it transduces the effector function signal. The term intracellular signaling domain is thus meant to include any truncated portion of the intracellular signaling domain sufficient to transduce the effector function signal.

[0218] The intracellular signaling domain generates a signal that promotes an immune effector function of the CAR containing cell, e.g., a CART cell. Examples of immune effector function, e.g., in a CART cell, include cytolytic activity and helper activity, including the secretion of cytokines.

[0219] In an embodiment, the intracellular signaling domain can comprise a primary intracellular signaling domain. Exemplary primary intracellular signaling domains include those derived from the molecules responsible for primary stimulation, or antigen dependent simulation. In an embodiment, the intracellular signaling domain can comprise a costimulatory intracellular domain. Exemplary costimulatory intracellular signaling domains include those derived from molecules responsible for costimulatory signals, or antigen independent stimulation. For example, in the case of a CART, a primary intracellular signaling domain can comprise a cytoplasmic sequence of a T cell receptor, and a costimulatory intracellular signaling domain can comprise cytoplasmic sequence from co-receptor or costimulatory molecule.

[0220] In some embodiments, the primary cytoplasmic signaling sequences include, those derived from CD3 zeta, FcR gamma, FcR beta, CD3 gamma, CD3 delta, CD3 epsilon, CD5, CD22, CD79a, CD79b, CD278 (also known as "ICOS"), FceRI, CD66d, DAP10 and DAP12. In one embodiment, the cytoplasmic signaling molecule in the CAR comprises a cytoplasmic signaling sequence derived from CD3 zeta.

[0221] In one embodiment, a primary signaling domain comprises a modified ITAM domain, e.g., a mutated ITAM domain which has altered (e.g., increased or decreased) activity as compared to the native ITAM domain. In one embodiment, a primary signaling

domain comprises a modified ITAM-containing primary intracellular signaling domain, e.g., an optimized and/or truncated ITAM-containing primary intracellular signaling domain. In an embodiment, a primary signaling domain comprises one, two, three, four or more ITAM motifs.

[0222] In some embodiments, the intracellular domain of the CAR can be designed to comprise the CD3-zeta signaling domain by itself or combined with any other desired cytoplasmic domain(s) useful in the context of the CAR. For example, the intracellular domain of the CAR can comprise a CD3 zeta chain portion and a costimulatory signaling region. The costimulatory signaling region refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or their ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such costimulatory molecules include CD27, CD28, 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. In some embodiments, the costimulatory domain comprises a functional signaling domain of a protein selected from the group consisting of 0X40, CD2, CD27, CD28, CDS, ICAM-1, LFA-1 (CDlla/CD18) and 4-1BB (CD137)

[0223] Specific, non-limiting examples, of such costimulatory molecules include peptides having sequences of amino acid numbers 236 to 351 of CD2 (NCBI RefSeq: NP.sub.--001758.2), amino acid numbers 421 to 458 of CD4 (NCBI RefSeq: NP.sub.--000607.1), amino acid numbers 402 to 495 of CD5 (NCBI RefSeq: NP.sub.--055022.2), amino acid numbers 207 to 235 of CD8.alpha. (NCBI RefSeq: NP.sub.--001759.3), amino acid numbers 196 to 210 of CD83 (GenBank: AAA35664.1), amino acid numbers 181 to 220 of CD28 (NCBI RefSeq: NP.sub.--006130.1), amino acid numbers 214 to 255 of CD137 (4-1BB, NCBI RefSeq: NP.sub.--001552.2), amino acid numbers 241 to 277 of CD134 (OX40, NCBI RefSeq: NP.sub.--003318.1), and amino acid numbers 166 to 199 of ICOS (NCBI RefSeq: NP.sub.--036224.1), and their variants having the same function as these peptides have. Thus, while the disclosure herein is exemplified primarily with 4-1BB as the co-stimulatory signaling element, other costimulatory elements are within the scope of the disclosure.

[0224] The intracellular signalling domain of the CAR can comprise the primary signaling domain, e.g., CD3-zeta signaling domain, by itself or it can be combined with any other desired intracellular signaling domain(s) useful in the context of a CAR of the invention. For example, the intracellular signaling domain of the CAR can comprise a primary signalling domain, e.g., CD3 zeta chain portion, and a costimulatory signaling domain. The costimulatory signaling domain refers to a portion of the CAR comprising the intracellular domain of a costimulatory molecule. A costimulatory molecule is a cell surface molecule other than an antigen receptor or its ligands that is required for an efficient response of lymphocytes to an antigen. Examples of such molecules include MHC class I molecule, TNF receptor proteins, Immunoglobulin-like proteins, cytokine receptors, integrins, signaling lymphocytic activation molecules (SLAM proteins), activating NK cell receptors, BTLA, a Toll ligand receptor, OX40, CD2, CD7, CD27, CD28, CD30, CD40, CDS, ICAM-1, LFA-1 (CDlla/CD18), 4-1BB (CD137), B7-H3, CDS, ICAM-1, ICOS (CD278), GITR, BAFFR, LIGHT, HVEM (LIGHTR), KIRDS2, SLAMF7, NKp80 (KLRF1), NKp44, NKp30, NKp46, CD19, CD4, CD8alpha, CD8beta, IL2R beta, IL2R gamma, IL7R alpha, ITGA4, VLAl, CD49a, ITGA4, IA4, CD49D, ITGA6, VLA-6, CD49f, ITGAD, CDlld, ITGAE, CD103, ITGAL, CDlla, LFA-1, ITGAM, CDllb, GG AC, CDllc, ITGB1, CD29, ITGB2, CD18, LFA-1, ITGB7, NKG2D, NKG2C, TNFR2, TRANCE/RANKL, DNAM1 (CD226), SLAMF4 (CD244, 2B4), CD84, CD96 (Tactile), CEACAM1, CRTAM, Ly9 (CD229), CD160 (BY55), PSGL1, CD100 (SEMA4D), CD69, SLAMF6 (NTB-A, Lyl08), SLAM (SLAMF1, CD150, IPO-3), BLAME (SLAMF8), SELPLG (CD162), LTBR, LAT, GADS, SLP-76, PAG/Cbp, CD19a, and a ligand that specifically binds with CD83, and the like. For example, CD27 costimulation has been demonstrated to enhance expansion, effector function, and survival of human CART cells in vitro and augments human T cell persistence and antitumor activity in vivo (Song et al. Blood. 2012; 119(3):696-706). The intracellular signaling sequences within the cytoplasmic portion of the CAR of the invention may be linked to each other in a random or specified order.

[0225] The cytoplasmic signaling sequences within the cytoplasmic signaling portion of the CAR may be linked to each other in a random or specified order. Optionally, a short oligo- or polypeptide linker, preferably between 2 and 10 amino acids in length may form the linkage. In some embodiments, the linker is a glycine-serine doublet or a triple alanine linker.

[0226] In some embodiments, the intracellular domain is designed to comprise a CD28 costimulatory signaling domain. In some embodiments, the intracellular domain of the CAR comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 32).

[0227] In some embodiments, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In some embodiments, the intracellular domain comprises a CD3-zeta with one or more modified immunoreceptor tyrosine based-activation motifs (ITAMs). In some embodiments, the intracellular domain comprises a CD3-zeta with the first of the three immunoreceptor tyrosine based-activation motifs (ITAMs) unmodified and the second and third ITAMs altered, named "1XX". In some embodiments, the intracellular domain of the CAR comprises an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% or 100% identical to RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLFNELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPP R. (SEQ ID NO: 33)

[0228] In another embodiment, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB. In yet another embodiment, the intracellular domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28 and 4-1BB.

[0229] In some embodiments, the intracellular domain of the CAR is designed to comprise the signaling domain of 4-1BB and the signaling domain of CD3-zeta, wherein the signaling domain of 4-1BB comprises KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 3)

and the signaling domain of CD3-zeta comprises the nucleic acid sequence set forth in RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR (SEQ ID NO: 4) or

RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQ EGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALP PR (SEQ ID NO: 71).

[0230] In some embodiments, the CAR domains are linked by a polypeptide linker. For example, a polypeptide linker can be between 2 and 10 amino acids (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids) in length may form the linkage between intracellular signaling sequence. In one embodiment, a glycine- serine doublet can be used as a suitable linker. In some embodiments, a single amino acid, e.g., an alanine, a glycine, can be used as a suitable linker.

[0231] In one aspect, the intracellular signaling domain is designed to comprise two or more, e.g., 2, 3, 4, 5, or more, costimulatory signaling domains. In an embodiment, the two or more. e.g., 2, 3, 4, 5, or more, costimulatory signaling domains, are separated by a linker molecule, e.g., a linker molecule described herein. In one embodiment, the intracellular signaling domain comprises two costimulatory signaling domains. In some embodiments, the linker molecule is a glycine residue. In some embodiments, the linker is an alanine residue.

[0232] In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of 4-1BB.

[0233] In one aspect, the intracellular signaling domain is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD27. In one aspect, the intracellular is designed to comprise the signaling domain of CD3-zeta and the signaling domain of CD28. In In one aspect, the intracellular is designed to comprise the signaling domain of CD3-zeta and the signaling domain of ICOS.

[0234] In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to an amino acid sequences provided in Table 4.

[0235]

Table 4. Amino Acid Sequences of Exemplary anti-GCC CARs

CAR	Sequence
V1-01 CAR	MGWSCIILFLVATATGVHSEVQLQESGPGLVKPSETLSLTCTVSGASISHYYWSWFRQPAGKGLEW IGRIYPSGSTSYNPSLKSRVAMSVDTPKNQFSLKLSSVTAADTAVYYCARDRSTGWSEWNSDLWG RGTLVTVSSRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYS LLVTVAFIIFWV RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQK DKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR (SEQ ID NO: 48)
V5 CAR	MELGLSWVFLVAILEGVQCQVQLVESGGGLVQPGGSLRLSCTASGFTFSRYWMSWVRQAPGKGL EWVAKIRHDGGEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCATDYTRDVWGQG TAVTVSSRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLL VTVAFIIFWV RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQK DKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR (SEQ ID NO: 49)
V36 CAR	MELGLSWVFLVAILEGVQCEVQLVESGGGLAQPGGSLRLSCAASGFTFSRYWMTWVRQAPGGRL EWVAKIKYDGSEKYYADSVKGRFTISRDNAKNSLYLQMDSLRAEDTAVYYCTRDYNKDYWGQG TLVTVSSRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLL VTVAFIIFWV RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQK DKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR (SEQ ID NO: 50)
V48 CAR	MELGLSWVFLVAILEGVQCEVQLVESGGGLVQPGGSLRLTCAASGFTFSRYWMTWVRQAPGKGL EWVAKIRHDGGEKYYPDSVKGRFTVSRDNAKNSLYLQMDNLRAEDTAMYYCTRDYNKDLWGQ GTLVTVSSRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSL LVTVAFIIFWV RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQK DKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR (SEQ ID NO: 51)
V51 CAR	MELGLSWVFLVAILEGVQCEVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWMTWVRQAPGKGL EWVAKIRHDGGEKYYADSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCTRDYNKDYWGQG TLVTVSSRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLL VTVAFIIFWV RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQK DKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR (SEQ ID NO: 52)
V1 CAR	MGWSCIILFLVATATGVHSQVQLQESGPGLVKPSETLSLTCTVSGASISHYYWSWFRQPAGKGLEW IGRIYPSGSTSYNPSLKSRVAMSVDTPKNQFSLNLSSVTAADTAVYYCARDRSTGWSEWNSDLWG RGTLVTVSSRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYS LLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAY QQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMK GERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR (SEQ ID NO: 47)

[0236] In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 47.

[0237] In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 48.

[0238] In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 49.

[0239] In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 50.

[0240] In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 51.

[0241] In some embodiments, the anti-GCC CAR comprises an amino acid sequence that is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to SEQ ID NO: 52.

Functional Features of CARs

[0242] Also expressly included within the scope of the invention are functional portions of the CARs disclosed herein. The term "functional portion" when used in reference to a CAR refers to any part or fragment of one or more of the CARs disclosed herein, which part or fragment retains the biological activity of the CAR of which it is a part (the parent CAR). Functional portions encompass, for example, those parts of a CAR that retain the ability to recognize target cells, or detect, treat, or prevent a disease, to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional portion can comprise, for instance, about 10%, 25%, 30%, 50%, 68%, 80%, 90%, 95%, or more, of the parent CAR.

[0243] The functional portion can comprise additional amino acids at the amino or carboxy terminus of the portion, or at both termini, which additional amino acids are not found in the amino acid sequence of the parent CAR. Desirably, the additional amino acids do not interfere with the biological function of the functional portion, e.g., recognize target cells, detect cancer, treat or prevent cancer, etc. More desirably, the additional amino acids enhance the biological activity, as compared to the biological activity of the parent CAR.

[0244] Included in the scope of the disclosure are functional variants of the CARs disclosed herein. The term "functional variant" as used herein refers to a CAR, polypeptide, or protein having substantial or significant sequence identity or similarity to a parent CAR, which functional variant retains the biological activity of the CAR of which it is a variant. Functional variants encompass, for example, those variants of the CAR described herein (the parent CAR) that retain the ability to recognize target cells to a similar extent, the same extent, or to a higher extent, as the parent CAR. In reference to the parent CAR, the functional variant can, for instance, be at least about 30%, 50%, 75%, 80%, 90%, 98% or more identical in amino acid sequence to the parent CAR.

[0245] A functional variant can, for example, comprise the amino acid sequence of the parent CAR with at least one conservative amino acid substitution. Alternatively or additionally, the functional variants can comprise the amino acid sequence of the parent CAR with at least one non-conservative amino acid substitution. In this case, it is preferable for the non-conservative amino acid substitution to not interfere with or inhibit the biological activity of the functional variant. The non-conservative amino acid substitution may enhance the biological activity of the functional variant, such that the biological activity of the functional variant is increased as compared to the parent CAR.

[0246] Amino acid substitutions of the CARs are preferably conservative amino acid substitutions. Conservative amino acid substitutions are known in the art, and include amino acid substitutions in which one amino acid having certain physical and/or chemical properties is exchanged for another amino acid that has the same or similar chemical or physical properties. For instance, the conservative amino acid substitution can be an acidic/negatively charged polar amino acid substituted for another acidic/negatively charged polar amino acid (e.g., Asp or Glu), an amino acid with a nonpolar side chain substituted for another amino

acid with a nonpolar side chain (e.g., Ala, Gly, Val, He, Leu, Met, Phe, Pro, Trp, Cys, Val, etc.), a basic/positively charged polar amino acid substituted for another basic/positively charged polar amino acid (e.g. Lys, His, Arg, etc.), an uncharged amino acid with a polar side chain substituted for another uncharged amino acid with a polar side chain (e.g., Asn, Gin, Ser, Thr, Tyr, etc.), an amino acid with a beta-branched side-chain substituted for another amino acid with a beta-branched side-chain (e.g., He, Thr, and Val), an amino acid with an aromatic side-chain substituted for another amino acid with an aromatic side chain (e.g., His, Phe, Trp, and Tyr), etc.

[0247] The CAR can consist essentially of the specified amino acid sequence or sequences described herein, such that other components, e.g., other amino acids, do not materially change the biological activity of the functional variant.

[0248] The CARs (including functional portions and functional variants) can be of any length, i.e., can comprise any number of amino acids, provided that the CARs (or functional portions or functional variants thereof) retain their biological activity, e.g., the ability to specifically bind to antigen, detect diseased cells in a mammal, or treat or prevent disease in a mammal, etc. For example, the CAR can be about 50 to about 5000 amino acids long, such as 50, 70, 75, 100, 125, 150, 175, 200, 300, 400, 500, 600, 700, 800, 900, 1000 or more amino acids in length.

[0249] The CARs (including functional portions and functional variants of the invention) can comprise synthetic amino acids in place of one or more naturally-occurring amino acids. Such synthetic amino acids are known in the art, and include, for example, aminocyclohexane carboxylic acid, norleucine, -amino n-decanoic acid, homoserine, S-acetylaminomethyl-cysteine, trans-3- and trans-4-hydroxyproline, 4-aminophenylalanine, 4-nitrophenylalanine, 4-chlorophenylalanine, 4-carboxyphenylalanine, β-phenylserine β-hydroxyphenylalanine, phenylglycine, a-naphthylalanine, cyclohexylalanine, cyclohexylglycine, indoline-2-carboxylic acid, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid, aminomalonic acid, aminomalonic acid monoamide, N'-benzyl-N'-methyl-lysine, N',N'-dibenzyl-lysine, 6-hydroxylysine, omithine, -aminocyclopentane carboxylic acid, a-aminocyclohexane carboxylic acid, a-aminocycloheptane carboxylic acid, a-(2-amino-2-

norbornane)-carboxylic acid, γ-diaminobutyric acid, β-diaminopropionic acid, homophenylalanine, and a-tert-butylglycine.

[0250] The CARs (including functional portions and functional variants) can be glycosylated, amidated, carboxylated, phosphorylated, esterified, N-acylated, cyclized via, e.g., a disulfide bridge, or converted into an acid addition salt and/or optionally dimerized or polymerized, or conjugated.

Substitutions and Variants

[0251] In some embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleic acid sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, e.g., antigen-binding.

a) Substitution, Insertion, and Deletion Variants

[0252] In some embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. As further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, e.g., retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

[0253] Amino acids may be grouped according to common side-chain properties:

[0254] (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

[0255] (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

[0256] (3) acidic: Asp, Glu;

[0257] (4) basic: His, Lys, Arg;

[0258] (5) residues that influence chain orientation: Gly, Pro;

[0259] (6) aromatic: Trp, Tyr, Phe.

[0260] Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

[0261] In some aspects,the antigen binding domain is humanized. A non-human antibody is humanized, where specific sequences or regions of the antibody are modified to increase similarity to an antibody naturally produced in a human or fragment thereof. In one aspect, the antigen binding domain is humanized. A humanized antibody (or antigen binding fragment) can be produced using a variety of techniques known in the art, including but not limited to, CDR-grafting (see, e.g., European Patent No. EP 239,400; International Publication No. WO 91/09967; and U.S. Pat. Nos. 5,225,539, 5,530,101, and 5,585,089, each of which is incorporated herein in its entirety by reference), veneering or resurfacing (see, e.g., European Patent Nos. EP 592,106 and EP 519,596; Padlan, 1991, Molecular Immunology, 28(4/5):489-498; Studnicka et al., 1994, Protein Engineering, 7(6):805-814; and Roguska et al., 1994, PNAS, 91:969-973, each of which is incorporated herein by its entirety by reference), chain shuffling (see, e.g., U.S. Pat. No. 5,565,332, which is incorporated herein in its entirety by reference), and techniques disclosed in, e.g., U.S. Patent Application Publication No. US2005/0042664, U.S. Patent Application Publication No. US2005/0048617, U.S. Pat. No. 6,407,213, U.S. Pat. No. 5,766,886, International Publication No. WO 9317105, Tan et al., J . Immunol., 169:1119-25 (2002), Caldas et al., Protein Eng., 13(5):353-60 (2000), Morea et al., Methods, 20(3):267-79 (2000), Baca et al., J . Biol. Chem., 272(16): 10678-84 (1997), Roguska et al., Protein Eng., 9(10):895-904 (1996), Couto et al., Cancer Res., 55 (23 Supp):5973s-5977s (1995), Couto et al., Cancer Res., 55(8):1717-22 (1995), Sandhu J S, Gene, 150(2):409-10 (1994), and Pedersen et al., J . Mol. Biol., 235(3):959-73 (1994), each of which is incorporated herein in its entirety by reference. Often, framework residues in the framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, for example improve, antigen binding. These framework substitutions, e.g., conservative substitutions are identified by methods well-known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework

residues at particular positions. (See, e.g., Queen et al., U.S. Pat. No. 5,585,089; and Riechmann et al., 1988, Nature, 332:323, which are incorporated herein by reference in their entireties.)

[0262] A humanized antibody or antibody fragment has one or more amino acid residues remaining in it from a source which is nonhuman. These nonhuman amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. As provided herein, humanized antibodies or antibody fragments comprise one or more CDRs from nonhuman immunoglobulin molecules and framework regions wherein the amino acid residues comprising the framework are derived completely or mostly from human germline. Multiple techniques for humanization of antibodies or antibody fragments are well known in the art.

[0263] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant (s) selected for further study will have modifications (e.g., improvements) in certain biological properties (e.g., increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody, which may be conveniently generated, e.g., using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies displayed on phage and screened for a particular biological activity (e.g. binding affinity).

[0264] Alterations (e.g., substitutions) may be made in HVRs, e.g., to improve antibody affinity. Such alterations may be made in HVR "hotspots, " i.e., residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, e.g., Chowdhury, Methods Mol. Biol. 207: 179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, e.g., in Hoogenboom et al. in Methods in Molecular Biology 178: 1-37 (O' Brien et al., ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (e.g.,

error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (e.g., 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, e.g., using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

[0265] In some embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (e.g., conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or CDRs. In some embodiments of the variant VH sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

[0266] A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244: 1081-1085. In this method, a residue or group of target residues (e.g., charged residues such as Arg, Asp, His, Lys, and Glu) are identified and replaced by a neutral or negatively charged amino acid (e.g., alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

[0267] Amino acid sequence insertions include amino-and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N-or C-terminus

of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

b) Glycosylation variants

[0268] In some embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

[0269] Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, e.g., Wright et al. TIBTECH 15: 26-32 (1997). The oligosaccharide may include various carbohydrates, e.g., mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the present application may be made in order to create antibody variants with certain improved properties.

[0270] In some embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1%to 80%, from 1%to 65%, from 5%to 65%or from 20%to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g., complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (EU numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, e.g., US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246;

US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki et al. J. Mol. Biol. 336: 1239-1249 (2004); Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka et al. Arch. Biochem. Biophys. 249: 533-545 (1986); US Patent Application No. US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams et al.,), and knockout cell lines, such as alpha-1, 6-fucosyltransferase gene, FUT8, knockout CHO cells (see, e.g., Yamane-Ohnuki et al. Biotech. Bioeng. 87: 614 (2004); Kanda, Y. et al., Biotechnol. Bioeng., 94 (4):680-688 (2006); and WO2003/085107).

[0271] Antibody variants are further provided with bisected oligosaccharides, e.g., in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, e.g., in WO 2003/011878 (Jean-Mairet et al.); US Patent No. 6,602,684 (Umana et al.); and US 2005/0123546 (Umana et al.). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, e.g., in WO 1997/30087 (Patel et al.); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

[0272] The CARs (including functional portions and functional variants thereof) can be obtained by methods known in the art. The CARs may be made by any suitable method of making polypeptides or proteins. Suitable methods of *de novo* synthesizing polypeptides and proteins are described in references, such as Chan et al., Fmoc Solid Phase Peptide Synthesis, Oxford University Press, Oxford, United Kingdom, 2000; Peptide and Protein Drug Analysis, ed. Reid, R., Marcel Dekker, Inc., 2000; Epitope Mapping, ed. Westwood et al., Oxford University Press, Oxford, United Kingdom, 2001; and U.S. Pat. No. 5,449,752. Also, polypeptides and proteins can be recombinantly produced using the nucleic acids described herein using standard recombinant methods. See, for instance, Sambrook et al., Molecular Cloning: A Laboratory Manual, 3rd ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 2001; and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, N Y, 1994. Further, some of the CARs (including

functional portions and functional variants thereof) can be isolated and/or purified from a source, such as a plant, a bacterium, an insect, a mammal, e.g., a rat, a human, etc. Methods of isolation and purification are well-known in the art. Alternatively, the CARs described herein (including functional portions and functional variants thereof) can be commercially synthesized by companies. In this respect, the CARs can be synthetic, recombinant, isolated, and/or purified.

Detectable Markers and Tags

[0273] A CAR, a T cell expressing a CAR, monoclonal antibodies, antigen binding fragments thereof, specific for one or more of the antigens disclosed herein, can also expressed with (e.g., co-expressed) with a tag protein. In some embodiments, a furin recognition site and downstream 2A self-cleaving peptide sequence, designed for simultaneous bicistronic expression of the tag sequence and the CAR sequence. In some embodiments, the 2A sequence comprises the nucleic acid sequence of GSGATNFSLLKQAGDVEENPGP SEQ ID NO: 44. In some embodiments, furin and P2A sequence comprises the nucleic acid sequence that encodes the amino acid sequence of SEQ ID NO: 44. In some embodiments, the P2A tag comprises the amino acid sequence of SEQ ID NO: 44 or a sequence with at least 95%, at least 96%, at least 97%, at least 98%, at least 99% identity thereof.

[0274] In some embodiments, a CAR, a T cell expressing a CAR, monoclonal antibodies, antigen binding fragments thereof, specific for one or more of the antigens disclosed herein, can also expressed with EGFR. In some embodiments, the CAR, T cell expressing a CAR, monoclonal antibodies, antigen binding fragments thereof, specific for one or more of the antigens disclosed herein, are expressed with (e.g., co-expressed) truncated EGFR (tEGFR). In some embodiments, tEGFR comprises an amino acid sequence that is at least 95% identical, at least 96% identical, at least 97% identical, at least 98% identical, at least 99% identical or 100% identical to SEQ ID NO: 43.

tEGFR:
MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDSLSINATNIKHFKNCTSISGDLHIL
PVAFRGDSFTHTPPLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFENLEIIRGRTKQ
HGQFSLAVVSLNITSLGLRSLKEISDGDVIISGNKNLCYANTINWKKLFGTSGQKTKIIS

NRGENSCKATGQVCHALCSPEGCWGPEPRDCVSCRNVSRGRECVDKCNLLEGEPRE FVENSECIQCHPECLPQAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAGVMGENNT LVWKYADAGHVCHLCHPNCTYGCTGPGLEGCPTNGPKIPSIATGMVGALLLLLVVA LGIGLFM (SEQ ID NO: 43)

[0275] A CAR, a T cell expressing a CAR, monoclonal antibodies, antigen binding fragments thereof, specific for one or more of the antigens disclosed herein, can also be conjugated with a detectable marker; for example, a detectable marker capable of detection by ELISA, spectrophotometry, flow cytometry, microscopy or diagnostic imaging techniques (such as computed tomography (CT), computed axial tomography (CAT) scans, magnetic resonance imaging (MRI), nuclear magnetic resonance imaging NMRI), magnetic resonance tomography (MTR), ultrasound, fiberoptic examination, and laparoscopic examination). Specific, non-limiting examples of detectable markers include fluorophores, chemiluminescent agents, enzymatic linkages, radioactive isotopes and heavy metals or compounds (for example super paramagnetic iron oxide nanocrystals for detection by MRI). For example, useful detectable markers include fluorescent compounds, including fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin, lanthanide phosphors and the like. Bioluminescent markers are also of use, such as luciferase, Green fluorescent protein (GFP), Yellow fluorescent protein (YFP). A CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, can also be conjugated with enzymes that are useful for detection, such as horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase, glucose oxidase and the like. When a CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, is conjugated with a detectable enzyme, it can be detected by adding additional reagents that the enzyme uses to produce a reaction product that can be discerned. For example, when the agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is visually detectable. A CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, may also be conjugated with biotin, and detected through indirect measurement of avidin or streptavidin binding. It should be noted that the avidin itself can be conjugated with an enzyme or a fluorescent label.

[0276] A CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, may be conjugated with a paramagnetic agent, such as gadolinium. Paramagnetic

agents such as superparamagnetic iron oxide are also of use as labels. Antibodies can also be conjugated with lanthanides (such as europium and dysprosium), and manganese. An antibody or antigen binding fragment may also be labeled with a predetermined polypeptide epitopes recognized by a secondary reporter (such as leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags).

[0277] A CAR, a T cell expressing a CAR, an antibody, or antigen binding portion thereof, can also be conjugated with a radiolabeled amino acid. The radiolabel may be used for both diagnostic and therapeutic purposes. For instance, the radiolabel may be used to detect one or more of the antigens disclosed herein and antigen expressing cells by x-ray, emission spectra, or other diagnostic techniques. Further, the radiolabel may be used therapeutically as a toxin for treatment of tumors in a subject, for example for treatment of a neuroblastoma. Examples of labels for polypeptides include, but are not limited to, the following radioisotopes or radionucleotides: ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I.

[0278] Means of detecting such detectable markers are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted illumination. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

Nucleic acids, Expression vectors, and Host Cells

[0279] Further provided by an embodiment of the invention is a nucleic acid comprising a nucleotide sequence encoding any of the CARs, an antibody, or antigen binding portion thereof, described herein (including functional portions and functional variants thereof). The nucleic acids of the invention may comprise a nucleotide sequence encoding any of the leader sequences, antigen binding domains, transmembrane domains, and/or intracellular T cell signaling domains described herein.

[0280] In one aspect, the present invention encompasses a recombinant nucleic acid construct comprising a nucleic acid molecule encoding a CAR, wherein the nucleic acid

molecule comprises the nucleic acid sequence encoding an anti-GCC binding domain, e.g., described herein, that is contiguous with and in the same reading frame as a nucleic acid sequence encoding an intracellular signaling domain. An exemplary intracellular signaling domain that can be used in the CAR includes, but is not limited to, one or more intracellular signaling domains of, e.g., CD3-zeta, CD28, 4-1BB, and the like. In some embodiments, the CAR can comprise any combination of CD3-zeta, CD28, 4-IBB, and the like.

[0281] In one aspect, the present invention provides a nucleic acid comprising a nucleotide sequence that is at least about 70% or more, e.g., about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to a the nucleic acid encoding a CAR construct described herein.

[0282] In some embodiments, the nucleic acid encodes an anti-GCC binding domain selected from an anti-GCC VH sequence provided in Tables 1-3 or Table 7. In some embodiments, the nucleic acid comprises a sequence that is at least about 70% or more, e.g., about 80%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to any one of SEQ ID NOs: 61-70. In some embodiments, the nucleic acid comprises a sequence identical to any one of SEQ ID NOs: 61-70

[0283] In some embodiments, the nucleotide sequence may be codon-modified. Without being bound to a particular theory, it is believed that codon optimization of the nucleotide sequence increases the translation efficiency of the mRNA transcripts. Codon optimization of the nucleotide sequence may involve substituting a native codon for another codon that encodes the same amino acid, but can be translated by tRNA that is more readily available within a cell, thus increasing translation efficiency. Optimization of the nucleotide sequence may also reduce secondary mRNA structures that would interfere with translation, thus increasing translation efficiency.

[0284] In an embodiment of the invention, the nucleic acid may comprise a codon-modified nucleotide sequence that encodes the antigen binding domain of the inventive CAR. In another embodiment of the invention, the nucleic acid may comprise a codon-modified nucleotide sequence that encodes any of the CARs described herein (including functional portions and functional variants thereof).

[0285] In one aspect aspect, the invention pertains to a vector comprising a nucleic acid molecule described herein, e.g., a nucleic acid molecule encoding a CAR described herein. In one embodiment, the vector is selected from the group consisting of a DNA, a RNA, a plasmid, a lentiviral vector, adenoviral vector, or a retrovirus vector.

[0286] In one embodiment, the vector is a lentiviral vector. In one embodiment, the vector further comprises a promoter. In one embodiment, the promoter is an EF-1 promoter.

[0287] Expression vectors include plasmids, retroviruses, cosmids, YACs, EBV derived episomes, and the like. A convenient vector is one that encodes a functionally complete human CH or CL immunoglobulin sequence, with appropriate restriction sites engineered so that any VH or VL sequence can be easily inserted and expressed. In such vectors, splicing usually occurs between the splice donor site in the inserted J region and the splice acceptor site preceding the human C region, and also at the splice regions that occur within the human CH exons. Suitable expression vectors can contain a number of components, for example, an origin of replication, a selectable marker gene, one or more expression control elements, such as a transcription control element (e.g., promoter, enhancer, or terminator) and/or one or more translation signals, a signal sequence or leader sequence, and the like. Polyadenylation and transcription termination occur at native chromosomal sites downstream of the coding regions. The resulting chimeric antibody may be joined to any strong promoter. Examples of suitable vectors that can be used include those that are suitable for mammalian hosts and based on viral replication systems, such as simian virus 40 (SV40), Rous sarcoma virus (RSV), adenovirus 2, bovine papilloma virus (BPV), papovavirus BK mutant (BKV), or mouse and human cytomegalovirus (CMV), and moloney murine leukemia virus (MMLV), native Ig promoters, etc. A variety of suitable vectors are known in the art, including vectors which are maintained in single copy or multiple copies, or which become integrated into the host cell chromosome, e.g., via LTRs, or via artificial chromosomes engineered with multiple integration sites (Lindenbaum et al. *Nucleic Acids Res.* 32:e172 (2004), Kennard et al. *Biotechnol. Bioeng.* Online May 20, 2009). Additional examples of suitable vectors are listed in a later section.

[0288] The present invention also includes an RNA construct that can be directly transfected into a cell. A method for generating mRNA for use in transfection involves in

vitro transcription (IVT) of a template with specially designed primers, followed by polyA addition, to produce a construct containing 3' and 5' untranslated sequence ("UTR"), a 5' cap and/or Internal Ribosome Entry Site (IRES), the nucleic acid to be expressed, and a polyA tail, typically 50-2000 bases in length. RNA so produced can efficiently transfect different kinds of cells. In one embodiment, the template includes sequences for the CAR. In an embodiment, an RNA CAR vector is transduced into a cell, e.g., T cell or NK cell, by electroporation.

[0289] Thus, the invention provides an expression vector comprising a nucleic acid encoding an antibody, antigen-binding fragment of an antibody (e.g., a human, humanized, chimeric antibody or antigen-binding fragment of any of the foregoing), antibody chain (e.g., heavy chain, light chain) or antigen-binding portion of an antibody chain that binds a GCC protein.

[0290] Expression in eukaryotic host cells is useful because such cells are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. However, any antibody produced that is inactive due to improper folding may be renaturable according to known methods (Kim and Baldwin, "Specific Intermediates in the Folding Reactions of Small Proteins and the Mechanism of Protein Folding", *Ann. Rev. Biochem.* 51, pp. 459-89 (1982)). It is possible that the host cells will produce portions of intact antibodies, such as light chain dimers or heavy chain dimers, which also are antibody homologs according to the present invention.

[0291] In an embodiment, the nucleic acids can be incorporated into a recombinant expression vector. In this regard, an embodiment provides recombinant expression vectors comprising any of the nucleic acids. For purposes herein, the term "recombinant expression vector" means a genetically-modified oligonucleotide or polynucleotide construct that permits the expression of an mRNA, protein, polypeptide, or peptide by a host cell, when the construct comprises a nucleotide sequence encoding the mRNA, protein, polypeptide, or peptide, and the vector is contacted with the cell under conditions sufficient to have the mRNA, protein, polypeptide, or peptide expressed within the cell. The vectors are not naturally-occurring as a whole.

[0292] However, parts of the vectors can be naturally-occurring. The recombinant expression vectors can comprise any type of nucleotides, including, but not limited to DNA and RNA, which can be single-stranded or double-stranded, synthesized or obtained in part from natural sources, and which can contain natural, non-natural or altered nucleotides. The recombinant expression vectors can comprise naturally-occurring or non-naturally-occurring internucleotide linkages, or both types of linkages. Preferably, the non-naturally occurring or altered nucleotides or internucleotide linkages do not hinder the transcription or replication of the vector.

[0293] In an embodiment, the recombinant expression vector can be any suitable recombinant expression vector, and can be used to transform or transfect any suitable host cell. Suitable vectors include those designed for propagation and expansion or for expression or both, such as plasmids and viruses. The vector can be selected from the group consisting of the pUC series (Fermentas Life Sciences, Glen Burnie, Md.), the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, Calif.).

[0294] Bacteriophage vectors, such as λ, λZapII (Stratagene), EMBL4, and λNMI 149, also can be used. Examples of plant expression vectors include pBIO1, pBI101.2, pBHO1.3, pBI121 and pBIN19 (Clontech). Examples of animal expression vectors include pEUK-C1, pMAM, and pMAMneo (Clontech). The recombinant expression vector may be a viral vector, e.g., a retroviral vector or a lentiviral vector. A lentiviral vector is a vector derived from at least a portion of a lentivirus genome, including especially a self-inactivating lentiviral vector as provided in Milone et al., Mol. Ther. 17(8): 1453-1464 (2009). Other examples of lentivirus vectors that may be used in the clinic, include, for example, and not by way of limitation, the LENTIVECTOR® gene delivery technology from Oxford BioMedica plc, the LENTIMAX™ vector system from Lentigen and the like. Nonclinical types of lentiviral vectors are also available and would be known to one skilled in the art.

[0295] A number of transfection techniques are generally known in the art (see, e.g., Graham et al., Virology, 52: 456-467 (1973); Sambrook et al., supra; Davis et al., Basic Methods in Molecular Biology, Elsevier (1986); and Chu et al, Gene, 13: 97 (1981).

[0296] Transfection methods include calcium phosphate co-precipitation (see, e.g., Graham et al., supra), direct micro injection into cultured cells (see, e.g., Capecchi, Cell, 22: 479-488 (1980)), electroporation (see, e.g., Shigekawa et al., BioTechniques, 6: 742-751 (1988)), liposome mediated gene transfer (see, e.g., Mannino et al., BioTechniques, 6: 682-690 (1988)), lipid mediated transduction (see, e.g., Feigner et al., Proc. Natl. Acad. Sci. USA, 84: 7413-7417 (1987)), and nucleic acid delivery using high velocity microprojectiles (see, e.g., Klein et al, Nature, 327: 70-73 (1987)).

[0297] In an embodiment, the recombinant expression vectors can be prepared using standard recombinant DNA techniques described in, for example, Sambrook et al., supra, and Ausubel et al., supra. Constructs of expression vectors, which are circular or linear, can be prepared to contain a replication system functional in a prokaryotic or eukaryotic host cell. Replication systems can be derived, e.g., from ColE1, 2μ plasmid, λ, SV40, bovine papilloma virus, and the like.

[0298] The recombinant expression vector may comprise regulatory sequences, such as transcription and translation initiation and termination codons, which are specific to the type of host cell (e.g., bacterium, fungus, plant, or animal) into which the vector is to be introduced, as appropriate, and taking into consideration whether the vector is DNA- or RNA-based. The recombinant expression vector may comprise restriction sites to facilitate cloning.

[0299] The recombinant expression vector can include one or more marker genes, which allow for selection of transformed or transfected host cells. Marker genes include biocide resistance, e.g., resistance to antibiotics, heavy metals, etc., complementation in an auxotrophic host to provide prototrophy, and the like. Suitable marker genes for the inventive expression vectors include, for instance, neomycin/G418 resistance genes, hygromycin resistance genes, histidinol resistance genes, tetracycline resistance genes, and ampicillin resistance genes.

[0300] The recombinant expression vector can comprise a native or nonnative promoter operably linked to the nucleotide sequence encoding the CAR (including functional portions and functional variants thereof), or to the nucleotide sequence which is complementary to or which hybridizes to the nucleotide sequence encoding the CAR. The selection of promoters, e.g., strong, weak, inducible, tissue-specific and developmental-

specific, is within the ordinary skill of the artisan. Similarly, the combining of a nucleotide sequence with a promoter is also within the skill of the artisan. The promoter can be a non-viral promoter or a viral promoter, e.g., a cytomegalovirus (CMV) promoter, an SV40 promoter, an RSV promoter, EFl alpha promoter or a promoter found in the long-terminal repeat of the murine stem cell virus.

[0301] The recombinant expression vectors can be designed for either transient expression, for stable expression, or for both. Also, the recombinant expression vectors can be made for constitutive expression or for inducible expression.

[0302] Further, the recombinant expression vectors can be made to include a suicide gene. As used herein, the term "suicide gene" refers to a gene that causes the cell expressing the suicide gene to die. The suicide gene can be a gene that confers sensitivity to an agent, e.g., a drug, upon the cell in which the gene is expressed, and causes the cell to die when the cell is contacted with or exposed to the agent. Suicide genes are known in the art (see, for example, Suicide Gene Therapy: Methods and Reviews, Springer, Caroline J. (Cancer Research UK Centre for Cancer Therapeutics at the Institute of Cancer Research, Sutton, Surrey, UK), Humana Press, 2004) and include, for example, the Herpes Simplex Virus (HSV) thymidine kinase (TK) gene, cytosine daminase, purine nucleoside phosphorylase, and nitroreductase.

[0303] An embodiment further provides a host cell comprising any of the recombinant expression vectors described herein. As used herein, the term "host cell" refers to any type of cell that can contain the inventive recombinant expression vector. The host cell can be a eukaryotic cell, e.g., plant, animal, fungi, or algae, or can be a prokaryotic cell, e.g., bacteria or protozoa. The host cell can be a cultured cell or a primary cell, i.e., isolated directly from an organism, e.g., a human. The host cell can be an adherent cell or a suspended cell, i.e., a cell that grows in suspension. Suitable host cells are known in the art and include, for instance, DH5a E. coli cells, Chinese hamster ovarian cells, monkey VERO cells, COS cells, HEK293 cells, HEK293T cells, and the like. For purposes of amplifying or replicating the recombinant expression vector, the host cell may be a prokaryotic cell, e.g., a DH5a cell. For purposes of producing a recombinant CAR, the host cell may be a mammalian cell. The host cell may be a human cell. While the host cell can be of any cell type, can originate from any

type of tissue, and can be of any developmental stage, the host cell may be a peripheral blood lymphocyte (PBL) or a peripheral blood mononuclear cell (PBMC). The host cell may be a T cell.

[0304] For purposes herein, the T cell can be any T cell, such as a cultured T cell, e.g., a primary T cell, or a T cell from a cultured T cell line, e.g., Jurkat, SupTl, etc., or a T cell obtained from a mammal. If obtained from a mammal, the T cell can be obtained from numerous sources, including but not limited to blood, bone marrow, lymph node, the thymus, or other tissues or fluids. T cells can also be enriched for or purified. The T cell may be a human T cell. The T cell may be a T cell isolated from a human. The T cell can be any type of T cell and can be of any developmental stage, including but not limited to, CD4+/CD8+ double positive T cells, CD4+ helper T cells, e.g., Th1 and Th2 cells, CD8+ T cells (e.g., cytotoxic T cells), tumor infiltrating cells, memory T cells, memory stem cells, i.e. Tscm, naive T cells, and the like. The T cell may be a CD8+ T cell or a CD4+ T cell.

[0305] In an embodiment, the CARs as described herein can be used in suitable non-T cells. Such cells are those with an immune-effector function, such as, for example, NK cells, and T-like cells generated from pluripotent stem cells.

[0306] One aspect of the present application provides an engineered immune effector cell, comprising any one of the CARs described herein, or any one of the isolated nucleic acids described above, or any one of the vectors described above. In some embodiments, the immune effector cell is a T cell, an NK cell, a peripheral blood mononuclear cell (PBMC), a hematopoietic stem cell, a pluripotent stem cell, or an embryonic stem cell. In some embodiments, the immune effector cell is a T cell.

[0307] Also provided by an embodiment is a population of cells comprising at least one host cell described herein. The population of cells can be a heterogeneous population comprising the host cell comprising any of the recombinant expression vectors described, in addition to at least one other cell, e.g., a host cell (e.g., a T cell), which does not comprise any of the recombinant expression vectors, or a cell other than a T cell, e.g., a B cell, a macrophage, a neutrophil, an erythrocyte, a hepatocyte, an endothelial cell, an epithelial cell, a muscle cell, a brain cell, etc. Alternatively, the population of cells can be a substantially homogeneous population, in which the population comprises mainly host cells (e.g.,

consisting essentially of) comprising the recombinant expression vector. The population also can be a clonal population of cells, in which all cells of the population are clones of a single host cell comprising a recombinant expression vector, such that all cells of the population comprise the recombinant expression vector. In one embodiment of the invention, the population of cells is a clonal population comprising host cells comprising a recombinant expression vector as described herein.

[0308] CARs (including functional portions and variants thereof), nucleic acids, recombinant expression vectors, host cells (including populations thereof), and antibodies (including antigen binding portions thereof), can be isolated and/or purified. For example, a purified (or isolated) host cell preparation is one in which the host cell is more pure than cells in their natural environment within the body. Such host cells may be produced, for example, by standard purification techniques. In some embodiments, a preparation of a host cell is purified such that the host cell represents at least about 50%, for example at least about 70%, of the total cell content of the preparation. For example, the purity can be at least about 50%, can be greater than about 60%, about 70% or about 80%, or can be about 100%.

[0309] The nucleic acid sequences coding for the desired molecules can be obtained using recombinant methods known in the art, such as, for example by screening libraries from cells expressing the gene, by deriving the gene from a vector known to include the same, or by isolating directly from cells and tissues containing the same, using standard techniques.

[0310] Alternatively, the gene of interest can be produced synthetically, rather than cloned. The present invention also provides vectors in which a DNA of the present invention is inserted. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity.

[0311] The expression of natural or synthetic nucleic acids encoding CARs may be achieved by operably linking a nucleic acid encoding the CAR polypeptide or portions thereof to a promoter, and incorporating the construct into an expression vector. The vectors can be

suitable for replication and integration eukaryotes. Typical cloning vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the desired nucleic acid sequence.

[0312] The expression constructs of the present invention may also be used for nucleic acid immunization and gene therapy, using standard gene delivery protocols. Methods for gene delivery are known in the art. See, e.g., U.S. Pat. Nos. 5,399,346, 5,580,859, 5,589,466 incorporated by reference herein in their entireties. In another embodiment, the invention provides a gene therapy vector.

[0313] The nucleic acid can be cloned into a number of types of vectors. For example, the nucleic acid can be cloned into a vector including, but not limited to a plasmid, a phagemid, a phage derivative, an animal vims, and a cosmid. Vectors of particular interest include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. Further, the expression vector may be provided to a cell in the form of a viral vector.

[0314] Viral vector technology is well known in the art and is described, for example, in Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York), and in other virology and molecular biology manuals. Viruses, which are useful as vectors include, but are not limited to, retroviruses, adenoviruses, adeno-associated viruses, herpes viruses, and lentivimses. In general, a suitable vector contains an origin of replication functional in at least one organism, a promoter sequence, convenient restriction endonuclease sites, and one or more selectable markers, (e.g., WO 01/96584; WO 01/29058; and U.S. Pat. No. 6,326,193).

[0315] A number of viral based systems have been developed for gene transfer into mammalian cells. For example, retroviruses provide a convenient platform for gene delivery systems. A selected gene can be inserted into a vector and packaged in retroviral particles using techniques known in the art. The recombinant vims can then be isolated and delivered to cells of the subject either in vivo or ex vivo. A number of retroviral systems are known in the art. In some embodiments, adenovirus vectors are used. A number of adenovirus vectors are known in the art. In one embodiment, lentivims vectors are used.

[0316] Additional promoter elements, e.g., enhancers, regulate the frequency of transcriptional initiation. Typically, these are located in the region 30-110 bp upstream of the

start site, although a number of promoters have recently been shown to contain functional elements downstream of the start site as well. The spacing between promoter elements frequently is flexible, so that promoter function is preserved when elements are inverted or moved relative to one another. In the thymidine kinase (tk) promoter, the spacing between promoter elements can be increased to 50 bp apart before activity begins to decline. Depending on the promoter, it appears that individual elements can function either cooperatively or independently to activate transcription.

[0317] One example of a suitable promoter is the immediate early cytomegalovirus (CMV) promoter sequence. This promoter sequence is a strong constitutive promoter sequence capable of driving high levels of expression of any polynucleotide sequence operatively linked thereto.

[0318] Another example of a suitable promoter is Elongation Growth Factor -l a (EF-la). However, other constitutive promoter sequences may also be used, including, but not limited to the simian vims 40 (SV40) early promoter, mouse mammary tumor virus (MMTV), human immunodeficiency vims (HIV) long terminal repeat (LTR) promoter, MoMuLV promoter, an avian leukemia vims promoter, an Epstein-Barr vims immediate early promoter, a Rous sarcoma vims promoter, as well as human gene promoters such as, but not limited to, the actin promoter, the myosin promoter, the hemoglobin promoter, and the creatine kinase promoter. Further, the invention should not be limited to the use of constitutive promoters. Inducible promoters are also contemplated as part of the invention. The use of an inducible promoter provides a molecular switch capable of turning on expression of the polynucleotide sequence which it is operatively linked when such expression is desired, or turning off the expression when expression is not desired. Examples of inducible promoters include, but are not limited to a metallothionine promoter, a glucocorticoid promoter, a progesterone promoter, and a tetracycline promoter.

[0319] In order to assess the expression of a CAR polypeptide or portions thereof, the expression vector to be introduced into a cell can also contain either a selectable marker gene or a reporter gene or both to facilitate identification and selection of expressing cells from the population of cells sought to be transfected or infected through viral vectors. In other aspects,

the selectable marker may be carried on a separate piece of DNA and used in a co-transfection procedure.

[0320] Both selectable markers and reporter genes may be flanked with appropriate regulatory sequences to enable expression in the host cells. Useful selectable markers include, for example, antibiotic-resistance genes, such as neo and the like. Reporter genes are used for identifying potentially transfected cells and for evaluating the functionality of regulatory sequences. In general, a reporter gene is a gene that is not present in or expressed by the recipient organism or tissue and that encodes a polypeptide whose expression is manifested by some easily detectable property, e.g., enzymatic activity. Expression of the reporter gene is assayed at a suitable time after the DNA has been introduced into the recipient cells. Suitable reporter genes may include genes encoding luciferase, beta-galactosidase, chloramphenicol acetyl transferase, secreted alkaline phosphatase, or the green fluorescent protein gene (e.g., Ui-Tei et al., 2000 FEBS Letters 479: 79-82). Suitable expression systems are well known and may be prepared using known techniques or obtained commercially. In general, the construct with the minimal 5' flanking region showing the highest level of expression of reporter gene is identified as the promoter. Such promoter regions may be linked to a reporter gene and used to evaluate agents for the ability to modulate promoter- driven transcription.

[0321] Methods of introducing and expressing genes into a cell are known in the art. In the context of an expression vector, the vector can be readily introduced into a host cell, e.g., mammalian, bacterial, yeast, or insect cell by any method in the art. For example, the expression vector can be transferred into a host cell by physical, chemical, or biological means. Physical methods for introducing a polynucleotide into a host cell include calcium phosphate precipitation, lipofection, particle bombardment, microinjection, electroporation, and the like. Methods for producing cells comprising vectors and/or exogenous nucleic acids are well-known in the art. See, for example, Sambrook et al. (2001, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York). A preferred method for the introduction of a polynucleotide into a host cell is calcium phosphate transfection.

[0322] Biological methods for introducing a polynucleotide of interest into a host cell include the use of DNA and RNA vectors. Viral vectors, and especially retroviral vectors, have become the most widely used method for inserting genes into mammalian, e.g., human

cells. Other viral vectors can be derived from lentivirus, poxviruses, herpes simplex vims I, adenoviruses and adeno-associated viruses, and the like. See, for example, U.S. Pat. Nos. 5,350,674 and 5,585,362. Chemical means for introducing a polynucleotide into a host cell include colloidal dispersion systems, such as macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes.

[0323] An exemplary colloidal system for use as a delivery vehicle in vitro and in vivo is a liposome (e.g., an artificial membrane vesicle). In the case where a non-viral delivery system is utilized, an exemplary delivery vehicle is a nanoparticle, e.g., a liposome or other suitable sub-micron sized delivery system. The use of lipid formulations is contemplated for the introduction of the nucleic acids into a host cell (in vitro, ex vivo or in vivo). In another aspect, the nucleic acid may be associated with a lipid. The nucleic acid associated with a lipid may be encapsulated in the aqueous interior of a liposome, interspersed within the lipid bilayer of a liposome, attached to a liposome via a linking molecule that is associated with both the liposome and the oligonucleotide, entrapped in a liposome, complexed with a liposome, dispersed in a solution containing a lipid, mixed with a lipid, combined with a lipid, contained as a suspension in a lipid, contained or complexed with a micelle, or otherwise associated with a lipid. Lipid, lipid/DNA or lipid/expression vector associated compositions are not limited to any particular structure in solution. For example, they may be present in a bilayer structure, as micelles, or with a "collapsed" structure. They may also simply be interspersed in a solution, possibly forming aggregates that are not uniform in size or shape. Lipids are fatty substances which may be naturally occurring or synthetic lipids. For example, lipids include the fatty droplets that naturally occur in the cytoplasm as well as the class of compounds which contain long-chain aliphatic hydrocarbons and their derivatives, such as fatty acids, alcohols, amines, amino alcohols, and aldehydes.

[0324] Lipids suitable for use can be obtained from commercial sources. For example, dimyristyl phosphatidylcholine ("DMPC") can be obtained from Sigma, St. Louis, MO; dicetyl phosphate ("DCP") can be obtained from K & K Laboratories (Plainview, NY); cholesterol ("Choi") can be obtained from Calbiochem-Behring; dimyristyl phosphatidylglycerol ("DMPG") and other lipids may be obtained from Avanti Polar Lipids, Inc. (Birmingham, AL). Stock solutions of lipids in chloroform or chloroform/methanol can

be stored at about -20°C. Chloroform is used as the only solvent since it is more readily evaporated than methanol. "Liposome" is a generic term encompassing a variety of single and multilamellar lipid vehicles formed by the generation of enclosed lipid bilayers or aggregates. Liposomes can be characterized as having vesicular structures with a phospholipid bilayer membrane and an inner aqueous medium. Multilamellar liposomes have multiple lipid layers separated by aqueous medium. They form spontaneously when phospholipids are suspended in an excess of aqueous solution. The lipid components undergo self-rearrangement before the formation of closed structures and entrap water and dissolved solutes between the lipid bilayers (Ghosh et a , 1991 Glycobiology 5: 505-10).

[0325] However, compositions that have different structures in solution than the normal vesicular structure are also encompassed. For example, the lipids may assume a micellar structure or merely exist as nonuniform aggregates of lipid molecules. Also contemplated are lipofectaminenucleic acid complexes. Regardless of the method used to introduce exogenous nucleic acids into a host cell or otherwise expose a cell to the inhibitor of the present invention, in order to confirm the presence of the recombinant DNA sequence in the host cell, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays well known to those of skill in the art, such as Southern and Northern blotting, RT-PCR and PCR; "biochemical" assays, such as detecting the presence or absence of a particular peptide, e.g., by immunological means (ELISAs and Western blots) or by assays described herein to identify agents falling within the scope of the invention.

[0326] The present invention further provides a vector comprising a CAR encoding nucleic acid molecule. In one aspect, a CAR vector can be directly transduced into a cell, *e.g.*, a T cell. In one aspect, the vector is a cloning or expression vector, *e.g.*, a vector including, but not limited to, one or more plasmids *(e.g.*, expression plasmids, cloning vectors, minicircles, minivectors, double minute chromosomes), retroviral and lentiviral vector constructs. In one aspect, the vector is capable of expressing the CAR construct in mammalian T cells. In one aspect, the mammalian T cell is a human T cell.

[0327] In some aspects, non-viral methods can be used to deliver a nucleic acid encoding a CAR described herein into a cell or tissue or a subject. In some embodiments, the non-viral method includes the use of a transposon (also called a transposable element). In

some embodiments, a transposon is a piece of DNA that can insert itself at a location in a genome, for example, a piece of DNA that is capable of self-replicating and inserting its copy into a genome, or a piece of DNA that can be spliced out of a longer nucleic acid and inserted into another place in a genome.

[0328] Additional and exemplary transposons and non-viral delivery methods are described on pages196-198 of International Application WO 2016/164731, filed April 8, 2016, which is incorporated by reference in its entirety.

Sources of T cells

[0329] Prior to expansion and genetic modification, e.g., to express a CAR described herein, a source of cells, e.g., T cell or NK cells, can be obtained from a subject. The term "subject" is ntended to include living organisms in which an immune response can be elicited (e.g., mammals). Examples of subjects include humans, dogs, cats, mice, rats, and transgenic species thereof.

[0330] In embodiments, immune effector cells (e.g., a population of immune effector cells), e.g., T cells, are derived from (e.g., differentiated from) a stem cell, e.g., an embryonic stem cell or a pluripotent stem cell, e.g., an induced pluripotent stem cell (iPSC). In embodiments, the cells are autologous or allogeneic. In embodiments wherein the cells are allogeneic, the cells, e.g., derived from stem cells (e.g., iPSCs), are modified to reduce their alloreactivity. For example, the cells can be modified to reduce alloreactivity, e.g., by modifying (e.g., disrupting) their T cell receptor. In embodiments, a site specific nuclease can be used to disrupt the T cell receptor, e.g., after T-cell differentiation. In other examples, cells, e.g., T cells derived from iPSCs, can be generated from virus-specific T cells, which are less likely to cause graft-versus-host disease because of their recognition of a pathogen-derived antigen. In yet other examples, alloreactivity can be reduced, e.g., minimized, by generating iPSCs from common HLA haplotypes such that they are histocompatible with matched, unrelated recipient subjects.

[0331] In yet other examples, alloreactivity can be reduced, e.g., minimized, by repressing HLA expression through genetic modification. For example, T cells derived from iPSCs can be processed as described in, e.g., Themeli et al. Nat. Biotechnol. 31.10(2013):928-35, incorporated herein by reference. In some examples, immune effector cells, e.g., T cells,

derived from stem cells, can be processed/generated using methods described in WO2014/165707, incorporated herein by reference.

[0332] T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain aspects of the present disclosure, any number of T cell lines available in the art, may be used. In certain aspects of the present disclosure, T cells can be obtained from a unit of blood collected from a subject using any number of techniques known to the skilled artisan, such as Ficoll™ separation. In one preferred aspect, cells from the circulating blood of an individual are obtained by apheresis. The apheresis product typically contains lymphocytes, including T cells, monocytes, granulocytes, B cells, other nucleated white blood cells, red blood cells, and platelets. In one aspect, the cells collected by apheresis may be washed to remove the plasma fraction and to place the cells in an appropriate buffer or media for subsequent processing steps. In one aspect of the invention, the cells are washed with phosphate buffered saline (PBS). In an alternative aspect, the wash solution lacks calcium and may lack magnesium or may lack many if not all divalent cations.

[0333] Initial activation steps in the absence of calcium can lead to magnified activation. As those of ordinary skill in the art would readily appreciate a washing step may be accomplished by methods known to those in the art, such as by using a semi-automated "flow-through" centrifuge (for example, the Cobe 2991 cell processor, the Baxter CytoMate, or the Haemonetics Cell Saver 5) according to the manufacturer's instructions. After washing, the cells may be resuspended in a variety of biocompatible buffers, such as, for example, Ca-free, Mg-free PBS, PlasmaLyte A, or other saline solution with or without buffer. Alternatively, the undesirable components of the apheresis sample may be removed and the cells directly resuspended in culture media.

[0334] It is recognized that the methods of the application can utilize culture media conditions comprising 5% or less, for example 2%, human AB serum, and employ known culture media conditions and compositions, for example those described in Smith et al., "Ex vivo expansion of human T cells for adoptive immunotherapy using the novel Xeno-free CTS

Immune Cell Serum Replacement" Clinical & Translational Immunology (2015) 4, e31; doi:10.1038/cti.2014.31.

[0335] In one aspect, T cells are isolated from peripheral blood lymphocytes by lysing the red blood cells and depleting the monocytes, for example, by centrifugation through a PERCOLL™ gradient or by counterflow centrifugal elutriation. A specific subpopulation of T cells, such as CD3+, CD28+, CD4+, CD8+, CD45RA+, and CD45RO+T cells, can be further isolated by positive or negative selection techniques. For example, in one aspect, T cells are isolated by incubation with anti-CD3/anti-CD28 (e.g., 3x28)-conjugated beads, such as DYNABEADS® M-450 CD3/CD28 T, for a time period sufficient for positive selection of the desired T cells. In one aspect, the time period is about 30 minutes. In a further aspect, the time period ranges from 30 minutes to 36 hours or longer and all integer values there between. In a further aspect, the time period is at least 1, 2, 3, 4, 5, or 6 hours. In yet another preferred aspect, the time period is 10 to 24 hours. In one aspect, the incubation time period is 24 hours. Longer incubation times may be used to isolate T cells in any situation where there are few T cells as compared to other cell types, such in isolating tumor infiltrating lymphocytes (TIL) from tumor tissue or from immunocompromised individuals. Further, use of longer incubation times can increase the efficiency of capture of CD8+ T cells. Thus, by simply shortening or lengthening the time T cells are allowed to bind to the CD3/CD28 beads and/or by increasing or decreasing the ratio of beads to T cells (as described further herein), subpopulations of T cells can be preferentially selected for or against at culture initiation or at other time points during the process. Additionally, by increasing or decreasing the ratio of anti-CD3 and/or anti-CD28 antibodies on the beads o r other surface, subpopulations of T cells can be preferentially selected for or against at culture initiation or at other desired time points. The skilled artisan would recognize that multiple rounds of selection can also be used in the context of this invention. In certain aspects, it may be desirable to perform the selection procedure and use the "unselected" cells in the activation and expansion process. "Unselected" cells can also be subjected to further rounds of selection.

[0336] Enrichment of a T cell population by negative selection can be accomplished with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers

present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail typically includes antibodies to CD14, CD20, CDllb, CD16, HLA-DR, and CD8. In certain aspects, it may be desirable to enrich for or positively select for regulatory T cells which typically express CD4+, CD25+, CD62Lhi, GITR+, and FoxP3+.

[0337] Alternatively, in certain aspects, T regulatory cells are depleted by anti-C25 conjugated beads or other similar method of selection. The methods described herein can include, e.g., selection of a specific subpopulation of immune effector cells, e.g., T cells, that are a T regulatory cell-depleted population, CD25+ depleted cells, using, e.g., a negative selection technique, e.g., described herein. Preferably, the population of T regulatory depleted cells contains less than 30%, 25%, 20%, 15%, 10%, 5%, 4%, 3%, 2%, 1% of CD25+ cells.

[0338] In one embodiment, T regulatory cells, e.g., CD25+ T cells, are removed from the population using an anti-CD25 antibody, or fragment thereof, or a CD25-binding ligand, IL-2. In one embodiment, the anti-CD25 antibody, or fragment thereof, or CD25-binding ligand is conjugated to a substrate, e.g., a bead, or is otherwise coated on a substrate, e.g., a bead. In one embodiment, the anti-CD25 antibody, or fragment thereof, is conjugated to a substrate as described herein.

[0339] In one embodiment, the T regulatory cells, e.g., CD25+ T cells, are removed from the population using CD25 depletion reagent from Miltenyi™. In one embodiment, the ratio of cells to CD25 depletion reagent is le7 cells to 20 uL, or le7 cells to 15 uL, or le7 cells to 10 uL, or le7 cells to 5 uL, or le7 cells to 2.5 uL, or le7 cells to 1.25 uL. In one embodiment, e.g., for T regulatory cells, e.g., CD25+ depletion, greater than 500 million cells/ml is used. In a further aspect, a concentration of cells of 600, 700, 800, or 900 million cells/ml is used. In one embodiment, the population of immune effector cells to be depleted includes about 6 x 109 CD25+ T cells. In other aspects, the population of immune effector cells to be depleted include about 1 x 109 to lx 10 10 CD25+ T cell, and any integer value in between. In one embodiment, the resulting population T regulatory depleted cells has 2 x 109T regulatory cells, e.g., CD25+ cells, or less (e.g., 1 x 109, 5 x 108, 1 x 108, 5 x 107, 1 x 107, or less CD25+ cells).

[0340] In one embodiment, the T regulatory cells, e.g., CD25+ cells, are removed from the population using the CliniMAC system with a depletion tubing set, such as, e.g., tubing 162-01. In one embodiment, the CliniMAC system is run on a depletion setting such as, e.g., DEPLETION2.1.

[0341] Without wishing to be bound by a particular theory, decreasing the level of negative regulators of immune cells (e.g., decreasing the number of unwanted immune cells, e.g., TREG cells), in a subject prior to apheresis or during manufacturing of a CAR-expressing cell product can reduce the risk of subject relapse. For example, methods of depleting TREG cells are known in the art. Methods of decreasing TREG cells include, but are not limited to, cyclophosphamide, anti-GITR antibody (an anti-GITR antibody described herein), CD25-depletion, and combinations thereof.

[0342] In some embodiments, the manufacturing methods comprise reducing the number of (e.g., depleting) TREG cells prior to manufacturing of the CAR-expressing cell. For example, manufacturing methods comprise contacting the sample, e.g., the apheresis sample, with an anti- GITR antibody and/or an anti-CD25 antibody (or fragment thereof, or a CD25-binding ligand), e.g., to deplete TREG cells prior to manufacturing of the CAR-expressing cell (e.g., T cell, NK cell) product.

[0343] In an embodiment, a subject is pre-treated with one or more therapies that reduce TREG cells prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment. In an embodiment, methods of decreasing TREG cells include, but are not limited to, administration to the subject of one or more of cyclophosphamide, anti-GITR antibody, CD25-depletion, or a combination thereof. Administration of one or more of cyclophosphamide, anti-GITR antibody, CD25- depletion, or a combination thereof, can occur before, during or after an infusion of the CARexpressing cell product.

[0344] In an embodiment, a subject is pre-treated with cyclophosphamide prior to collection of cells for CAR-expressing cell product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment. In an embodiment, a subject is pre-treated with an anti-GITR antibody prior to collection of cells for CAR-expressing cell

product manufacturing, thereby reducing the risk of subject relapse to CAR-expressing cell treatment.

[0345] In one embodiment, the population of cells to be removed are neither the regulatory T cells or tumor cells, but cells that otherwise negatively affect the expansion and/or function of CART cells, e.g. cells expressing CD14, CDllb, CD33, CD15, or other markers expressed by potentially immune suppressive cells. In one embodiment, such cells are envisioned to be removed concurrently with regulatory T cells and/or tumor cells, or following said depletion, or in another order.

[0346] The methods described herein can include more than one selection step, e.g., more than one depletion step. Enrichment of a T cell population by negative selection can be accomplished, e.g., with a combination of antibodies directed to surface markers unique to the negatively selected cells. One method is cell sorting and/or selection via negative magnetic immunoadherence or flow cytometry that uses a cocktail of monoclonal antibodies directed to cell surface markers present on the cells negatively selected. For example, to enrich for CD4+ cells by negative selection, a monoclonal antibody cocktail can include antibodies to CD14, CD20, CDllb, CD16, HLA-DR, and CD8.

[0347] The methods described herein can further include removing cells from the population which express a tumor antigen, e.g., a tumor antigen that does not comprise CD25, e.g., CD19, CD30, CD38, CD123, CD20, CD14 or CDllb, to thereby provide a population of T regulatory depleted, e.g., CD25+ depleted, and tumor antigen depleted cells that are suitable for expression of a CAR, e.g., a CAR described herein. In one embodiment, tumor antigen expressing cells are removed simultaneously with the T regulatory, e.g., CD25+ cells. For example, an anti-CD25 antibody, or fragment thereof, and an anti-tumor antigen antibody, or fragment thereof, can be attached to the same substrate, e.g., bead, which can be used to remove the cells or an anti-CD25 antibody, or fragment thereof, or the anti-tumor antigen antibody, or fragment thereof, can be attached to separate beads, a mixture of which can be used to remove the cells. In other embodiments, the removal of T regulatory cells, e.g., CD25+ cells, and the removal of the tumor antigen expressing cells is sequential, and can occur, e.g., in either order.

[0348] Also provided are methods that include removing cells from the population which express a check point inhibitor, e.g., a check point inhibitor described herein, e.g., one or more of PD1+ cells, FAG3+ cells, and TIM3+ cells, to thereby provide a population of T regulatory depleted, e.g., CD25+ depleted cells, and check point inhibitor depleted cells, e.g., PD1+, FAG3+ and/or TIM3+ depleted cells. Exemplary check point inhibitors include B7-H1, B7-1, CD160, P1H, 2B4, PD1, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM 5), LAG3, TIGIT, CTLA-4, BTLA and LAIR1. In one embodiment, check point inhibitor expressing cells are removed simultaneously with the T regulatory, e.g., CD25+ cells. For example, an anti-CD25 antibody, or fragment thereof, and an anti-check point inhibitor antibody, or fragment thereof, can be attached to the same bead which can be used to remove the cells, or an anti-CD25 antibody, or fragment thereof, and the anti-check point inhibitor antibody, or fragment there, can be attached to separate beads, a mixture of which can be used to remove the cells. In other embodiments, the removal of T regulatory cells, e.g., CD25+ cells, and the removal of the check point inhibitor expressing cells is sequential, and can occur, e.g., in either order.

[0349] In one embodiment, a T cell population can be selected that expresses one or more of IFN-g, TNFa, IL-17A, IL-2, IL-3, IL-4, GM-CSF, IL-10, IL-13, granzyme B, and perforin, or other appropriate molecules, e.g., other cytokines. Methods for screening for cell expression can be determined, e.g., by the methods described in PCT Publication No.: WO 2013/126712.

[0350] For isolation of a desired population of cells by positive or negative selection, the concentration of cells and surface (e.g., particles such as beads) can be varied. In certain aspects, it may be desirable to significantly decrease the volume in which beads and cells are mixed together (e.g., increase the concentration of cells), to ensure maximum contact of cells and beads. For example, in one aspect, a concentration of 2 billion cells/ml is used. In one aspect, a concentration of 1 billion cells/ml is used. In a further aspect, greater than 100 million cells/ml is used. In a further aspect, a concentration of cells of 10, 15, 20, 25, 30, 35, 40, 45, or 50 million cells/ml is used. In yet one aspect, a concentration of cells from 75, 80, 85, 90, 95, or 100 million cells/ml is used. In further aspects, concentrations of 125 or 150 million cells/ml can be used.

[0351] Using high concentrations can result in increased cell yield, cell activation, and cell expansion. Further, use of high cell concentrations allows more efficient capture of cells that may weakly express target antigens of interest, such as CD28-negative T cells, or from samples where there are many tumor cells present (e.g., leukemic blood, tumor tissue, etc.). Such populations of cells may have therapeutic value and would be desirable to obtain. For example, using high concentration of cells allows more efficient selection of CD8+ T cells that normally have weaker CD28 expression.

[0352] In a related aspect, it may be desirable to use lower concentrations of cells. By significantly diluting the mixture of T cells and surface (e.g., particles such as beads) interactions between the particles and cells is minimized. This selects for cells that express high amounts of desired antigens to be bound to the particles. For example, CD4+ T cells express higher levels of CD28 and are more efficiently captured than CD8+ T cells in dilute concentrations.

[0353] In one aspect, the concentration of cells used is 5 X 10e6/ml. In other aspects, the concentration used can be from about 1X 105/ml to 1X 106/ml, and any integer value in between.

[0354] In other aspects, the cells may be incubated on a rotator for varying lengths of time at varying speeds at either 2-10°C or at room temperature. T cells for stimulation can also be frozen after a washing step. Wishing not to be bound by theory, the freeze and subsequent thaw step provides a more uniform product by removing granulocytes and to some extent monocytes in the cell population. After the washing step that removes plasma and platelets, the cells may be suspended in a freezing solution. While many freezing solutions and parameters are known in the art and will be useful in this context, one method involves using PBS containing 20% DMSO and 8% human serum albumin, or culture media containing 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin and 7.5% DMSO, or 31.25% Plasmalyte-A, 31.25% Dextrose 5%, 0.45% NaCl, 10% Dextran 40 and 5% Dextrose, 20% Human Serum Albumin, and 7.5% DMSO or other suitable cell freezing media containing for example, Hespan and PlasmaLyte A, the cells then are frozen to -80°C at a rate of 1° per minute and stored in the vapor phase of a liquid nitrogen storage tank. Other

methods of controlled freezing may be used as well as uncontrolled freezing immediately at -20° C or in liquid nitrogen.

[0355] In certain aspects, cryopreserved cells are thawed and washed as described herein and allowed to rest for one hour at room temperature prior to activation using the methods of the present disclosure.

[0356] Also contemplated in the context of the invention is the collection of blood samples or apheresis product from a subject at a time period prior to when the expanded cells as described herein might be needed. As such, the source of the cells to be expanded can be collected at any time point necessary, and desired cells, such as T cells, isolated and frozen for later use in T cell therapy for any number of diseases or conditions that would benefit from T cell therapy, such as those described herein.

[0357] In one aspect a blood sample or an apheresis is taken from a generally healthy subject. In certain aspects, a blood sample or an apheresis is taken from a generally healthy subject who is at risk of developing a disease, but who has not yet developed a disease, and the cells of interest are isolated and frozen for later use. In certain aspects, the T cells may be expanded, frozen, and used at a later time. In certain aspects, samples are collected from a patient shortly after diagnosis of a particular disease as described herein but prior to any treatments.

[0358] In a further aspect, the cells are isolated from a blood sample or an apheresis from a subject prior to any number of relevant treatment modalities, including but not limited to treatment with agents such as natalizumab, efalizumab, antiviral agents, chemotherapy, radiation, immunosuppressive agents, such as cyclosporin, azathioprine, methotrexate, mycophenolate, and FK506, antibodies, or other immunoablative agents such as CAMPATH, anti-CD3 antibodies, cytoxan, fludarabine, cyclosporin, FK506, rapamycin, mycophenolic acid, steroids, FR901228, and irradiation.

[0359] In a further aspect of the present disclosure, T cells are obtained from a patient directly following treatment that leaves the subject with functional T cells. In this regard, it has been observed that following certain cancer treatments, in particular treatments with drugs that damage the immune system, shortly after treatment during the period when patients

would normally be recovering from the treatment, the quality of T cells obtained may be optimal or improved for their ability to expand ex vivo.

[0360] Likewise, following ex vivo manipulation using the methods described herein, these cells may be in a preferred state for enhanced engraftment and in vivo expansion. Thus, it is contemplated within the context of the present disclosure to collect blood cells, including T cells, dendritic cells, or other cells of the hematopoietic lineage, during this recovery phase. Further, in certain aspects, mobilization (for example, mobilization with GM-CSF) and conditioning regimens can be used to create a condition in a subject wherein repopulation, recirculation, regeneration, and/or expansion of particular cell types is favored, especially during a defined window of time following therapy. Illustrative cell types include T cells, B cells, dendritic cells, and other cells of the immune system.

[0361] In some embodiments, the T cell population is diaglycerol kinase (DGK)-deficient. DGKdeficient cells include cells that do not express DGK RNA or protein, or have reduced or inhibited DGK activity. DGK-deficient cells can be generated by genetic approaches, e.g., administering RNA-interfering agents, e.g., siRNA, shRNA, miRNA, to reduce or prevent DGK expression. Alternatively, DGK-deficient cells can be generated by treatment with DGK inhibitors described herein.

[0362] In one embodiment, a T cell population is Ikaros-deficient. Ikaros-deficient cells include cells that do not express Ikaros RNA or protein, or have reduced or inhibited Ikaros activity, Ikaros-deficient cells can be generated by genetic approaches, e.g., administering RNAinterfering agents, e.g., siRNA, shRNA, miRNA, to reduce or prevent Ikaros expression.

[0363] Alternatively, Ikaros-deficient cells can be generated by treatment with Ikaros inhibitors, e.g., lenalidomide. In embodiments, a T cell population is DGK-deficient and Ikaros-deficient, e.g., does not express DGK and Ikaros, or has reduced or inhibited DGK and Ikaros activity. Such DGK and Ikaros-deficient cells can be generated by any of the methods described herein. In an embodiment, the NK cells are obtained from the subject. In another embodiment, the NK cells are an NK cell line, e.g., NK-92 cell line (Conkwest).

Allogeneic CAR Immune Effector Cells

[0364] In embodiments described herein, the immune effector cell can be an allogeneic immune effector cell, e.g., T cell or NK cell. For example, the cell can be an allogeneic T cell, e.g., an allogeneic T cell lacking expression of a functional T cell receptor (TCR) and/or human leukocyte antigen (HLA), e.g., HLA class I and/or HLA class II.

[0365] A T cell lacking a functional TCR can be, e.g., engineered such that it does not express any functional TCR on its surface, engineered such that it does not express one or more subunits that comprise a functional TCR or engineered such that it produces very little functional TCR on its surface. Alternatively, the T cell can express a substantially impaired TCR, e.g., by expression of mutated or truncated forms of one or more of the subunits of the TCR. The term "substantially impaired TCR" means that this TCR will not elicit an adverse immune reaction in a host.

[0366] A T cell described herein can be, e.g., engineered such that it does not express a functional HLA on its surface. For example, a T cell described herein, can be engineered such that cell surface expression HLA, e.g., HLA class I and/or HLA class II, is downregulated.

[0367] In some embodiments, the T cell can lack a functional TCR and a functional HLA, e.g., HLA class I and/or HLA class II. Modified T cells that lack expression of a functional TCR and/or HLA can be obtained by any suitable means, including a knock out or knock down of one or more subunit of TCR or HLA. For example, the T cell can include a knock down of TCR and/or HLA using siRNA, shRNA, clustered regularly interspaced short palindromic repeats (CRISPR) transcriptionactivator like effector nuclease (TALEN), or zinc finger endonuclease (ZFN).

[0368] In some embodiments, the allogeneic cell can be a cell which does not expresses or expresses at low levels an inhibitory molecule, e.g. by any method described herein. For example, the cell can be a cell that does not express or expresses at low levels an inhibitory molecule, e.g., that can decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD1, PD-L1, CTLA4, TIM3, LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, and adenosine. Inhibition of an inhibitory molecule, e.g., by inhibition at the DNA,

RNA or protein level, can optimize a CAR-expressing cell performance. In embodiments, an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), e.g., as described herein, can be used.

siRNA and shRNA to inhibit TCR or HLA

[0369] In some embodiments, TCR expression and/or HLA expression can be inhibited using siRNA or shRNA that targets a nucleic acid encoding a TCR and/or HLA, and/or an inhibitory molecule (e.g., PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, and adenosine), in a cell, e.g., T cell.

[0370] Expression systems for siRNA and shRNAs, and exemplary shRNAs, are described, e.g., in paragraphs 649 and 650 of International Publication WO2015/142675, filed March 13, 2015, which is incorporated by reference in its entirety.

CRISPR to inhibit TCR or HLA

[0371] "CRISPR" or "CRISPR to TCR and/or HLA" or "CRISPR to inhibit TCR and/or HLA" as used herein refers to a set of clustered regularly interspaced short palindromic repeats, or a system comprising such a set of repeats. "Cas", as used herein, refers to a CRISPR-associated protein.

[0372] A "CRISPR/Cas" system refers to a system derived from CRISPR and Cas which can be used to silence or mutate a TCR and/or HLA gene, and/or an inhibitory molecule (e.g., PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, and adenosine), in a cell, e.g., T cell. The CRISPR/Cas system, and uses thereof, are described, e.g., in paragraphs 651-658 of

International Publication WO2015/142675, filed March 13, 2015, which is incorporated by reference in its entirety.

TALEN to inhibit TCR and/or HLA

[0373] "TALEN" or "TALEN to HLA and/or TCR" or "TALEN to inhibit HLA and/or TCR" refers to a transcription activator-like effector nuclease, an artificial nuclease which can be used to edit the HLA and/or TCR gene, and/or an inhibitory molecule (e.g., PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, and adenosine), in a cell, e.g., T cell.

[0374] TALENs, and uses thereof, are described, e.g., in paragraphs 659-665 of International Publication WO20 15/142675, filed March 13, 2015, which is incorporated by reference in its entirety.

Zinc finger nuclease to inhibit HLA and/or TCR

[0375] "ZFN" or "Zinc Finger Nuclease" or "ZFN to HLA and/or TCR" or "ZFN to inhibit HLA and/or TCR" refer to a zinc finger nuclease, an artificial nuclease which can be used to edit the HLA and/or TCR gene, and/or an inhibitory molecule (e.g., PD1, PD-L1, PD-L2, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, and adenosine), in a cell, e.g., T cell.

[0376] ZFNs, and uses thereof, are described, e.g., in paragraphs 666-671 of International Publication WO20 15/142675, filed March 13, 2015, which is incorporated by reference in its entirety.

Telomerase expression

[0377] While not wishing to be bound by any particular theory, in some embodiments, a therapeutic T cell has short term persistence in a patient, due to shortened telomeres in the T cell; accordingly, transfection with a telomerase gene can lengthen the telomeres of the T cell and improve persistence of the T cell in the patient. See Carl June, "Adoptive T cell therapy

for cancer in the clinic", Journal of Clinical Investigation, 117:1466-1476 (2007). Thus, in an embodiment, an immune effector cell, e.g., a T cell, ectopically expresses a telomerase subunit, e.g., the catalytic subunit of telomerase, e.g., TERT, e.g., hTERT. In some aspects, this disclosure provides a method of producing a CAR-expressing cell, comprising contacting a cell with a nucleic acid encoding a telomerase subunit, e.g., the catalytic subunit of telomerase, e.g., TERT, e.g., hTERT. The cell may be contacted with the nucleic acid before, simultaneous with, or after being contacted with a construct encoding a CAR.

[0378] In one aspect, the disclosure features a method of making a population of immune effector cells (e.g., T cells, NK cells). In an embodiment, the method comprises: providing a population of immune effector cells (e.g., T cells or NK cells), contacting the population of immune effector cells with a nucleic acid encoding a CAR; and contacting the population of immune effector cells with a nucleic acid encoding a telomerase subunit, e.g., hTERT, under conditions that allow for CAR and telomerase expression.

[0379] In an embodiment, the nucleic acid encoding the telomerase subunit is DNA. In an embodiment, the nucleic acid encoding the telomerase subunit comprises a promoter capable of driving expression of the telomerase subunit AAC5 1724.1 (Meyerson et ah, "hEST2, the Putative Human Telomerase Catalytic Subunit Gene, Is Up-Regulated in Tumor Cells and during Immortalization" Cell Volume 90, Issue 4, 22 August 1997, Pages 785-795).

Methods of Treatment

[0380] The present invention relates methods of treatment comprising administering an anti-GCC antigen binding molecule to a subject. In some embodiments, anti-GCC CARs and antigen binding molecules disclosed herein can be used in methods of treating or preventing a disease in a mammal. In this regard, an embodiment provides a method of treating or preventing cancer in a mammal, comprising administering to the mammal the CARs, the nucleic acids, the recombinant expression vectors, the host cells, the population of cells, the antibodies and/or the antigen binding portions thereof, and/or the pharmaceutical compositions in an amount effective to treat or prevent cancer in the mammal. The invention also relates to an anti-GCC antigen binding molecule as described herein (e.g. a sdAb or CAR) for use in the treatment of a disease. The invention also relates to an anti-GCC antigen

binding molecule as described herein (e.g. a sdAb or CAR) for use in the treatment of cancer. The invention also relates to an anti-GCC antigen binding molecule as described herein (e.g. a sdAb or CAR) for use in the manufacture of a medicament for the treatment of cancer.

[0381] The administration of the compositions described herein may 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 transarterially, subcutaneously, intradermally, intratumorally, intranodally, intramedullary, intramuscularly, by intravenous (i.v.) injection, or intraperitoneally. In one embodiment, the compositions described herein, e.g., comprising a CAR-expressing cell, are administered to a patient by intradermal or subcutaneous injection. In one embodiment, the the compositions described herein, e.g., comprising a CAR-expressing cell, are administered by i.v. injection. The compositions described herein, e.g., comprising a CAR-expressing cell, may be injected directly into a tumor, lymph node, or site of infection.

[0382] It can generally be stated that a pharmaceutical composition comprising the immune effector cells described herein may be administered at a dosage of 104 to 109 cells/kg body weight, in some instances 105 to 106 cells/kg body weight, including all integer values within those ranges. The immune effector cell compositions may also be administered multiple times at these dosages. The cells can be administered by using infusion techniques that are commonly known in immunotherapy (see, e.g., Rosenberg et al., New Eng. J . of Med. 319:1676, 1988).

[0383] In certain aspects, it may be desired to administer activated immune effector cells to a subject and then subsequently redraw blood (or have an apheresis performed), activate the cells therefrom according to the present invention, and reinfuse the patient with these activated and expanded cells. This process can be carried out multiple times every few weeks. In certain aspects, the cells can be activated from blood draws of from 10cc to 400cc. In certain aspects, the cells are activated from blood draws of 20cc, 30cc, 40cc, 50cc, 60cc, 70cc, 80cc, 90cc, or 100cc.

[0384] In some embodiments, the anti-GCC CAR is expressed on donor cells. In some embodiments the donor T cells for use in the T cell therapy are obtained from a patient (e.g., for an autologous T cell therapy). In other embodiments, the donor T cells for use in the T cell

therapy are obtained from a subject that is not the patient (e.g., an allogeneic T cell therapy). The CAR+ T cells may be administered at a therapeutically effective amount. For example, a therapeutically effective amount of the T cells may be at least about 10^4 cells, at least about 10^5 cells, at least about 10^6 cells, at least about 10^7 cells, at least about 10^8 cells, at least about 10^9, or at least about 10^{10}.

[0385] In some embodiments, the therapeutically effective amount of the T cells is about 10^4 cells, about 10^5 cells, about 10^6 cells, about 10^7 cells, or about 10^8 cells. In some embodiments, the therapeutically effective amount of the CAR T cells is about 2 X 10^6 cells/kg, about 3 X 10^6 cells/kg, about 4 X 10^6 cells/kg, about 5 X 10^6 cells/kg, about 6 X 10^6 cells/kg, about 7 X 10^6 cells/kg, about 8 X 10^6 cells/kg, about 9 X 10^6 cells/kg, about 1 X 10^7 cells/kg, about 2 X 10^7 cells/kg, about 3 X 10^7 cells/kg, about 4 X 10^7 cells/kg, about 5 X 10^7 cells/kg, about 6 X 10^7 cells/kg, about 7 X 10^7 cells/kg, about 8 X 10^7 cells/kg, or about 9 X 10^7 cells/kg. In some embodiments, the therapeutically effective amount of the CAR-positive viable T cells is between about 1 X 10^6 and about 2 X 10^6 CAR-positive viable T cells per kg body weight up to a maximum dose of about 1 x 10^8 CAR-positive viable T cells.

[0386] In some embodiments, the therapeutically effective amount of the CAR-positive viable T cells is between about 0.25 X 10^6 and 2 X 10^6. In some embodiments, the therapeutically effective amount of the CAR-positive viable T cells is about 0.25 x 10^6, 0.3 x 10^6, 0.4 x 10^6, about 0.5 x 10^6, about 0.6 x 10^6, about 0.7 x 10^6, about 0.8 x 10^6, about 0.9 x 10^6, about 1.0 x 10^6, about 1.1 x 10^6, about 1.2 x 10^6, about 1.3 x 10^6, about 1.4 x 10^6, about 1.5 x 10^6, about 1.6 x 10^6, about 1.7 x 10^6, about 1.8 x 10^6, about 1.9 x 10^6, or about 2.0 x 10^6 CAR-positive viable T cells.

[0387] In some embodiments, the therapeutically effective amount of the CAR-positive viable T cells is between about 0.4 x 10^8 and about 2 x 10^8 CAR-positive viable T cells. In some embodiments, the therapeutically effective amount of the CAR-positive viable T cells is about 0.4 x 10^8, about 0.5 x 10^8, about 0.6 x 10^8, about 0.7 x 10^8, about 0.8 x 10^8, about 0.9 x 10^8, about 1.0 x 10^8, about 1.1 x 10^8, about 1.2 x 10^8, about 1.3 x 10^8, about 1.4 x 10^8, about 1.5 x 10^8, about 1.6 x 10^8, about 1.7 x 10^8, about 1.8 x 10^8, about 1.9 x 10^8, or about 2.0 x 10^8 CAR-positive viable T cells.

[0388] In some embodiments, a dose of CAR-expressing cells (e.g., CAR-expressing cells described herein, e.g,. GCC CAR-expressing cells described herein) comprises about 1 x 106 cells/m2 to about 1 x 109 cells/m2, e.g., about 1 x 107 cells/m2 to about 5 x 108 cells/m2, e.g., about 1.5 x 107 cells/m2, about 2 x 107 cells/m2, about 4.5 x 107 cells/m2, about 108 cells/m2, about 1.2 x 108 cells/m2, or about 2 x 108 cells/m2.

[0389] The disclosure includes (among other things) a type of cellular therapy where T cells are genetically modified to express a chimeric antigen receptor (CAR) and the CAR T cell is infused to a recipient in need thereof. The infused cell is able to kill tumor cells in the recipient. Unlike antibody therapies, CAR-modified T cells are able to replicate in vivo resulting in long-term persistence that can lead to sustained tumor control. In various aspects, the T cells administered to the patient, persist in the patient for at least four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, twelve months, thirteen months, fourteen month, fifteen months, sixteen months, seventeen months, eighteen months, nineteen months, twenty months, twenty-one months, twenty-two months, twenty-three months, two years, three years, four years, or five years after administration of the T cell to the patient.

[0390] In some embodiments, the GCC CAR-expressing cells are administered in a plurality of doses, e.g., a first dose, a second dose, and optionally a third dose. In embodiments, the method comprises treating a subject (e.g., an adult subject) having a cancer (e.g., colorectal cancer), comprising administering to the subject a first dose, a second dose, and optionally one or more additional doses, each dose comprising immune effector cells expressing a CAR molecule, e.g., an GCC CAR molecule, e.g., a CAR molecule provided in Table 7.

[0391] In embodiments, the method comprises administering a dose of 2-5x10^6 viable CARexpressing cells/kg, wherein the subject has a body mass of less than 50 kg; or administering a dose of 1.0 -2.5 x10^8 viable CAR-expressing cells, wherein the subject has a body mass of at least 50 kg.

[0392] In embodiments, a single dose is administered to the subject, e.g., a pediatric or an adult subject.

[0393] In embodiments, the doses are administered on sequential days, e.g., the first dose is administered on day 1, the second dose is administered on day 2, and the optional third dose (if administered) is administered on day 3.

[0394] In embodiments, a fourth, fifth, or sixth dose, or more doses, are administered.

[0395] In embodiments, the first dose comprises about 10% of the total dose, the second dose comprises about 30% of the total dose, and the third dose comprises about 60% of the total dose, wherein the aforementioned percentages have a sum of 100%. In embodiments, the first dose comprises about 9-11%, 8-12%, 7-13%, or 5-15% of the total dose. In embodiments, the second dose comprises about 29-31%, 28-32%, 27-33%, 26-34%, 25-35%, 24-36%, 23-37%, 22-38%, 21-39%, or 20-40% of the total dose. In embodiments, the third dose comprises about 55-65%, 50-70%, 45-75%, or 40-80% of the total dose. In embodiments, the total dose refers to the total number of viable CAR-expressing cells administered over the course of 1 week, 2 weeks, 3 weeks, or 4 weeks. In some embodiments wherein two doses are administered, the total dose refers to the sum of the number of viable CAR-expressing cells administered to the subject in the first and second doses. In some embodiments wherein three doses are administered, the total dose refers to the sum of the number of viable CAR-expressing cells administered to the subject in the first, second, and third doses.

[0396] In embodiments, the dose is measured according to the number of viable CAR-expressing cells therein. CAR expression can be measured, e.g., by flow cytometry using an antibody molecule that binds the CAR molecule and a detectable label. Viability can be measured, e.g., by Cellometer.

[0397] The invention also includes a type of cellular therapy where immune effector cells, e.g., NK cells or T cells are modified, e.g., by in vitro transcribed RNA, to transiently express a chimeric antigen receptor (CAR) and the CAR-expressing (e.g., CART) cell is infused to a recipient in need thereof. The infused cell is able to kill cancer cells in the recipient. Thus, in various aspects, the CAR-expressing cells, e.g., T cells, administered to the patient, is present for less than one month, e.g., three weeks, two weeks, one week, after administration of the CAR expressing cell, e.g., T cell, to the patient.

[0398] Without wishing to be bound by any particular theory, the anti-cancer immunity response elicited by the CAR-modified T cells may be an active or a passive immune response, or alternatively may be due to a direct vs indirect immune response. In one aspect, the CAR (e.g., GCC -CAR) transduced T cells exhibit specific proinflammatory cytokine secretion and potent cytolytic activity in response to human cancer cells expressing the target antigen (e.g., GCC), resist soluble target antigen inhibition, mediate bystander killing and mediate regression of an established human cancer. For example, antigen-less cancer cells within a heterogeneous field of target antigen-expressing cancer may be susceptible to indirect destruction by target antigen-redirected T cells that has previously reacted against adjacent antigen-positive cancer cells.

[0399] In one aspect, the disclosure features a method of treating cancer in a subject. The method comprises administering to the subject a therapy that includes administering a CAR-expressing cell (e.g., GCC CAR-expressing cell) such that the cancer is treated in the subject. In one embodiment, the therapy of a CAR-expressing cell (e.g., GCC CAR-expressing cell) described herein results in one or more of: improved or increased anti-tumor activity of the CAR-expressing cell (e.g., GCC CAR-expressing cell); increased proliferation or persistence of the CAR-expressing cell; improved or increased infiltration of the CAR-expressing cell; improved inhibition of tumor progression; delay of tumor progression; inhibition or reduction in cancer cell proliferation; and/or reduction in tumor burden, e.g., tumor volume, or size. In one embodiment, the therapy results in increased persistence of the CAR-expressing cell and/or increased persistence of the CAR-expressing cell and a lower, e.g., reduced, risk of relapse.

[0400] The present invention provides methods for inhibiting the proliferation of or reducing an antigen-expressing (e.g., GCC -expressing) cell population. In one embodiment, the methods comprise administering a CAR therapy (e.g., a population of CAR expressing cells). In certain embodiments, the therapy described herein reduces the quantity, number, amount or percentage of cells and/or cancer cells by at least at least 5% , 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% in a subject with or animal model of an antigen (e.g., GCC) or another cancer associated with antigen-expressing (e.g. GCC -expressing) cells

relative to the quantity, number, amount, or percentage of cells and/or cancer cells in a subject treated with a different cancer therapy. In one embodiment, the subject is a mammal. In some embdiments, the subject is a human.

[0401] The invention also provides methods for preventing, treating and/or managing a disorder, e.g., a disorder associated with antigen-expressing cells (e.g., GCC -expressing cells) (e.g., a cancer described herein), the methods comprising administering to a subject in need a CAR expressing cell (e.g., GCC CAR-expressing cell, a population of CAR-expressing cells). In one aspect, the subject is a human.

[0402] In one aspect, the invention pertains to a method of inhibiting growth of a cancer cell, (e.g., an antigen-expressing, e.g., GCC -expressing, cancer cell), comprising contacting the cancer cell with a CAR-expressing (e.g., GCC CAR expressing) cell, e.g., an GCC CART cell, described herein, such that the CART is activated in response to the antigen and targets the cancer cell, wherein the growth of the cancer is inhibited.

[0403] The present disclosure also provides methods for preventing, treating and/or managing a disease, e.g., a disease associated with antigen-expressing (e.g., GCC -expressing) cells (e.g., a cancer expressing the antigen, e.g., GCC), the methods comprising administering to a subject in need an CAR-expressing (e.g., GCC CAR-expressing) cell that binds to the antigen-expressing cell. In some embodimenbts, the subject is a human.

[0404] The present disclosure also provides methods for preventing, treating and/or managing a disease associated with antigen-expressing (e.g., GCC -expressing) cells, the methods comprising administering to a subject in need a CART cell (e.g., an anti- GCC CART cell) of the invention that binds to the antigen-expressing (e.g., GCC -expressing) cell. In one aspect, the subject is a human.

[0405] The present disclosure also provides methods for preventing relapse of cancer, e.g.,associated with antigen-expressing (e.g., GCC -expressing) cells, the methods comprising administering to a subject in need thereof a CART cell (e.g., an anti-GCC CART cell) of the invention that binds to the antigen-expressing (e.g., GCC -expressing) cell. In one aspect, the methods comprise administering to the subject in need thereof an effective amount of a CART cell (e.g., an anti- GCC CART cell) described herein that binds to the antigen expressing (e.g., GCC -expressing) cell in combination with an effective amount of another therapy.

[0406] An embodiment further comprises lymphodepleting the mammal prior to administering the CARs disclosed herein. Examples of lymphodepletion include, but may not be limited to, nonmyeloablative lymphodepleting chemotherapy, myeloablative lymphodepleting chemotherapy, total body irradiation, etc.

[0407] In a particular exemplary aspect, subjects may undergo leukapheresis, wherein leukocytes are collected, enriched, or depleted ex vivo to select and/or isolate the cells of interest, e.g., T cells. These T cell isolates may be expanded by methods known in the art and treated such that one or more CAR constructs of the invention may be introduced, thereby creating a CAR T cell of the invention. Subjects in need thereof may subsequently undergo standard treatment with high dose chemotherapy followed by peripheral blood stem cell transplantation. In certain aspects, following or concurrent with the transplant, subjects receive an infusion of the expanded CAR expressing cells of the present invention. In an additional aspect, expanded cells are administered before or following surgery.

[0408] For purposes of the methods, wherein host cells or populations of cells are administered, the cells can be cells that are allogeneic or autologous to the mammal. Preferably, the cells are autologous to the mammal. As used herein, allogeneic means any material derived from a different animal of the same species as the individual to whom the material is introduced. Two or more individuals are said to be allogeneic to one another when the genes at one or more loci are not identical. In some aspects, allogeneic material from individuals of the same species may be sufficiently unlike genetically to interact antigenically. As used herein, "autologous" means any material derived from the same individual to whom it is later to be re-introduced into the individual.

[0409] The mammal referred to herein can be any mammal. As used herein, the term "mammal" refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. The mammals may be from the order Carnivora, including Felines (cats) and Canines (dogs). The mammals may be from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). The mammals may be of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). In some embodiments, the mammal is a human.

[0410] With respect to the methods, the cancer can be any cancer, including any of acute lymphocytic cancer, acute myeloid leukemia, alveolar rhabdomyosarcoma, bladder cancer (e.g., bladder carcinoma), bone cancer, brain cancer (e.g., meduloblastoma), breast cancer, cancer of the anus, anal canal, or anorectum, cancer of the eye, cancer of the intrahepatic bile duct, cancer of the joints, cancer of the neck, gallbladder, or pleura, cancer of the nose, nasal cavity, or middle ear, cancer of the oral cavity, cancer of the vulva, chronic lymphocytic leukemia, chronic myeloid cancer, colon cancer, esophageal cancer, cervical cancer, fibrosarcoma, gastrointestinal carcinoid tumor, head and neck cancer (e.g., head and neck squamous cell carcinoma), Hodgkin lymphoma, hypopharynx cancer, kidney cancer, larynx cancer, leukemia, liquid tumors, liver cancer, lung cancer (e.g., non-small cell lung carcinoma and lung adenocarcinoma), lymphoma, mesothelioma, mastocytoma, melanoma, multiple myeloma, nasopharynx cancer, non-Hodgkin lymphoma, B-chronic lymphocytic leukemia, hairy cell leukemia, acute lymphocytic leukemia (ALL), and Burkitt's lymphoma, ovarian cancer, pancreatic cancer, peritoneum, omentum, and mesentery cancer, pharynx cancer, prostate cancer, rectal cancer, renal cancer, skin cancer, small intestine cancer, soft tissue cancer, solid tumors, synovial sarcoma, gastric cancer, testicular cancer, thyroid cancer, and ureter cancer.

[0411] In certain embodiments, the cancer is a gastrointestinal cancer. In some embodiments, the cancer is gastric cancer. In some embodiments, the cancer is colorectal cancer. In some embodiments, the cancer is colon cancer. In some embodiments, the cancer has abnormal expression of GCC.

[0412] The terms "treat," and "prevent" as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment or prevention. Rather, there are varying degrees of treatment or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the methods can provide any amount or any level of treatment or prevention of cancer in a mammal.

[0413] Furthermore, the treatment or prevention provided by the method can include treatment or prevention of one or more conditions or symptoms of the disease, e.g., cancer, being treated or prevented. Also, for purposes herein, "prevention" can encompass delaying the onset of the disease, or a symptom or condition thereof.

[0414] Another embodiment provides a method of detecting the presence of cancer in a mammal, comprising: (a) contacting a sample comprising one or more cells from the mammal with the CARs, the nucleic acids, the recombinant expression vectors, the host cells, the population of cells, the antibodies, and/or the antigen binding portions thereof, or the pharmaceutical compositions, thereby forming a complex, (b) and detecting the complex, wherein detection of the complex is indicative of the presence of cancer in the mammal. In some embodiments, the contacting can take place in vitro or in vivo with respect to the mammal. In some embodiments, the contacting is *in vitro*.

[0415]

[0416] The sample may be obtained by any suitable method, e.g., biopsy or necropsy. A biopsy is the removal of tissue and/or cells from an individual. Such removal may be to collect tissue and/or cells from the individual in order to perform experimentation on the removed tissue and/or cells. This experimentation may include experiments to determine if the individual has and/or is suffering from a certain condition or disease-state. The condition or disease may be, e.g., cancer.

[0417] With respect to an embodiment of the method of detecting the presence of a proliferative disorder, e.g., cancer, in a mammal, the sample comprising cells of the mammal can be a sample comprising whole cells, lysates thereof, or a fraction of the whole cell lysates, e.g., a nuclear or cytoplasmic fraction, a whole protein fraction, or a nucleic acid fraction. If the sample comprises whole cells, the cells can be any cells of the mammal, e.g., the cells of any organ or tissue, including blood cells or endothelial cells.

[0418] Also, detection of the complex can occur through any number of ways known in the art. For instance, the CARs disclosed herein, polypeptides, proteins, nucleic acids, recombinant expression vectors, host cells, populations of cells, or antibodies, or antigen binding portions thereof, described herein, can be labeled with a detectable label such as, for instance, a radioisotope, a fluorophore (e.g., fluorescein isothiocyanate (FITC), phycoerythrin (PE)), an enzyme (e.g., alkaline phosphatase, horseradish peroxidase), and element particles (e.g., gold particles) as disclosed supra.

[0419] Methods of testing a CAR for the ability to recognize target cells and for antigen specificity are known in the art. For instance, Clay et al., J. Immunol, 163: 507-513

(1999), teaches methods of measuring the release of cytokines (e.g., interferon-γ, granulocyte/monocyte colony stimulating factor (GM-CSF), tumor necrosis factor a (TNF-α) or interleukin 2 (IL-2)). In addition, CAR function can be evaluated by measurement of cellular cytotoxicity, as described in Zhao et al, J. Immunol, 174: 4415-4423 (2005).

[0420] Another embodiment provides for the use of the CARs, nucleic acids, recombinant expression vectors, host cells, populations of cells, antibodies, or antigen binding portions thereof, and/or pharmaceutical compositions of the invention, for the treatment or prevention of a proliferative disorder, e.g., cancer, in a mammal. The cancer may be any of the cancers described herein.

[0421] Any method of administration can be used for the disclosed therapeutic agents, including local and systemic administration. For example topical, oral, intravascular such as intravenous, intramuscular, intraperitoneal, intranasal, intradermal, intrathecal and subcutaneous administration can be used. The particular mode of administration and the dosage regimen will be selected by the attending clinician, taking into account the particulars of the case (for example the subject, the disease, the disease state involved, and whether the treatment is prophylactic). In cases in which more than one agent or composition is being administered, one or more routes of administration may be used; for example, a chemotherapeutic agent may be administered orally and an antibody or antigen binding fragment or conjugate or composition may be administered intravenously. Methods of administration include injection for which the CAR, CAR T Cell, conjugates, antibodies, antigen binding fragments, or compositions are provided in a nontoxic pharmaceutically acceptable carrier such as water, saline, Ringer's solution, dextrose solution, 5% human serum albumin, fixed oils, ethyl oleate, or liposomes. In some embodiments, local administration of the disclosed compounds can be used, for instance by applying the antibody or antigen binding fragment to a region of tissue from which a tumor has been removed, or a region suspected of being prone to tumor development. In some embodiments, sustained intra-tumoral (or near-tumoral) release of the pharmaceutical preparation that includes a therapeutically effective amount of the antibody or antigen binding fragment may be beneficial. In other examples, the conjugate is applied as an eye drop topically to the cornea, or intravitreally into the eye.

[0422] The disclosed therapeutic agents can be formulated in unit dosage form suitable for individual administration of precise dosages. In addition, the disclosed therapeutic agents may be administered in a single dose or in a multiple dose schedule. A multiple dose schedule is one in which a primary course of treatment may be with more than one separate dose, for instance 1-10 doses, followed by other doses given at subsequent time intervals as needed to maintain or reinforce the action of the compositions. Treatment can involve daily or multi-daily doses of compound(s) over a period of a few days to months, or even years. Thus, the dosage regime will also, at least in part, be determined based on the particular needs of the subject to be treated and will be dependent upon the judgment of the administering practitioner.

[0423] In one embodiment, the CAR is introduced into immune effector cells, e.g., using in vitro transcription, and the subject (e.g., human) receives an initial administration of CAR-expressing cells of the invention, and one or more subsequent administrations of the CAR-expressing cells of the invention, wherein the one or more subsequent administrations are administered less than 15 days, e.g., 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, or 2 days after the previous administration. In one embodiment, more than one administration of the CAR-expressing cells of the invention are administered to the subject (e.g., human) per week, e.g., 2, 3, or 4 administrations of the CARexpressing cells of the invention are administered per week. In one embodiment, the subject (e.g., human subject) receives more than one administration of the CAR-expressing cells per week (e.g., 2, 3 or 4 administrations per week) (also referred to herein as a cycle), followed by a week of no CAR-expressing cells administration, and then one or more additional administration of the CAR-expressing cells (e.g., more than one administration of the CAR-expressing cells per week) is administered to the subject. In another embodiment, the subject (e.g., human subject) receives more than one cycle of CAR-expressing cells, and the time between each cycle is less than 10, 9, 8, 7, 6, 5, 4, or 3 days. In one embodiment, the CAR-expressing cells are administered every other day for 3 administrations per week. In one embodiment, the CARexpressing cells of the invention are administered for at least two, three, four, five, six, seven, eight or more weeks.

[0424] Typical dosages of the antibodies or conjugates can range from about 0.01 to about 30 mg/kg, such as from about 0.1 to about 10 mg/kg.

[0425] In particular examples, the subject is administered a therapeutic composition that includes one or more of the conjugates, antibodies, compositions, CARs, CAR T cells or additional agents, on a multiple daily dosing schedule, such as at least two consecutive days, 10 consecutive days, and so forth, for example for a period of weeks, months, or years. In one example, the subject is administered the conjugates, antibodies, compositions or additional agents for a period of at least 30 days, such as at least 2 months, at least 4 months, at least 6 months, at least 12 months, at least 24 months, or at least 36 months.

[0426] In some embodiments, the disclosed methods include providing surgery, radiation therapy, and/or chemotherapeutics to the subject in combination with a disclosed antibody, antigen binding fragment, conjugate, CAR or T cell expressing a CAR (for example, sequentially, substantially simultaneously, or simultaneously). Methods and therapeutic dosages of such agents and treatments are known to those skilled in the art, and can be determined by a skilled clinician. Preparation and dosing schedules for the additional agent may be used according to manufacturer's instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in Chemotherapy Service, (1992) Ed., M. C. Perry, Williams & Wilkins, Baltimore, Md.

[0427] In some embodiments, the combination therapy can include administration of a therapeutically effective amount of an additional cancer inhibitor to a subject. Non-limiting examples of additional therapeutic agents that can be used with the combination therapy include microtubule binding agents, DNA intercalators or cross-linkers, DNA synthesis inhibitors, DNA and RNA transcription inhibitors, antibodies, enzymes, enzyme inhibitors, gene regulators, and angiogenesis inhibitors. These agents (which are administered at a therapeutically effective amount) and treatments can be used alone or in combination. For example, any suitable anti-cancer or anti-angiogenic agent can be administered in combination with the CARS, CAR-T cells, antibodies, antigen binding fragment, or conjugates disclosed herein. Methods and therapeutic dosages of such agents are known to those skilled in the art, and can be determined by a skilled clinician.

[0428] Additional chemotherapeutic agents include, but are not limited to alkylating agents, such as nitrogen mustards (for example, chlorambucil, chlormethine,

cyclophosphamide, ifosfamide, and melphalan), nitrosoureas (for example, carmustine, fotemustine, lomustine, and streptozocin), platinum compounds (for example, carboplatin, cisplatin, oxaliplatin, and BBR3464), busulfan, dacarbazine, mechlorethamine, procarbazine, temozolomide, thiotepa, and uramustine; antimetabolites, such as folic acid (for example, methotrexate, pemetrexed, and raltitrexed), purine (for example, cladribine, clofarabine, fludarabine, mercaptopurine, and tioguanine), pyrimidine (for example, capecitabine), cytarabine, fluorouracil, and gemcitabine; plant alkaloids, such as podophyllum (for example, etoposide, and teniposide), taxane (for example, docetaxel and paclitaxel), vinca (for example, vinblastine, vincristine, vindesine, and vinorelbine); cytotoxic/antitumor antibiotics, such as anthracycline family members (for example, daunorubicin, doxorubicin, epirubicin, idarubicin, mitoxantrone, and valrubicin), bleomycin, rifampicin, hydroxyurea, and mitomycin; topoisomerase inhibitors, such as topotecan and irinotecan; monoclonal antibodies, such as alemtuzumab, bevacizumab, cetuximab, gemtuzumab, rituximab, panitumumab, pertuzumab, and trastuzumab; photosensitizers, such as aminolevulinic acid, methyl aminolevulinate, porfimer sodium, and verteporfin; and other agents, such as alitretinoin, altretamine, amsacrine, anagrelide, arsenic trioxide, asparaginase, axitinib, bexarotene, bevacizumab, bortezomib, celecoxib, denileukin diftitox, erlotinib, estramustine, gefitinib, hydroxycarbamide, imatinib, lapatinib, pazopanib, pentostatin, masoprocol, mitotane, pegaspargase, tamoxifen, sorafenib, sunitinib, vemurafinib, vandetanib, and tretinoin. Selection and therapeutic dosages of such agents are known to those skilled in the art, and can be determined by a skilled clinician.

[0429] General Chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex®), bicalutamide (Casodex®), bleomycin sulfate (Blenoxane®), busulfan (Myleran®), busulfan injection (Busulfex®), capecitabine (Xeloda®), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin®), carmustine (BiCNU®), chlorambucil (Leukeran®), cisplatin (Platinol®), cladribine (Leustatin®), cyclophosphamide (Cytoxan® or Neosar®), cytarabine, cytosine arabinoside (Cytosar-U®), cytarabine liposome injection (DepoCyt®), dacarbazine (DTIC-Dome®), dactinomycin (Actinomycin D, Cosmegan), daunombicin hydrochloride (Cembidine®), daunombicin citrate liposome injection (DaunoXome®), dexamethasone, docetaxel (Taxotere®), doxorubicin hydrochloride (Adriamycin®, Rubex®), etoposide (Vepesid®),

fludarabine phosphate (Fludara®), 5-fluorouracil (Adrucil®, Efudex®), flutamide (Eulexin®), tezacitibine, Gemcitabine (difluorodeoxycitidine), hydroxyurea (Hydrea®), Idarubicin (Idamycin®), ifosfamide (IFEX®), irinotecan (Camptosar®), L-asparaginase (ELSPAR®), leucovorin calcium, melphalan (Alkeran®), 6-mercaptopurine (Purinethol®), methotrexate (Folex®), mitoxantrone (Novantrone®), mylotarg, paclitaxel (Taxol®), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel®), tamoxifen citrate (Nolvadex®), teniposide (Vumon®), 6-thioguanine, thiotepa, tirapazamine (Tirazone®), topotecan hydrochloride for injection (Hycamptin®), vinblastine (Velban®), vincristine (Oncovin®), and vinorelbine (Navelbine®).

[0430] Exemplary alkylating agents are disclosed on pages 270-271 of International Application WO 2016/164731, filed April 8, 2016, which is incorporated by reference in its entirety. Further examples include include, without limitation, nitrogen mustards, ethylenimine derivatives, alkyl sulfonates, nitrosoureas and triazenes): uracil mustard (Aminouracil Mustard®, Chlorethaminacil®, Demethyldopan®, Desmethyldopan®, Haemanthamine®, Nordopan®, Uracil nitrogen mustard®, Uracillost®, Uracilmostaza®, Uramustin®, Uramustine®), chlormethine (Mustargen®), cyclophosphamide (Cytoxan®, Neosar®, Clafen®, Endoxan®, Procytox®, Revimmune™), ifosfamide (Mitoxana®), melphalan (Alkeran®), Chlorambucil (Leukeran®), pipobroman (Amedel®, Vercyte®), triethylenemelamine (Hemel®, Hexalen®, Hexastat®), triethylenethiophosphoramine, Temozolomide (Temodar®), thiotepa (Thioplex®), busulfan (Busilvex®, Myleran®), carmustine (BiCNU®), lomustine (CeeNU®), streptozocin (Zanosar®), and Dacarbazine (DTIC-Dome®). Additional exemplary alkylating agents include, without limitation, Oxaliplatin (Eloxatin®); Temozolomide (Temodar® and Temodal®); Dactinomycin (also known as actinomycin-D, Cosmegen®); Melphalan (also known as L-PAM, L-sarcolysin, and phenylalanine mustard, Alkeran®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Carmustine (BiCNU®); Bendamustine (Treanda®); Busulfan (Busulfex® and Myleran®); Carboplatin (Paraplatin®); Lomustine (also known as CCNU, CeeNU®); Cisplatin (also known as CDDP, Platinol® and Platinol®-AQ); Chlorambucil (Leukeran®); Cyclophosphamide (Cytoxan® and Neosar®); Dacarbazine (also known as DTIC, DIC and imidazole carboxamide, DTIC-Dome®); Altretamine (also known as hexamethylmelamine (HMM), Hexalen®); Ifosfamide (Ifex®); Prednumustine; Procarbazine

(Matulane®); Mechlorethamine (also known as nitrogen mustard, mustine and mechloroethamine hydrochloride, Mustargen®); Streptozocin (Zanosar®); Thiotepa (also known as thiophosphoamide, TESPA and TSPA, Thioplex®); Cyclophosphamide (Endoxan®, Cytoxan®, Neosar®, Procytox®, Revimmune®); and Bendamustine HC1 (Treanda®).

[0431] Exemplary platinum based agents include, without limitation, carboplatin, cisplatin, and oxaliplatin.

[0432] Exemplary angiogenesis inhibitors include, without limitation A6 (Angstrom Pharmacueticals), ABT-510 (Abbott Laboratories), ABT-627 (Atrasentan) (Abbott Laboratories/Xinlay), ABT-869 (Abbott Laboratories), Actimid (CC4047, Pomalidomide) (Celgene Corporation), AdGVPEDF.llD (GenVec), ADH-1 (Exherin) (Adherex Technologies), AEE788 (Novartis), AG-013736 (Axitinib) (Pfizer), AG3340 (Prinomastat) (Agouron Pharmaceuticals), AGX1053 (AngioGenex), AGX51 (AngioGenex), ALN-VSP (ALN-VSP 02) (Alnylam Pharmaceuticals), AMG 386 (Amgen), AMG706 (Amgen), Apatinib (YN968D1) (Jiangsu Hengrui Medicine), AP23573 (Ridaforolimus/MK8669) (Ariad Pharmaceuticals), AQ4N (Novavea), ARQ 197 (ArQule), ASA404 (Novartis/Antisoma), Atiprimod (Callisto Pharmaceuticals), ATN-161 (Attenuon), AV-412 (Aveo Pharmaceuticals), AV-951 (Aveo Pharmaceuticals), Avastin (Bevacizumab) (Genentech), AZD2171 (Cediranib/Recentin) (AstraZeneca), BAY 57-9352 (Telatinib) (Bayer), BEZ235 (Novartis), BIBF1120 (Boehringer Ingelheim Pharmaceuticals), BIBW 2992 (Boehringer Ingelheim Pharmaceuticals), BMS-275291 (Bristol-Myers Squibb), BMS-582664 (Brivanib) (Bristol-Myers Squibb), BMS-690514 (Bristol-Myers Squibb), Calcitriol, CCI-779 (Torisel) (Wyeth), CDP-791 (ImClone Systems), Ceflatonin (Homoharringtonine/HHT) (ChemGenex Therapeutics), Celebrex (Celecoxib) (Pfizer), CEP-7055 (Cephalon/Sanofi), CHIR-265 (Chiron Corporation), NGR-TNF, COL-3 (Metastat) (Collagenex Pharaceuticals), Combretastatin (Oxigene), CP-751, 87 l(Figitumumab) (Pfizer), CP-547,632 (Pfizer), CS-7017 (Daiichi Sankyo Pharma), CT-322 (Angiocept) (Adnexus), Curcumin, Dalteparin (Fragmin) (Pfizer), Disulfiram (Antabuse), E7820 (Eisai Limited), E7080 (Eisai Limited), EMD 121974(Cilengitide) (EMD Pharmaceuticals), ENMD-1198 (EntreMed), ENMD-2076 (EntreMed), Endostar (Simcere), Erbitux (ImClone/Bristol-Myers Squibb), EZN-2208 (Enzon Pharmaceuticals), EZN-2968 (Enzon Pharmaceuticals), GC1008 (Genzyme), Genistein,

GSK1363089(Foretinib) (GlaxoSmithKline), GW786034 (Pazopanib) (GlaxoSmithKline), GT-111 (Vascular Biogenics Ltd.), IMC—1121B (Ramucirumab) (ImClone Systems), IMC-18F1 (ImClone Systems), IMC-3G3 (ImClone LLC), INCB007839 (Incyte Corporation), INGN 241 (Introgen Therapeutics), Iressa (ZD1839/Gefitinib), LBH589 (Faridak/Panobinostst) (Novartis), Lucentis (Ranibizumab) (Genentech/Novartis), LY3 17615 (Enzastaurin) (Eli Lilly and Company), Macugen (Pegaptanib) (Pfizer), MED1522 (Abegrin) (MedImmune), MLN518(Tandutinib) (Millennium), Neovastat (AE941/Benefin) (Aeterna Zentaris), Nexavar (Bayer/Onyx), NM-3 (Genzyme Corporation), Noscapine (Cougar Biotechnology), NPT2358 (Nereus Pharmaceuticals), OSI-930 (OSI), Palomid 529 (Paloma Pharmaceuticals, Inc.), Panzem Capsules (2ME2) (EntreMed), Panzem NCD (2ME2) (EntreMed), PF-02341066 (Pfizer), PF-04554878 (Pfizer), PI-88 (Progen Industries/Medigen Biotechnology), PKC412 (Novartis), Polyphenon E (Green Tea Extract) (Polypheno E International, Inc), PPI-2458 (Praecis Pharmaceuticals), PTC299 (PTC Therapeutics), PTK787 (Vatalanib) (Novartis), PXD101 (Belinostat) (CuraGen Corporation), RAD001 (Everolimus) (Novartis), RAF265 (Novartis), Regorafenib (BAY73-4506) (Bayer), Revlimid (Celgene), Retaane (Alcon Research), SN38 (Liposomal) (Neopharm), SNS-032 (BMS-387032) (Sunesis), SOM230(Pasireotide) (Novartis), Squalamine (Genaera), Suramin, Sutent (Pfizer), Tarceva (Genentech), TB-403 (Thrombogenics), Tempostatin (Collard Biopharmaceuticals), Tetrathiomolybdate (Sigma-Aldrich), TG100801 (TargeGen), Thalidomide (Celgene Corporation), Tinzaparin Sodium, TKI258 (Novartis), TRC093 (Tracon Pharmaceuticals Inc.), VEGF Trap (Aflibercept) (Regeneron Pharmaceuticals), VEGF Trap-Eye (Regeneron Pharmaceuticals), Veglin (VasGene Therapeutics), Bortezomib (Millennium), XL184 (Exelixis), XL647 (Exelixis), XL784 (Exelixis), XL820 (Exelixis), XL999 (Exelixis), ZD6474 (AstraZeneca), Vorinostat (Merck), and ZSTK474.

[0433] Exemplary Vascular Endothelial Growth Factor (VEGF) receptor inhibitors include, but are not limited to, Bevacizumab (Avastin®), axitinib (Inlyta®); Brivanib alaninate (BMS-582664, (S)-((R)-l-(4-(4-Fluoro-2-methyl-17/-indol-5-yloxy)-5-methylpyrrolo[2,l-/][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate); Sorafenib (Nexavar®); Pazopanib (Votrient®); Sunitinib malate (Sutent®); Cediranib (AZD2171, CAS 288383-20-1); Vargatef (BIBF1120, CAS 928326-83-4); Foretinib (GSK1363089); Telatinib (BAY57-9352, CAS 332012-40-5); Apatinib (YN968D1, CAS 811803-05-1); Imatinib

(Gleevec®); Ponatinib (AP24534, CAS 943319-70-8); Tivozanib (AV951, CAS 475108-18-0); Regorafenib (BAY73-4506, CAS 755037-03-7); Vatalanib dihydrochloride (PTK787, CAS 212141-51-0); Brivanib (BMS-540215, CAS 649735-46-6); Vandetanib (Caprelsa® or AZD6474); Motesanib diphosphate (AMG706, CAS 857876-30-3, N-(2,3-dihydro-3, 3-dimethyl- lH-indol-6-yl)-2-[(4-pyridinylmethyl)amino]-3-pyridinecarboxamide, described in PCT Publication No. WO 02/066470); Dovitinib dilactic acid (TKI258, CAS 852433-84-2); Linfanib (ABT869, CAS 796967-16-3); Cabozantinib (XL184, CAS 849217-68-1); Lestaurtinib (CAS 111358-88-4); N-[5-[[[5-(1,1-Dimethylethyl)-2-oxazolyl] methyl]thio] -2-thiazolyl] -4-piperidinecarboxamide (BMS38703, CAS 345627-80-7); (3R,4R)-4-Amino-l-((4-((3-methoxyphenyl)amino)pyrrolo [2, 1-f] [1,2,4] triazin-5-yl)methyl)piperidin-3 -ol (BMS6905 14); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7- [[(3aa,5P ,6aa)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]- 4-quinazolinamine (XL647, CAS 781613-23-8); 4-Methyl-3-[[l-methyl-6-(3-pyridinyl)-l//-pyrazolo[3,4-i/]pyrimidin-4-yl]amino]-/V-[3-(trifluoromethyl)phenyl]-benzamide (BHG712, CAS 940310-85-0); . and Aflibercept (Eylea®). Exemplary EGF pathway inhibitors include, without limitation tyrphostin 46, EKB-569, erlotinib (Tarceva®), gefitinib (Iressa®), erbitux, nimotuzumab, lapatinib (Tykerb®), cetuximab (anti-EGFR mAb), 188Re-labeled nimotuzumab (anti-EGFR mAb), and those compounds that are generically and specifically disclosed in WO 97/02266, EP 0 564 409, WO 99/03854, EP 0 520 722, EP 0 566 226, EP 0 787 722, EP 0 837 063, US 5,747,498, WO 98/10767, WO 97/30034, WO 97/49688, WO 97/38983 and WO 96/33980.

[0434] Exemplary EGFR antibodies include, but are not limited to, Cetuximab (Erbitux®); Panitumumab (Vectibix®); Matuzumab (EMD-72000); Trastuzumab (Herceptin®); Nimotuzumab (hR3); Zaluturnumab; TheraCIM h-R3; MDX0447 (CAS 339151-96-1); and ch806 (mAb-806, CAS 946414-09-1). Exemplary Epidermal growth factor receptor (EGFR) inhibitors include, but not limited to, Erlotinib hydrochloride (Tarceva®), Gefitnib (Iressa®); N-[4-[(3-Chloro-4-fluorophenyl)amino] -7- [[(3 "S")-tetrahydro-3 -furanyl] oxy] -6-quinazolinyl] -4(dimethylamino)-2-butenamide, Tovok®); Vandetanib (Caprelsa®); Lapatinib (Tykerb®); (3R,4R)-4-Amino-l- ((4-((3 -methoxyphenyl)amino)pyrrolo [2, 1-f] [1,2,4] triazin-5-yl)methyl)piperidin-3 –ol (BMS690514); Canertinib dihydrochloride (CI-1033); 6-[4-[(4-Ethyl-lpiperazinyl) methyl]phenyl]-/V-[(lR)-l-phenylethyl]- 7/ -Pyrro1o[2 3- 7]pyri in idin-4-ain inc (AEE788,

CAS 497839-62-0); Mubritinib (TAK165); Pelitinib (EKB569); Afatinib (BIBW2992); Neratinib (HKI-272); A-[4-[[l-[(3-Fluorophenyl)methyl]-l//-indazol-5-yl]amino]-5-methylpyrrolo[2,l-/][l,2,4]triazin-6-yl]-carbamic acid, 3)-3-in orpho1iny1in cthy1 ester (BMS599626); /V-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[(3aa,5p,6aa)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]- 4-quinazolinamine (XL647, CAS 781613-23-8); and 4-[4- [[(1R)- 1-Phenylethyl] amino] -7A/-pyrrolo[2,3-z/J pyri midi n-6-y 1J-phenol (PKI1 66, CAS 187724-61-4).

[0435] Exemplary mTOR inhibitors include, without limitation, rapamycin (Rapamune®), and analogs and derivatives thereof; SDZ-RAD; Temsirolimus (Torisel®; also known as CCI-779); Ridaforolimus (formally known as deferolimus, (lR,2R,4S)-4-[(2R)-2 [(IR,9S, 125, 15R,16E,1SR, 19R,21R, 23S,24£,26£,28Z,30S,32S,35R)- 1,18-dihydroxy- 19,30-dimethoxy-15,17,21,23, 29,35-hexamethyl-2,3,10,14,20-pentaoxo-ll,36-dioxa-4-azatricyclo[30.3.1.0 4,9] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); Everolimus (Afinitor® or RAD001); Rapamycin (AY22989, Sirolimus®); Simapimod (CAS 164301-51-3); (5-{2,4-Bis[(3S)-3-methylmorpholin-4- yl]pyrido[2,3-i/]pyrimidin-7-yl]-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8- [trans-A- (2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-i/]pyrimidin-7(8)-one (PF04691502, CAS 1013101-36-4); and 2-[l,4-dioxo-4-[[4-(4-oxo-8-phenyl-4//-l- benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L-a-aspartylL-serine (SEQ ID NO: 73), inner salt (SF1126, CAS 936487-67-1).

[0436] Exemplary immunomodulators include, e.g., afutuzumab (available from Roche®); pegfilgrastim (Neulasta®); lenalidomide (CC-5013, Revlimid®); thalidomide (Thalomid®), actimid (CC4047); and IRX-2 (mixture of human cytokines including interleukin 1, interleukin 2, and interferon g , CAS 951209-71-5, available from IRX Therapeutics). Exemplary anthracyclines include, e.g., doxorubicin (Adriamycin® and Rubex®); bleomycin (lenoxane®); daunorubicin (dauombicin hydrochloride, daunomycin, and rubidomycin hydrochloride, Cembidine®); daunorubicin liposomal (daunorubicin citrate liposome, DaunoXome®); mitoxantrone (DHAD, Novantrone®); epirubicin (Ellence™); idambicin (Idamycin®, Idamycin PFS®); mitomycin C (Mutamycin®); geldanamycin; herbimycin; ravidomycin; and desacetylravidomycin. Exemplary vinca alkaloids include, e.g.,

vinorelbine tartrate (Navelbine®), Vincristine (Oncovin®), and Vindesine (Eldisine®)); vinblastine (also known as vinblastine sulfate, vincaleukoblastine and VLB, Alkaban-AQ® and Velban®); and vinorelbine (Navelbine®). Exemplary proteosome inhibitors include bortezomib (Velcade®); carfilzomib (PX-171-007, (S)-4-Methyl-A/-((S)-l-(((S)-4-methyl-l-((R)-2-methyloxiran-2-yl)-l-oxopentan-2-yl)amino)-l-oxo-3-phenylpropan-2-yl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-pentanamide); marizomib (NPI-0052); ixazomib citrate (MLN-9708); delanzomib (CEP-18770); and C-Mcthy l- V- (2- mcthy 1-5-thiazol y1)carbony 1J-L-scry 1- C-mcthy l- V- (5)-2-[(2/^)-2- methyl-2-oxiranyl] -2-oxo- 1-(phenylmethyl)ethyl] - L-serinamide (ONX-09 12).

[0437] Exemplary Phosphoinositide 3-kinase (PI3K) inhibitors include, but are not limited to, 4-[2-(1H-Indazol-4-yl)-6- [[4-(methylsulfonyl)piperazin- 1-yl]methyl] thieno [3,2-d]pyrimidin-4-yl]morpholine (also known as GDC 0941 and described in PCT Publication Nos. WO 09/036082 and WO 09/055730); 2-Methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-l-yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in PCT Publication No. WO 06/122806); 4-(trifluoromethyl)-5-(2,6-dimorpholinopyrimidin-4-yl)pyridin-2-amine (also known as BKM120 or NVP-BKM120, and described in PCT Publication No. W02007/084786); Tozasertib (VX680 or MK-0457, CAS 639089-54-6); (5Z)- 5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidinedione (GSK1059615, CAS 958852- 01-2); (1E,4S ,4aR,5R,6aS ,9aR)-5-(Acetyloxy)- 1-[(di-2-propenylamino)methylene] - 4,4a,5,6,6a,8,9,9a-octahydro-ll-hydroxy-4-(methoxymethyl)-4a,6a-dimethylcyclopenta[5,6]naphtho[l,2-c]pyran-2,7,10(lH)-trione (PX866, CAS 502632-66-8); and 8-Phenyl-2-(morpholin-4-yl)-chromen-4-one (LY294002, CAS 154447-36-6).

[0438] Exemplary Protein Kinase B (PKB) or AKT inhibitors include, but are not limited to. 8-[4-(l-Aminocyclobutyl)phenyl]-9-phenyl-l,2,4-triazolo[3,4-f][l,6]naphthyridin-3(2H)-one (MK-2206, CAS 1032349-93-1); Perifosine (KRX0401); 4-Dodecyl-N-l,3,4-thiadiazol-2-ylbenzenesulfonamide (PHT-427, CAS 1191951-57-1); 4-[2-(4-Amino-l,2,5-oxadiazol-3-yl)-lethyl-7-[(3S)-3-piperidinylmethoxy]-lH-imidazo[4,5-c]pyridin-4-yl]-2-methyl-3-butyn-2-ol (GSK690693, CAS 937174-76-0); 8-(l-Hydroxyethyl)-2-methoxy-3-[(4-methoxyphenyl)methoxy]- 6H-dibenzo[b,d]pyran-6-one (palomid 529, P529, or SG-00529); Tricirbine (6-Amino-4-methyl-8-(P-D-ribofuranosyl)-4H,8H-pyrrolo[4,3,2-de]pyrimido[4,5-

c]pyridazine); (aS)-a-[[[5-(3-Methyl-lH-indazol-5-yl)-3-pyridinyl]oxy]methyl]-benzeneethanamine (A674563, CAS 552325-73-2); 4-[(4-Chlorophenyl)methyl]-l-(7Hpyrrolo[2,3-d]pyrimidin-4-yl)- 4-piperidinamine (CCT128930, CAS 885499-61-6); 4-(4-Chlorophenyl)-4-[4-(lH pyrazol-4-yl)phenyl] -piperidine (AT7867, CAS 857531-00-1); and Archexin (RX-0201, CAS 663232-27-7).

[0439] Drugs that inhibit either the calcium dependent phosphatase calcineurin (cyclosporine and FK506) or inhibit the p70S6 kinase that is important for growth factor induced signaling (rapamycin). (Liu et al., Cell 66:807-815, 1991; Henderson et al., Immun. 73:316-321, 1991; Bierer et al., Curr. Opin. Immun. 5:763-773, 1993) can also be used. In a further aspect, the cell compositions of the present invention may be administered to a patient in conjunction with (e.g., before, simultaneously or following) bone marrow transplantation, T cell ablative therapy using chemotherapy agents such as, fludarabine, external-beam radiation therapy (XRT), cyclophosphamide, and/or antibodies such as OKT3 or CAMPATH. In one aspect, 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.

[0440] The combination therapy may provide synergy and prove synergistic, that is, the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect may be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation, a synergistic effect may be attained when the compounds are administered or delivered sequentially, for example by different injections in separate syringes. In general, during alternation, an effective dosage of each active ingredient is administered sequentially, i.e. serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

[0441] In one embodiment, an effective amount of an antibody or antigen binding fragment that specifically binds to one or more of the antigens disclosed herein or a conjugate thereof is administered to a subject having a tumor following anti-cancer treatment. After a sufficient amount of time has elapsed to allow for the administered antibody or antigen binding fragment or conjugate to form an immune complex with the antigen expressed on the respective cancer cell, the immune complex is detected. The presence (or absence) of the immune complex indicates the effectiveness of the treatment. For example, an increase in the immune complex compared to a control taken prior to the treatment indicates that the treatment is not effective, whereas a decrease in the immune complex compared to a control taken prior to the treatment indicates that the treatment is effective.

[0442] In various embodiments, an anti-GCC antigen binding agent as described herein may be included in a course of treatment that further includes administration of at least one additional agent to a subject. In various embodiments, an additional agent administered in combination with an anti-GCC antigen binding agent as described herein may be chemotherapy agent. In various embodiments, an additional agent administered in combination with an antigen binding agent as described herein may be an agent that inhibits inflammation.

[0443] In some embodiments, anti-GCC antigen binding agent is a single domain antibody with specificity for human GCC. In some embodiments, the anti- GCC single domain antibody can be conjugated (e.g., linked to) to a therapeutic agent (e.g., a chemotherapeutic agent and a radioactive atom) for binding to a cancer cell, delivering therapeutic agent to the cancer cell, and killing the cancer cell which expresses human GCC. In some embodiments, an anti-GCC binding agent is linked to a therapeutic agent. In some embodiments, therapeutic agent is a chemotherapeutic agent, a cytokine, a radioactive atom, an siRNA, or a toxin. In some embodiments, therapeutic agent is a chemotherapeutic agent. In some embodiments, the agent is a radioactive atom.

[0444] In some embodiments, the methods can be performed in conjunction with other therapies for GCC-associated disorders. For example, the composition can be administered to a subject at the same time, prior to, or after, chemotherapy. In some embodiments, the

composition can be administered to a subject at the same time, prior to, or after, an adoptive therapy method.

[0445] In various embodiments, an additional agent administered in combination with an anti-GCC binding agent as described herein may be administered at the same time as an anti- GCC binding agent, on the same day as an anti- GCC binding agent, or in the same week as an anti- GCC binding agent. In various embodiments, an additional agent administered in combination with an anti- GCC binding agent as described herein may be administered in a single formulation with an anti- GCC binding agent. In certain embodiments, an additional agent administered in a manner temporally separated from administration of an anti-GCC binding agent as described herein, e.g., one or more hours before or after, one or more days before or after, one or more weeks before or after, or one or more months before or after administration of an anti- GCC binding agent. In various embodiments, the administration frequency of one or more additional agents may be the same as, similar to, or different from the administration frequency of an anti- GCC binding agent as described herein.

[0446] Encompassed within combination therapy is the a treatment regimen that includes administration of two distinct antibodies as described herein and/or a treatment regimen that includes administration of an antibody as described herein by a plurality of formulations and/or routes of administration.

[0447] In some embodiments, compositions can be formulated with one or more additional therapeutic agents, e.g., additional therapies for treating or preventing a GCC-associated disorder (e.g., a cancer or autoimmune disorder) in a subject. Additional agents for treating a GCC-associated disorder in a subject will vary depending on the particular disorder being treated, but can include, without limitation, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone, osfamide, carboplatin, etoposide, dexamethasone, cytarabine, cisplatin, cyclophosphamide, or fludarabine.

[0448] A composition described herein can replace or augment a previously or currently administered therapy. For example, upon treating with a composition described herein, administration of the one or more additional active agents can cease or diminish, e.g., be administered at lower levels, e.g., lower levels of a reference antibody that cross-competes for GCC binding) following administration of an anti-GCC binding agent described herein.

In some embodiments, administration of the previous therapy can be maintained. In some embodiments, a previous therapy will be maintained until the level of the composition reaches a level sufficient to provide a therapeutic effect. The two therapies can be administered in combination.

[0449] In some embodiments, the subject can be administered an agent which reduces or ameliorates a side effect associated with the administration of the therapy or combination described herein, e.g., a CAR-expressing cell (e.g., GCC CAR-expressing cell) and an additional agent. Side effects associated with the administration of a CAR-expressing cell include, but are not limited to CRS, and hemophagocytic lymphohistiocytosis (HLH), also termed Macrophage Activation Syndrome (MAS). Symptoms of CRS include high fevers, nausea, transient hypotension, hypoxia, and the like. Accordingly, the methods described herein can comprise administering a therapy described herein, e.g., a CAR-expressing cell (e.g., GCC CAR-expressing cell), to a subject and further administering an agent to manage elevated levels of a soluble factor resulting from treatment with a CAR-expressing cell. In one embodiment, the soluble factor elevated in the subject is one or more of IFN-g, TNFa, IL-2 and IL-6. Therefore, an agent administered to treat this side effect can be an agent that neutralizes one or more of these soluble factors. Such agents include, but are not limited to a steroid, an inhibitor of TNFa, and an inhibitor of IL-6. An example of a TNFa inhibitor is entanercept. An example of an IL-6 inhibitor is Tocilizumab.

[0450] In one embodiment, the subject can be administered an agent which enhances the activity of the therapy described herein, e.g., a CAR-expressing cell (e.g., GCC CAR expressing cell). For example, in one embodiment, the agent can be an agent which inhibits an inhibitory molecule. Additional inhibitory molecules, e.g., can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD1, PD-F1, CTFA4, TIM3, FAG3, VISTA, BTFA, TIGIT, FAIR1, CD160, and 2B4.

[0451] Inhibition of an inhibitory molecule, e.g., by inhibition at the DNA, RNA or protein level, can optimize a CAR-expressing cell performance. In embodiments, an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, can be used to inhibit expression of an inhibitory molecule in the CAR-expressing

cell. In an embodiment the inhibitor is an shRNA. In an embodiment, the inhibitory molecule is inhibited within a CAR-expressing cell. In these embodiments, a dsRNA molecule that inhibits expression of the inhibitory molecule is linked to the nucleic acid that encodes a component, e.g., all of the components, of the CAR.

[0452] In some embodiments, the inhibitor of an inhibitory signal can be, e.g., an antibody or antibody fragment that binds to an inhibitory molecule. For example, the agent can be an antibody or antibody fragment that binds to PD1, PD-L1, PD-L2 or CTLA4 (e.g., ipilimumab (also referred to as MDX-010 and MDX-101, and marketed as Yervoy®; Bristol-Myers Squibb; Tremelimumab (IgG2 monoclonal antibody available from Pfizer, formerly known as ticilimumab, CP-675,206).)· The agent which enhances the activity of e.g., a CAR-expressing cell (e.g., GCC CAR-expressing cell) can be, e.g., a fusion protein comprising a first domain and a second domain, wherein the first domain is an inhibitory molecule, or fragment thereof, and the second domain is a polypeptide that is associated with a positive signal, e.g., the polypeptide that is associated with a positive signal is CD28, ICOS, and fragments thereof, e.g., an intracellular signaling domain of CD28 and/or ICOS. In one embodiment, the fusion protein is expressed by the same cell that expressed the CAR. In another embodiment, the fusion protein is expressed by a cell, e.g., a T cell that does not express an anti-GCC CAR.

[0453] In another embodiment, the subjects receive an infusion of the CAR-expressing cell, e.g.,compositions of the present disclosure prior to transplantation, e.g., allogeneic stem cell transplant, of cells. In a preferred embodiment, CAR expressing cells transiently express CAR, e.g., by electroporation of an mRNA encoding a CAR, whereby the expression of the CAR is terminated prior to infusion of donor stem cells to avoid engraftment failure.

[0454] Some patients may experience allergic reactions to the compounds of the present disclosure and/or other anti-cancer agent(s) during or after administration; therefore, anti-allergic agents are often administered to minimize the risk of an allergic reaction. Suitable anti-allergic agents include corticosteroids, such as dexamethasone (e.g., Decadron®), beclomethasone (e.g., Beclovent®), hydrocortisone (also known as cortisone, hydrocortisone sodium succinate, hydrocortisone sodium phosphate, and sold under the

tradenames Ala-Cort®, hydrocortisone phosphate, Solu-Cortef®, Hydrocort Acetate® and Lanacort®), prednisolone (sold under the tradenames Delta-Cortel®, Orapred®, Pediapred® and Prelone®), prednisone (sold under the tradenames Deltasone®, Liquid Red®, Meticorten® and Orasone®), methylprednisolone (also known as 6-methylprednisolone, methylprednisolone acetate, methylprednisolone sodium succinate, sold under the tradenames Duralone®, Medralone®, Medrol®, M-Prednisol® and Solu-Medrol®); antihistamines, such as diphenhydramine (e.g., Benadryl®), hydroxyzine, and cyproheptadine; and bronchodilators, such as the beta-adrenergic receptor agonists, albuterol (e.g., Proventil®), and terbutaline (Brethine®). Some patients may experience nausea during and after administration of the compound of the present disclosure and/or other anti-cancer agent(s); therefore, anti-emetics are used in preventing nausea (upper stomach) and vomiting. Suitable anti-emetics include aprepitant (Emend®), ondansetron (Zofran®), granisetron HC1 (Kytril®), lorazepam (Ativan® dexamethasone (Decadron®), prochlorperazine (Compazine®), casopitant (Rezonic® and Zunrisa®), and combinations thereof.

[0455] Medication to alleviate the pain experienced during the treatment period is often prescribed to make the patient more comfortable. Common over-the-counter analgesics, such Tylenol®, are often used. However, opioid analgesic drugs such as hydrocodone/paracetamol or hydrocodone/acetaminophen (e.g., Vicodin®), morphine (e.g., Astramorph® or Avinza®), oxycodone (e.g., OxyContin® or Percocet®), oxymorphone hydrochloride (Opana®), and fentanyl (e.g., Duragesic®) are also useful for moderate or severe pain.

[0456] In an effort to protect normal cells from treatment toxicity and to limit organ toxicities, cytoprotective agents (such as neuroprotectants, free-radical scavengers, cardioprotectors, anthracycline extravasation neutralizers, nutrients and the like) may be used as an adjunct therapy. Suitable cytoprotective agents include Amifostine (Ethyol®), glutamine, dimesna (Tavocept®), mesna (Mesnex®), dexrazoxane (Zinecard® or Totect®), xaliproden (Xaprila®), and leucovorin (also known as calcium leucovorin, citrovorum factor and folinic acid).

[0457] The structure of the active compounds identified by code numbers, generic or trade names may be taken from the actual edition of the standard compendium "The Merck

Index" or from databases, e.g. Patents International (e.g. IMS World Publications).The above-mentioned compounds, which can be used in combination with a compound of the present disclosure, can be prepared and administered as described in the art, such as in the documents cited above.

[0458] In one embodiment, the present disclosure provides pharmaceutical compositions comprising at least one therapeutic agent of the present disclosure (e.g., a therapeutic agent of the present disclosure) or a pharmaceutically acceptable salt thereof together with a pharmaceutically acceptable carrier suitable for administration to a human or animal subject, either alone or together with other anti-cancer agents.

[0459] In one embodiment, the present disclosure provides methods of treating human or animal subjects suffering from a cellular proliferative disease, such as cancer. The present disclosure provides methods of treating a human or animal subject in need of such treatment, comprising administering to the subject a therapeutically effective amount of a therapeutic agent of the present disclosure or a pharmaceutically acceptable salt thereof, either alone or in combination with other anti-cancer agents.

[0460] In particular, compositions will either be formulated together as a combination therapeutic or administered separately. In combination therapy, the compound of the present disclosure and other anti-cancer agent(s) may be administered either simultaneously, concurrently or sequentially with no specific time limits, wherein such administration provides therapeutically effective levels of the two compounds in the body of the patient.

[0461] In some embodiments, the compound of the present disclosure and the other anti-cancer agent(s) is generally administered sequentially in any order by infusion or orally. The dosing regimen may vary depending upon the stage of the disease, physical fitness of the patient, safety profiles of the individual drugs, and tolerance of the individual drugs, as well as other criteria well-known to the attending physician and medical practitioner(s) administering the combination. The therapeutic agent of the present disclosure (e.g., GCC CAR) and other anti-cancer agent(s) may be administered within minutes of each other, hours, days, or even weeks apart depending upon the particular cycle being used for treatment. In addition, the cycle could include administration of one drug more often than the other during the treatment cycle and at different doses per administration of the drug.

[0462] A therapeutic agent (e.g., GCC CAR) of the present disclosure may also be used to advantage in combination with known therapeutic processes, for example, the administration of hormones or especially radiation. A compound of the present disclosure may in particular be used as a radiosensitizer, especially for the treatment of tumors which exhibit poor sensitivity to radiotherapy.

[0463] In embodiments, the subject can be administered an agent which reduces or ameliorates a side effect associated with the administration of a CAR-expressing cell. Side effects associated with the administration of a CAR-expressing cell include, but are not limited to CRS, and hemophagocytic lymphohistiocytosis (HLH), also termed Macrophage Activation Syndrome (MAS).

[0464] Accordingly, the methods described herein can comprise administering a CAR expressing cell described herein to a subject and further administering one or more agents to manage elevated levels of a soluble factor resulting from treatment with a CAR-expressing cell. In one embodiment, the soluble factor elevated in the subject is one or more of IFN-g, TNFa, IL- 2 and IL-6. In an embodiment, the factor elevated in the subject is one or more of IL-1, GMCSF, IL-10, IL-8, IL-5 and fraktalkine. Therefore, an agent administered to treat this side effect can be an agent that neutralizes one or more of these soluble factors.

[0465] In one embodiment, the agent that neutralizes one or more of these soluble forms is an antibody or antigen binding fragment thereof. Examples of such agents include, but are not limited to a steroid (e.g., corticosteroid), an inhibitor of TNFa, and an inhibitor of IL-6. An example of a TNFa inhibitor is an anti-TNFa antibody molecule such as, infliximab, adalimumab, certolizumab pegol, and golimumab. Another example of a TNFa inhibitor is a fusion protein such as entanercept. Small molecule inhibitor of TNFa include, but are not limited to, xanthine derivatives (e.g. pentoxifylline) and bupropion. An example of an IL-6 inhibitor is an anti-IL-6 antibody molecule such as tocilizumab (toe), sarilumab, elsilimomab, CNTO 328, ALD518/BMS-945429, CNTO 136, CPSI-2364, CDP6038, VX30, ARGX-109, FE301, and FM101. In one embodiment, the anti-IL-6 antibody molecule is tocilizumab. An example of an IL-1R based inhibitor is anakinra.

[0466] In some embodiment, the subject is administered a corticosteroid, such as, e.g., methylprednisolone, hydrocortisone, among others. In some embodiments, the subject is

administered a vasopressor, such as, e.g., norepinephrine, dopamine, phenylephrine, epinephrine, vasopressin, or a combination thereof.

[0467] In an embodiment, the subject can be administered an antipyretic agent. In an embodiment, the subject can be administered an analgesic agent.

[0468] In one embodiment, the subject can be further administered an agent which enhances the activity or fitness of a CAR-expressing cell. For example, in one embodiment, the agent can be an agent which inhibits a molecule that modulates or regulates, e.g., inhibits, T cell function. In some embodiments, the molecule that modulates or regulates T cell function is an inhibitory molecule. Inhibitory molecules, e.g., Programmed Death 1 (PD-1) or PD-1 ligand (PD-L1), can, in some embodiments, decrease the ability of a CAR-expressing cell to mount an immune effector response. Examples of inhibitory molecules include PD-1, PD-L1, CTLA4, TIM3, CEACAM (e.g., CEACAM-1, CEACAM-3 and/or CEACAM-5), LAG3, VISTA, BTLA, TIGIT, LAIR1, CD160, 2B4, CD80, CD86, B7-H3 (CD276), B7-H4 (VTCN1), HVEM (TNFRSF14 or CD270), KIR, A2aR, MHC class I, MHC class II, GAL9, and adenosine. Inhibition of a molecule that modulates or regulates, e.g., inhibits, T cell function, e.g., by inhibition at the DNA, RNA or protein level, can optimize a CAR-expressing cell performance. In embodiments, an agent, e.g., an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., an inhibitory nucleic acid, e.g., a dsRNA, e.g., an siRNA or shRNA, a clustered regularly interspaced short palindromic repeats (CRISPR), a transcription-activator like effector nuclease (TALEN), or a zinc finger endonuclease (ZFN), e.g., as described herein, can be used to inhibit expression of an inhibitory molecule in the CAR-expressing cell. In an embodiment, the inhibitor is an shRNA. In an embodiment, the agent that modulates or regulates, e.g., inhibits, T-cell function is inhibited within a CAR-expressing cell. In these embodiments, a dsRNA molecule that inhibits expression of a molecule that modulates or regulates, e.g., inhibits, T-cell function is linked to the nucleic acid that encodes a component, e.g., all of the components, of the CAR.

[0469] In an embodiment, a nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is operably linked to a promoter, e.g., a HI- or a U6-derived promoter such that the dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g.,

inhibits, T-cell function is expressed, e.g., is expressed within a CAR-expressing cell. See e.g., Tiscomia G., "Development of Lentiviral Vectors Expressing siRNA," Chapter 3, in Gene Transfer: Delivery and Expression of DNA and RNA (eds. Friedmann and Rossi). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA, 2007; Brummelkamp TR, et al. (2002) Science 296: 550-553; Miyagishi M, et al. (2002) Nat. Biotechnol. 19: 497-500.

[0470] In an embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is present on the same vector, e.g., a lentiviral vector, that comprises a nucleic acid molecule that encodes a component, e.g., all of the components, of the CAR. In such an embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is located on the vector, e.g., the lentiviral vector, 5'- or 3'- to the nucleic acid that encodes a component, e.g., all of the components, of the CAR. The nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function can be transcribed in the same or different direction as the nucleic acid that encodes a component, e.g., all of the components, of the CAR. In an embodiment the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is present on a vector other than the vector that comprises a nucleic acid molecule that encodes a component, e.g., all of the components, of the CAR. In an embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function it transiently expressed within a CAR-expressing cell. In an embodiment, the nucleic acid molecule that encodes a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function is stably integrated into the genome of a CAR-expressing cell. Configurations of exemplary vectors for expressing a component, e.g., all of the components, of the CAR with a dsRNA molecule that inhibits expression of the molecule that modulates or regulates, e.g., inhibits, T-cell function, is provided, e.g., in Figure 47 of International Publication WO20 15/090230, filed December 19, 2014, which is herein incorporated by reference.

[0471] In embodiments, the therapy described herein, e.g., a CAR-expressing cell (e.g., GCC CAR-expressing cell), is administered in combination with an anti-epileptic agent,

e.g., acetazolamide, bivaracetam, carbamazepine, clobazam, clonazepam, eslicarbazepine acetate, ethosuximide, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, perampanel, phenobarbital, phenytoin, piracetam, pregabalin, primidone, rudinamide, sodium valproate, stiripentol, tiagabine, topiramate, valporic acid, vigabatrin, zonisamide. See, Weller et al. Lancet 13.9 (2012):e375-e382. In embodiments, the anti epileptic agent is administered in an amount effective to prevent seizures before the therapy described herein, e.g., a CAR-expressing cell (e.g. GCC CAR-expressing cell). In embodiments, administration of the anti-epileptic agent is optionally continued throughout and after administration the therapy described herein, e.g., a CAR-expressing cell (e.g. GCC CAR-expressing cell). In embodiments, the therapy described herein, e.g., a CAR-expressing cell (e.g. GCC CAR-expressing cell), may be used in a treatment in combination with an anti-epileptic agent and radiation.

[0472] Cytokine Release Syndrome (CRS)

[0473] Cytokine release syndrome (CRS) is a potentially life-threatening cytokine-associated toxicity that can occur as a result of cancer immunotherapy, e.g., cancer antibody therapies or T cell immunotherapies (e.g., CAR T cells). CRS results from high-level immune activation when large numbers of lymphocytes and/or myeloid cells release inflammatory cytokines upon activation. The severity of CRS and the timing of onset of symptoms can vary depending on the magnitude of immune cell activation, the type of therapy administered, and/or the extent of tumor burden in a subject. In the case of T-cell therapy for cancer, symptom onset is typically days to weeks after administration of the T-cell therapy, e.g., when there is peak in vivo T-cell expansion. See, e.g., Lee et al. Blood. 124.2(2014): 188-95.

[0474] Symptoms of CRS can include neurologic toxicity, disseminated intravascular coagulation, cardiac dysfunction, adult respiratory distress syndrome, renal failure, and/or hepatic failure. For example, symptoms of CRS include high fevers, nausea, transient hypotension, hypoxia, and the like. CRS may include clinical constitutional signs and symptoms such as fever, fatigue, anorexia, myalgias, arthalgias, nausea, vomiting, and headache. CRS may include clinical skin signs and symptoms such as rash. CRS may include clinical gastrointestinal signs and symsptoms such as nausea, vomiting and diarrhea. CRS may include clinical respiratory signs and symptoms such as tachypnea and hypoxemia. CRS

may include clinical cardiovascular signs and symptoms such as tachycardia, widened pulse pressure, hypotension, increased cardac output (early) and potentially diminished cardiac output (late). CRS may include clinical coagulation signs and symptoms such as elevated d-dimer, hypofibrinogenemia with or without bleeding. CRS may include clinical renal signs and symptoms such as azotemia. CRS may include clinical hepatic signs and symptoms such as transaminitis and hyperbilirubinemia. CRS may include clinical neurologic signs and symptoms such as headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dymetria, altered gait, and seizures.

[0475] IL-6 is thought to be a mediator of CRS toxicity. See, e.g., id. High IL-6 levels may initiate a proinflammatory IL-6 signaling cascade, leading to one or more of the CRS symptoms. In some cases, the level of C-reactive protein (CRP) (a biomolecule produced by the liver, e.g., in response to IL-6) can be a measure of IL-6 activity. In some cases, CRP levels may increase several fold (e.g., several logs) during CRS. CRP levels can be measured using methods described herein, and/or standard methods available in the art.

[0476] CRS Grading

[0477] In some embodiments, CRS can be graded in severity from 1-5 as follows. Grades 1-3 are less than severe CRS. Grades 4-5 are severe CRS. For Grade 1 CRS, only symptomatic treatment is needed (e.g., nausea, fever, fatigue, myalgias, malaise, headache) and symptoms are not life threatening. For Grade 2 CRS, the symptoms require moderate intervention and generally respond to moderate intervention. Subjects having Grade 2 CRS develop hypotension that is responsive to either fluids or one low-dose vasopressor; or they develop grade 2 organ toxicity or mild respiratory symptoms that are responsive to low flow oxygen (<40% oxygen).

[0478] In Grade 3 CRS subjects, hypotension generally cannot be reversed by fluid therapy or one low dose vasopressor. These subjects generally require more than low flow oxygen and have grade 3 organ toxicity (e.g., renal or cardiac dysfunction or coagulopathy) and/or grade 4 transaminitis. Grade 3 CRS subjects require more aggressive intervention, e.g., oxygen of 40% or higher, high dose vasopressor(s), and/or multiple vasopressors. Grade 4 CRS subjects suffer from immediately life-threatening symptoms, including grade 4 organ toxicity or a need for mechanical ventilation. Grade 4 CRS subjects generally do not have

transaminitis. In Grade 5 CRS subjects, the toxicity causes death. For example, criteria for grading CRS is provided herein as Table 9. Unless otherwise specified, CRS as used herein refers to CRS according to the criteria of Table 9.

Table 9. CRS Grading

Grl	Supportive care only
Gr2	IV therapies+/- hospitalization.
Gr3	Hypotension requiring IV fluids or low-dose vasoactives or hypoxemia requiring oxygen, CPAP, or BIPAP.
Gr4	Hypotension requiring high-dose vasoactives or hypoxemia requiring mechanical
Gr5	Death

CRS Therapies

[0479] Therapies for CRS include IL-6 inhibitor or IL-6 receptor (IL-6R) inhibitors (e.g., tocilizumab or siltuximab), sgpl30 blockers, vasoactive medications, corticosteroids, immunosuppressive agents, and mechanical ventilation. Exemplary therapies for CRS are described in International Application W02014011984, which is hereby incorporated by reference.

[0480] Tocilizumab is a humanized, immunoglobulin Glkappa anti-human IL-6R monoclonal antibody. See, e.g., id. Tocilizumab blocks binding of IL-6 to soluble and membrane bound IL- 6 receptors (IL-6Rs) and thus inhibitos classical and trans-IL-6 signaling. In embodiments, tocilizumab is administered at a dose of about 4-12 mg/kg, e.g., about 4-8 mg/kg for adults and about 8-12 mg/kg for pediatric subjects, e.g., administered over the course of 1 hour. In some embodiments, the CRS therapeutic is an inhibitor of IL-6 signalling, e.g., an inhibitor of IL-6 or IL-6 receptor. In one embodiment, the inhibitor is an anti-IL-6 antibody, e.g., an anti-IL-6 chimeric monoclonal antibody such as siltuximab. In other embodiments, the inhibitor comprises a soluble gpl30 or a fragment thereof that is capable of blocking IL-6 signalling. In some embodiments, the sgpl30 or fragment thereof is

fused to a heterologous domain, e.g., an Lc domain, e.g., is a gp130-Lc fusion protein such as LE301. In embodiments, the inhibitor of IL-6 signalling comprises an antibody, e.g., an antibody to the IL-6 receptor, such as sarilumab, olokizumab (CDP6038), elsilimomab, simkumab (CNTO 136), ALD518/BMS- 945429, ARGX-109, or LM101. In some embodiments, the inhibitor of IL-6 signaling comprises a small molecule such as CPST2364.

[0481] Exemplary vasoactive medications include but are not limited to angiotensin-1 1, endothelin-1, alpha adrenergic agonists, rostanoids, phosphodiesterase inhibitors, endothelin antagonists, inotropes (e.g., adrenaline, dobutamine, isoprenaline, ephedrine), vasopressors (e.g., noradrenaline, vasopressin, metaraminol, vasopressin, methylene blue), inodilators (e.g., milrinone, levosimendan), and dopamine. Exemplary vasopressors include but are not limited to norepinephrine, dopamine, phenylephrine, epinephrine, and vasopressin. In some embodiments, a high-dose vasopressor includes one or more of the following: norpepinephrine monotherapy at >20 ug/min, dopamine monotherapy at >10 ug/kg/min, phenylephrine monotherapy at >200 ug/min, and/or epinephrine monotherapy at >10 ug/min. In some embodiments, if the subject is on vasopressin, a high-dose vasopressor includes vasopressin + norepinephrine equivalent of >10 ug/min, where the norepinephrine equivalent dose = [norepinephrine (ug/min)] + [dopamine (ug/kg/min) / 2] + [epinephrine (ug/min)] + [phenylephrine (ug/min) / 10]. In some embodiments, if the subject is on combination vasopressors (not vasopressin), a high-dose vasopressor includes norepinephrine equivalent of >20 ug/min, where the norepinephrine equivalent dose = [norepinephrine (ug/min)] + [dopamine (ug/kg/min) / 2] + [epinephrine (ug/min)] + [phenylephrine (ug/min) / 10]. See e.g., Id.

[0482] In some embodiments, a low-dose vasopressor is a vasopressor administered at a dose less than one or more of the doses listed above for high-dose vasopressors. Exemplary corticosteroids include but are not limited to dexamethasone, hydrocortisone, and methylprednisolone. In embodiments, a dose of dexamethasone of 0.5 mg/kg is used. In embodiments, a maximum dose of dexamethasone of 10 mg/dose is used. In embodiments, a dose of methylprednisolone of 2 mg/kg/day is used.

[0483] Exemplary immunosuppressive agents include but are not limited to an inhibitor of TNFa or an inhibitor of IL-1. In embodiments, an inhibitor of TNFa comprises an

anti-TNFa antibody, e.g., monoclonal antibody, e.g., infliximab. In embodiments, an inhibitor of TNFa comprises a soluble TNFa receptor (e.g., etanercept). In embodiments, an IL-1 or IL-1R inhibitor comprises anakinra.

[0484] In some embodiments, the subject at risk of developing severe CRS is administered an anti-IFN-gamma or anti-sIF2Ra therapy, e.g., an antibody molecule directed against IFN-gamma or sIF2Ra.

[0485] In embodiments, for a subject who has received a therapeutic antibody molecule such as blinatumomab and who has CRS or is at risk of developing CRS, the therapeutic antibody molecule is administered at a lower dose and/or a lower frequency, or administration of the therapeutic antibody molecule is halted.

[0486] In embodiments, a subject who has CRS or is at risk of developing CRS is treated with a fever reducing medication such as acetaminophen. embodiments, a subject herein is administered or provided one or more therapies for CRS described herein, e.g., one or more of IL-6 inhibitors or IL-6 receptor (IL-6R) inhibitors (e.g., tocilizumab), vasoactive medications, corticosteroids, immunosuppressive agents, or mechanical ventilation, in any combination, e.g., in combination with a CAR-expressing cell described herein.

[0487] In embodiments, a subject at risk of developing CRS (e.g., severe CRS) (e.g., identified as having a high risk status for developing severe CRS) is administered one or more therapies for CRS described herein, e.g., one or more of IL-6 inhibitor or IL-6 receptor (IL-6R) inhibitors (e.g., tocilizumab), vasoactive medications, corticosteroids, immunosuppressive agents, or mechanical ventilation, in any combination, e.g., in combination with a CAR-expressing cell described herein.

[0488] In embodiments, a subject herein (e.g., a subject at risk of developing severe CRS or a subject identified as at risk of developing severe CRS) is transferred to an intensive care unit. In some embodiments, a subject herein (e.g., a subject at risk of developing severe CRS or a subject identified as at risk of developing severe CRS) is monitored for one ore more symptoms or conditions associated with CRS, such as fever, elevated heart rate, coagulopathy, MODS (multiple organ dysfunction syndrome), cardiovascular dysfunction, distributive shock, cardiomyopathy, hepatic dysfunction, renal dysfunction, encephalopathy, clinical seizures, respiratory failure, or tachycardia. In some embodiments, the methods herein

comprise administering a therapy for one of the symptoms or conditions associated with CRS. For instance, in embodiments, e.g., if the subject develops coagulopathy, the method comprises administering cryoprecipitate. In some embodiments, e.g., if the subject develops cardiovascular dysfunction, the method comprises administering vasoactive infusion support. In some embodiments, e.g., if the subject develops distributive shock, the method comprises administering alpha-agonist therapy. In some embodiments, e.g., if the subject develops cardiomyopathy, the method comprises administering milrinone therapy. In some embodiments, e.g., if the subject develops respiratory failure, the method comprises performing mechanical ventilation (e.g., invasive mechanical ventilation or noninvasive mechanical ventilation). In some embodiments, e.g., if the subject develops shock, the method comprises administering crystalloid and/or colloid fluids. In embodiments, the CAR-expressing cell is administered prior to, concurrently with, or subsequent to administration of one or more therapies for CRS described herein, e.g., one or more of IL-6 inhibitor or IL-6 receptor (IL-6R) inhibitors (e.g., tocilizumab), vasoactive medications, corticosteroids, immunosuppressive agents, or mechanical ventilation. In embodiments, the CAR-expressing cell is administered within 2 weeks (e.g., within 2 or 1 week, or within 14 days, e.g., within 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 day or less) of administration of one or more therapies for CRS described herein, e.g., one or more of IL-6 inhibitors or IL-6 receptor (IL-6R) inhibitors (e.g., tocilizumab), vasoactive medications, corticosteroids, immunosuppressive agents, or mechanical ventilation. In embodiments, the CAR-expressing cell is administered at least 1 day (e.g., at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 1, week, 2 weeks, 3 weeks, 4 weeks, 1 month, 2 months, 3 months, 3 months, or more) before or after administration of one or more therapies for CRS described herein, e.g., one or more of IL-6 inhibitors or IL-6 receptor (IL-6R) inhibitors (e.g., tocilizumab), vasoactive medications, corticosteroids, immunosuppressive agents, or mechanical ventilation.

[0489] In embodiments, a subject herein (e.g., a subject at risk of developing severe CRS or a subject identified as at risk of developing severe CRS) is administered a single dose of an IL-6 inhibitor or IL-6 receptor (IL-6R) inhibitor (e.g., tocilizumab). In embodiments, the subject is administered a plurality of doses (e.g., 2, 3, 4, 5, 6, or more doses) of an IL-6 inhibitor or IL-6 receptor (IL-6R) inhibitor (e.g., tocilizumab).

[0490] In embodiments, a subject at low or no risk of developing CRS (e.g., severe CRS) (e.g., identified as having a low risk status for developing severe CRS) is not administered a therapy for CRS described herein, e.g., one or more of IL-6 inhibitor or IL-6 receptor (IL-6R) inhibitors (e.g., tocilizumab), vasoactive medications, corticosteroids, immunosuppressive agents, or mechanical ventilation.

[0491] In some embodiments, the subject treated by the methods disclosed herein has a low severity of CRS, e.g., grade 1, grade 2 or grade 3.

[0492] In embodiments, the viable CAR-expressing cells are administered in ascending doses. In embodiments, the second dose is larger than the first dose, e.g., larger by 10%, 20%, 30%, or 50%. In embodiments, the second dose is twice, three times, four times, or five times the size of the first dose. In embodiments, the third dose is larger than the second dose, e.g., larger by 10%, 20%, 30%, or 50%. In embodiments, the third dose is twice, three times, four times, or five times the size of the second dose.

[0493] In certain embodiments, the method includes one, two, three, four, five, six, seven or all of a)-h) of the following:

[0494] a) the number of CAR-expressing, viable cells administered in the first dose is no more than 1/3, of the number of CAR-expressing, viable cells administered in the second dose;

[0495] b) the number of CAR-expressing, viable cells administered in the first dose is no more than 1/X, wherein X is 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40 or 50, of the total number of CARexpressing, viable cells administered;

[0496] c) the number of CAR-expressing, viable cells administered in the first dose is no more than 1 x 107, 2 x 107, 3 x 107, 4 x 107, 5 x 107, 6 x 107, 7 x 107, 8 x 107, 9 x 107, 1 x 108, 1.75 x 108, 2 x 108, 3 x 108, 4 x 108, or 5 x 108 CAR-expressing, viable cells, and the second dose is greater than the first dose;

[0497] d) the number of CAR-expressing, viable cells administered in the second dose is no more than 1/2, of the number of CAR-expressing, viable cells administered in the third dose;

[0498] e) the number of CAR-expressing, viable cells administered in the second dose is no more than 1/Y, wherein Y is 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40 or 50, of the total number of CAR-expressing, viable cells administered;

[0499] f) the number of CAR-expressing, viable cells administered in the second dose is no more than 1 x 107, 2 x 107, 3 x 107, 4 x 107, 5 x 107, 6 x 107, 7 x 107, 8 x 107, 9 x 107, 1 x 108, 1.75 x 108, 2 x 108, 3 x 108, 4 x 108, or 5 x 108 CAR-expressing, viable cells, and the third dose is greater than the second dose;

[0500] h) the dosages and time periods of administration of the first, second, and optionally third doses are selected such that the subject experiences CRS at a level no greater than 4, 3, 2, or 1.

[0501] In embodiments, the total dose is about 5 x 108 CAR-expressing, viable cells. In embodiments, the total dose is about 5 x 107 - 5 x 108 CAR-expressing, viable cells. In embodiments, the first dose is about 5 x 107 (e.g., ± 10%, 20%, or 30%) CAR-expressing, viable cells, the second dose is about 1.5 x 108 (e.g., ± 10%, 20%, or 30%) CAR-expressing, viable cells, and the third dose is about 3 x 108 (e.g., ± 10%, 20%, or 30%) CAR-expressing, viable cells.

[0502] In embodiments, the subject is evaluated for CRS after receiving a dose, e.g., after receiving the first dose, the second dose, and/or the third dose.

[0503] In embodiments, the subject receives a CRS treatment, e.g., tocilizumab, a corticosteroid, etanercept, or siltuximab. In embodiments, the CRS treatment is administered before or after the first dose of cells comprising the CAR molecule. In embodiments, the CRS treatment is administered before or after the second dose of cells comprising the CAR molecule. In embodiments, the CRS treatment is administered before or after the third dose of cells comprising the CAR molecule. In embodiments, the CRS treatment is administered between the first and second doses of cells comprising the CAR molecule, and/or between the second and third doses of cells comprising the CAR molecule.

[0504] In embodiments, in a subject having CRS after the first dose, e.g., CRS grade 1, 2, 3, or 4, the second dose is administered at least 2, 3, 4, or 5 days after the first dose. In embodiments, in a subject having CRS after the second dose, e.g., CRS grade 1, 2, 3, or 4, the

third dose is administered at least 2, 3, 4, or 5 days after the second dose. In embodiments, in a subject having CRS after the first dose, the second dose of CAR-expressing cells is delayed relative to when the second dose would have been administered had the subject not had CRS. In embodiments, in a subject having CRS after the second dose, the third dose of CAR-expressing cells is delayed relative to when the third dose would have been administered had the subject not had CRS.

[0505] In embodiments, the subject has a cancer with a high disease burden before the first dose is administered. In embodiments, the subject has bone marrow blast levels of at least 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, or 50%, e.g., at least 5%. In embodiments, the subject has a cancer in stage I, II, III, or IV. In embodiments, the subject has a tumor mass of at least 1, 2, 5, 10, 20, 50, 100, 200, 500, or 1000 g, e.g., in a single tumor or a plurality of tumors.

[0506] In some embodiments, the subject has cancer (e.g., a colorectal cancer). In some embodiments, the cancer is a disease associated with GCC expression, e.g., as described herein. In embodiments, the CAR molecule is a CAR molecule as described herein.

[0507] In one aspect, CAR-expressing cells, e.g., GCC CAR-expressing cells, are generated using lentiviral viral vectors, such as lentivirus. CAR-expressing cells generated that way will have stable CAR expression. Vectors derived from retroviruses such as the lentivirus are suitable tools to achieve long-term gene transfer since they allow long-term, stable integration of a transgene and its propagation in daughter cells. Lentiviral vectors have the added advantage over vectors derived from onco-retroviruses such as murine leukemia viruses in that they can transduce non proliferating cells, such as hepatocytes. They also have the added advantage of low immunogenicity. A retroviral vector may also be, *e.g.*, a gammaretroviral vector. A gammaretroviral vector may include, *e.g.*, a promoter, a packaging signal (y), a primer binding site (PBS), one or more *(e.g.*, two) long terminal repeats (LTR), and a transgene of interest, *e.g.*, a gene encoding a CAR. A gammaretroviral vector may lack viral structural gens such as gag, pol, and env. Exemplary gammaretroviral vectors include Murine Leukemia Virus (MLV), Spleen- Focus Forming Virus (SFFV), and Myeloproliferative Sarcoma Virus (MPSV), and vectors derived therefrom. Other

gammaretroviral vectors are described, *e.g.*, in Tobias Maetzig et a , "Gammaretroviral Vectors: Biology, Technology and Application" Viruses. 2011 Jun; 3(6): 677-713.

[0508] In one aspect, the CAR-expressing cells transiently express CAR vectors for 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 days after transduction. Transient expression of CARs can be effected by RNA CAR vector delivery. In one aspect, the CAR RNA is transduced into the T cell by electroporation.

[0509] A potential issue that can arise in patients being treated using transiently expressing CAR-expressing cells (particularly with murine scFv bearing CAR-expressing cells) is anaphylaxis after multiple treatments. Without being bound by this theory, it is believed that such an anaphylactic response might be caused by a patient developing humoral anti-CAR response, i.e., anti-CAR antibodies having an anti-IgE isotype. It is thought that a patient's antibody producing cells undergo a class switch from IgG isotype (that does not cause anaphylaxis) to IgE isotype when there is a ten to fourteen day break in exposure to antigen.

[0510] If a patient is at high risk of generating an anti-CAR antibody response during the course of transient CAR therapy (such as those generated by RNA transductions), CAR-expressing cell infusion breaks should not last more than ten to fourteen days.

Compositions

[0511] Compositions are provided herein for use in gene therapy, immunotherapy and/or cell therapy that include one or more of the disclosed CARs, or T cells expressing a CAR, antibodies, antigen binding fragments, conjugates, CARs, or T cells expressing a CAR that specifically bind to one or more antigens disclosed herein, in a carrier (such as a pharmaceutically acceptable carrier). The compositions can be prepared in unit dosage forms for administration to a subject. The amount and timing of administration are at the discretion of the treating clinician to achieve the desired outcome. The compositions can be formulated for systemic (such as intravenous) or local (such as intra-tumor) administration. In one example, a disclosed CARs, or T cells expressing a CAR, antibody, antigen binding fragment, conjugate, is formulated for parenteral administration, such as intravenous administration. Compositions including a CAR, or T cell expressing a CAR, a conjugate, antibody or antigen binding fragment as disclosed herein are of use, for example, for the treatment and detection

of a tumor, for example, and not by way of limitation, a neuroblastoma. In some examples, the compositions are useful for the treatment or detection of a carcinoma. The compositions including a CAR, or T cell expressing a CAR, a conjugate, antibody or antigen binding fragment as disclosed herein are also of use, for example, for the detection of pathological angiogenesis.

[0512] The compositions for administration can include a solution of the CAR, or T cell expressing a CAR, conjugate, antibody or antigen binding fragment dissolved in a pharmaceutically acceptable carrier, such as an aqueous carrier. A variety of aqueous carriers can be used, for example, buffered saline and the like. These solutions are sterile and generally free of undesirable matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents, adjuvant agents, and the like, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate and the like. The concentration of a CAR, or T cell expressing a CAR, antibody or antigen binding fragment or conjugate in these formulations can vary widely, and will be selected primarily based on fluid volumes, viscosities, body weight and the like in accordance with the particular mode of administration selected and the subject's needs. Actual methods of preparing such dosage forms for use in gene therapy, immunotherapy and/or cell therapy are known, or will be apparent, to those skilled in the art.

[0513] In some embodiments, the composition comprises a GCC CAR in combination with one or more pharmaceutically or physiologically acceptable carriers, diluents or excipients. Such compositions may comprise buffers such as neutral buffered saline, phosphate buffered saline and the like; carbohydrates such as glucose, mannose, sucrose or dextrans, mannitol; proteins; polypeptides or amino acids such as glycine; antioxidants; chelating agents such as EDTA or glutathione; adjuvants (e.g., aluminum hydroxide); and preservatives. Compositions of the present invention are in one aspect formulated for intravenous administration.

[0514] Pharmaceutical compositions of the present invention may be administered in a manner appropriate to the disease to be treated (or prevented). The quantity and frequency of

administration will be determined by such factors as the condition of the patient, and the type and severity of the patient's disease, although appropriate dosages may be determined by clinical trials.

[0515] In one embodiment, the pharmaceutical composition is substantially free of, e.g., there are no detectable levels of a contaminant, e.g., selected from the group consisting of endotoxin, mycoplasma, replication competent lentivirus (RCL), p24, VSV-G nucleic acid, HIV gag, residual anti-CD3/anti-CD28 coated beads, mouse antibodies, pooled human serum, bovine serum albumin, bovine serum, culture media components, vector packaging cell or plasmid components, a bacterium and a fungus. In one embodiment, the bacterium is at least one selected from the group consisting of Alcaligenes faecalis, Candida albicans, Escherichia coli, Haemophilus influenza, Neisseria meningitides, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia, and Streptococcus pyogenes group A.

[0516] In one aspect, the present invention provides a population of CAR-expressing cells, e.g., CART cells or CAR-expressing NK cells. In one aspect, the present invention provides methods comprising administering a population of CAR-expressing cells (e.g., CART cells or CAR-expressing NK cells), in combination with another agent, e.g., a kinase inhibitor, such as a kinase inhibitor described herein.

[0517] In another aspect, the invention pertains to a cell comprising a vector described herein. In one embodiment, the cell is a cell described herein, e.g., a human T cell, e.g., a human T cell described herein. In one embodiment, the human T cell is a CD8+ T cell.

[0518] In another aspect, the invention pertains to a method of making a cell comprising transducing a cell described herein, e.g., a T cell described herein, with a vector of comprising a nucleic acid encoding a CAR, e.g., a CAR described herein.

[0519] The present invention also provides a method of generating a population of RNA engineered cells, e.g., cells described herein, e.g., T cells, transiently expressing exogenous RNA. The method comprises introducing an in vitro transcribed RNA or synthetic RNA into a cell, where the RNA comprises a nucleic acid encoding a CAR molecule described herein.

[0520] In embodiments of any of the methods and compositions described herein, the cell comprising a CAR comprises a nucleic acid encoding the CAR. In one embodiment, the nucleic acid encoding the CAR is a lentiviral vector. In one embodiment, the nucleic acid encoding the CAR is introduced into the cells by lentiviral transduction. In one embodiment, the nucleic acid encoding the CAR is an RNA, e.g., an in vitro transcribed RNA. In one embodiment, the nucleic acid encoding the CAR is introduced into the cells by electroporation.

[0521] In embodiments of any of the methods and compositions described herein, the cell is a T cell or an NK cell. In one embodiment, the T cell is an autologous or allogeneic T cell.

[0522] A typical composition for intravenous administration includes about 0.01 to about 30 mg/kg of antibody or antigen binding fragment or conjugate per subject per day (or the corresponding dose of a CAR, or T cell expressing a CAR, conjugate including the antibody or antigen binding fragment). Actual methods for preparing administrable compositions will be known or apparent to those skilled in the art and are described in more detail in such publications as Remington's Pharmaceutical Science, 19th ed., Mack Publishing Company, Easton, Pa. (1995).

[0523] A CAR, or T cell expressing a CAR, antibodies, antigen binding fragments, or conjugates may be provided in lyophilized form and rehydrated with sterile water before administration, although they are also provided in sterile solutions of known concentration. The CARs, or T cells expressing a CAR, antibody or antigen binding fragment or conjugate solution is then added to an infusion bag containing 0.9% sodium chloride, USP, and in some cases administered at a dosage of from 0.5 to 15 mg/kg of body weight. Considerable experience is available in the art in the administration of antibody or antigen binding fragment and conjugate drugs; for example, antibody drugs have been marketed in the U.S. since the approval of RITUXAN® in 1997. A CAR, or T cell expressing a CAR, antibodies, antigen binding fragments and conjugates thereof can be administered by slow infusion, rather than in an intravenous push or bolus. In one example, a higher loading dose is administered, with subsequent, maintenance doses being administered at a lower level. For example, an initial loading dose of 4 mg/kg antibody or antigen binding fragment (or the corresponding dose of a

conjugate including the antibody or antigen binding fragment) may be infused over a period of some 90 minutes, followed by weekly maintenance doses for 4-8 weeks of 2 mg/kg infused over a 30 minute period if the previous dose was well tolerated.

[0524] Controlled release parenteral formulations can be made as implants, oily injections, or as particulate systems. For a broad overview of protein delivery systems see, Banga, A. J., Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems, Technomic Publishing Company, Inc., Lancaster, Pa., (1995). Particulate systems include microspheres, microparticles, microcapsules, nanocapsules, nanospheres, and nanoparticles. Microcapsules contain the therapeutic protein, such as a cytotoxin or a drug, as a central core. In microspheres, the therapeutic is dispersed throughout the particle. Particles, microspheres, and microcapsules smaller than about 1 μm are generally referred to as nanoparticles, nanospheres, and nanocapsules, respectively. Capillaries have a diameter of approximately 5 μm so that only nanoparticles are administered intravenously. Microparticles are typically around 100 μm in diameter and are administered subcutaneously or intramuscularly. See, for example, Kreuter, J., Colloidal Drug Delivery Systems, J. Kreuter, ed., Marcel Dekker, Inc., New York, N.Y., pp. 219-342 (1994); and Tice & Tabibi, Treatise on Controlled Drug Delivery, A. Kydonieus, ed., Marcel Dekker, Inc. New York, N.Y., pp. 315-339, (1992).

[0525] Polymers can be used for ion-controlled release of the CARs, or T cells expressing a CAR, antibody or antigen binding fragment or conjugate compositions disclosed herein. Various degradable and nondegradable polymeric matrices for use in controlled drug delivery are known in the art (Langer, Accounts Chem. Res. 26:537-542, 1993). For example, the block copolymer, polaxamer 407, exists as a viscous yet mobile liquid at low temperatures but forms a semisolid gel at body temperature. It has been shown to be an effective vehicle for formulation and sustained delivery of recombinant interleukin-2 and urease (Johnston et al., Pharm. Res. 9:425-434, 1992; and Pec et al., J. Parent. Sci. Tech. 44(2):58-65, 1990). Alternatively, hydroxyapatite has been used as a microcarrier for controlled release of proteins (Ijntema et al., Int. J. Pharm. 112:215-224, 1994). In yet another aspect, liposomes are used for controlled release as well as drug targeting of the lipid-capsulated drug (Betageri et al., Liposome Drug Delivery Systems, Technomic Publishing Co., Inc., Lancaster, Pa. (1993)). Numerous additional systems for controlled delivery of therapeutic proteins are

known (see U.S. Pat. Nos. 5,055,303; 5,188,837; 4,235,871; 4,501,728; 4,837,028; 4,957,735; 5,019,369; 5,055,303; 5,514,670; 5,413,797; 5,268,164; 5,004,697; 4,902,505; 5,506,206; 5,271,961; 5,254,342 and 5,534,496).

Kits

[0526] In one aspect, kits employing the CARs disclosed herein are also provided. For example, kits for treating a tumor in a subject, or making a CAR T cell that expresses one or more of the CARs disclosed herein. The kits will typically include a disclosed antibody, antigen binding fragment, conjugate, nucleic acid molecule, CAR or T cell expressing a CAR as disclosed herein. More than one of the disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, CARs or T cells expressing a CAR can be included in the kit.

[0527] The kit can include a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container typically holds a composition including one or more of the disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, CARs or T cells expressing a CAR. In several embodiments the container may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). A label or package insert indicates that the composition is used for treating the particular condition.

[0528] The label or package insert typically will further include instructions for use of a disclosed antibodies, antigen binding fragments, conjugates, nucleic acid molecules, CARs or T cells expressing a CAR, for example, in a method of treating or preventing a tumor or of making a CAR T cell. The package insert typically includes instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products. The instructional materials may be written, in an electronic form (such as a computer diskette or compact disk) or may be visual (such as video files). The kits may also include additional components to facilitate the particular application for which the kit is designed. Thus, for example, the kit may additionally contain means of detecting a

label (such as enzyme substrates for enzymatic labels, filter sets to detect fluorescent labels, appropriate secondary labels such as a secondary antibody, or the like). The kits may additionally include buffers and other reagents routinely used for the practice of a particular method. Such kits and appropriate contents are well known to those of skill in the art.

Strategies for Regulating Chimeric Antigen Receptors

[0529] There are many ways CAR activities can be regulated. In some embodiments, a regulatable CAR (RCAR) where the CAR activity can be controlled is desirable to optimize the safety and efficacy of a CAR therapy. For example, inducing apoptosis using, e.g., a caspase fused to a dimerization domain (see, e.g., Di et al., N Engl. J . Med. 2011 Nov. 3; 365(18):1673- 1683), can be used as a safety switch in the CAR therapy of the instant invention. In another example, CAR-expressing cells can also express an inducible Caspase-9 (iCaspase-9) molecule that, upon administration of a dimerizer drug (e.g., rimiducid (also called AP1903 (Bellicum Pharmaceuticals) or AP20187 (Ariad)) leads to activation of the Caspase-9 and apoptosis of the cells. The iCaspase-9 molecule contains a chemical inducer of dimerization (CID) binding domain that mediates dimerization in the presence of a CID. This results in inducible and selective depletion of CAR-expressing cells. In some cases, the iCaspase-9 molecule is encoded by a nucleic acid molecule separate from the CAR-encoding vector(s). In some cases, the iCaspase-9 molecule is encoded by the same nucleic acid molecule as the CAR-encoding vector. The iCaspase-9 can provide a safety switch to avoid any toxicity of CAR-expressing cells. See, e.g., Song et al. Cancer Gene Ther. 2008; 15(10):667-75; Clinical Trial Id. No. NCT02107963; and Di Stasi et al. N. Engl. J . Med. 2011; 365:1673-83.

[0530] Alternative strategies for regulating the CAR therapy of the instant invention include utilizing small molecules or antibodies that deactivate or turn off CAR activity, e.g., by deleting CAR-expressing cells, e.g., by inducing antibody dependent cell-mediated cytotoxicity (ADCC). For example, CAR-expressing cells described herein may also express an antigen that is recognized by molecules capable of inducing cell death, e.g., ADCC or compliment-induced cell death. For example, CAR expressing cells described herein may also express a receptor capable of being targeted by an antibody or antibody fragment. Examples of such receptors include EpCAM, VEGFR, integrins (e.g., integrins anb3, a4, aI¾b3, a4b7,

a5b1, anb3, an), members of the TNF receptor superfamily (e.g., TRAIL-R1 , TRAIL-R2), PDGF Receptor, interferon receptor, folate receptor, GPNMB, ICAM-1 , HLA-DR, CEA, CA-125, MUC1, TAG-72, IL-6 receptor, 5T4, GD2, GD3, CD2, CD3, CD4, CD5, CD1 1 , CD1 1 a/LFA-1, CD15, CD18/ITGB2, CD19, CD20, CD22, CD23/lgE Receptor, CD25, CD28, CD30, CD33, CD38, CD40, CD41 , CD44, CD51 , CD52, CD62L, CD74, CD80, CD125, CD147/basigin, CD152/CTLA-4, CD154/CD40L, CD195/CCR5, CD319/SLAMF7, and EGFR, and truncated versions thereof (e.g., versions preserving one or more extracellular epitopes but lacking one or more regions within the cytoplasmic domain). For example, CAR-expressing cells described herein may also express a truncated epidermal growth factor receptor (EGFR) which lacks signaling capacity but retains the epitope that is recognized by molecules capable of inducing ADCC, e.g., cetuximab (ERBITUX®), such that administration of cetuximab induces ADCC and subsequent depletion of the CAR-expressing cells (see, e.g., WO2011/056894, and Jonnalagadda et al., Gene Ther. 2013; 20(8)853-860).

[0531] Another strategy includes expressing a highly compact marker/suicide gene that combines target epitopes from both CD32 and CD20 antigens in the CAR-expressing cells described herein, which binds rituximab, resulting in selective depletion of the CAR-expressing cells, e.g., by ADCC (see, e.g., Philip et al., Blood. 2014; 124(8)1277-1287). Other methods for depleting CAR-expressing cells described herein include administration of CAMPATH®, a monoclonal anti-CD52 antibody that selectively binds and targets mature lymphocytes, e.g., CAR-expressing cells, for destruction, e.g., by inducing ADCC. In other embodiments, CAR-expressing cells can be selectively targeted using a CAR ligand, e.g., an anti-idiotypic antibody. In some embodiments, the anti-idiotypic antibody can cause effector cell activity, e.g, ADCC or ADC activities, thereby reducing the number of CAR-expressing cells. In other embodiments, the CAR ligand, e.g., the antiidiotypic antibody, can be coupled to an agent that induces cell killing, e.g., a toxin, thereby reducing the number of CAR-expressing cells. Alternatively, the CAR molecules themselves can be configured such that the activity can be regulated, e.g., turned on and off, as described below.

[0532] In some embodiments, a RCAR comprises a set of polypeptides, typically two in the simplest embodiments, in which the components of a standard CAR described herein, e.g., an antigen binding domain and an intracellular signaling domain, are partitioned on separate polypeptides or members. In some embodiments, the set of polypeptides include a

dimerization switch that, upon the presence of a dimerization molecule, can couple the polypeptides to one another, e.g., can couple an antigen binding domain to an intracellular signaling domain.

[0533] Additional description and exemplary configurations of such regulatable CARs are provided herein and in International Publiciation No. WO 2015/090229, hereby incorporated by reference in its entirety. In an embodiment, an RCAR comprises two polypeptides or members: 1) an intracellular signaling member comprising an intracellular signaling domain, e.g., a primary intracellular signaling domain described herein, and a first switch domain; 2) an antigen binding member comprising an antigen binding domain, e.g., that targets a tumor antigen described herein, as described herein and a second switch domain. Optionally, the RCAR comprises a transmembrane domain described herein. In an embodiment, a transmembrane domain can be disposed on the intracellular signaling member, on the antigen binding member, or on both. (Unless otherwise indicated, when members or elements of an RCAR are described herein, the order can be as provided, but other orders are included as well. In other words, in an embodiment, the order is as set out in the text, but in other embodiments, the order can be different. E.g., the order of elements on one side of a transmembrane region can be different from the example, e.g., the placement of a switch domain relative to a intracellular signaling domain can be different, e.g., reversed).

[0534] In an embodiment, the first and second switch domains can form an intracellular or an extracellular dimerization switch. In an embodiment, the dimerization switch can be a homodimerization switch, e.g., where the first and second switch domain are the same, or a heterodimerization switch, e.g., where the first and second switch domain are different from one another.

[0535] In embodiments, an RCAR can comprise a "multi switch." A multi switch can comprise heterodimerization switch domains or homodimerization switch domains. A multi switch comprises a plurality of, e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10, switch domains, independently, on a first member, e.g., an antigen binding member, and a second member, e.g., an intracellular signaling member. In an embodiment, the first member can comprise a plurality of first switch domains, and the second member can comprise a plurality of second

switch domains. In an embodiment, the first member can comprise a first and a second switch domain, and the second member can comprise a first and a second switch domain.

[0536] In an embodiment, the intracellular signaling member comprises one or more intracellular signaling domains, e.g., a primary intracellular signaling domain and one or more costimulatory signaling domains.

[0537] In an embodiment, the antigen binding member may comprise one or more intracellular signaling domains, e.g., one or more costimulatory signaling domains. In an embodiment, the antigen binding member comprises a plurality, e.g., 2 or 3 costimulatory signaling domains described herein, e.g., selected from 4-IBB, CD28, CD27, ICOS, and OX40, and in embodiments, no primary intracellular signaling domain. In an embodiment, the antigen binding member comprises the following costimulatory signaling domains, from the extracellular to intracellular direction: 4-1BB-CD27; 4-1BB-CD27; CD27-4-1BB; 4-1BBCD28; CD28-4-1BB; OX40-CD28; CD28-OX40; CD28-4-1BB; or 4-1BB-CD28. In such embodiments, the intracellular binding member comprises a CD3zeta domain. In one such embodiment the RCAR comprises (1) an antigen binding member comprising, an antigen binding domain, a transmembrane domain, and two costimulatory domains and a first switch domain; and (2) an intracellular signaling domain comprising a transmembrane domain or membrane tethering domain and at least one primary intracellular signaling domain, and a second switch domain.

[0538] An embodiment provides RCARs wherein the antigen binding member is not tethered to the surface of the CAR cell. This allows a cell having an intracellular signaling member to be conveniently paired with one or more antigen binding domains, without transforming the cell with a sequence that encodes the antigen binding member. In such embodiments, the RCAR comprises: 1) an intracellular signaling member comprising: a first switch domain, a transmembrane domain, an intracellular signaling domain, e.g., a primary intracellular signaling domain, and a first switch domain; and 2) an antigen binding member comprising: an antigen binding domain, and a second switch domain, wherein the antigen binding member does not comprise a transmembrane domain or membrane tethering domain, and, optionally, does not comprise an intracellular signaling domain. In some embodiments, the RCAR may further comprise 3) a second antigen binding member comprising: a second

antigen binding domain, e.g., a second antigen binding domain that binds a different antigen than is bound by the antigen binding domain; and a second switch domain.

[0539] Also provided herein are RCARs wherein the antigen binding member comprises bispecific activation and targeting capacity. In this embodiment, the antigen binding member can comprise a plurality, e.g., 2, 3, 4, or 5 antigen binding domains, e.g., scFvs, wherein each antigen binding domain binds to a target antigen, e.g. different antigens or the same antigen, e.g., the same or different epitopes on the same antigen. In an embodiment, the plurality of antigen binding domains are in tandem, and optionally, a linker or hinge region is disposed between each of the antigen binding domains. Suitable linkers and hinge regions are described herein.

[0540] An embodiment provides RCARs having a configuration that allows switching of proliferation. In this embodiment, the RCAR comprises: 1) an intracellular signaling member comprising: optionally, a transmembrane domain or membrane tethering domain; one or more co-stimulatory signaling domain, e.g., selected from 4-IBB, CD28, CD27, ICOS, and OX40, and a switch domain; and 2) an antigen binding member comprising: an antigen binding domain, a transmembrane domain, and a primary intracellular signaling domain, e.g., a CD3zeta domain, wherein the antigen binding member does not comprise a switch domain, or does not comprise a switch domain that dimerizes with a switch domain on the intracellular signaling member. In an embodiment, the antigen binding member does not comprise a costimulatory signaling domain. In an embodiment, the intracellular signaling member comprises a switch domain from a homodimerization switch. In an embodiment, the intracellular signaling member comprises a first switch domain of a heterodimerization switch and the RCAR comprises a second intracellular signaling member which comprises a second switch domain of the heterodimerization switch. In such embodiments, the second intracellular signaling member comprises the same intracellular signaling domains as the intracellular signaling member. In an embodiment, the dimerization switch is intracellular. In an embodiment, the dimerization switch is extracellular.

[0541] Also provided herein are nucleic acids and vectors comprising RCAR encoding sequences. Sequence encoding various elements of an RCAR can be disposed on the

same nucleic acid molecule, e.g., the same plasmid or vector, e.g., viral vector, e.g., lentiviral vector.

[0542] In an embodiment, (i) sequence encoding an antigen binding member and (ii) sequence encoding an intracellular signaling member, can be present on the same nucleic acid, e.g., vector. Production of the corresponding proteins can be achieved, e.g., by the use of separate promoters, or by the use of a bicistronic transcription product (which can result in the production of two proteins by cleavage of a single translation product or by the translation of two separate protein products). In an embodiment, a sequence encoding a cleavable peptide, e.g., a P2A or F2A sequence, is disposed between (i) and (ii). In an embodiment, a sequence encoding an IRES, e.g., an EMCV or EV71 IRES, is disposed between (i) and (ii). In these embodiments, (i) and (ii) are transcribed as a single RNA. In an embodiment, a first promoter is operably linked to (i) and a second promoter is operably linked to (ii), such that (i) and (ii) are transcribed as separate mRNAs.

[0543] Alternatively, the sequence encoding various elements of an RCAR can be disposed on the different nucleic acid molecules, e.g., different plasmids or vectors, e.g., viral vector, e.g., lentiviral vector. E.g., the (i) sequence encoding an antigen binding member can be present on a first nucleic acid, e.g., a first vector, and the (ii) sequence encoding an intracellular signaling member can be present on the second nucleic acid, e.g., the second vector.

[0544] Unless stated otherwise, all technical and scientific terms and phrases used herein have the same meaning as commonly understood by one of ordinary skill in the art. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference.

[0545] Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are

cited and discussed throughout the present specification. See e.g., Sambrook et al. Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

[0546] All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The present invention will be more fully understood by reference to the following Examples.

EXAMPLES

[0547] These Examples are set forth to aid in the understanding of the invention but are not intended to, and should not be construed to limit its scope in any way. The Examples do not include detailed descriptions of conventional methods that would be well known to those of ordinary skill in the art (molecular cloning techniques, etc.).

Example 1. Isolation and selection of GCC VH antibodies

[0548] Single domain heavy chain antibodies (vH) were generated that specifically target human GCC (e.g., anti-GCC binders provided in Tables 1-3). As shown in Table 5, recombinant single domain anti-GCC VH constructs were assessed for binding affinity to GCC *in vitro*. Binding kinetics were determined using an Octet binding assay. These were measured in real-time bio-layer interferometer based biosensor Octet (ForteBio). All the binding studies were performed in HBS-ET Octet kinetics buffer. Biosensors were always washed in Octet kinetics buffer in between different steps. A seven point, two-fold dilution series of each VH was made. The contact time for each of the association steps was 300 seconds and the dissociation step was varied between 400-600 seconds. Kinetic association (ka) and dissociation (kd) rate constants were determined by processing and fitting the data to a 1:1 binding model using ForteBio Analysis software. The calculated affinity and kinetic constants are shown in Table 5.

[0549] Binding of single domain antibodies to CT26 cells was measured using flow cytometry. CT26 cells were incubated with serially diluted VH and fluorescence was measured by flow cytometry. Anti-GCC VH antibodies were confirmed for binding to CT26

cells by a FACS dose response assay to obtain an on cell binding EC50 (nM) as shown in Table 5.

Table 5. Binding Affinity of GCC VH antibodies

GCC Antigen Binding agent	**Koff (1/s)**	**KD(M)**	**on cell binding EC50 (M)**
V1	0.00020795	3.2935E-10	7.2E-10
V5	0.000718667	7.713E-09	1.52E-09
V36	0.0035	0.000000105	N/A
V48	0.000341	1.375E-09	1.01E-09
V51	0.00185	9.544E-09	N/A

[0550] To determine stability, VH constructs were subjected to size exclusion chromatography (SEC). Briefly, purified VH were stored at varied concentrations in PBS buffer overnight at 4°C, and then analysed at various time points using a SEC column. Samples were injected in a sodium phosphate buffer. Data were collected over time and the area of monomer peak remaining after storage as compared to that present at the start (T=0) was calculated. Stability results were obtained as shown in Table 6 below.

Table 6. Overnight stability using SEC

Anti-GCC VH antibody	% 4°C Monomer (%)	Purity (%)	RT (min)
V1	108.4	91.5	3.586
V5	123.04	79.2	3.17
V36	92.43	79.36	3.08
V48	104.54	73.91	3.208
V51	109.2	59.22	3.304

[0551] ELISA were performed to measure VH binding to human light chains. Binding values for each of the VH (measured as OD450nm) were less than 0.1, which is the background signal for this assay.

Example 2. Design and characterization of GCC Chimeric Antigen Receptors

[0552] Chimeric antigen receptor constructs are engineered to include an extracellular binding domain (e.g., anti-GCC binder sequence) comprising the single domain antibodies described above (e.g., a VH provided in Table 2 or Table 3). CAR T constructs are generated by linking the binder sequence in frame to CD28 hinge/transmembrane domains and costimulatory domain and CD3 zeta-1xx signaling domain. Schematics of exemplary CAR constructs are shown in Fig. 1A and 1B. Exemplary CAR construct sequences tested in this example are provided in Table 4. V1 (SEQ ID NO:1) and V1-01 (SEQ ID NO: 20) are expected to work in the examples below.

[0553] Nucleic acids encoding the CAR construct sequences (SEQ ID Nos: 61-70) were cloned into a retroviral plasmid backbone. Retroviral vector containing supernatants were generated by transient transfection of phenix ampho cells (ATCC CRL-3213) and retroviral vector-containing supernatants were harvested, and stored at −80° C.

[0554] Human primary T cells from healthy donors were purified from Peripheral blood mononuclear cell (PBMC) isolated from leukopaks (purchased from commercial provider with donor's written consent) using immunomagnetic bead selection of CD3+ cells according to manufacturer's protocol (EasySep™ Human T Cell Isolation Kit, Stem Cell Technologies #17951). T cells were cultured in X-vivo 15 medium (Lonza #04-744Q) supplemented with 10% Penicillin-Streptomycin (Gibco 15140-122) and 2ng/ml IL-2 (Milteyni 130-097-743) at a density of 1 million cells/ml. Cells were activated with CD3/CD28 MACS® T Cell TransAct reagent (Miltenyi Biotec MACS #130-111-160) and transduced on day 2 or day 3 with retroviral vectors encoding CAR constructs overnight. The next day, CAR-T cell cultures were transferred to a G-Rex6® Well Plate (WilsonWolf P/N 80240M) and propagated in X-vivo 15 medium (Lonza #04-744Q) supplemented with 10% Penicillin-Streptomycin (Gibco 15140-122) and 2ng/ml IL-2 (Milteyni 130-097-743) until harvest on day 7-10. Medium change and IL-2 replenishment were performed every 2-3 days.

[0555] CAR T cell expression was evaluated by flow cytometry using an anti-EGFR antibody (R&D systems: FAB9577R) or soluble GCC extracellar domain recombinant protein for CAR surface expression.

Example 3. GCC CAR T cell activity *in vitro*

[0556] The present Example describes anti-GCC CAR T cell activity *in vitro*. CAR-T cells expressing anti-GCC CARs (SEQ ID NO: 48-52) were examined for cytotoxicity against GCC-expressing and GCC-negative target cancer cell lines. The target cancer cell lines included GSU, LS1034 and HT55, which endogenously express GCC, as well as HT29-GCC, a human colorectal cancer cell line engineered to stably express GCC, and its vector control cell line that is GCC-negative, HT29-vec. Each target cell line was seeded in a 384-well plate, and GCC CAR-T or non-targeting CAR-T cells (negative control) were added at effector-to-target (E:T) ratios of 10:1, 3:1, 1:1 and 0.3:1.Wells with target cells only and wells with effector cells only were included as controls. After two days, cell viability was measured using CellTiter-Glo® One Solution Assay (Promega, G8462). Percent viability of target cells was calculated from the luminescence signals of the co-culture wells, after first subtracting the signals of the effector-cells-only wells, then dividing by the signals of the target-cell-only wells. Percent killing was calculated by subtracting the percent viability of target cells from 100% GCC CAR-T cells.

[0557] VH anti-GCC binders exhibited cell killing against GCC-expressing target cell lines, in contrast to non-targeting CD19 CAR-T cells (1928z-1xx) used as a control. As shown in Fig. 2A-2D, CAR-T cells expressing anti-GCC CARs in the absence of truncated EGFR (tEGFR) demonstrated *in vitro* cytotoxicity against GCC expressing cells HT29-GCC cells, human colorectal cancer cell line HT29 engineered to stably express GCC) (Fig 2A); and endogenously expressing GCC cell lines GSU (Fig 2C) and LS1034 (Fig 2D). As shown in Fig. 3A-3D, CAR-T cells expressing anti-GCC CARs in the presence of truncated EGFR (tEGFR) (SEQ ID NOs: 56-60) also demonstrated *in vitro* cytotoxicity against GCC expressing cells HT29-GCC (Fig. 3A); GSU (Fig. 3C) and LS1034 (Fig. 3D). Bars represent mean+SD values from three technical replicates. Data are representative of >3 independent experiments performed with anti-GCC CAR T cells from >3 donors. GCC CAR-T cells did not exhibit cell killing against the GCC-negative HT29-vec cells (Fig 2B and Fig. 3B), indicating GCC CAR-T cell killing activity was antigen-dependent.

[0558] In addition to antigen-dependent cell killing, the *in vitro* activity of GCC CAR-T cells was also assessed by evaluating its antigen-dependent secretion of IFNγ and IL2. GCC

CAR-T cells with anti-GCC VH binders were co-cultured with GCC-expressing and GCC-negative target cancer cell lines at E:T ratios of 10:1, 3:1, 1:1 and 0.3:1. Supernatant was collected after two days of co-culture. Secreted IFNγ and IL2 in the supernatant were detected using the Intellicyt QBeads Human PlexScreen kit (Sartorius, 90702). GCC CAR-T cells with all VH binders secreted IFNγ both in the presence of tEGFR (5A-5D) and the absence of (4A-4D) when co-cultured with GCC-expressing target cells, but not when co-cultured with GCC-negative target cells (Fig. 4B and 5B), indicating antigen-dependent cytokine release. GCC CAR-T cells with all VH binders secreted IL2 both in the presence (7A-7D) and absence of tEGFR (6A-6D) when co-cultured with GCC-expressing target cells, but not when co-cultured with GCC-negative target cells (Fig. 6B and 7B), indicating antigen-dependent cytokine release.

Example 4. GCC CAR T cell activity *in vivo*

[0559] The present example describes anti-GCC CAR T cell activity *in vivo*. The antitumor activity of GCC CAR-T with different VH binders were studied in multiple human colorectal cancer tumor xenograft models expressing guanylyl cyclase C (GCC) endogenously. Non-obese diabetic/severe combined immunodeficient/γ-chain−/− (NSG) mice were inoculated subcutaneously with either HT55 cells, LS1034 cells, or GSU cells. When average tumor volume reached approximately 150 mm^3, GCC CAR-T cells or mock T cells (non targeted CAR-T) were administered intravenously (IV) acutely. Different dose levels and donor sources were evaluated and indicated as shown in Fig. 8A-18. Tumor volume and body weight were measured twice weekly for up to 50 days.

[0560] Change in average tumor volume (mm^3) over time (40 days) was determined in a HT55 model (endogenously expressing GCC expressing colorectal cancer, GCC H-score=300/300) treated with anti-GCC CAR-T cells manufactured from two different healthy donors D393 (Fig. 8A) and D686 (Fig. 8B). Change in average tumor volume (mm^3) over time (42 days) was determined in a HT55 model treated with anti-GCC CAR-T cells manufactured from three different healthy donors D393 (Fig. 9A), D797 (Fig. 9B) and D954 (Fig. 9C). Change in average tumor volume (mm^3) over time (42 days) was determined in a

HT55 model treated with anti-GCC CAR-T cells co-expressing tEGFR manufactured from healthy donors D393 (Fig. 10A), D797 (Fig. 10B) and D954 (Fig. 10C) and treated with anti-GCC CAR-T cells in the absence of tEGFR manufactured from healthy donors D393. Change in average tumor volume (mm^3) over time (42 days) was determined in a HT55 model treated with anti-GCC CAR-T cells manufactured from three different healthy donors D393 (Fig. 11A), D954 (Fig. 11B) and D686 (Fig. 11C). Change in average tumor volume (mm^3) over time (42 days) was determined in a HT55 model treated with v48 anti-GCC CAR-T cells co-expressing tEGFR manufactured from healthy donors D393 (Fig. 12A), D954 (Fig. 12B) and D686 (Fig. 12C) at doses of 0.25×10^6 CAR+ cells, 0.5×10^6 CAR+ cells, or 1×10^6 CAR+ cells.

[0561] Change in average tumor volume (mm3) over time (42 days) was determined in a GSU model (endogenously GCC expressing gastric cancer, GCC H-score=170/300) treated with anti-GCC CAR-T cells manufactured from two healthy donors D393 (Fig. 13A), D954 (Fig. 13B), D393 (Fig. 15A), D954 (Fig. 15B) and D686 (Fig. 15C) in the absence of tEGFR. Change in average tumor volume (mm3) over time (42 days) was determined in a GSU model (endogenously GCC expressing gastric cancer, GCC H-score=170/300) treated with anti-GCC CAR-T cells co-expressing tEGFR manufactured from different healthy donors D393 (Fig. 14A) and D954 (Fig. 14B), D393 (Fig. 16A), D954 (Fig. 16B) and D686 (Fig. 16C).

[0562] Change in average tumor volume (mm3) over time (42 days) was determined in a LS1034 model (endogenously GCC expressing colorectal cancer, GCC H-score=300/300) treated with anti-GCC CAR-T cells manufactured from a healthy donor co-expressing tEGFR (Fig. 18) or in the absence of tEGFR (Fig. 17).

[0563] Acute IV administration of GCC CAR-T cells from multiple donors led to significant inhibition of tumor growth in all tumor models compared to their respective control group. There was no significant body weight loss observed in LS1034 or GSU tumor bearing NSG mice treated with GCC CAR T cells compared with their respective control group. In HT-55 tumor model, body weight loss observed is due to tumor burden. Tumor burden reduction post GCC CAR-T cell treatment results in body weight gain.

[0564] Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated that various alterations, modifications, and improvements will readily be apparent to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawings are by way of example only and the invention is described in detail by the claims that follow.

Table of Sequences

[0565] Table 7 below provides descriptions and sequences disclosed herein.

Table 7. Table of Sequences

SEQ ID NO	Description	Sequence
1	V1 vH	QVQLQESGPGLVKPSETLSLTCTVSGASISHYYWS WFRQPAGKGLEWIGRIYPSGSTSYNPSLKSRVAMS VDTPKNQFSLNLSSVTAADTAVYYCARDRSTGWS EWNSDLWGRGTLVTVSS
2	CD8 transmembrane domain	IYIWAPLAGTCGVLLLSLVITLYC
3	4-1BB Intracellular domain	KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEE EEGGCEL
4	CD3-zeta signaling domain	RVKFSRSADAPAYKQGQNQLYNELNLGRREEYDV LDKRRGRDPEMGGKPRRKNPQEGLYNELQKDKM AEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTY DALHMQALPPR
5	Hinge sequence	TTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVH TRGLDFACD

6	Leader Sequence (v1)	MKHLWFFLLLVAAPRWVLS
7	Leader Sequence (v5, v36, v48, v51)	MELGLSWVFLVAILEGVQC
8	V1 HCDR1	HYYWS
9	V5 HCDR1	RYWMS
10	V36 HCDR1	RYWMT
10	V48 HCDR1	RYWMT
10	V51 HCDR1	RYWMT
11	V1 HCDR2	RIYPSGSTSYNPSLKS
12	V5 HCDR2	KIRHDGGEKYYVDSVKG
13	V36 HCDR2	KIKYDGSEKYYADSVKG
14	V48 HCDR2	KIRHDGGEKYYPDSVKG
15	V51 HCDR2	KIRHDGGEKYYADSVKG
16	V1 HCDR3	DRSTGWSEWNSDL
17	V5 HCDR3	DYTRDV
18	V36 HCDR3	DYNKDY
19	V48 HCDR3	DYNKDL
18	V51 HCDR3	DYNKDY
20	V1	EVQLQESGPGLVKPSETLSLTCTVSGASISHYYWS WFRQPAGKGLEWIGRIYPSGSTSYNPSLKSRVAMS VDTPKNQFSLKLSSVTAADTAVYYCARDRSTGWS EWNSDLWGRGTLVTVSS
21	V5	QVQLVESGGGLVQPGGSLRLSCTASGFTFSRYWM SWVRQAPGKGLEWVAKIRHDGGEKYYVDSVKGR FTISRDNAKNSLYLQMNSLRAEDTAVYYCATDYT RDVWGQGTAVTVSS
22	V8	QVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWM SWVRQAPGKGLEWVAKIKYDGSEKYYVDSVKGR

		FTISRDNAKNSVYLQMNSLRAEDTGVYYCATDFT RDVWGQGTTVTVSS
23	V9	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWM TWVRQAPGRGLEWVAKIRYDGGEKYYVDSVKGR FTISRDNAKNSLYLQMNSLRAEDTAVYYCATDFT RDVWGQGTTVTVSS
24	V30	QVQLVESGGGLVQPGGSLRLSCAASGFNFGRYWM SWVRQAPGKGREWVAKIKYDGSEKYYVDSVKGR FTISRDNAKNSLYLQMNSLRAEDTAVYYCATDFT RDVWGQGTTVTVSS
25	V31	QVQLVESGGGVVRPGGSLRLSCAASGFTFSRYWM SWVRQAPGKGREWVAKIKYDGSEKYYADSVKGR FTISRDNAKNSLYLQMNSLRADDTAVYYCATDFT RDVWGQGTTVTVSS
26	V36	EVQLVESGGGLAQPGGSLRLSCAASGFTFSRYWM TWVRQAPGGRLEWVAKIKYDGSEKYYADSVKGR FTISRDNAKNSLYLQMDSLRAEDTAVYYCTRDYN KDYWGQGTLVTVSS
27	V48	EVQLVESGGGLVQPGGSLRLTCAASGFTFSRYWM TWVRQAPGKGLEWVAKIRHDGGEKYYPDSVKGR FTVSRDNAKNSLYLQMDNLRAEDTAMYYCTRDY NKDLWGQGTLVTVSS
28	V51	EVQLVESGGGLVQPGGSLRLSCAASGFTFSRYWM TWVRQAPGKGLEWVAKIRHDGGEKYYADSVKGR FTISRDNAKNSLYLQMNSLRAEDTAVYYCTRDYN KDYWGQGTLVTVSS
29	CD28 hinge domain	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP SKP
30	CD28 Transmembrane domain	FWVLVVVGGVLACYSLLVTVAFIIFWV

31	CD28 hinge/transmembrane domain	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWV
32	CD28 signal domain	RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS
33	CD3z-1XX mutant signaling domain	RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR
34	COMBINED CAR transmembrane and intracellular domains	IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR
35	V1-01 CAR	MGWSCIILFLVATATGVHSEVQLQESGPGLVKPSETLSLTCTVSGASISHYYWSWFRQPAGKGLEWIGRIYPSGSTSYNPSLKSRVAMSVDTPKNQFSLKLSSVTAADTAVYYCARDRSTGWSEWNSDLWGRGTLVTVSSIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR
36	V5 CAR	MELGLSWVFLVAILEGVQCQVQLVESGGGLVQPGGSLRLSCTASGFTFSRYWMSWVRQAPGKGLEWVAKIRHDGGEKYYVDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCATDYTRDVWGQGTAVTVSS

		IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP SKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRS RLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAY RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKD KMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKD TFDALHMQALPPR
37	V36 CAR	MELGLSWVFLVAILEGVQCEVQLVESGGGLAQPG GSLRLSCAASGFTFSRYWMTWVRQAPGGRLEWV AKIKYDGSEKYYADSVKGRFTISRDNAKNSLYLQ MDSLRAEDTAVYYCTRDYNKDYWGQGTLVTVSS IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP SKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRS RLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAY RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKD KMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKD TFDALHMQALPPR
38	V48 CAR	MELGLSWVFLVAILEGVQCEVQLVESGGGLVQPG GSLRLTCAASGFTFSRYWMTWVRQAPGKGLEWV AKIRHDGGEKYYPDSVKGRFTVSRDNAKNSLYLQ MDNLRAEDTAMYYCTRDYNKDLWGQGTLVTVSS IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP SKPFWVLVVVGGVLACYSLLVTVAFIIFWVRSKRS RLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAY RSRVKFSRSADAPAYQQGQNQLYNELNLGRREEY DVLDKRRGRDPEMGGKPRRKNPQEGLFNELQKD KMAEAFSEIGMKGERRRGKGHDGLFQGLSTATKD TFDALHMQALPPR
39	V51 CAR	MELGLSWVFLVAILEGVQCEVQLVESGGGLVQPG GSLRLSCAASGFTFSRYWMTWVRQAPGKGLEWV

		AKIRHDGGEKYYADSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCTRDYNKDYWGQGTLVTVSS IEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGP SKPFWVLVVVGGVLACYSLLVTVAFIIFWV RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPR DFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNE LQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLST ATKDTFDALHMQALPPR
40	heat-stable enterotoxin	NTFYCCELCCNPACAGCY
41	GCC AA Sequence	MKTLLLDLALWSLLFQPGWLSFSSQVSQNCHNGS YEISVLMMGNSAFAEPLKNLEDAVNEGLEIVRGRL QNAGLNVTVNATFMYSDGLIHNSGDCRSSTCEGL DLLRKISNAQRMGCVLIGPSCTYSTFQMYLDTELS YPMISAGSFGLSCDYKETLTRLMSPARKLMYFLVN FWKTNDLPFKTYSWSTSYVYKNGTETEDCFWYLN ALEASVSYFSHELGFKVVLRQDKEFQDILMDHNR KSNVIIMCGGPEFLYKLKGDRAVAEDIVIILVDLFN DQYFEDNVTAPDYMKNVLVLTLSPGNSLLNSSFSR NLSPTKRDFALAYLNGILLFGHMLKIFLENGENITT PKFAHAFRNLTFEGYDGPVTLDDWGDVDSTMVLL YTSVDTKKYKVLLTYDTHVNKTYPVDMSPTFTW KNSKLPNDITGRGPQILMIAVFTLTGAVVLLLLVAL LMLRKYRKDYELRQKKWSHIPPENIFPLETNETNH VSLKIDDDKRRDTIQRLRQCKYDKKRVILKDLKHN DGNFTEKQKIELNKLLQIDYYNLTKFYGTVKLDT MIFGVIEYCERGSLREVLNDTISYPDGTFMDWEFKI SVLYDIAKGMSYLHSSKTEVHGRLKSTNCVVDSR MVVKITDFGCNSILPPKKDLWTAPEHLRQANISQK GDVYSYGIIAQEIILRKETFYTLSCRDRNEKIFRVEN

		SNGMKPFRPDLFLETAEEKELEVYLLVKNCWEED PEKRPDFKKIETTLAKIFGLFHDQKNESYMDTLIRR LQLYSRNLEHLVEERTQLYKAERDRADRLNFMLL PRLVVKSLKEKGFVEPELYEEVTIYFSDIVGFTTICK YSTPMEVVDMLNDIYKSFDHIVDHHDVYKVETIG DAYMVASGLPKRNGNRHAIDIAKMALEILSFMGT FELEHLPGLPIWIRIGVHSGPCAAGVVGIKMPRYCL FGDTVNTASRMESTGLPLRIHVSGSTIAILKRTECQ FLYEVRGETYLKGRGNETTYWLTGMKDQKFNLPT PPTVENQQRLQAEFSDMIANSLQKRQAAGIRSQKP RRVASYKKGTLEYLQLNTTDKESTYF
42	v31 leader	MEFGLSWVFLVAIIKGVQC
43	tEGFR	MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDS LSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTP PLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFE NLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISD GDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNR GENSCKATGQVCHALCSPEGCWGPEPRDCVSCRN VSRGRECVDKCNLLEGEPREFVENSECIQCHPECLP QAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAG VMGENNTLVWKYADAGHVCHLCHPNCTYGCTGP GLEGCPTNGPKIPSIATGMVGALLLLLVVALGIGLF M
44	P2A	GSGATNFSLLKQAGDVEENPGP
45	Leader sequence	MALPVTALLLPLALLLHAARP
46	V1 CAR	MGWSCIILFLVATATGVHS QVQLQESGPGLVKPSETLSLTCTVSGASISHYYWS WFRQPAGKGLEWIGRIYPSGSTSYNPSLKSRVAMS VDTPKNQFSLNLSSVTAADTAVYYCARDRSTGWS EWNSDLWGRGTLVTVSSIEVMYPPPYLDNEKSNG

		TIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLAC YSLLVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPG PTRKHYQPYAPPRDFAAYRSRVKFSRSADAPAYQ QGQNQLYNELNLGRREEYDVLDKRRGRDPEMGG KPRRKNPQEGLFNELQKDKMAEAFSEIGMKGERR RGKGHDGLFQGLSTATKDTFDALHMQALPPR
47	V1 CAR	MGWSCIILFLVATATGVHS QVQLQESGPGLVKPSETLSLTCTVSGASISHYYWS WFRQPAGKGLEWIGRIYPSGSTSYNPSLKSRVAMS VDTPKNQFSLNLSSVTAADTAVYYCARDRSTGWS EWNSDLWGRGTLVTVSSRAAAIEVMYPPPYLDNE KSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGG VLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTP RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAP AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMKG ERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR
48	V1-01 CAR	MGWSCIILFLVATATGVHSEVQLQESGPGLVKPSE TLSLTCTVSGASISHYYWSWFRQPAGKGLEWIGRI YPSGSTSYNPSLKSRVAMSVDTPKNQFSLKLSSVT AADTAVYYCARDRSTGWSEWNSDLWGRGTLVTV SSRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPS PLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPP RDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFN ELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLS TATKDTFDALHMQALPPR
49	V5 CAR	MELGLSWVFLVAILEGVQCQVQLVESGGGLVQPG GSLRLSCTASGFTFSRYWMSWVRQAPGKGLEWV AKIRHDGGEKYYVDSVKGRFTISRDNAKNSLYLQ

		MNSLRAEDTAVYYCATDYTRDVWGQGTAVTVSS RAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPL FPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRD FAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNEL QKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTA TKDTFDALHMQALPPR
50	V36 CAR	MELGLSWVFLVAILEGVQCEVQLVESGGGLAQPG GSLRLSCAASGFTFSRYWMTWVRQAPGGRLEWV AKIKYDGSEKYYADSVKGRFTISRDNAKNSLYLQ MDSLRAEDTAVYYCTRDYNKDYWGQGTLVTVSS RAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPL FPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRD FAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNEL QKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTA TKDTFDALHMQALPPR
51	V48 CAR	MELGLSWVFLVAILEGVQCEVQLVESGGGLVQPG GSLRLTCAASGFTFSRYWMTWVRQAPGKGLEWV AKIRHDGGEKYYPDSVKGRFTVSRDNAKNSLYLQ MDNLRAEDTAMYYCTRDYNKDLWGQGTLVTVSS RAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPL FPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRD FAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNEL QKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTA TKDTFDALHMQALPPR

52	V51 CAR	MELGLSWVFLVAILEGVQCEVQLVESGGGLVQPG GSLRLSCAASGFTFSRYWMTWVRQAPGKGLEWV AKIRHDGGEKYYADSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCTRDYNKDYWGQGTLVTVSS RAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPL FPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWV RSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPR DFAAYRSRVKFSRSADAPAYQQGQNQLYNELNLG RREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNE LQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLST ATKDTFDALHMQALPPR
53	Linker sequence	RAAA
54	Linker Sequence	GGGGS
55	V1 CAR (28z1xx-tEGFR)	MGWSCIILFLVATATGVHS QVQLQESGPGLVKPSETLSLTCTVSGASISHYYWS WFRQPAGKGLEWIGRIYPSGSTSYNPSLKSRVAMS VDTPKNQFSLNLSSVTAADTAVYYCARDRSTGWS EWNSDLWGRGTLVTVSSRAAAIEVMYPPPYLDNE KSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGG VLACYSLLVTVAFIIFWVRSKRSRLLHSDYMNMTP RRPGPTRKHYQPYAPPRDFAAYRSRVKFSRSADAP AYQQGQNQLYNELNLGRREEYDVLDKRRGRDPE MGGKPRRKNPQEGLFNELQKDKMAEAFSEIGMKG ERRRGKGHDGLFQGLSTATKDTFDALHMQALPPR GSGATNFSLLKQAGDVEENPGP MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDS LSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTP PLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFE NLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISD

		GDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNR GENSCKATGQVCHALCSPEGCWGPEPRDCVSCRN VSRGRECVDKCNLLEGEPREFVENSECIQCHPECLP QAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAG VMGENNTLVWKYADAGHVCHLCHPNCTYGCTGP GLEGCPTNGP KIPSIATGMVGALLLLLVVALGIGLFM
56	V1-01 CAR (28z1xx-tEGFR)	MGWSCIILFLVATATGVHSEVQLQESGPGLVKPSE TLSLTCTVSGASISHYYWSWFRQPAGKGLEWIGRI YPSGSTSYNPSLKSRVAMSVDTPKNQFSLKLSSVT AADTAVYYCARDRSTGWSEWNSDLWGRGTLVTV SSRAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPS PLFPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFW VRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPP RDFAAYRSRVKFSRSADAPAYQQGQNQLYNELNL GRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLFN ELQKDKMAEAFSEIGMKGERRRGKGHDGLFQGLS TATKDTFDALHMQALPPR GSGATNFSLLKQAGDVEENPGP MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDS LSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTP PLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFE NLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISD GDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNR GENSCKATGQVCHALCSPEGCWGPEPRDCVSCRN VSRGRECVDKCNLLEGEPREFVENSECIQCHPECLP QAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAG VMGENNTLVWKYADAGHVCHLCHPNCTYGCTGP GLEGCPTNGP KIPSIATGMVGALLLLLVVALGIGLFM

57	V5 CAR (28z1xx-tEGFR)	MELGLSWVFLVAILEGVQCQVQLVESGGGLVQPG GSLRLSCTASGFTFSRYWMSWVRQAPGKGLEWV AKIRHDGGEKYYVDSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCATDYTRDVWGQGTAVTVSS RAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPL FPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRD FAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNEL QKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTA TKDTFDALHMQALPPR GSGATNFSLLKQAGDVEENPGP MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDS LSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTP PLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFE NLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISD GDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNR GENSCKATGQVCHALCSPEGCWGPEPRDCVSCRN VSRGRECVDKCNLLEGEPREFVENSECIQCHPECLP QAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAG VMGENNTLVWKYADAGHVCHLCHPNCTYGCTGP GLEGCPTNGP KIPSIATGMVGALLLLLVVALGIGLFM
58	V36 CAR (28z1xx-tEGFR)	MELGLSWVFLVAILEGVQCEVQLVESGGGLAQPG GSLRLSCAASGFTFSRYWMTWVRQAPGGRLEWV AKIKYDGSEKYYADSVKGRFTISRDNAKNSLYLQ MDSLRAEDTAVYYCTRDYNKDYWGQGTLVTVSS RAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPL FPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR

		SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRD FAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNEL QKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTA TKDTFDALHMQALPPR GSGATNFSLLKQAGDVEENPGP MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDS LSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTP PLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFE NLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISD GDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNR GENSCKATGQVCHALCSPEGCWGPEPRDCVSCRN VSRGRECVDKCNLLEGEPREFVENSECIQCHPECLP QAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAG VMGENNTLVWKYADAGHVCHLCHPNCTYGCTGP GLEGCPTNGP KIPSIATGMVGALLLLLVVALGIGLFM
59	V48 CAR (28z1xx- tEGFR)	MELGLSWVFLVAILEGVQCEVQLVESGGGLVQPG GSLRLTCAASGFTFSRYWMTWVRQAPGKGLEWV AKIRHDGGEKYYPDSVKGRFTVSRDNAKNSLYLQ MDNLRAEDTAMYYCTRDYNKDLWGQGTLVTVSS RAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPL FPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRD FAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNEL QKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTA TKDTFDALHMQALPPR GSGATNFSLLKQAGDVEENPGP

		MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDS LSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTP PLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFE NLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISD GDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNR GENSCKATGQVCHALCSPEGCWGPEPRDCVSCRN VSRGRECVDKCNLLEGEPREFVENSECIQCHPECLP QAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAG VMGENNTLVWKYADAGHVCHLCHPNCTYGCTGP GLEGCPTNGP KIPSIATGMVGALLLLLVVALGIGLFM
60	V51 CAR (28z1xx- tEGFR)	MELGLSWVFLVAILEGVQCEVQLVESGGGLVQPG GSLRLSCAASGFTFSRYWMTWVRQAPGKGLEWV AKIRHDGGEKYYADSVKGRFTISRDNAKNSLYLQ MNSLRAEDTAVYYCTRDYNKDYWGQGTLVTVSS RAAAIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPL FPGPSKPFWVLVVVGGVLACYSLLVTVAFIIFWVR SKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRD FAAYRSRVKFSRSADAPAYQQGQNQLYNELNLGR REEYDVLDKRRGRDPEMGGKPRRKNPQEGLFNEL QKDKMAEAFSEIGMKGERRRGKGHDGLFQGLSTA TKDTFDALHMQALPPR GSGATNFSLLKQAGDVEENPGP MLLLVTSLLLCELPHPAFLLIPRKVCNGIGIGEFKDS LSINATNIKHFKNCTSISGDLHILPVAFRGDSFTHTP PLDPQELDILKTVKEITGFLLIQAWPENRTDLHAFE NLEIIRGRTKQHGQFSLAVVSLNITSLGLRSLKEISD GDVIISGNKNLCYANTINWKKLFGTSGQKTKIISNR GENSCKATGQVCHALCSPEGCWGPEPRDCVSCRN VSRGRECVDKCNLLEGEPREFVENSECIQCHPECLP

		QAMNITCTGRGPDNCIQCAHYIDGPHCVKTCPAG VMGENNTLVWKYADAGHVCHLCHPNCTYGCTGP GLEGCPTNGP KIPSIATGMVGALLLLLVVALGIGLFM
61	V1-01 CAR Nucleic acid sequence	ATGGGCTGGTCTTGTATTATCTTGTTTCTCGTCG CAACTGCTACAGGCGTTCATTCTGAAGTTCAACT CCAAGAATCTGGTCCTGGCCTGGTGAAACCTTCC GAAACTCTCTCACTCACCTGTACCGTCTCAGGCG CTTCAATATCACACTATTATTGGTCTTGGTTCCG GCAACCTGCAGGCAAAGGTCTCGAATGGATAGG CAGAATATATCCCAGTGGAAGCACCTCCTATAA TCCCTCTCTCAAGTCAAGAGTTGCAATGAGCGTT GACACACCCAAGAACCAATTCTCCCTCAAGCTG TCATCTGTAACTGCCGCCGATACTGCCGTTTACT ACTGCGCTAGAGATAGATCAACCGGATGGTCAG AATGGAATTCAGATCTGTGGGGACGGGGAACCC TCGTGACCGTATCTTCTCGGGCGGCCGCAATTGA AGTTATGTATCCTCCTCCTTACCTAGACAATGAG AAGAGCAATGGAACCATTATCCATGTGAAAGGG AAACACCTTTGTCCAAGTCCCCTATTTCCCGGAC

		CTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGG TGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACA GTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGA GGAGCAGGCTCCTGCACAGTGACTACATGAACA TGACTCCCCGCCGCCCCGGGCCCACCCGCAAGC ATTACCAGCCCTATGCCCCACCACGCGACTTCGC AGCCTATCGCTCCAGAGTGAAGTTCAGCAGGAG CGCAGACGCCCCCGCGTACCAGCAGGGCCAGAA CCAGCTCTATAACGAGCTCAATCTAGGACGAAG AGAGGAGTACGATGTTTTGGACAAGAGACGTGG CCGGGACCCTGAGATGGGGGGAAAGCCGAGAA GGAAGAACCCTCAGGAAGGCCTGTTCAATGAAC TGCAGAAAGATAAGATGGCGGAGGCCTTCAGTG AGATTGGGATGAAAGGCGAGCGCCGGAGGGGC AAGGGGCACGATGGCCTTTTCCAGGGTCTCAGT ACAGCCACCAAGGACACCTTCGACGCCCTTCAC ATGCAGGCCCTGCCCCCTCGC
62	V5 CAR Nucleic acid sequence	ATGGAACTGGGACTGTCTTGGGTGTTCCTGGTCG CTATATTGGAAGGAGTACAGTGCCAGGTCCAGC TCGTCGAGTCCGGGGGTGGCCTGGTGCAGCCCG GCGGCAGCCTCCGGCTGAGCTGCACAGCCTCAG GGTTTACATTCAGCAGGTACTGGATGAGTTGGG TTAGGCAAGCCCCTGGCAAAGGCCTGGAGTGGG TGGCCAAAATCCGACATGATGGGGGCGAAAAGT ACTATGTGGATAGTGTGAAGGGACGGTTCACAA TATCACGAGACAATGCCAAAAACTCTTTGTACCT GCAAATGAACTCCCTGCGCGCCGAAGACACAGC TGTGTACTACTGCGCTACAGACTACACTAGGGA CGTCTGGGGTCAAGGAACAGCCGTCACCGTGAG TAGTCGGGCGGCCGCAATTGAAGTTATGTATCCT CCTCCTTACCTAGACAATGAGAAGAGCAATGGA

		ACCATTATCCATGTGAAAGGGAAACACCTTTGT CCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCT TTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGC TTGCTATAGCTTGCTAGTAACAGTGGCCTTTATT ATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTC CTGCACAGTGACTACATGAACATGACTCCCCGC CGCCCCGGGCCCACCCGCAAGCATTACCAGCCC TATGCCCCACCACGCGACTTCGCAGCCTATCGCT CCAGAGTGAAGTTCAGCAGGAGCGCAGACGCCC CCGCGTACCAGCAGGGCCAGAACCAGCTCTATA ACGAGCTCAATCTAGGACGAAGAGAGGAGTACG ATGTTTTGGACAAGAGACGTGGCCGGGACCCTG AGATGGGGGGAAAGCCGAGAAGGAAGAACCCT CAGGAAGGCCTGTTCAATGAACTGCAGAAAGAT AAGATGGCGGAGGCCTTCAGTGAGATTGGGATG AAAGGCGAGCGCCGGAGGGGCAAGGGGCACGA TGGCCTTTTCCAGGGTCTCAGTACAGCCACCAAG GACACCTTCGACGCCCTTCACATGCAGGCCCTGC CCCCTCGCTAA
63	V36 CAR Nucleic acid sequence	ATGGAGCTGGGATTGTCCTGGGTTTTCCTGGTGG CTATACTCGAAGGCGTACAGTGTGAAGTGCAGT TGGTGGAGAGTGGCGGTGGCCTGGCCCAGCCGG GAGGCTCTTTGAGACTCTCCTGCGCTGCCTCCGG CTTCACTTTCTCCCGCTATTGGATGACCTGGGTC CGGCAGGCGCCCGGCGGACGCCTGGAGTGGGTG GCTAAGATCAAGTATGATGGATCAGAAAAATAT TACGCAGATAGCGTAAAAGGCCGGTTCACAATA TCCAGGGATAATGCAAAAAACTCCCTGTATCTG CAGATGGATAGCCTGCGCGCTGAAGACACCGCC GTATATTATTGCACAAGAGACTACAATAAAGAT TACTGGGGCCAGGGAACCCTGGTTACGGTGAGC

		TCACGGGCGGCCGCAATTGAAGTTATGTATCCTC CTCCTTACCTAGACAATGAGAAGAGCAATGGAA CCATTATCCATGTGAAAGGGAAACACCTTTGTCC AAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTT TGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTT GCTATAGCTTGCTAGTAACAGTGGCCTTTATTAT TTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCT GCACAGTGACTACATGAACATGACTCCCCGCCG CCCCGGGCCCACCCGCAAGCATTACCAGCCCTA TGCCCCACCACGCGACTTCGCAGCCTATCGCTCC AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCC GCGTACCAGCAGGGCCAGAACCAGCTCTATAAC GAGCTCAATCTAGGACGAAGAGAGGAGTACGAT GTTTTGGACAAGAGACGTGGCCGGGACCCTGAG ATGGGGGGAAAGCCGAGAAGGAAGAACCCTCA GGAAGGCCTGTTCAATGAACTGCAGAAAGATAA GATGGCGGAGGCCTTCAGTGAGATTGGGATGAA AGGCGAGCGCCGGAGGGGCAAGGGGCACGATG GCCTTTTCCAGGGTCTCAGTACAGCCACCAAGG ACACCTTCGACGCCCTTCACATGCAGGCCCTGCC CCCTCGC
64	V48 CAR Nucleic acid sequence	ATGGAGCTGGGGCTTTCTTGGGTGTTTCTGGTAG CCATCCTCGAGGGAGTCCAGTGCGAGGTCCAGC TCGTCGAATCTGGCGGGGGGCTGGTCCAGCCTG GCGGTTCTCTCCGCCTGACCTGTGCGGCCTCAGG GTTCACTTTCAGCCGGTACTGGATGACATGGGTG AGACAGGCCCCCGGCAAGGGACTGGAATGGGTA GCAAAGATTAGGCACGACGGCGGTGAGAAATAC TATCCCGACAGTGTCAAGGGGCGGTTTACTGTCT CCCGAGATAATGCCAAAAACTCACTCTACCTGC AGATGGATAATCTGCGAGCGGAGGATACTGCTA

		TGTACTACTGTACTCGAGACTACAACAAGGACC TGTGGGGGCAGGGGACACTGGTGACGGTTAGTT CTCGGGCGGCCGCAATTGAAGTTATGTATCCTCC TCCTTACCTAGACAATGAGAAGAGCAATGGAAC CATTATCCATGTGAAAGGGAAACACCTTTGTCC AAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTT TGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTT GCTATAGCTTGCTAGTAACAGTGGCCTTTATTAT TTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCT GCACAGTGACTACATGAACATGACTCCCCGCCG CCCCGGGCCCACCCGCAAGCATTACCAGCCCTA TGCCCCACCACGCGACTTCGCAGCCTATCGCTCC AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCC GCGTACCAGCAGGGCCAGAACCAGCTCTATAAC GAGCTCAATCTAGGACGAAGAGAGGAGTACGAT GTTTTGGACAAGAGACGTGGCCGGGACCCTGAG ATGGGGGGAAAGCCGAGAAGGAAGAACCCTCA GGAAGGCCTGTTCAATGAACTGCAGAAAGATAA GATGGCGGAGGCCTTCAGTGAGATTGGGATGAA AGGCGAGCGCCGGAGGGGCAAGGGGCACGATG GCCTTTTCCAGGGTCTCAGTACAGCCACCAAGG ACACCTTCGACGCCCTTCACATGCAGGCCCTGCC CCCTCGC
65	V51 CAR Nucleic acid sequence	ATGGAACTGGGACTGTCATGGGTCTTTCTCGTGG CCATTCTCGAGGGGGTCCAGTGTGAGGTTCAGC TGGTGGAGAGCGGGGGGGGTCTGGTTCAGCCAG GTGGCAGTCTTAGGTTGTCATGTGCCGCGAGCG GGTTCACGTTCTCACGATATTGGATGACCTGGGT TCGCCAGGCACCAGGGAAGGGGCTGGAGTGGGT CGCCAAGATCAGGCACGACGGCGGAGAAAAAT ATTACGCGGATTCCGTGAAAGGCAGATTCACAA

		TCTCTAGGGATAACGCCAAAAATTCCCTTTATCT TCAGATGAATAGCCTGAGGGCTGAAGACACTGC CGTGTACTACTGCACGCGGGATTACAACAAAGA TTATTGGGGCCAGGGAACACTGGTGACCGTCAG CTCTCGGGCGGCCGCAATTGAAGTTATGTATCCT CCTCCTTACCTAGACAATGAGAAGAGCAATGGA ACCATTATCCATGTGAAAGGGAAACACCTTTGT CCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCT TTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGC TTGCTATAGCTTGCTAGTAACAGTGGCCTTTATT ATTTTCTGGGTGAGGAGTAAGAGGAGCAGGCTC CTGCACAGTGACTACATGAACATGACTCCCCGC CGCCCCGGGCCCACCCGCAAGCATTACCAGCCC TATGCCCCACCACGCGACTTCGCAGCCTATCGCT CCAGAGTGAAGTTCAGCAGGAGCGCAGACGCCC CCGCGTACCAGCAGGGCCAGAACCAGCTCTATA ACGAGCTCAATCTAGGACGAAGAGAGGAGTACG ATGTTTTGGACAAGAGACGTGGCCGGGACCCTG AGATGGGGGGAAAGCCGAGAAGGAAGAACCCT CAGGAAGGCCTGTTCAATGAACTGCAGAAAGAT AAGATGGCGGAGGCCTTCAGTGAGATTGGGATG AAAGGCGAGCGCCGGAGGGGCAAGGGGCACGA TGGCCTTTTCCAGGGTCTCAGTACAGCCACCAAG GACACCTTCGACGCCCTTCACATGCAGGCCCTGC CCCCTCGC
66	V1-01 CAR + tEGFR Nucleic Acid Sequence	ATGGGCTGGTCTTGTATTATCTTGTTTCTCGTCG CAACTGCTACAGGCGTTCATTCTGAAGTTCAACT CCAAGAATCTGGTCCTGGCCTGGTGAAACCTTCC GAAACTCTCTCACTCACCTGTACCGTCTCAGGCG CTTCAATATCACACTATTATTGGTCTTGGTTCCG GCAACCTGCAGGCAAAGGTCTCGAATGGATAGG

		CAGAATATATCCCAGTGGAAGCACCTCCTATAA
		TCCCTCTCTCAAGTCAAGAGTTGCAATGAGCGTT
		GACACACCCAAGAACCAATTCTCCCTCAAGCTG
		TCATCTGTAACTGCCGCCGATACTGCCGTTTACT
		ACTGCGCTAGAGATAGATCAACCGGATGGTCAG
		AATGGAATTCAGATCTGTGGGGACGGGGAACCC
		TCGTGACCGTATCTTCTCGGGCGGCCGCAATTGA
		AGTTATGTATCCTCCTCCTTACCTAGACAATGAG
		AAGAGCAATGGAACCATTATCCATGTGAAAGGG
		AAACACCTTTGTCCAAGTCCCCTATTTCCCGGAC
		CTTCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGG
		TGGAGTCCTGGCTTGCTATAGCTTGCTAGTAACA
		GTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGA
		GGAGCAGGCTCCTGCACAGTGACTACATGAACA
		TGACTCCCCGCCGCCCCGGGCCCACCCGCAAGC
		ATTACCAGCCCTATGCCCCACCACGCGACTTCGC
		AGCCTATCGCTCCAGAGTGAAGTTCAGCAGGAG
		CGCAGACGCCCCCGCGTACCAGCAGGGCCAGAA
		CCAGCTCTATAACGAGCTCAATCTAGGACGAAG
		AGAGGAGTACGATGTTTTGGACAAGAGACGTGG
		CCGGGACCCTGAGATGGGGGGAAAGCCGAGAA
		GGAAGAACCCTCAGGAAGGCCTGTTCAATGAAC
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		AGATTGGGATGAAAGGCGAGCGCCGGAGGGGC
		AAGGGGCACGATGGCCTTTTCCAGGGTCTCAGT
		ACAGCCACCAAGGACACCTTCGACGCCCTTCAC
		ATGCAGGCCCTGCCCCCTCGCGGAAGCGGAGCT
		ACTAACTTCAGCCTGCTGAAGCAGGCTGGAGAC
		GTGGAGGAGAACCCTGGACCCATGCTTCTCCTG
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		CGGAATAGGTATTGGTGAATTTAAAGACTCACT CTCCATAAATGCTACGAATATTAAACACTTCAA AAACTGCACCTCCATCAGTGGCGATCTCCACATC CTGCCGGTGGCATTTAGGGGTGACTCCTTCACAC ATACTCCTCCTCTGGACCCACAGGAACTGGATAT TCTGAAAACCGTAAAGGAAATCACAGGGTTTTT GCTGATTCAGGCTTGGCCTGAAAACAGGACGGA CCTCCATGCCTTTGAGAACCTAGAAATCATACGC GGCAGGACCAAGCAACATGGTCAGTTTTCTCTT GCAGTCGTCAGCCTGAACATAACATCCTTGGGA TTACGCTCCCTCAAGGAGATAAGTGATGGAGAT GTGATAATTTCAGGAAACAAAAATTTGTGCTAT GCAAATACAATAAACTGGAAAAAACTGTTTGGG ACCTCCGGTCAGAAAACCAAAATTATAAGCAAC AGAGGTGAAAACAGCTGCAAGGCCACAGGCCA GGTCTGCCATGCCTTGTGCTCCCCCGAGGGCTGC TGGGGCCCGGAGCCCAGGGACTGCGTCTCTTGC CGGAATGTCAGCCGAGGCAGGGAATGCGTGGAC AAGTGCAACCTTCTGGAGGGTGAGCCAAGGGAG TTTGTGGAGAACTCTGAGTGCATACAGTGCCAC CCAGAGTGCCTGCCTCAGGCCATGAACATCACC TGCACAGGACGGGGACCAGACAACTGTATCCAG TGTGCCCACTACATTGACGGCCCCCACTGCGTCA AGACCTGCCCGGCAGGAGTCATGGGAGAAAACA ACACCCTGGTCTGGAAGTACGCAGACGCCGGCC ATGTGTGCCACCTGTGCCATCCAAACTGCACCTA CGGATGCACTGGGCCAGGTCTTGAAGGCTGTCC CACGAATGGGCCTAAGATCCCGTCCATCGCCAC TGGGATGGTGGGGGCCCTCCTCTTGCTGCTGGTG GTGGCCCTGGGGATCGGCCTCTTCATG

67	V5 CAR + tEGFR Nucleic Acid Sequence	ATGGAACTGGGACTGTCTTGGGTGTTCCTGGTCG CTATATTGGAAGGAGTACAGTGCCAGGTCCAGC TCGTCGAGTCCGGGGGTGGCCTGGTGCAGCCCG GCGGCAGCCTCCGGCTGAGCTGCACAGCCTCAG GGTTTACATTCAGCAGGTACTGGATGAGTTGGG TTAGGCAAGCCCCTGGCAAAGGCCTGGAGTGGG TGGCCAAAATCCGACATGATGGGGGCGAAAAGT ACTATGTGGATAGTGTGAAGGGACGGTTCACAA TATCACGAGACAATGCCAAAAACTCTTTGTACCT GCAAATGAACTCCCTGCGCGCCGAAGACACAGC TGTGTACTACTGCGCTACAGACTACACTAGGGA CGTCTGGGGTCAAGGAACAGCCGTCACCGTGAG TAGTGCGGCCGCAATTGAAGTTATGTATCCTCCT CCTTACCTAGACAATGAGAAGAGCAATGGAACC ATTATCCATGTGAAAGGGAAACACCTTTGTCCA AGTCCCCTATTTCCCGGACCTTCTAAGCCCTTTT GGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTT GCTATAGCTTGCTAGTAACAGTGGCCTTTATTAT TTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCT GCACAGTGACTACATGAACATGACTCCCCGCCG CCCCGGGCCCACCCGCAAGCATTACCAGCCCTA TGCCCCACCACGCGACTTCGCAGCCTATCGCTCC AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCC GCGTACCAGCAGGGCCAGAACCAGCTCTATAAC GAGCTCAATCTAGGACGAAGAGAGGAGTACGAT GTTTTGGACAAGAGACGTGGCCGGGACCCTGAG ATGGGGGGAAAGCCGAGAAGGAAGAACCCTCA GGAAGGCCTGTTCAATGAACTGCAGAAAGATAA GATGGCGGAGGCCTTCAGTGAGATTGGGATGAA AGGCGAGCGCCGGAGGGGCAAGGGGCACGATG GCCTTTTCCAGGGTCTCAGTACAGCCACCAAGG

		ACACCTTCGACGCCCTTCACATGCAGGCCCTGCC
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		GCTGAAGCAGGCTGGAGACGTGGAGGAGAACC
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		GCTCTGTGAGTTACCACACCCAGCATTCCTCCTG
		ATCCCACGCAAAGTGTGTAACGGAATAGGTATT
		GGTGAATTTAAAGACTCACTCTCCATAAATGCTA
		CGAATATTAAACACTTCAAAAACTGCACCTCCA
		TCAGTGGCGATCTCCACATCCTGCCGGTGGCATT
		TAGGGGTGACTCCTTCACACATACTCCTCCTCTG
		GACCCACAGGAACTGGATATTCTGAAAACCGTA
		AAGGAAATCACAGGGTTTTTGCTGATTCAGGCTT
		GGCCTGAAAACAGGACGGACCTCCATGCCTTTG
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		GAACATAACATCCTTGGGATTACGCTCCCTCAA
		GGAGATAAGTGATGGAGATGTGATAATTTCAGG
		AAACAAAAATTTGTGCTATGCAAATACAATAAA
		CTGGAAAAAACTGTTTGGGACCTCCGGTCAGAA
		AACCAAAATTATAAGCAACAGAGGTGAAAACA
		GCTGCAAGGCCACAGGCCAGGTCTGCCATGCCT
		TGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAGC
		CCAGGGACTGCGTCTCTTGCCGGAATGTCAGCC
		GAGGCAGGGAATGCGTGGACAAGTGCAACCTTC
		TGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACT
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		CTCAGGCCATGAACATCACCTGCACAGGACGGG
		GACCAGACAACTGTATCCAGTGTGCCCACTACA
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		CAGGAGTCATGGGAGAAAACAACACCCTGGTCT
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		TGTGCCATCCAAACTGCACCTACGGATGCACTG GGCCAGGTCTTGAAGGCTGTCCCACGAATGGGC CTAAGATCCCGTCCATCGCCACTGGGATGGTGG GGGCCCTCCTCTTGCTGCTGGTGGTGGCCCTGGG GATCGGCCTCTTCATG
68	V36 CAR + tEGFR Nucleic Acid Sequence	ATGGAGCTGGGATTGTCCTGGGTTTTCCTGGTGG CTATACTCGAAGGCGTACAGTGTGAAGTGCAGT TGGTGGAGAGTGGCGGTGGCCTGGCCCAGCCGG GAGGCTCTTTGAGACTCTCCTGCGCTGCCTCCGG CTTCACTTTCTCCCGCTATTGGATGACCTGGGTC CGGCAGGCGCCCGGCGGACGCCTGGAGTGGGTG GCTAAGATCAAGTATGATGGATCAGAAAAATAT TACGCAGATAGCGTAAAAGGCCGGTTCACAATA TCCAGGGATAATGCAAAAAACTCCCTGTATCTG CAGATGGATAGCCTGCGCGCTGAAGACACCGCC GTATATTATTGCACAAGAGACTACAATAAAGAT TACTGGGGCCAGGGAACCCTGGTTACGGTGAGC TCACGGGCGGCCGCAATTGAAGTTATGTATCCTC CTCCTTACCTAGACAATGAGAAGAGCAATGGAA CCATTATCCATGTGAAAGGGAAACACCTTTGTCC AAGTCCCCTATTTCCCGGACCTTCTAAGCCCTTT TGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTT GCTATAGCTTGCTAGTAACAGTGGCCTTTATTAT TTTCTGGGTGAGGAGTAAGAGGAGCAGGCTCCT GCACAGTGACTACATGAACATGACTCCCCGCCG CCCCGGGCCCACCCGCAAGCATTACCAGCCCTA TGCCCCACCACGCGACTTCGCAGCCTATCGCTCC AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCC GCGTACCAGCAGGGCCAGAACCAGCTCTATAAC GAGCTCAATCTAGGACGAAGAGAGGAGTACGAT GTTTTGGACAAGAGACGTGGCCGGGACCCTGAG

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		AGGCGAGCGCCGGAGGGGCAAGGGGCACGATG
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		GCTGAAGCAGGCTGGAGACGTGGAGGAGAACC
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		GCTCTGTGAGTTACCACACCCAGCATTCCTCCTG
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		TGCCCCACCACGCGACTTCGCAGCCTATCGCTCC AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCC GCGTACCAGCAGGGCCAGAACCAGCTCTATAAC GAGCTCAATCTAGGACGAAGAGAGGAGTACGAT GTTTTGGACAAGAGACGTGGCCGGGACCCTGAG ATGGGGGGAAAGCCGAGAAGGAAGAACCCTCA GGAAGGCCTGTTCAATGAACTGCAGAAAGATAA GATGGCGGAGGCCTTCAGTGAGATTGGGATGAA AGGCGAGCGCCGGAGGGGCAAGGGGCACGATG GCCTTTTCCAGGGTCTCAGTACAGCCACCAAGG ACACCTTCGACGCCCTTCACATGCAGGCCCTGCC CCCTCGCGGAAGCGGAGCTACTAACTTCAGCCT GCTGAAGCAGGCTGGAGACGTGGAGGAGAACC CTGGACCCATGCTTCTCCTGGTGACAAGCCTTCT GCTCTGTGAGTTACCACACCCAGCATTCCTCCTG ATCCCACGCAAAGTGTGTAACGGAATAGGTATT GGTGAATTTAAAGACTCACTCTCCATAAATGCTA CGAATATTAAACACTTCAAAAACTGCACCTCCA TCAGTGGCGATCTCCACATCCTGCCGGTGGCATT TAGGGGTGACTCCTTCACACATACTCCTCCTCTG GACCCACAGGAACTGGATATTCTGAAAACCGTA AAGGAAATCACAGGGTTTTTGCTGATTCAGGCTT GGCCTGAAAACAGGACGGACCTCCATGCCTTTG AGAACCTAGAAATCATACGCGGCAGGACCAAGC AACATGGTCAGTTTTCTCTTGCAGTCGTCAGCCT GAACATAACATCCTTGGGATTACGCTCCCTCAA GGAGATAAGTGATGGAGATGTGATAATTTCAGG AAACAAAAATTTGTGCTATGCAAATACAATAAA CTGGAAAAAACTGTTTGGGACCTCCGGTCAGAA AACCAAAATTATAAGCAACAGAGGTGAAAACA GCTGCAAGGCCACAGGCCAGGTCTGCCATGCCT

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70	V51 CAR + tEGFR Nucleic Acid Sequence	ATGGAACTGGGACTGTCATGGGTCTTTCTCGTGG CCATTCTCGAGGGGGTCCAGTGTGAGGTTCAGC TGGTGGAGAGCGGGGGGGGTCTGGTTCAGCCAG GTGGCAGTCTTAGGTTGTCATGTGCCGCGAGCG GGTTCACGTTCTCACGATATTGGATGACCTGGGT TCGCCAGGCACCAGGGAAGGGGCTGGAGTGGGT CGCCAAGATCAGGCACGACGGCGGAGAAAAAT ATTACGCGGATTCCGTGAAAGGCAGATTCACAA TCTCTAGGGATAACGCCAAAAATTCCCTTTATCT TCAGATGAATAGCCTGAGGGCTGAAGACACTGC CGTGTACTACTGCACGCGGGATTACAACAAAGA TTATTGGGGCCAGGGAACACTGGTGACCGTCAG CTCTCGGGCGGCCGCAATTGAAGTTATGTATCCT CCTCCTTACCTAGACAATGAGAAGAGCAATGGA ACCATTATCCATGTGAAAGGGAAACACCTTTGT

		CCAAGTCCCCTATTTCCCGGACCTTCTAAGCCCT
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		CCGCGTACCAGCAGGGCCAGAACCAGCTCTATA
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		AGATGGGGGGAAAGCCGAGAAGGAAGAACCCT
		CAGGAAGGCCTGTTCAATGAACTGCAGAAAGAT
		AAGATGGCGGAGGCCTTCAGTGAGATTGGGATG
		AAAGGCGAGCGCCGGAGGGGCAAGGGGCACGA
		TGGCCTTTTCCAGGGTCTCAGTACAGCCACCAAG
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		CCCCTCGCGGAAGCGGAGCTACTAACTTCAGCC
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		CTGGACCCATGCTTCTCCTGGTGACAAGCCTTCT
		GCTCTGTGAGTTACCACACCCAGCATTCCTCCTG
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		GGTGAATTTAAAGACTCACTCTCCATAAATGCTA
		CGAATATTAAACACTTCAAAAACTGCACCTCCA
		TCAGTGGCGATCTCCACATCCTGCCGGTGGCATT
		TAGGGGTGACTCCTTCACACATACTCCTCCTCTG
		GACCCACAGGAACTGGATATTCTGAAAACCGTA
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		GGCCTGAAAACAGGACGGACCTCCATGCCTTTG
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		GAACATAACATCCTTGGGATTACGCTCCCTCAA GGAGATAAGTGATGGAGATGTGATAATTTCAGG AAACAAAAATTTGTGCTATGCAAATACAATAAA CTGGAAAAAACTGTTTGGGACCTCCGGTCAGAA AACCAAAATTATAAGCAACAGAGGTGAAAACA GCTGCAAGGCCACAGGCCAGGTCTGCCATGCCT TGTGCTCCCCCGAGGGCTGCTGGGGCCCGGAGC CCAGGGACTGCGTCTCTTGCCGGAATGTCAGCC GAGGCAGGGAATGCGTGGACAAGTGCAACCTTC TGGAGGGTGAGCCAAGGGAGTTTGTGGAGAACT CTGAGTGCATACAGTGCCACCCAGAGTGCCTGC CTCAGGCCATGAACATCACCTGCACAGGACGGG GACCAGACAACTGTATCCAGTGTGCCCACTACA TTGACGGCCCCCACTGCGTCAAGACCTGCCCGG CAGGAGTCATGGGAGAAAACAACACCCTGGTCT GGAAGTACGCAGACGCCGGCCATGTGTGCCACC TGTGCCATCCAAACTGCACCTACGGATGCACTG GGCCAGGTCTTGAAGGCTGTCCCACGAATGGGC CTAAGATCCCGTCCATCGCCACTGGGATGGTGG GGGCCCTCCTCTTGCTGCTGGTGGTGGCCCTGGG GATCGGCCTCTTCATG

Equivalents

[0566] Use of ordinal terms such as "first," "second," "third," etc., in the claims to modify a claim element does not by itself connote any priority, precedence, or order of one claim element over another or the temporal order in which acts of a method are performed, but are used merely as labels to distinguish one claim element having a certain name from another element having a same name (but for use of the ordinal term) to distinguish the claim elements.

[0567] The articles "a" and "an" as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to include the plural referents. Claims or descriptions that include "or" between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention also includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process. Furthermore, it is to be understood that the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, descriptive terms, etc., from one or more of the listed claims is introduced into another claim dependent on the same base claim (or, as relevant, any other claim) unless otherwise indicated or unless it would be evident to one of ordinary skill in the art that a contradiction or inconsistency would arise. Where elements are presented as lists, (e.g., in Markush group or similar format) it is to be understood that each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements, features, etc., certain embodiments of the invention or aspects of the invention consist, or consist essentially of, such elements, features, etc. For purposes of simplicity those embodiments have not in every case been specifically set forth in so many words herein. It should also be understood that any embodiment or aspect of the invention can be explicitly excluded from the claims, regardless of whether the specific exclusion is recited in the specification. The publications, websites and other reference materials referenced herein to

describe the background of the invention and to provide additional detail regarding its practice are hereby incorporated by reference.

We claim:

1. An anti-guanylyl cyclase C (GCC) chimeric antigen receptor (CAR) comprising an extracellular antigen binding domain that binds to guanylyl cyclase C (GCC), a transmembrane domain, and at least one intracellular signaling domain.

2. The anti-GCC CAR of claim 1, wherein the antigen binding domain comprises

a heavy chain variable region (V_H) with complementarity determining region (CDR) sequences of HYYWS (HCDR1) (SEQ ID NO: 8), RIYPSGSTSYNPSLKS (HCDR2) (SEQ ID NO: 11) and DRSTGWSEWNSDL (HCDR3) (SEQ ID NO: 16);

a heavy chain variable region (V_H) with complementarity determining region (CDR) sequences of RYWMS (HCDR1) (SEQ ID NO: 9), KIRHDGGEKYYVDSVKG (HCDR2) (SEQ ID NO: 12) and DYTRDV (HCDR3) (SEQ ID NO: 17);

a heavy chain variable region (V_H) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIKYDGSEKYYADSVKG (HCDR2) (SEQ ID NO: 13) and DYNKDY (HCDR3) (SEQ ID NO: 18);

a heavy chain variable region (V_H) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIRHDGGEKYYPDSVKG (HCDR2) (SEQ ID NO: 14) and DYNKDL (HCDR3) (SEQ ID NO: 19) or

a heavy chain variable region (V_H) with complementarity determining region (CDR) sequences of RYWMT (HCDR1) (SEQ ID NO: 10), KIRHDGGEKYYADSVKG (HCDR2) (SEQ ID NO: 15) and DYNKDY (HCDR3) (SEQ ID NO: 18).

3. The anti-GCC CAR of claim 1, wherein the antigen binding domain comprises a heavy chain variable region (VH) that is at least 90% identical to SEQ ID NO: 1 or SEQ ID NO: 20.

4. The anti-GCC CAR of claim 1, wherein the antigen binding domain comprises

an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 21;

an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 26;

an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 27; or

an immunoglobulin heavy chain variable (VH) region comprising an amino acid sequence that is at least 90% identical to SEQ ID NO: 28.

5. The anti-GCC CAR of any one of claims 1-4, wherein the antigen binding domain comprises an immunoglobulin variable heavy chain only anti-GCC antigen binding domain.

6. The anti-GCC CAR of any one of claims 1-5, wherein the extracellular anti-GCC antigen binding domain is preceded by a leader nucleotide sequence encoding a leader peptide.

7. The anti-GCC CAR of claim 28, wherein the leader peptide comprises SEQ ID NO: 6, SEQ ID NO: 7, or SEQ ID NO: 42.

8. The anti-GCC CAR of any one of claims 1-7, further comprising a hinge domain.

9. The anti-GCC CAR of claim 8, wherein the hinge domain is comprises a hinge domain of CD28.

10. The anti-GCC CAR of claim 9, wherein the CD28 hinge domain comprises SEQ ID NO: 29

11. The anti-GCC CAR of any one of claims 8-10, wherein the hinge domain is fused to the transmembrane domain.

12. The anti-GCC CAR of any one of claims 1-11, wherein the transmembrane domain comprises a transmembrane domain of a protein selected from the alpha, beta or zeta chain of the T-cell receptor, CD8, CD28, CD3 epsilon, CD45, CD4, CD5, CD8, CD9, CD16, CD22, CD33, CD37, CD64, CD80, CD83, CD86, CD134, CD137, CD154, and TNFRSF19, and any combination thereof.

13. The anti-GCC CAR of claim 12, wherein the transmembrane domain comprises a CD28 transmembrane domain.

14. The anti-GCC CAR of claim 13, wherein the CD28 transmembrane domain comprises SEQ ID NO: 30.

15. The anti-GCC CAR of any one of claims 1-14, wherein the at least one intracellular signaling domain comprises a costimulatory domain and a primary signaling domain.

16. The anti-GCC CAR of claim 15, wherein the costimulatory domain comprises a functional signaling domain of OX40, CD70, CD27, CD28, CD5, ICAM-1, LFA-1 (CD11a/CD18), ICOS (CD278), DAP10, DAP12, and 4-1BB (CD137), or any combination thereof.

17. The anti-GCC CAR of claim 16, wherein the costimulatory domain comprises a functional signaling domain of CD28.

18. The anti-GCC CAR of claim 17, wherein the CD28 costimulatory domain comprises SEQ ID NO: 32.

19. The anti-GCC CAR of claim 15-18, wherein the primary signaling domain comprises a CD3zeta signaling domain.

20. The anti-GCC CAR of claim 19, wherein the CD3 zeta signaling domain comprises SEQ ID NO: 33.
21. An anti-GCC CAR comprising a sequence selected from the group consisting of SEQ ID NO: 47-52.

22. An isolated polynucleotide encoding the anti-GCC CAR of any one of claims 1-21.

23. The isolated polynucleotide of claim 22, further comprising a truncated sequence of epidermal growth factor receptor (tEGFR).

24. The isolated polynucleotide of claim 23, wherein the tEGFR comprises a nucleic acid sequence that encodes an amino acid sequence that is at least 95%, 96%, 97%, 98%, 99% identical to SEQ ID NO: 43.

25. The isolated polynucleotide of claim 24, wherein the tEGFR comprises a nucleic acid sequence that encodes an amino acid sequence that is identical to SEQ ID NO: 43.

26. The isolated polynucleotide of any one of claims 22-25, further comprising a furin recognition site and downstream 2A self-cleaving peptide sequence, designed for simultaneous bicistronic expression of the tag sequence and the CAR sequence.

27. The isolated polynucleotide of claim 26, wherein the 2A self-cleaving peptide is selected from F2A, P2A, E2A and T2A.

28. The isolated polynucleotide of claim 27, wherein the 2A self-cleaving peptide is P2A.

29. A vector comprising the isolated polynucleotide of any one of claims 22-28.

30. The vector of claim 29, wherein the vector is an adenoviral vector, an adenovirus-associated vector, a DNA vector, a lentiviral vector, a plasmid, a retroviral vector, or an RNA vector.

31. A cell comprising the vector of claim 29 or 30.

32. The cell of claim 31, wherein the cell is a T cell, an allogeneic T cell, an autologous T cell, or a tumor-infiltrating lymphocyte (TIL).

33. A population of cells comprising the anti-GCC CAR of any one of claims 1-20or the nucleic acid of any one of claims 22-32.

34. A kit comprising the population of cells of claim 33.

35. A pharmaceutical composition comprising a population of the cells of claim 31 or 32.

36. The pharmaceutical composition of claim 35, wherein greater than 70%, 80%, 90%, or 95% of the cells in the population express the anti-GCC CAR.

37. A method of treating a cancer comprising administering a pharmaceutical composition or population of cells comprising the anti-GCC CAR of any one of claims 1-20 to a subject in need of treatment.

38. The method of claim 37, wherein the cancer is selected from gastrointestinal cancer, colorectal cancer, colorectal adenocarcinoma, colorectal leiomyosarcoma, colorectal lymphoma, colorectal melanoma, a colorectal neuroendocrine tumor, metastatic colon cancer, stomach cancer, gastric adenocarcinoma, gastric lymphoma, gastric sarcoma, esophageal cancer, squamous cell carcinoma, adenocarcinoma of the esophagus, or pancreatic cancer.

39. The method of claim 38, wherein the cancer is a gastrointestinal cancer.

40. The method of claim 39, wherein the gastrointestinal cancer is colon cancer, colorectal cancer, stomach cancer, or esophageal cancer.

41. A method of reducing tumor growth or tumor size comprising administering a pharmaceutical composition or population of cells comprising the anti-GCC CAR of any one of claims 1-21 to a subject in need of treatment


CD3 1XX
CD28 costimu
CD28 hinge/TM
VH

FIG. 1A

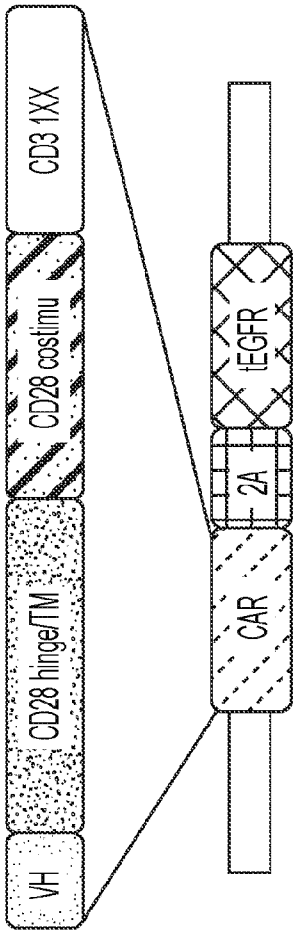

CD3 1XX
CD28 costimu
CD28 hinge/TM
VH
tEGFR
2A
CAR

FIG. 1B

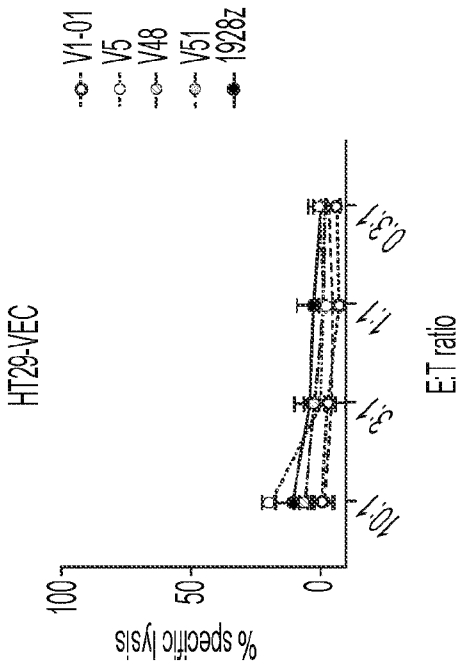


FIG. 2A

FIG. 2B

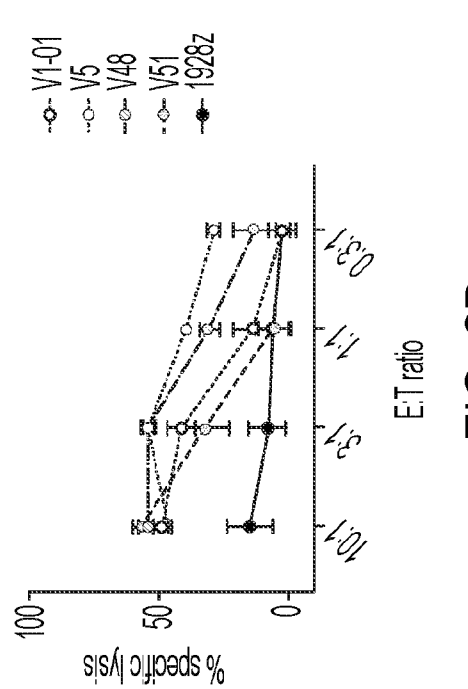


FIG. 2C

FIG. 2D



FIG. 3B

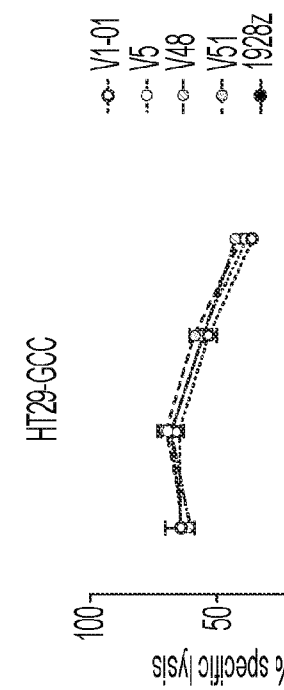


FIG. 3A

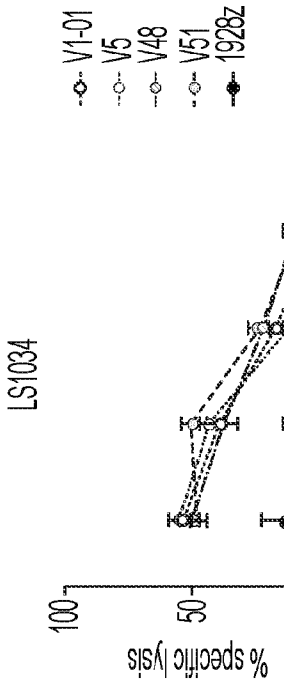


FIG. 3D

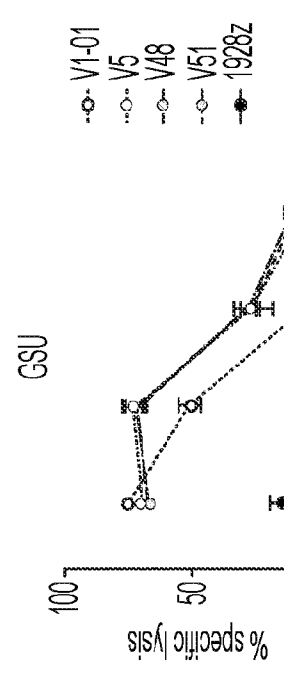


FIG. 3C

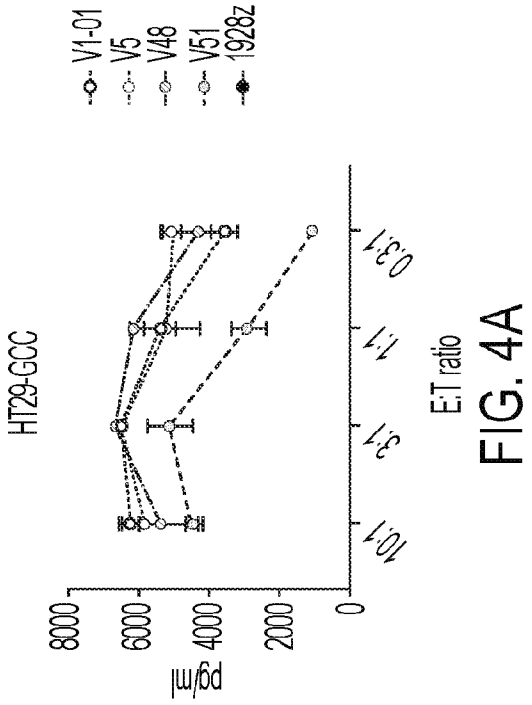


FIG. 4A

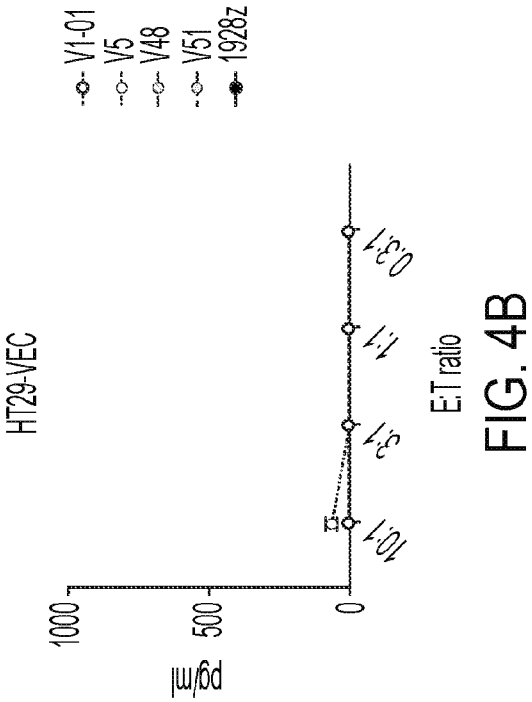


FIG. 4B

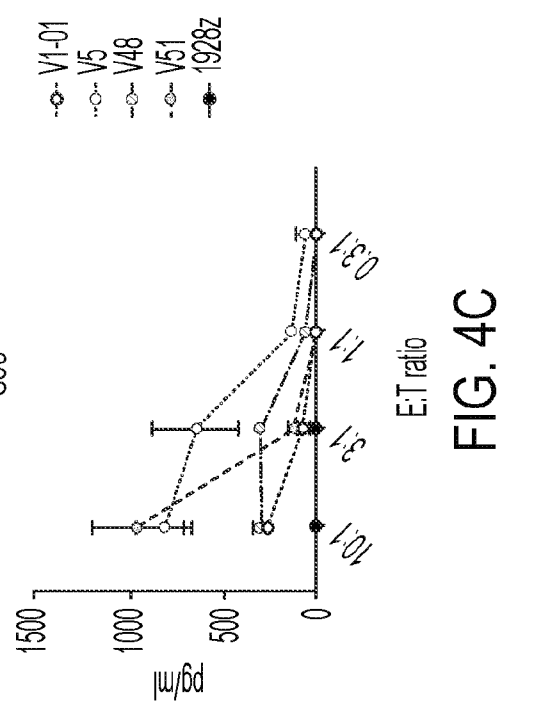


FIG. 4C

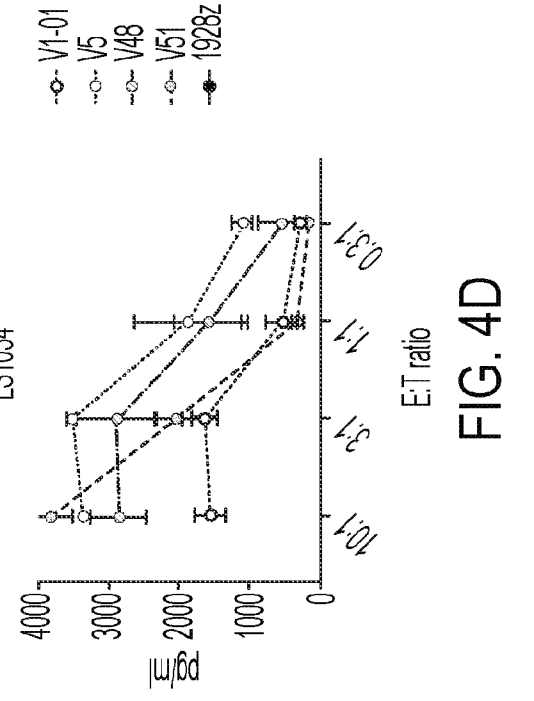


FIG. 4D

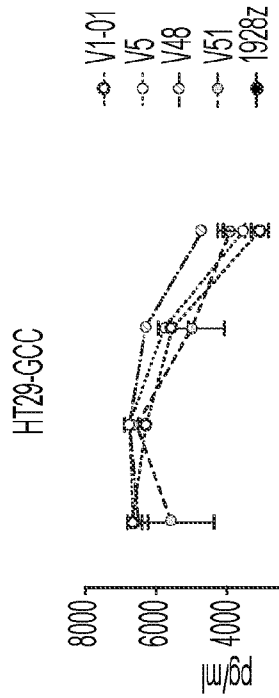


FIG. 5A



FIG. 5B

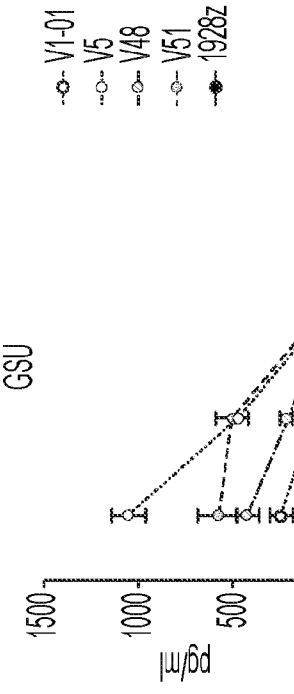


FIG. 5C

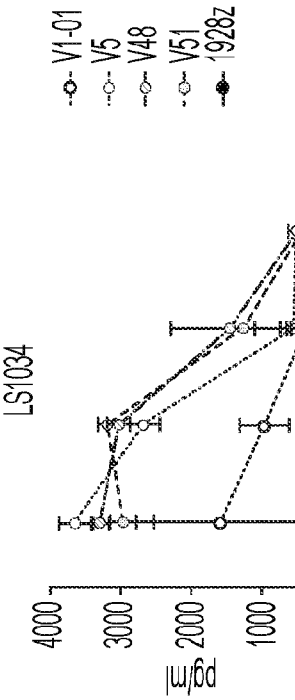


FIG. 5D

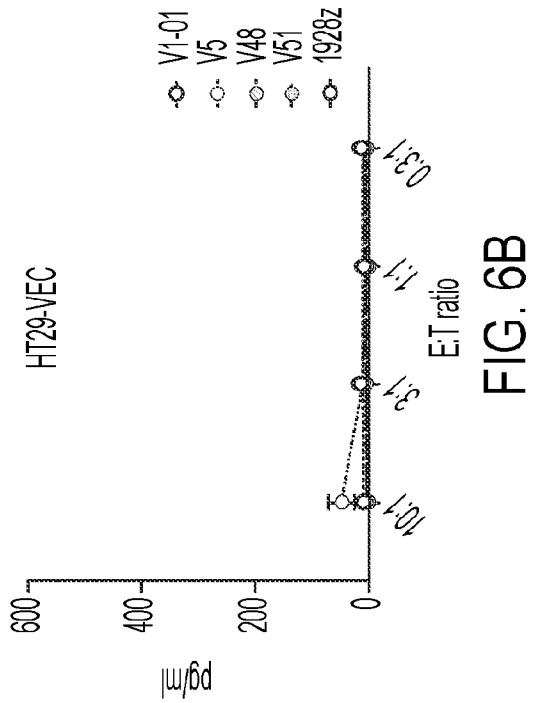


FIG. 6B

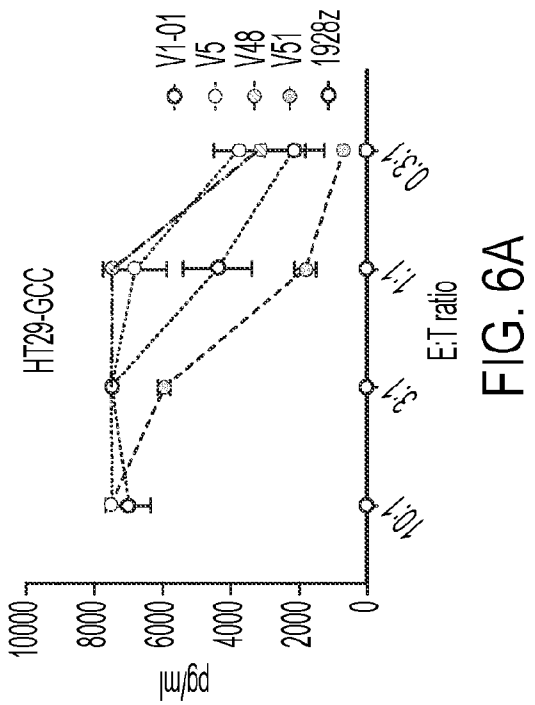


FIG. 6A

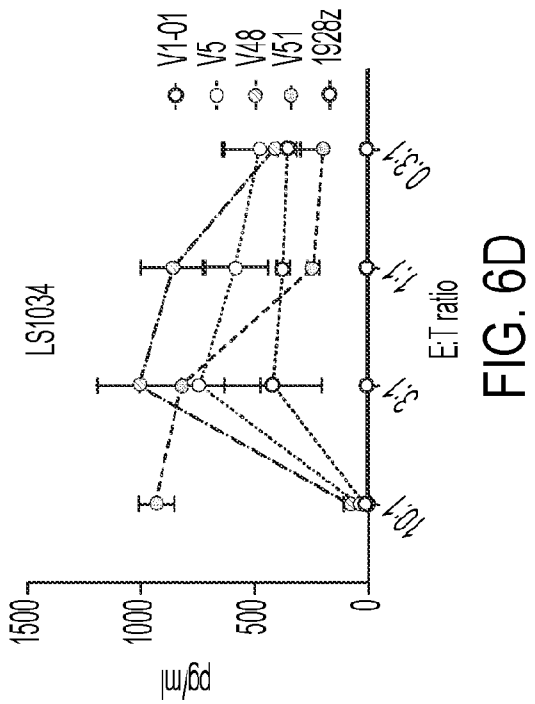


FIG. 6D

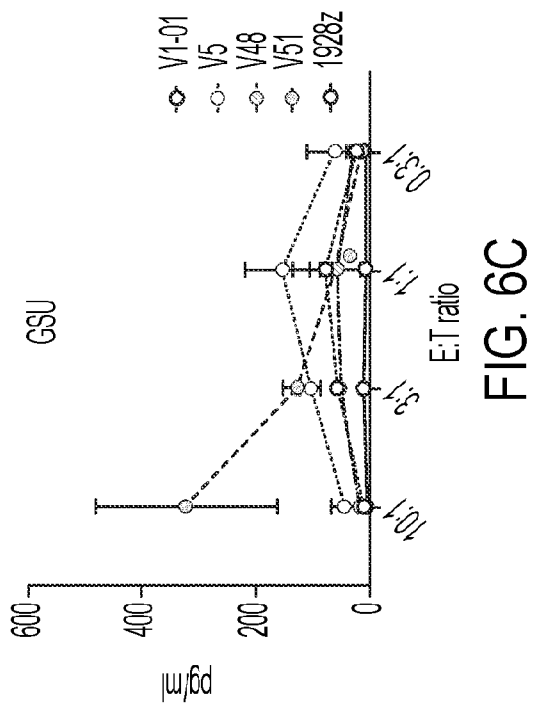


FIG. 6C

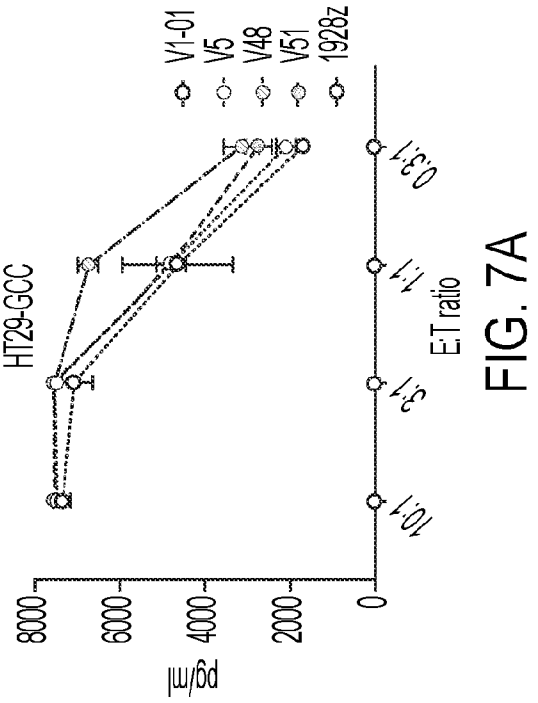


FIG. 7A

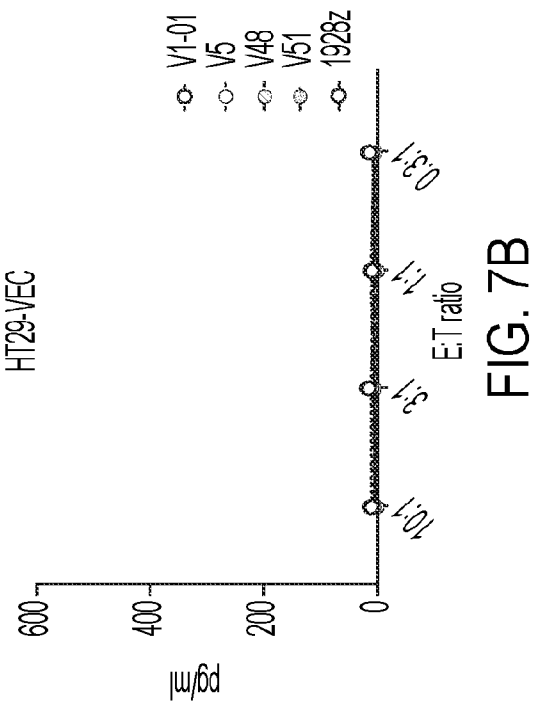


FIG. 7B

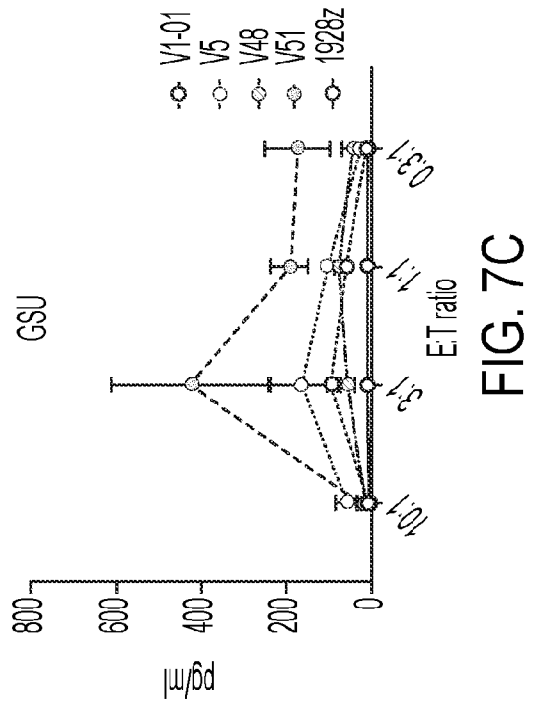


FIG. 7C

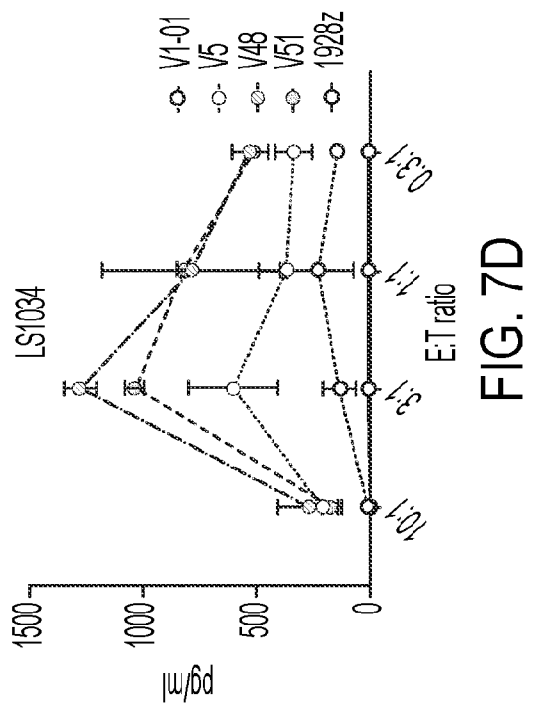


FIG. 7D

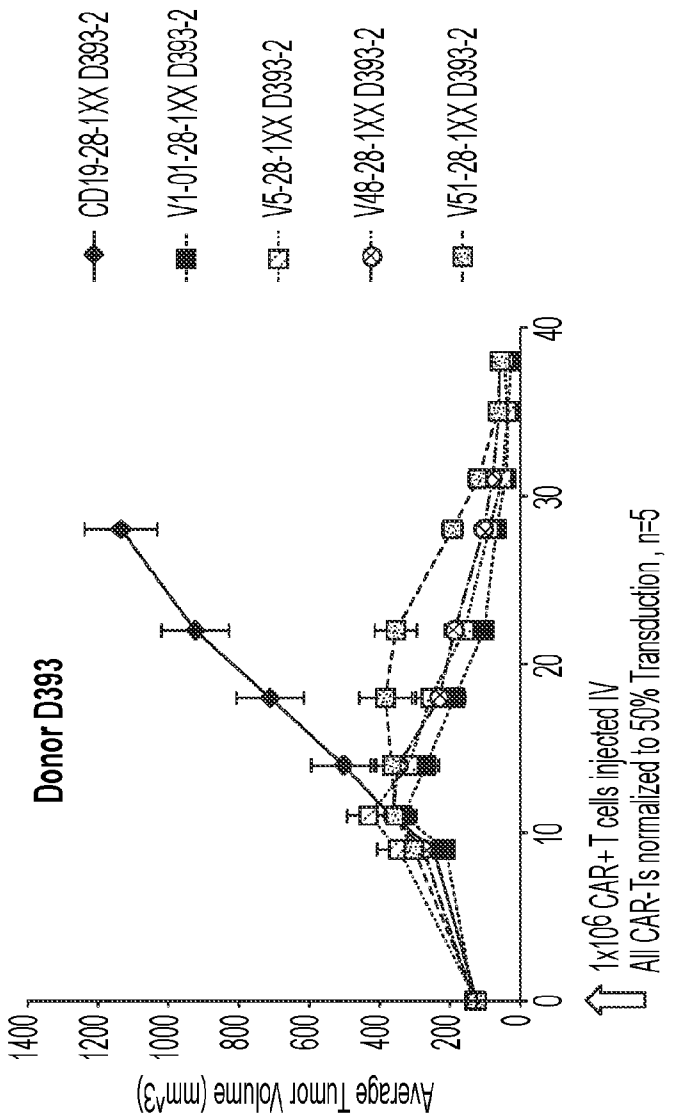

Donor D393
Average Tumor Volume (mm^3)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
1x10^6 CAR+ T cells injected IV
All CAR-Ts normalized to 50% Transduction , n=5
CD19-28-1XX D393-2
V1-01-28-1XX D393-2
V5-28-1XX D393-2
V48-28-1XX D393-2
V51-28-1XX D393-2

FIG. 8A

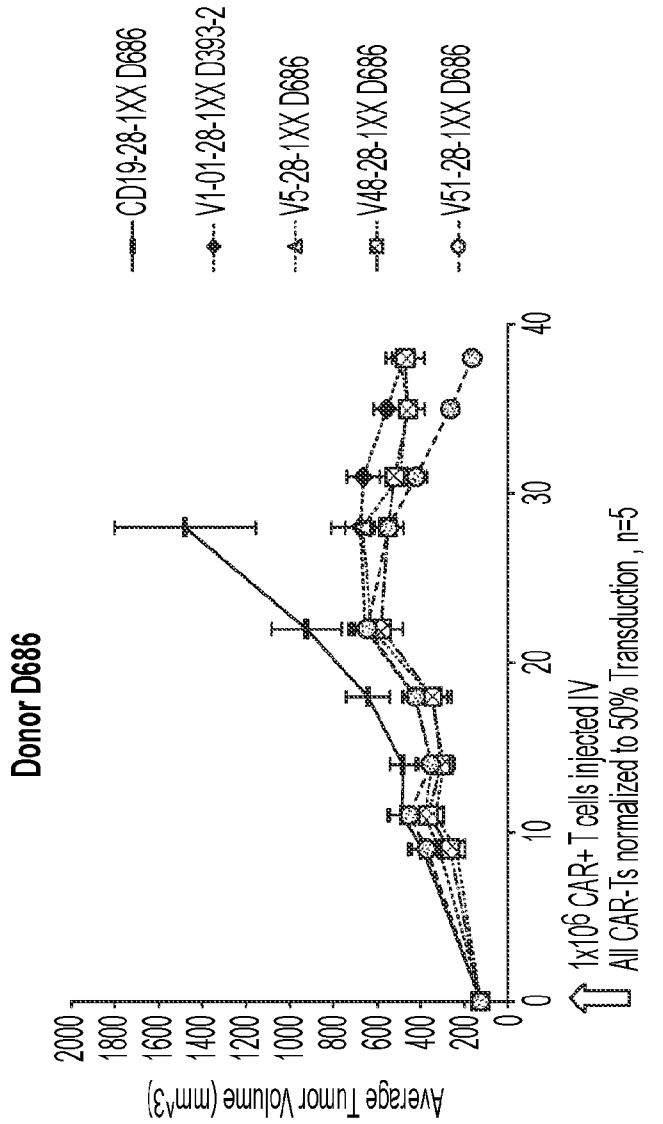

Donor D686
CD19-28-1XX D686
V1-01-28-1XX D393-2
V5-28-1XX D686
V48-28-1XX D686
V51-28-1XX D686
Average Tumor Volume (mm^3)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
1x10^6 CAR+ T cells injected IV
All CAR-Ts normalized to 50% Transduction , n=5

FIG. 8B

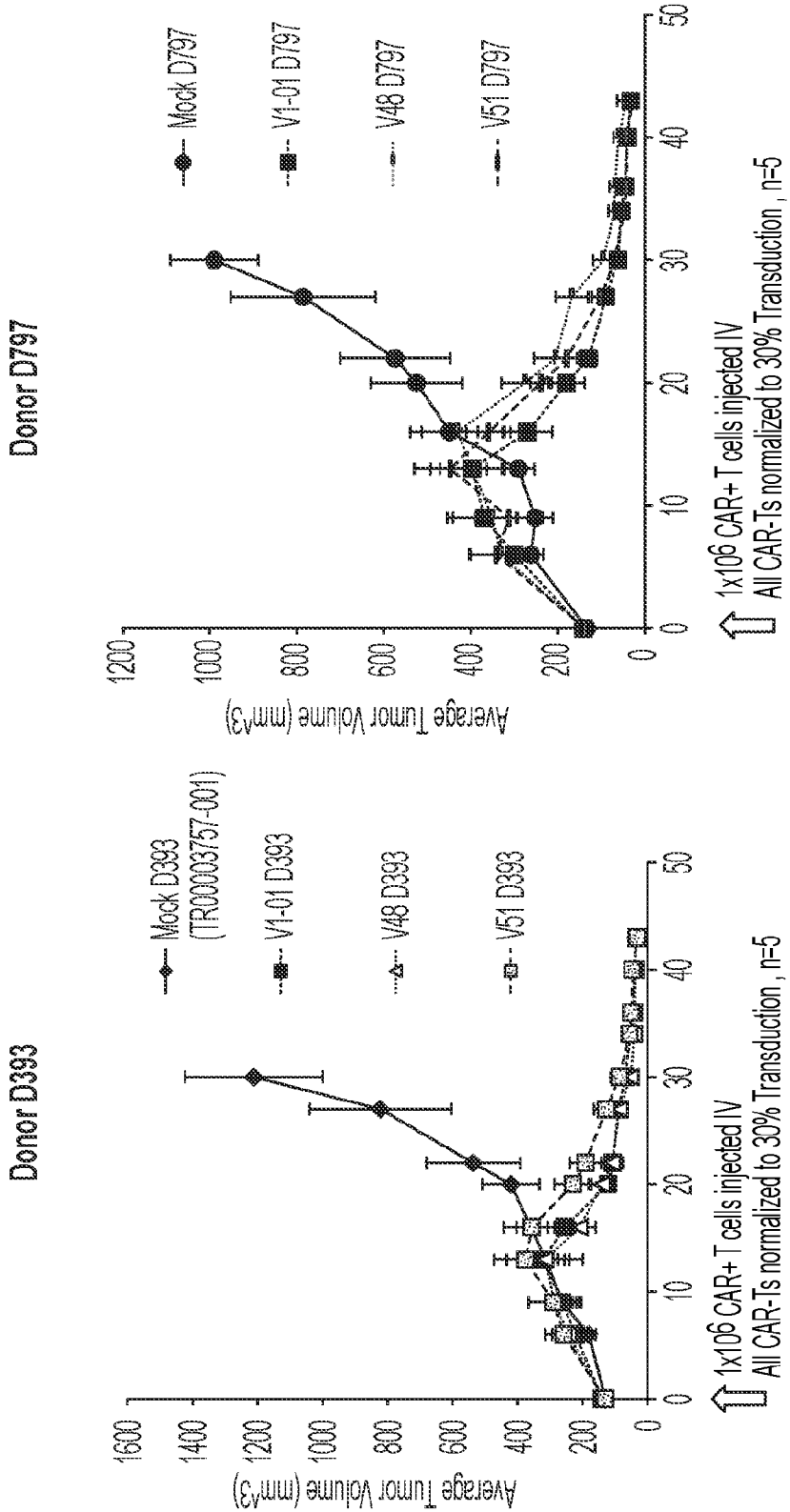

Donor D393
Mock D393 (TR0003757-001)
V1-01 D393
V48 D393
V51 D393
Average Tumor Volume (mm^3)
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
1x10^6 CAR+ T cells injected IV
All CAR-Ts normalized to 30% Transduction , n=5

FIG. 9A

Donor D797
Mock D797
V1-01 D797
V48 D797
V51 D797
Average Tumor Volume (mm^3)
1200
1000
800
600
400
200
0
0
10
20
30
40
50
1x10^6 CAR+ T cells injected IV
All CAR-Ts normalized to 30% Transduction , n=5

FIG. 9B

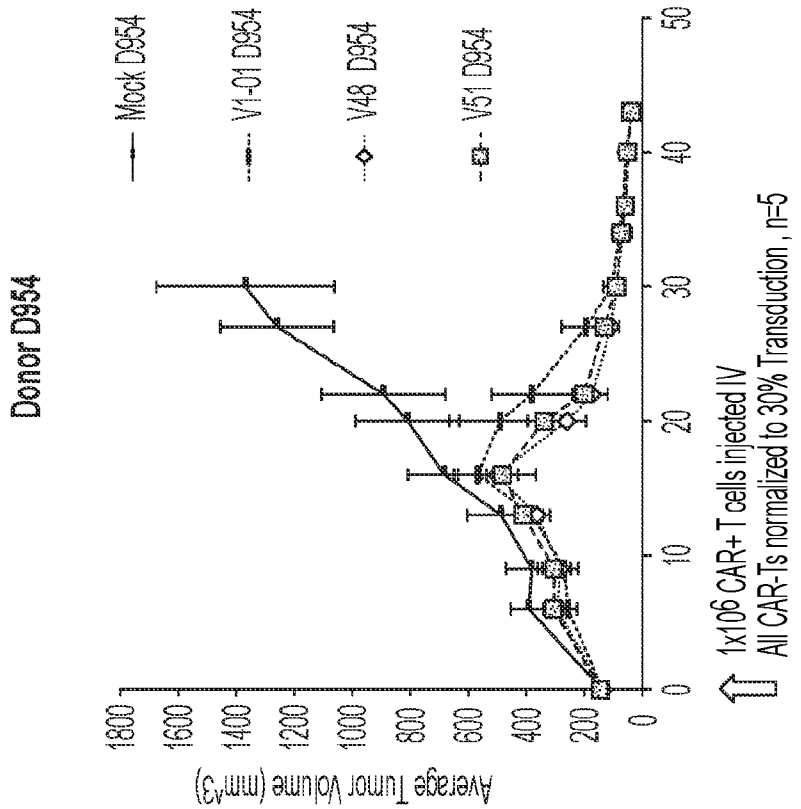

Donor D954
Mock D954
V1-01 D954
V48 D954
V51 D954
Average Tumor Volume (mm^3)
1800
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
1x10^6 CAR+ T cells injected IV
All CAR-Ts normalized to 30% Transduction , n=5

FIG. 9C

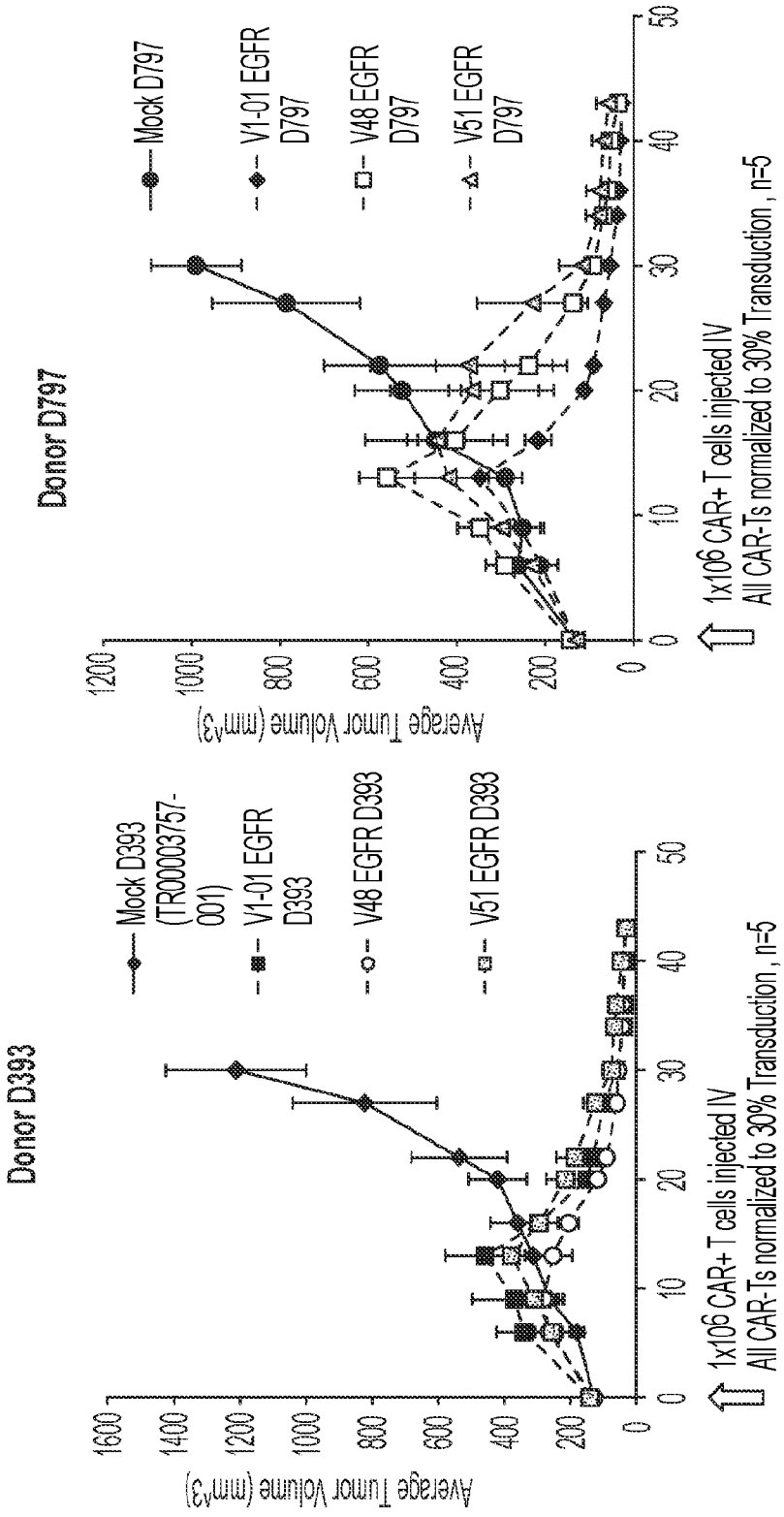


FIG. 10A

FIG. 10B

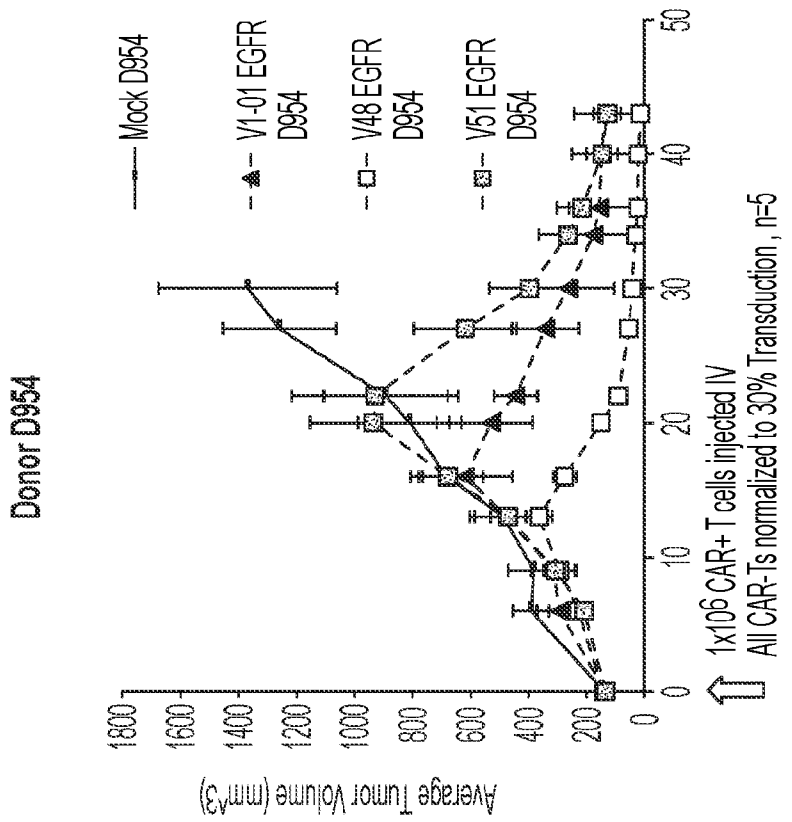

Donor D954
Mock D954
V1-01 EGFR D954
V48 EGFR D954
V51 EGFR D954
Average Tumor Volume (mm^3)
1800
1600
1400
1200
1000
800
600
400
200
0
10
20
30
40
50
1x10^6 CAR+ T cells injected IV
All CAR-Ts normalized to 30% Transduction , n=5

FIG. 10C

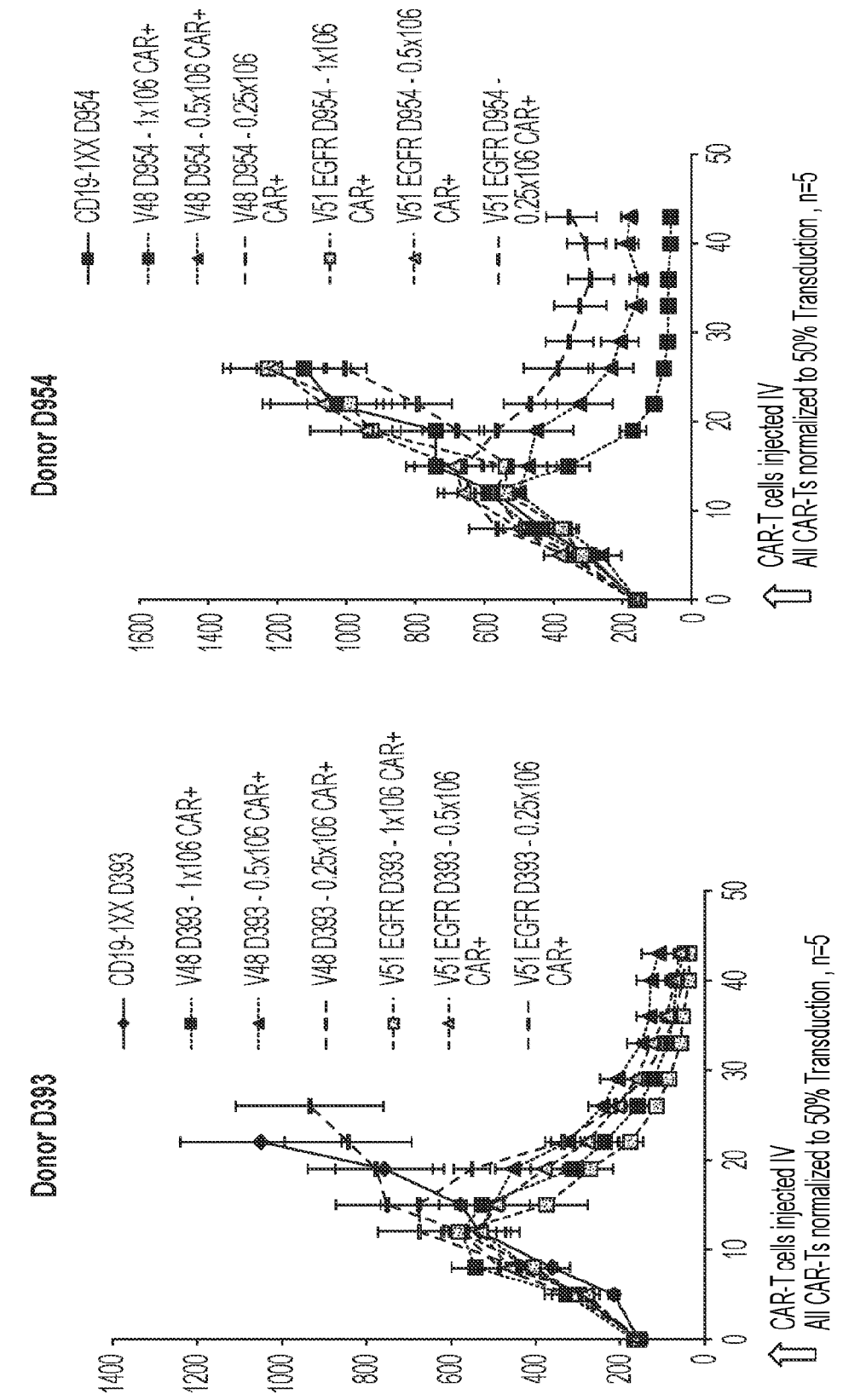


FIG. 11A

FIG. 11B

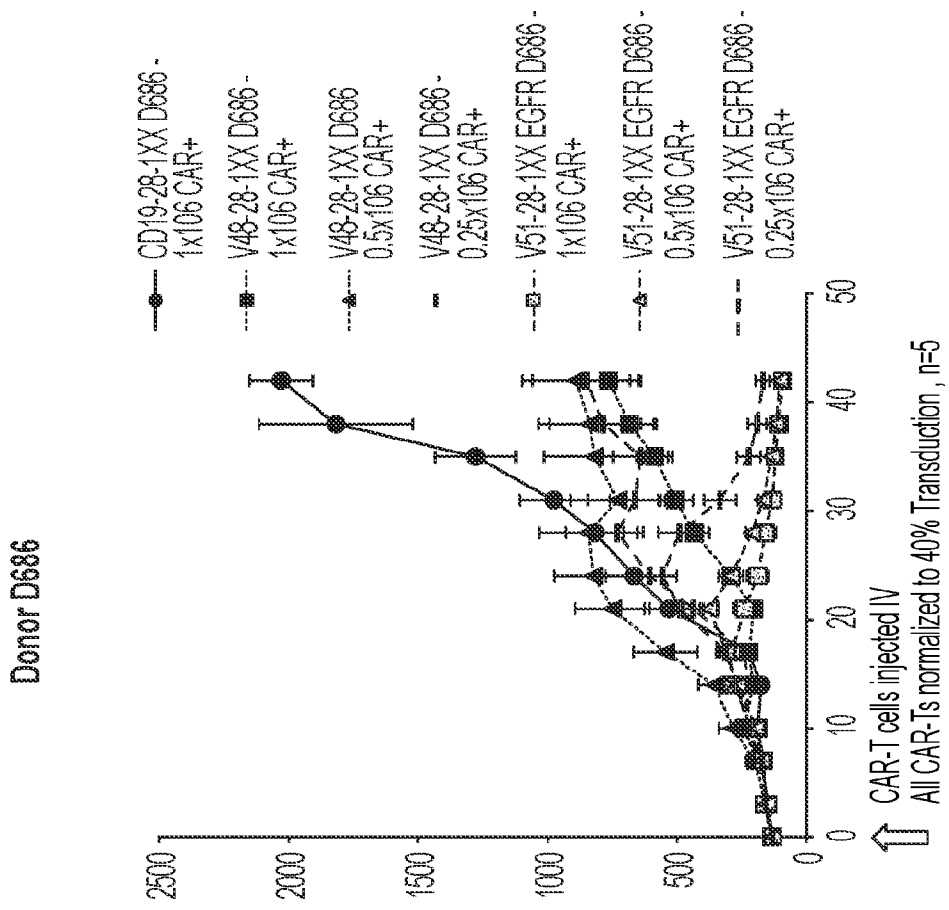

Donor D686
CD19-28-1XX D686 - 1x106 CAR+
V48-28-1XX D686 - 1x106 CAR+
V48-28-1XX D686 0.5x106 CAR+
V48-28-1XX D686 - 0.25x106 CAR+
V51-28-1XX EGFR D686 - 1x106 CAR+
V51-28-1XX EGFR D686 - 0.5x106 CAR+
V51-28-1XX EGFR D686 - 0.25x106 CAR+
2500
2000
1500
1000
500
0
0
10
20
30
40
50
CAR-T cells injected IV
All CAR-Ts normalized to 40% Transduction , n=5

FIG. 11C

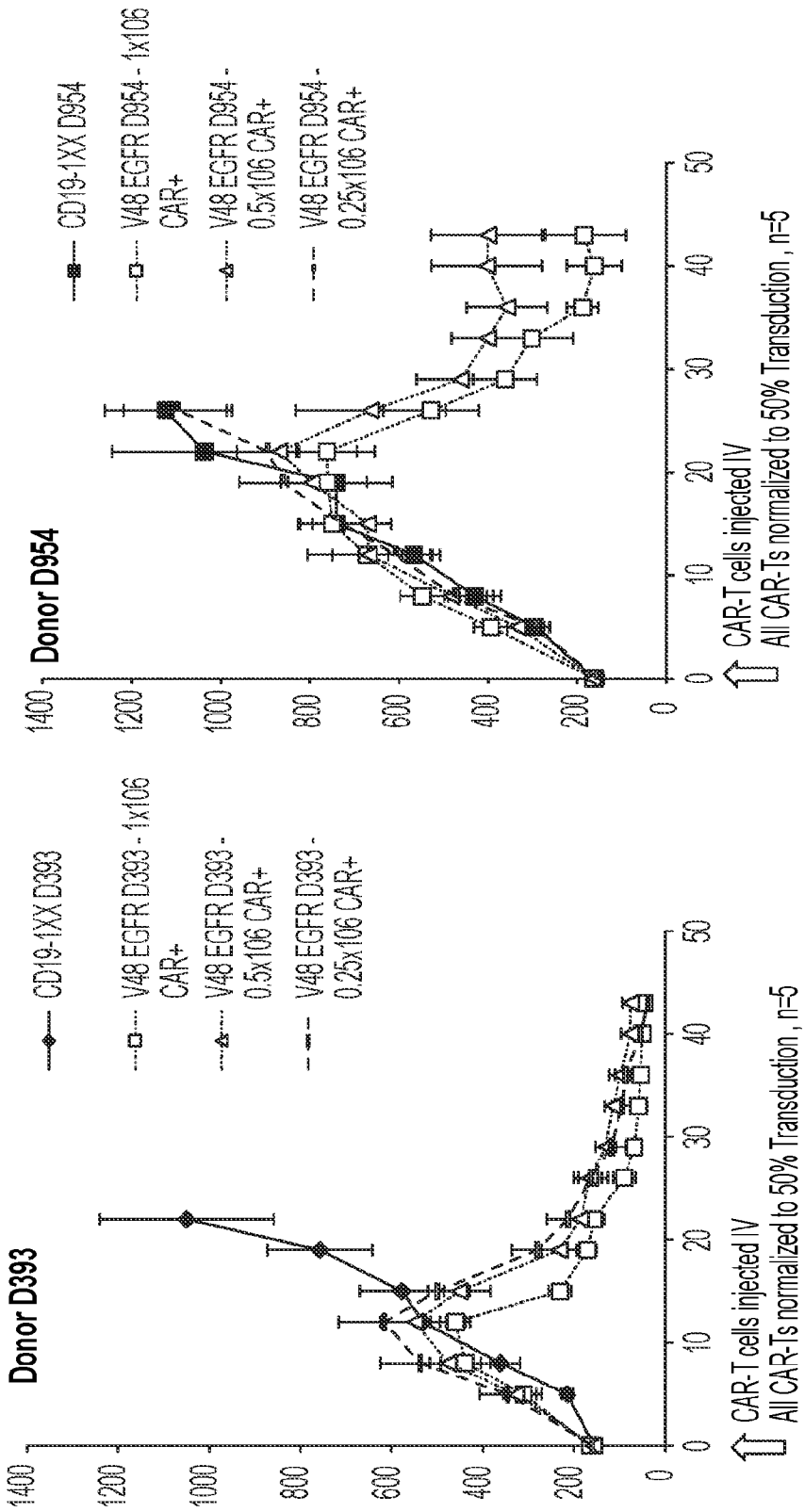


FIG. 12A

FIG. 12B

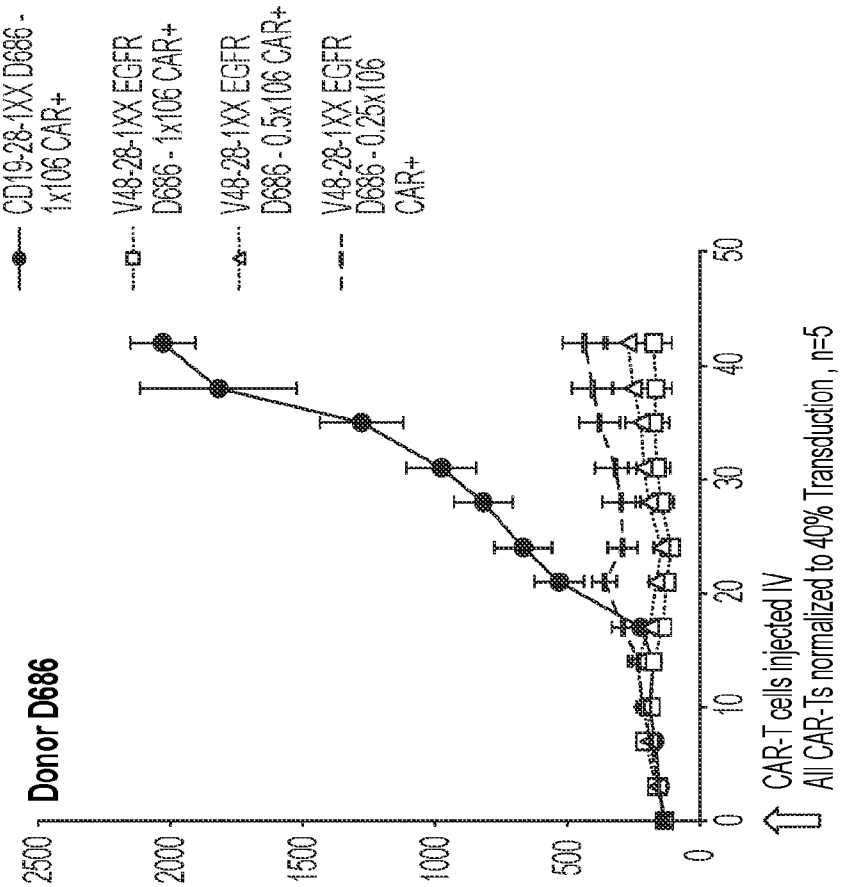

Donor D686
CD19-28-1XX D686 - 1x106 CAR+
V48-28-1XX EGFR D686 - 1x106 CAR+
V48-28-1XX EGFR D686 - 0.5x106 CAR+
V48-28-1XX EGFR D686 - 0.25x106 CAR+
2500
2000
1500
1000
500
0
0
10
20
30
40
50
CAR-T cells injected IV
All CAR-Ts normalized to 40% Transduction , n=5

FIG. 12C

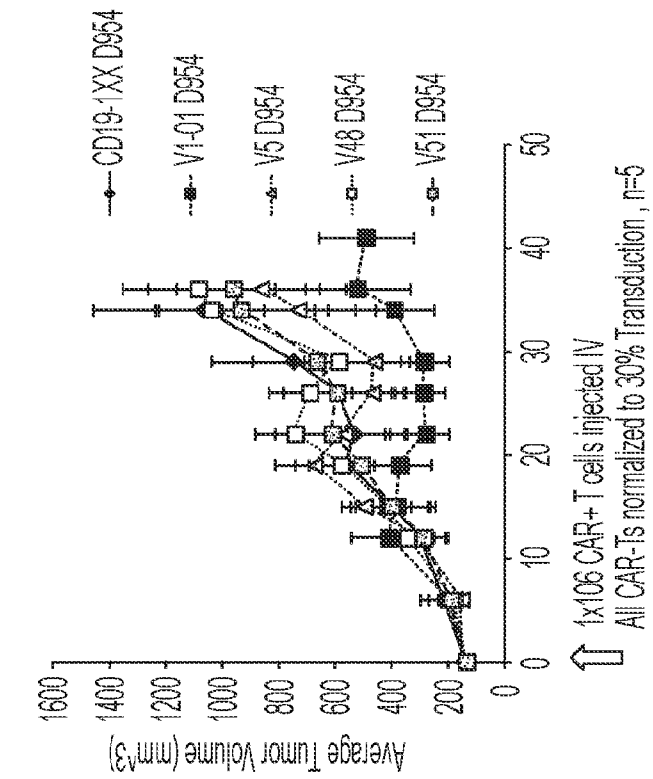


FIG. 13B

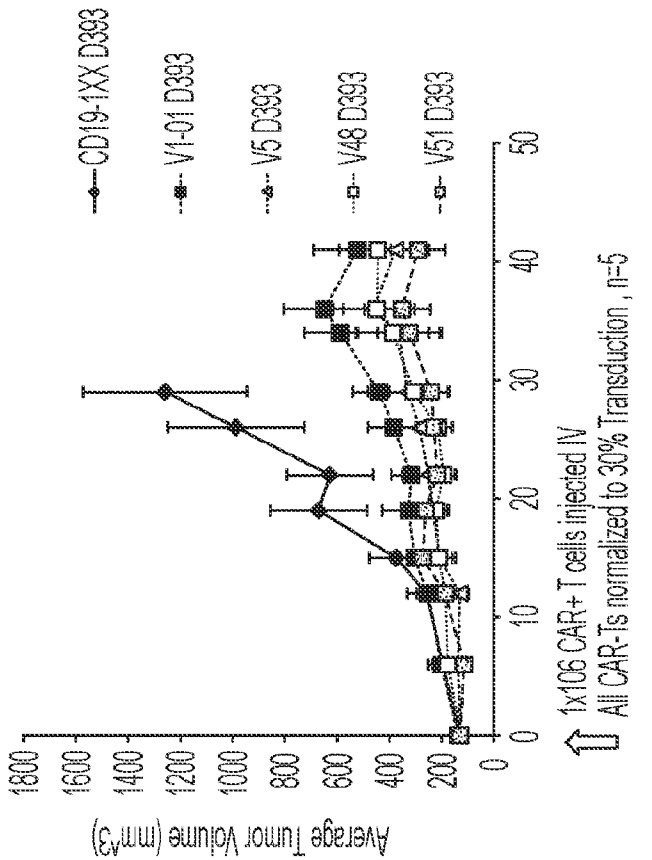


FIG. 13A

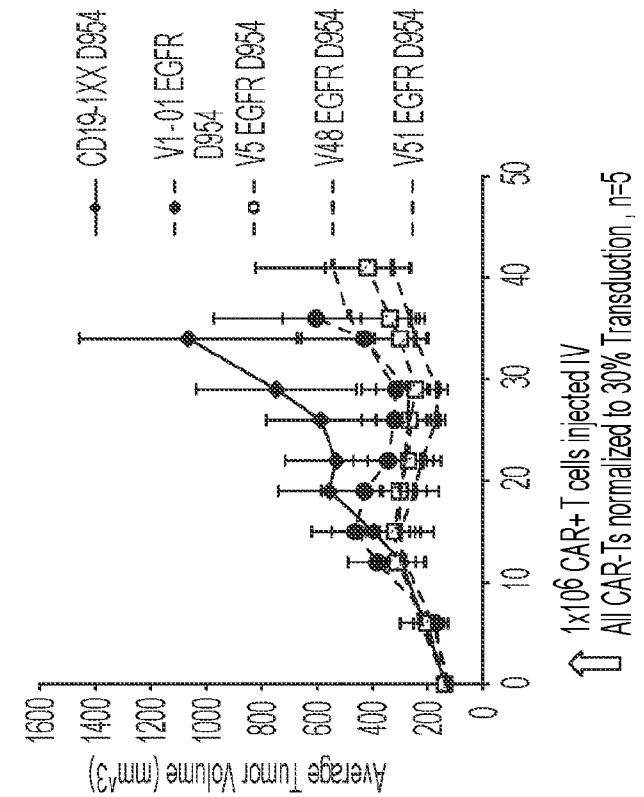


FIG. 14B

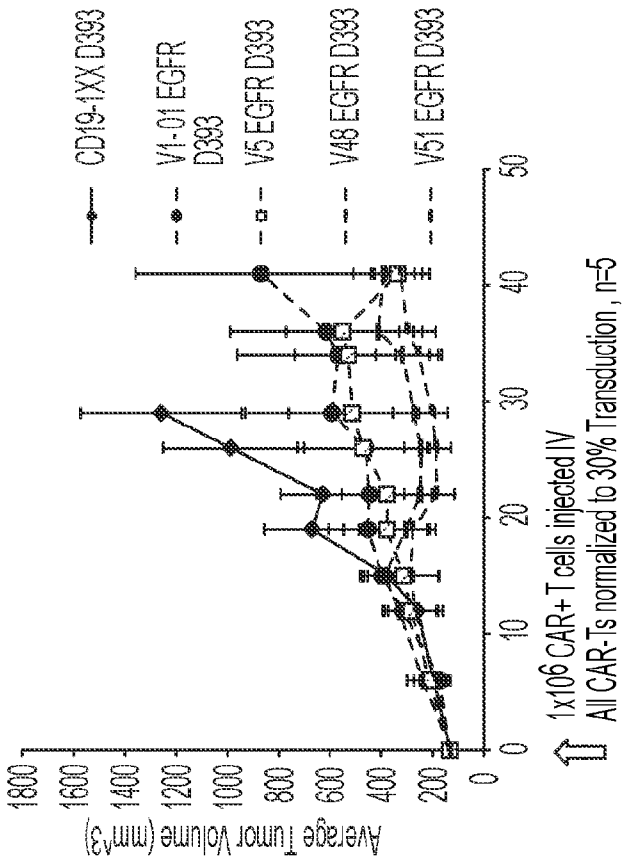


FIG. 14A

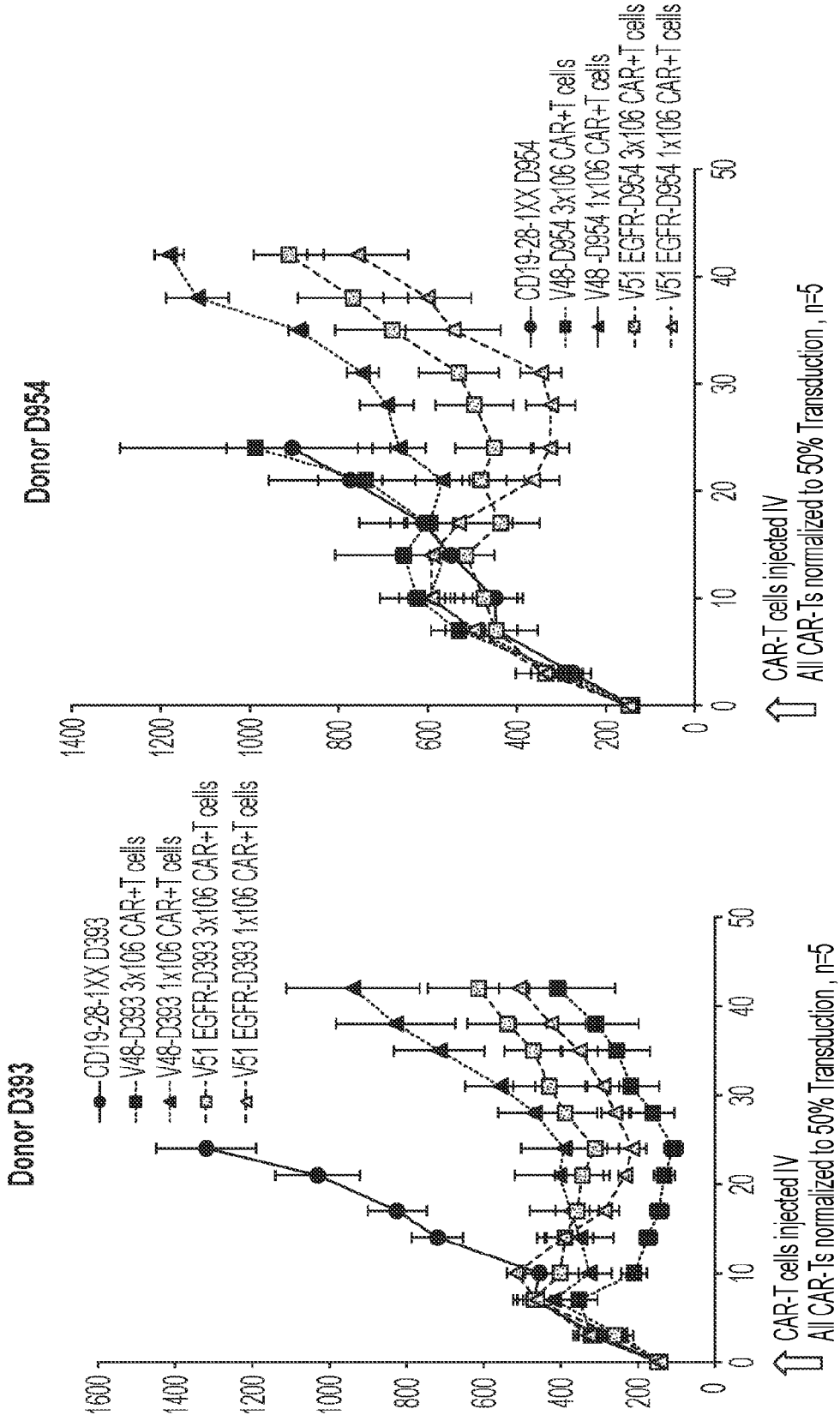


FIG. 15A

FIG. 15B

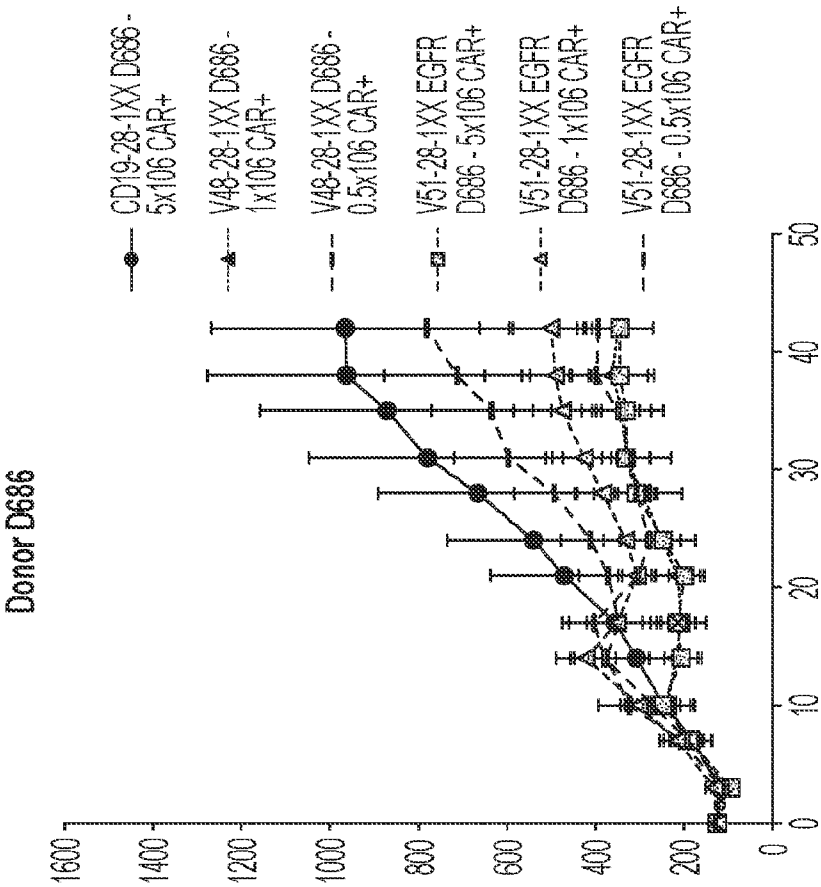

Donor D686
CD19-28-1XX D686 - 5x106 CAR+
V48-28-1XX D686 - 1x106 CAR+
V48-28-1XX D686 - 0.5x106 CAR+
V51-28-1XX EGFR D686 - 5x106 CAR+
V51-28-1XX EGFR D686 - 1x106 CAR+
V51-28-1XX EGFR D686 - 0.5x106 CAR+
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Day
CAR-T cells injected IV
All CAR-Ts normalized to 40% Transduction , n=5

FIG. 15C

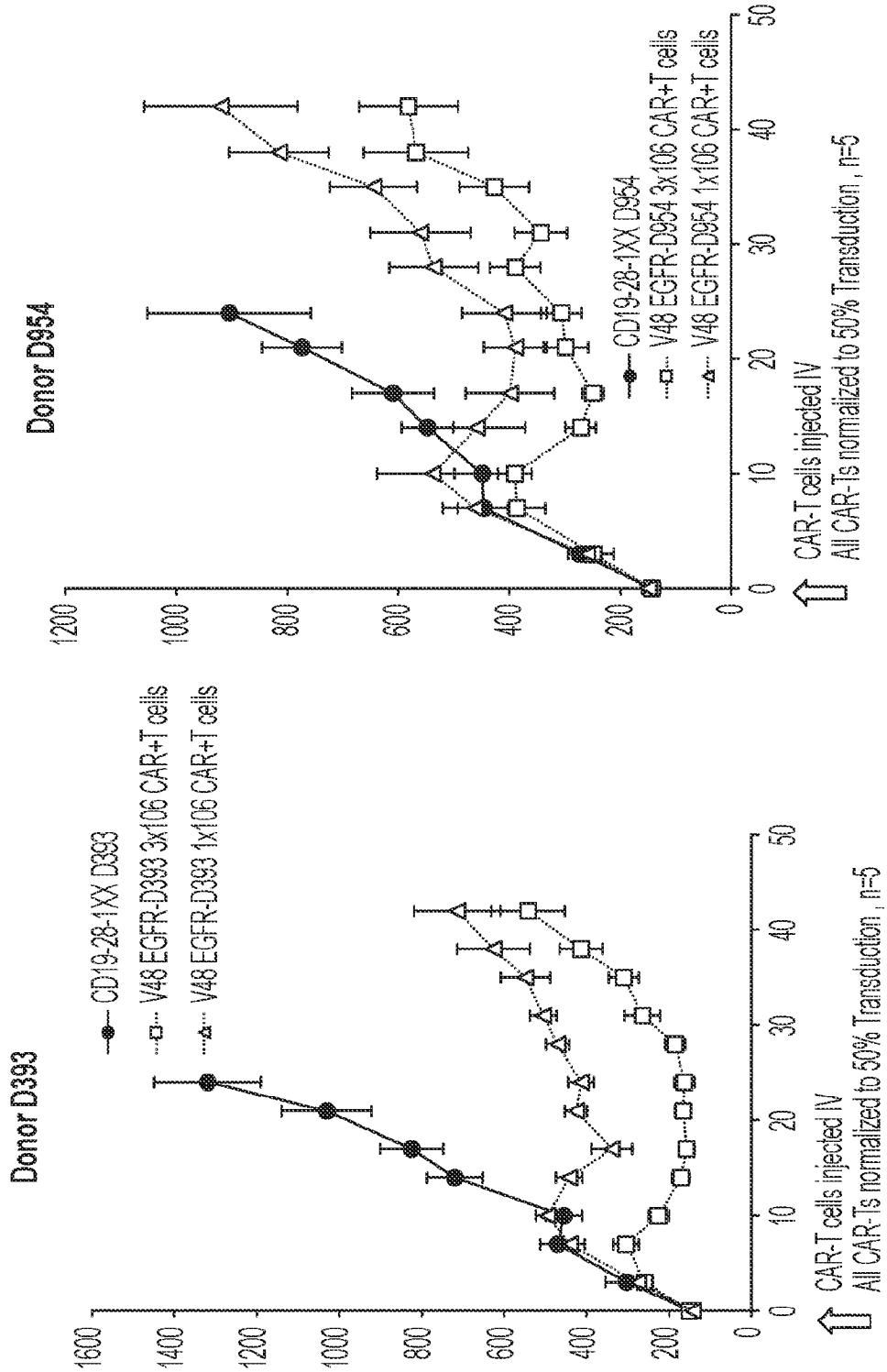


FIG. 16A

FIG. 16B

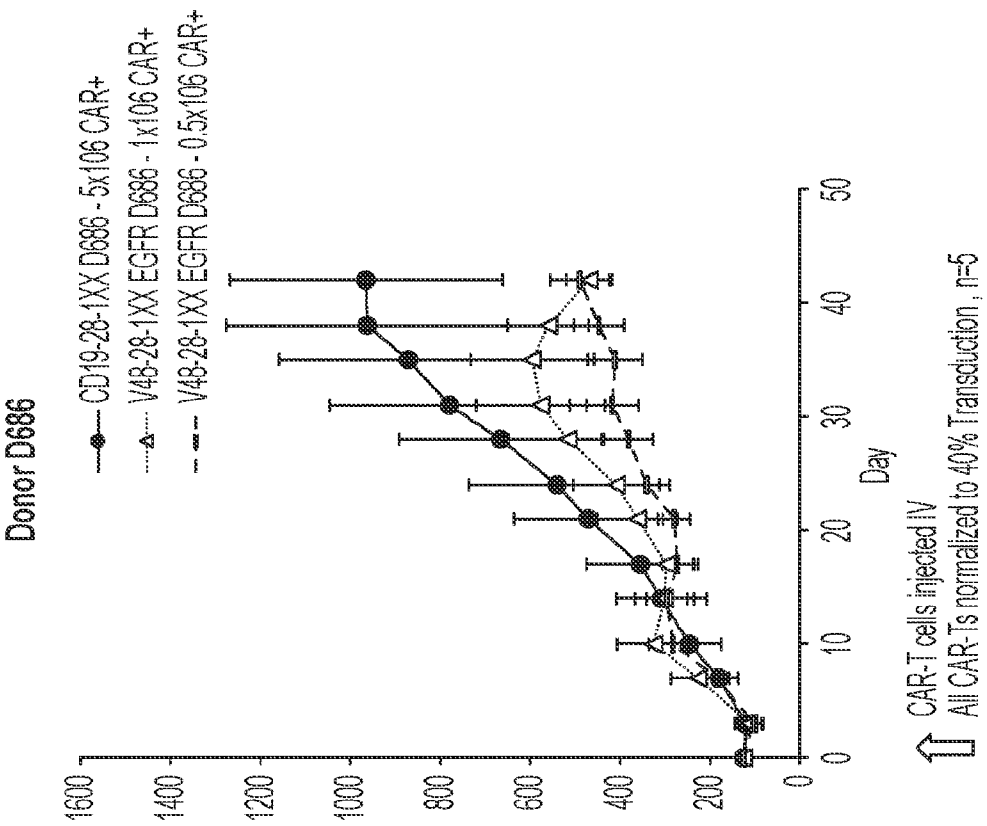

Donor D686
CD19-28-1XX D686 - 5x106 CAR+
V48-28-1XX EGFR D686 - 1x106 CAR+
V48-28-1XX EGFR D686 - 0.5x106 CAR+
1600
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Day
CAR-T cells injected IV
All CAR-Ts normalized to 40% Transduction , n=5

FIG. 16C

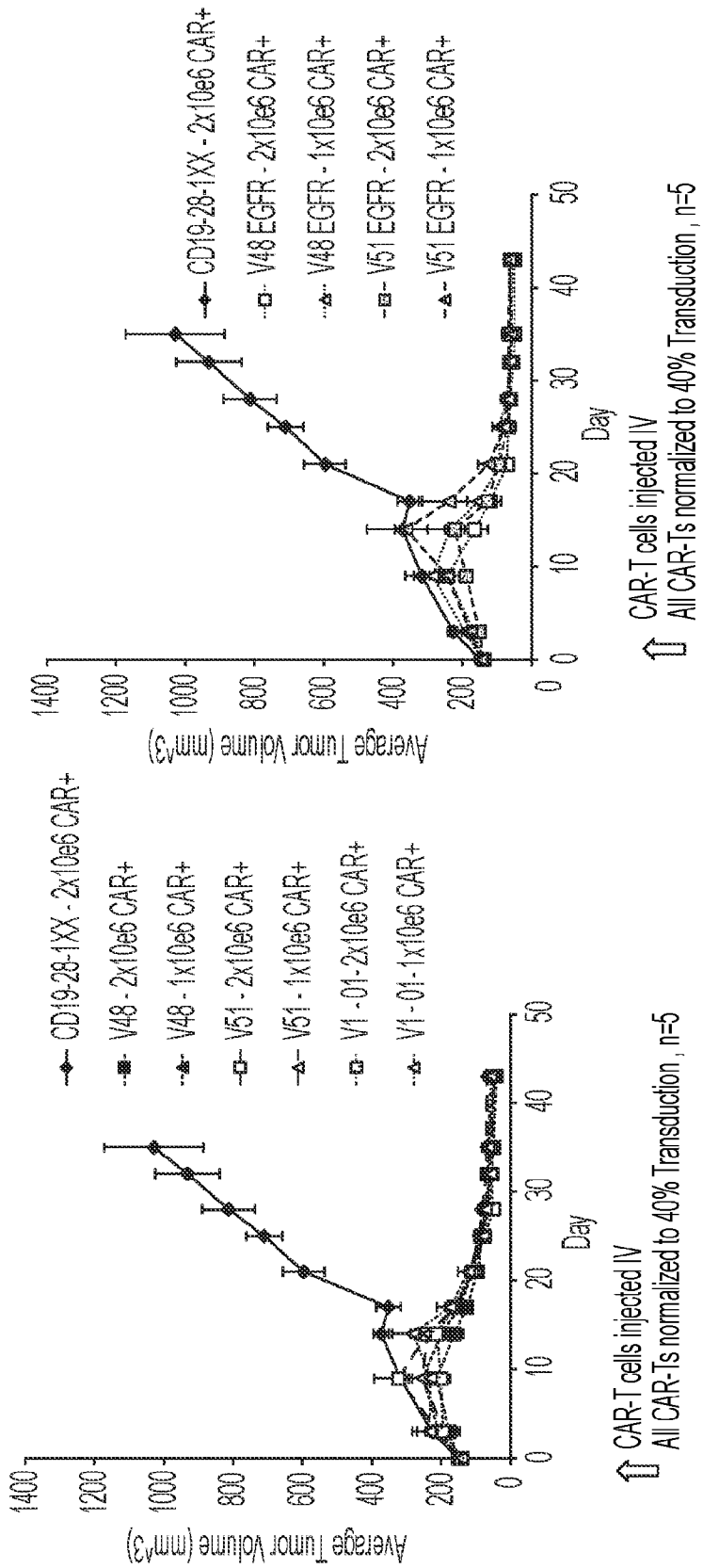

Average Tumor Volume (mm^3)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Day
CD19-28-1XX - 2x10e6 CAR+
V48 - 2x10e6 CAR+
V48 - 1x10e6 CAR+
V51 - 2x10e6 CAR+
V51 - 1x10e6 CAR+
V1 - 01- 2x10e6 CAR+
V1 - 01- 1x10e6 CAR+
CAR-T cells injected IV
All CAR-Ts normalized to 40% Transduction , n=5

FIG. 17

Average Tumor Volume (mm^3)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
Day
CD19-28-1XX - 2x10e6 CAR+
V48 EGFR - 2x10e6 CAR+
V48 EGFR - 1x10e6 CAR+
V51 EGFR - 2x10e6 CAR+
V51 EGFR - 1x10e6 CAR+
CAR-T cells injected IV
All CAR-Ts normalized to 40% Transduction , n=5

FIG. 18

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/000873

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 C07K14/725 A61K39/00 A61K35/17 A61P35/00
ADD.

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)
C07K A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2019/178580 A1 (UNIV JEFFERSON [US]) 19 September 2019 (2019-09-19)	1,6,8,9, 11-13, 15-17, 19,22, 29-33, 35-41
Y	the whole document in particular, pages 3, 8, 12-14, 16,17, 38	2-4,7, 10,14, 18,20,21

-/--

[X] Further documents are listed in the continuation of Box C.

[X] 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

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"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	Date of mailing of the international search report
6 April 2022	19/04/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pérez-Mato, Isabel

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2021/000873

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CN 108 949 789 A (SHANDONG XINRUI BIOTECHNOLOGY CO LTD) 7 December 2018 (2018-12-07)	1,6,8,9, 11-13, 15-17, 19,22, 26,27, 29-33, 35-41
Y	the whole document in particular, figure on page 13 and page 5	2-4,21

X	WO 2017/172981 A2 (UNIV SOUTHERN CALIFORNIA [US]) 5 October 2017 (2017-10-05)	1,5,6,8, 11-13, 15-17, 23-28,34
Y	the whole document in particular, 16-20, 134, 194-197, 203, 619 and 636-637, and page 230	2-4,21

X	MICHAEL S. MAGEE ET AL: "GUCY2C-directed CAR-T cells oppose colorectal cancer metastases without autoimmunity", ONCOIMMUNOLOGY, vol. 5, no. 10, 2 September 2016 (2016-09-02), page e1227897, XP055624726, DOI: 10.1080/2162402X.2016.1227897	1,6, 11-13, 15-17, 19,37-41
Y	the whole document in particular, figure 2 and page 3	2-4,21

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